

# Head Structures of *Priacma serrata* Leconte (Coleoptera, Archostemata) Inferred From X-ray Tomography

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**ABSTRACT** Internal and external features of the head of *Priacma serrata* were studied with X-ray microtomography and with histological methods. The comparison of both techniques shows that X-ray tomography is a promising new technique for the investigation of insect anatomy. The still somewhat coarse resolution of the X-ray data is compensated for by advantages like the nondestructive and artifact-free data acquisition. The head of *P. serrata* and other adults of Archostemata is characterized by many derived features. Muscular features of *Priacma*, especially muscles of the labium and pharynx, differ strongly from what is found in other groups of Coleoptera. Several character states are considered as autapomorphies of Archostemata: scale-like surface structures, constricted neck, strongly reduced tentorium, and the plate-like, enlarged prementum. The scales provide a protecting surface pattern and may have evolved with a more exposed lifestyle. The enlarged prementum forms a lid, which closes the mouth and covers the ligula when it is pulled back by contraction of the unusually strong submento-premental muscle. The presence of four cone-

shaped protuberances on the dorsal side of the head is considered an autapomorphy of Cupedidae. The galea with a narrow stalk and a round and pubescent distal galeomere is another autapomorphy of this family. It has probably evolved as an adaptation to pollen-feeding. The shape of the mandible of Cupedidae is plesiomorphic compared to what is found in adults of Ommatidae. The vertical arrangement of apical teeth is an autapomorphy of the latter family. The lateral insertion of the antenna in *Priacma* is a groundplan feature of Cupedidae. The dorsal shift is a synapomorphy of all other cupetid genera. A cladistic analysis of characters of the head and additional data resulted in the following branching pattern: ((*Crowsoniella* + (*Omma* + *Tetraphalerus*)) + (*Micromalthus* + (*Priacma* + (*Paracupes* + (*Cupes* + *Tenomerga* + *Prolixocupes* + *Rhipsideigma* + *Distocupes* + (*Adinolepis* + *Ascioplaga*)))))). *J. Morphol.* 252:298–314, 2002.

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*Priacma serrata* belongs to Cupedidae, one of four or five extant families of the Archostemata. The earliest fossil species of Cupedidae are approximately 250 million years old (Permian; Sakmarian; Labandeira, 1994). Cupedidae is the oldest extant beetle family according to the presently available fossil record and Archostemata the oldest suborder of Coleoptera (Lawrence, 1999). The extant Archostemata retain a number of primitive characters, the most obvious of which is the reticulation of the elytra. This feature was already present in the oldest known beetles from 280-million-year-old sediments of the lower Permian (Ponomarenko, 1969; Beutel, 1997). Other probably ancestral characters include the largely exposed propleura and prothoracic trochantin, the relatively large mesothorax with a transverse suture on the ventrite, and the presence of several thoracic muscles that are absent from all other beetles (Baehr, 1975; Beutel and Haas, 2000). Nevertheless, there are also derived characters, especially in the morphology of the larvae (Beutel, 1997) and in the male genital armatures (Edwards, 1953a,b; Neboiss, 1984), that allow us to identify the Archostemata as a monophyletic taxon.

Knowledge of Archostemata is very limited, despite the obvious phylogenetic importance of the group. Available data on internal morphology and biology are restricted to very few representatives. As far as adult morphology is concerned, *Priacma serrata* is the best investigated species because the males can easily be collected in large numbers (Edwards, 1951; Atkins, 1957). Besides the thoracic morphology (Baehr, 1975), the male genitalia were examined by Edwards (1953a,b) and the flight is also well documented (Atkins, 1958). Nevertheless,

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even for this species, considerable information is wanting. Females of *P. serrata* are very rare and their genitalia are yet undescribed. Only the first-instar larva is known (Ross and Potheary, 1970). From the publications of Edwards (1951, 1953a,b), Atkins (1957, 1958), Roth and Potheary (1970), and from the personal experience of the authors one can summarize the knowledge of the biology of *P. serrata* as follows: The species has an activity period of approximately 4 weeks each year. This period usually starts in spring or early summer with calm, sunny weather and temperatures above 15°C. During these 4 weeks *Priacma* males are attracted to solutions of sodium hypochlorite and sodium chlorate and can be collected in large numbers. It is unknown whether these 4 weeks are the pairing season of the species and adult beetles are around for a longer time of the year or whether adult beetles are only present during this period. There seems to be an affinity of *Priacma* to conifers, because wherever *Priacma* was collected there were conifers (often Douglas fir) in the vicinity. A direct proof of such an association is still missing. There is no information available about the food sources of larvae and adults.

Head morphology is also largely unknown, especially internal features. It was demonstrated in earlier studies that head structures are not only of great importance in terms of coordination of the central nervous system, sensory perception, and food processing, but also provide much phylogenetic information due to their complexity (e.g., Beutel, 1997). Therefore, the main goal of this contribution is to provide anatomical data on the head of *Priacma*, to compare the findings with characters of other archostematan representatives, to offer functional interpretations, and to give a preliminary phylogenetic interpretation.

To investigate head structures of *Priacma serrata*, an innovative technology—X-ray tomography—was applied for the first time in insect morphology and compared with other techniques. X-ray tomography, besides its application for medical purposes, has so far mainly been used to investigate bone and tooth structures in vertebrates (Rüegsegger et al., 1996; Bjørndal et al., 1999; Barou et al., 1999). The reasons for these restrictions are that mineralized tissues give distinctly better X-ray images than soft tissues and that the spatial resolution of the available X-ray equipment did not allow the investigation of smaller organisms. Due to the usage of phase contrast and improvements in the spatial resolution this method could also be used for studies of soft tissues (Beckmann et al., 1999) and very small structures like the inner ear of the mouse (Van Spaendonck et al., 2000). Important advantages of X-ray tomography over other methods are the possibility of a nondestructive investigation of internal structures and the avoidance of preparation artifacts. There are no such problems as the deforma-

tion of slices during the cutting process or the necessity to align the slices correctly. This makes it relatively easy to reconstruct the X-ray data into a three-dimensional model of the structures of interest. Further development in the achievable spatial resolution now makes it possible to use X-ray tomography for the study of insect morphology, as is demonstrated in the following.

## MATERIALS AND METHODS

### Materials: Fixed Specimens

*Priacma serrata* LeConte, 1861, from Montana, USA, collected by one of the authors (T.H.).

### Dried Specimens From Museum Collections

*Adinolepis youanga* (Neboiss, 1960), from Australia, Australian National Insect Collection, Australia.

*Distocupes varians* (Lea, 1902), from Australia, Australian National Insect Collection, Australia.

*Omma stanleyi* (Newman, 1839), from Australia, Australian National Insect Collection, Australia.

*Priacma serrata* (LeConte, 1861), from USA, Museum of Comparative Zoology, Cambridge, MA, USA; Los Angeles County Natural History Museum, USA; Field Museum of Natural History, Chicago, IL, USA.

*Prolixocupes latreillei* (Solier, 1894), from Chile, Staatliches Museum für Tierkunde, Dresden, Germany.

*Prolixocupes lobiceps* (LeConte, 1874), from Los Angeles County Natural History Museum, USA.

*Rhypsodeigma cretacaetincta* (Kolbe, 1897), from Tanzania, Africa, Field Museum of Natural History, Chicago, IL, USA.

*Rhypsodeigma lugubris* (Fairmaire, 1895), from Madagascar, Field Museum of Natural History, Chicago, IL, USA.

*Rhypsodeigma raffrayi* (Faimaire, 1884), from Madagascar, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany.

*Tetraphalerus bruchi* (Heller, 1913), from Argentina, holotype, Staatliches Museum für Tierkunde, Dresden, Germany.

Some of the dry specimens were dissected (*Omma stanleyi*, *Distocupes varians*). Others were used to compare the external morphology of head capsule and its appendages.

### Methods

All specimens of *Priacma serrata* were collected directly into alcoholic Bouin's solution (=Duboscq-Brasil) (Romeis, 1989: 97). After 2 days they were transferred to 80% ethanol. The specimens for sectioning were softened in Diaphanol (Romeis, 1968:

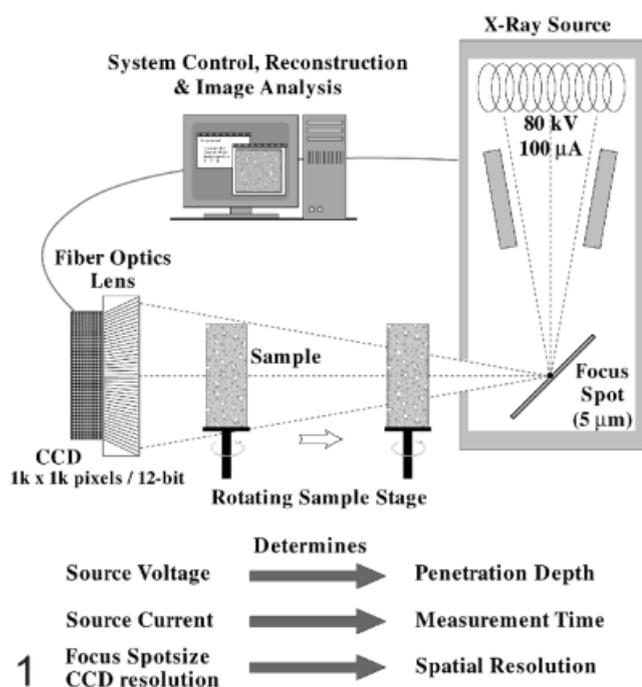


Fig. 1. System setup for X-ray computer tomography.

556) for 5–8 days and then transferred to Paraplast. Sectioning ( $5\ \mu\text{m}$ ) was carried out with a Jung-Universal-Mikrotom 1140/Autocut with C-steel knives. After mounting, slices were stained with a modified Azan-technique (Romeis, 1968: 245, 501). One set of longitudinal sections and one set of cross-sections were produced. The histological sections were used to corroborate and complete the X-ray data.

The specimens for the X-ray tomography were transferred to 100% ethanol and dried in a Balzer CPD 030 Critical Point Dryer. The dry specimens were mounted with soft wax to the sample stage of the X-ray machine.

All measurements and reconstructions were made with a Skyscan 1072 high-resolution Micro-CT system. MicroCT (computerized tomography) is a method for nondestructive 3D X-ray microscopy. The basic principles of the technique are similar to those used in medical CT-scanners. Recent improvements in the development of X-ray sources and CCD-detectors now make it possible to achieve a resolution down to a few microns.

The Skyscan Micro-CT system (Fig. 1) consists of a sealed microfocus X-ray tube (80 kV,  $100\ \mu\text{A}$ ,  $5\ \mu\text{m}$  spotsize), a fiber-optics coupled, cooled CCD-detector (field of view  $22 \times 22\ \text{mm}$ ,  $1024 \times 1024$  pixels, 12-bit A/D conversion), a precision object manipulator, and a dual-processor computer for system control and reconstruction of cross-sections.

The measurement is performed by placing the object on the specimen stage between the X-ray

source and the CCD detector. As the source produces a cone beam of X-rays, magnification is achieved by moving the object towards the X-ray source (Fig. 1). After positioning, 2D X-ray images (radiography) are acquired from 400 angles over  $180^\circ$  (total acquisition time about 30 min), and saved in the computer memory. Then cross-sections of the object are reconstructed by applying the “filtered back-projection algorithm for fan-beam geometries.” This algorithm combines all angular information for every line in the camera to generate a cross-section of the object (reconstruction time about 20 sec per cross-section). With this method one can reconstruct up to 1024 (size of the CCD detector) cross-sections of the object, with a lateral resolution, and a slice-to-slice distance down to a few microns. For the dataset used for this study the pixel size is  $5.429\ \mu\text{m}$  and the cross-section to cross-section distance is  $10.858\ \mu\text{m}$ . The magnification is  $44.07\times$ .

The 3D reconstruction and analysis of the X-ray data was done with the Research Systems NOESYS software package on a dual Pentium III 550 MHz computer. The NOESYS software allows the recombination of the X-ray sections into a 3D model of the object. This model can be manipulated in different ways. It is possible to rotate the model around all three axes and to change the magnification. One can also remove parts of the dataset to look into the beetle. Since the X-ray data only represent absorption values it is necessary to apply “false” color-tables to the dataset to produce grayscale or color images. Via the color-tables it is also possible to change the opacity of selected colors or absorption values.

Character analysis was carried out with PAUP (v. 3.1; Swofford, 1991). Minimal length cladograms were sought with the branch and bound method. The outgroup taxa (*Sialis* sp., *Trachypachus holmbergi* Mannerheim 1853) were treated as all other groups in the analysis (simultaneous analysis; Nixon and Carpenter, 1993). Analysis of character evolution was conducted in MacClade (v. 3; Maddison and Maddison, 1992).

The muscles are named and numbered according to the nomenclature of Kéler (1963).

## RESULTS

### Head capsule (Fig. 2)

The size of *Priacma serrata* varies from ca. 10–22 mm total length with head lengths between 1.5–2.8 mm ( $n = 70$ ). The head is approximately 1.2 times longer than wide and 1.6 times longer than high. The complete surface is densely covered with small, rounded tubercles, each with an articulated scale (Fig. 2A–C). The color of the scales is light to darker gray to gray-brown. The cuticle underneath is dark brown in most specimens examined. The postgenal area is prolonged and delimited from the anterior

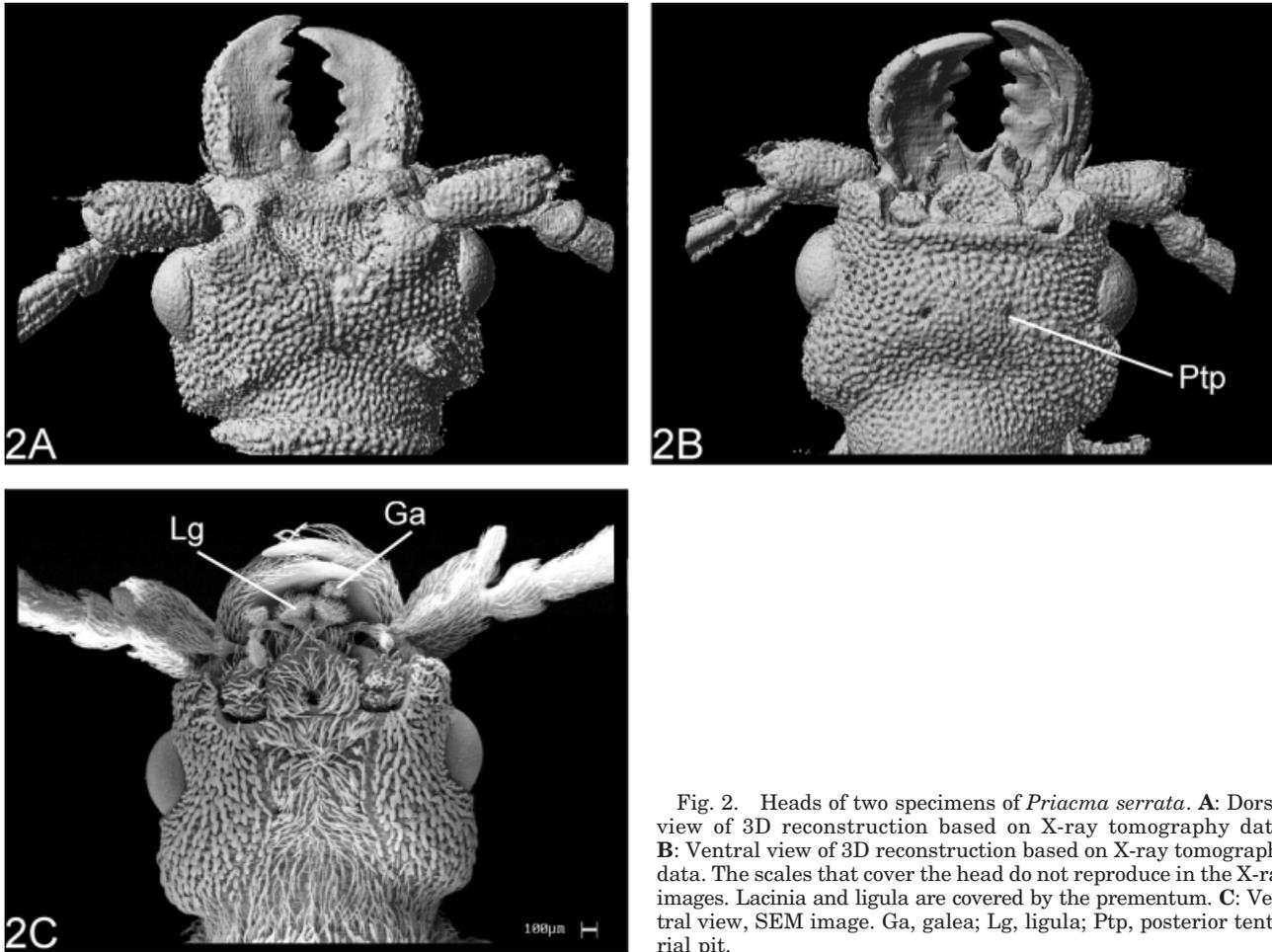


Fig. 2. Heads of two specimens of *Priacma serrata*. **A:** Dorsal view of 3D reconstruction based on X-ray tomography data. **B:** Ventral view of 3D reconstruction based on X-ray tomography data. The scales that cover the head do not reproduce in the X-ray images. Lacinia and ligula are covered by the prementum. **C:** Ventral view, SEM image. Ga, galea; Lg, ligula; Ptp, posterior tentorial pit.

part of the head by an abrupt constriction, thus creating a distinct neck region. The posterior part of the head forms a kind of ball-and-socket joint with the prothorax. The exposed part of the head (without mandibles) is slightly shorter than wide (length/width = 0.82). The genae are separated by a broad submental region that is completely integrated in the ventral head capsule. The eyes are round, with a diameter approximately equal to the length of the postocular genal region. The antennae are inserted laterally, above the dorsal joint of the mandible. Its articulation almost completely fills the space between the eyes and the anterior border of the head capsule. Supra- or subocular antennal furrows are absent. Two pairs of prominent, pointed protuberances are present posterior to the antennal bases and posterior to the compound eyes. The tentorium is strongly reduced. The posterior tentorial arms are short and membranous and connected with the gular ridges. The dorsal arms are also membranous and serve as attachment area for the antennal muscles. The anterior tentorial pits are very small and lie on the ventral rim of the antennal insertion. The anterior tentorial arms are thin, straight, sclerotized rods. They are strongly shortened, scarcely

extending beyond the antennal socket posteriorly and not connected with the posterior tentorium. No muscles are attached to the anterior tentorial arms. The gula is long, parallel-sided and moderately broad. A gular suture is not visible externally.

#### Labrum (Fig. 2A)

The labrum is moveably connected to the head-capsule. It is about one-third as wide as the head. Its anterior border is deeply indented medially. The surface is covered with long, anteriorly directed hairs.

*Musculus* (= *M.*) *labroepipharyngalis* (7). This is a pair of small muscles consisting of only a few muscle fibers. They originate laterally on the dorsal wall of the labrum and insert medially on the ventral wall (epipharynx). The fibers of each muscle are arranged in line from about the middle of the labrum to its base with the insertion more caudad than the origin (tilted forward). The muscle pair works as levator of the epipharynx.

*M. frontoepipharyngalis*: absent.

### Antenna (Figs. 2, 4, 7)

The antenna is inserted laterally and composed of 11 antennomeres, which are densely covered with hairs. The segments are all similar in size with the exception of scapus and pedicellus. The scapus is two to three times as long as the pedicellus and distinctly wider. The pedicellus is as wide as the following antennomeres and about half as long.

The antenna is moved by three muscles associated with the scapus and two muscles that are attached to the pedicellus (Figs. 4, 7, 9).

*M. tentorioscapalis anterior* (1). This muscle is the strongest of the three antennal muscles. It has its origin on the proximal end of the dorsal tentorial arm. It inserts via a thin tendon anteroventrally on the base of the scapus and functions as protractor of the antenna.

*M. tentorioscapalis posterior* (2). This muscle has its origin on the dorsal end of the dorsal tentorial arm dorsad of *M. ts. medialis*. It inserts with a short tendon on the base of the scapus. *M. ts. posterior* is the principal antennal retractor.

*M. tentorioscapalis medialis* (4). Originates on the dorsal tentorial arm dorsad of *M. ts. anterior*. It inserts dorsocaudally on the basal rim of the scapus. The function of this muscle is to move the antenna backward and upward.

*M. scapopedicellaris lateralis* (5). Originates on the posterior wall near the base of the scapus and inserts dorsocaudally at the base of the pedicellus. It moves the flagellum backward and upward.

*M. scapopedicellaris medialis* (6). This muscle is composed of two parts. One part is slightly longer and more slender than *M. sp. lateralis*. It originates on the anterior wall close to the base of the scapus and inserts anteroventrally on the base of the pedicellus. The second part originates on the posterior median to basal wall of the scapus. It inserts on the pedicellus together with part one. Both parts together move the flagellum anteriorly.

### Mandible (Figs. 2, 6, 8)

The mandible is nearly as long as the head capsule. In addition to the apex, four distinct teeth are arranged in a horizontal row along the mesal cutting edge. Their size decreases from anterior to posterior. Mola and lacinia mobilis are absent. The ventral and dorsal surface are smooth without hairs or scales. The outer surface is sculptured with small elevations and tubercles like most of the body parts and is densely covered with hairs, which increase in length towards the apex.

*M. craniomandibularis internus* (11). This muscle is the adductor of the mandible and the largest head muscle (Figs. 5, 6). It is connected to the mandible by a large, forked tendon-plate. The muscle is composed of a dorsal and a ventral portion. The dorsal portion is organized in two parts. The external part

of the dorsal portion originates on the lateral and dorsal area of the head from just behind the eye (circumocular ridge) to about the posterior third of the head. It inserts on the dorsal surface of the tendon-plate, which is connected via a broad base to the posterior median part of the mandible.

The internal part of the dorsal portion of *M. 11* has its origin on the dorsal and lateral area of the head caudally of the external part. It covers the area from about the middle of the head to the postoccipital ridge. The smaller part of it inserts directly on the median anterior corner of the tendon-plate. The rest is connected via the dorsal branch of the tendon-plate.

The ventral portion of *M. 11* originates on the lateroventral wall of the head from about the middle to the postoccipital ridge. Its median boundary is formed by the gula ridge. Laterodorsally it extends to just below the dorsal portion of *M. 11*. The ventral portion of *M. 11* inserts on the ventral surface of the tendon-plate.

*M. craniomandibularis externus* (12). The muscle is distinctly smaller than *M. 11*. It originates with a wide base on the ventral half of the lateral wall of the head from about the middle of the eye to the constriction of the head. It inserts via a long tendon at the lateral posterior corner of the mandible, halfway between the dorsal and ventral joints. *M. 12* is the abductor of the mandible.

### Maxilla (Figs. 2, 3B,C, 9A)

The externally visible part of the cardo is small and semicircular. It is inserted in a small and shallow maxillary groove. Scales and hairs are present but the surface is slightly smoother than that of the proximal stipes. The apodeme for attachment of the cranial cardinal extensor is short and Y-shaped. The stipes is separated by a distinct suture into two equally sized parts: the proximal basistipes, which is connected with the cardo, and the mediostipes with the lacinia, palpifer and the insertion of the galea. The basistipes is sculptured like the rest of the body surface with slender scales and hairs, whereas the surface of the mediostipes is largely smooth, with only a few short sensillae on the dorsal surface. The maxillary palpus is four-segmented and inserts on a large palpifer which is laterally connected with the mediostipes. All segments are about equally sized, cylindrical and about twice as long as wide. Palpomeres II–IV are covered with long strong setae, whereas only four to six setae are present on the dorsal and ventral side of the distal end of palpomere I. Two fields of sensillae are present on the apical palpomere: an apical field, with many short sensillae (not more than twice as long as wide) and a round, shallow pit approximately at half-length on the dorsal side of the palpomere. This area contains 11 sensillae, which are about eight times as long as wide. The galea is composed of a smooth, sclerotized stalk, about as long as palpomeres I+II and an

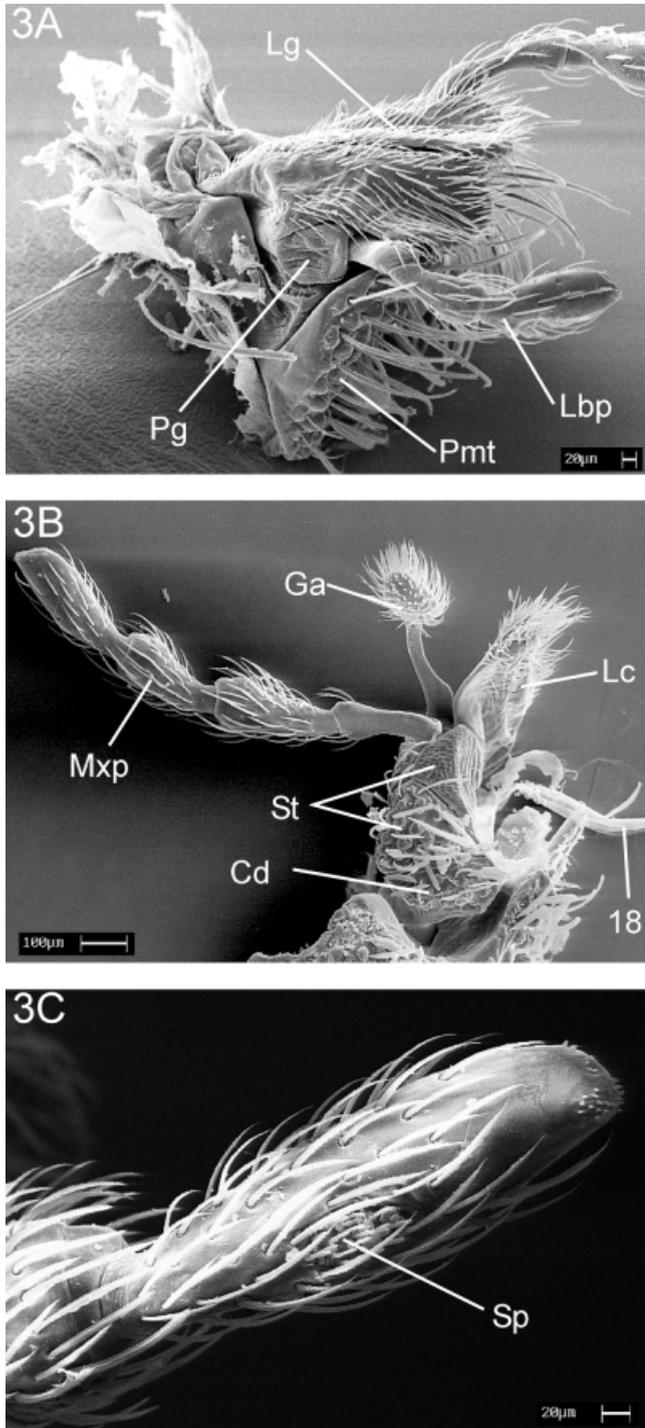


Fig. 3. SEM images of mouthparts of *Priacma serrata*. **A:** Isolated labium and hypopharynx, dorsolateral view. **B:** Isolated maxilla, ventral view. **C:** Apical maxillary palpomere with short apical sensillae and dorsolateral field of long sensillae. Cd, cardo; Ga, galea; Lbp, labial palp; Lc, lacinia; Lg, ligula; Mxp, maxillary palp; Pg, palpiger; Pmt, prementum; Sp, sensory pit; St, stipes.

onion-shaped, soft, pubescent club. The lacinia is as long as the galea and spade-shaped. The apical and oral surfaces are covered with long hairs.

*M. craniocardinalis externus* (15). A slender muscle with the origin on the head capsule exactly in front and laterad of the base of the posterior tentorial arm. Inserts on the distal tip of the apodeme of the cardo. Musculus craniocardinalis internus (16) is missing.

*M. tentoriocardinalis* (17). This muscle is stronger than M. 15. It has its origin on the lateral side of the base of the posterior tentorial arm. The insertion lies on the proximal tip of the cardo apodeme.

*M. tentoriostipitalis* (18). A slender muscle with the origin on the median side of the posterior tentorial arm and the insertion in the posterior median area of the stipes.

*M. craniolaciniialis* (19). Musculus craniolaciniialis is stronger than *M. tentoriostipitalis*. It has an unusual origin between M. 15 and the posterior tentorial arm. The muscle inserts on the lateral posterior corner of the lacinia.

There are no internal muscles of the lacinia [*M. stipitolaciniialis* (20)] or the galea [*M. stipitogalealis* (21)].

*M. stipitopalpalis externus/internus* (22/23). Only one muscle is present at the base of the palp. Considering its insertion on the external margin of the palpal base, homology with *M. s. externus* appears likely. The origin of the muscle lies on the posterior ventral wall of the stipes.

The distal muscles of the maxillary palp, *M. palpopalpalis tertius* (26) and *M. p. quartus* (27), are present. *M. p. maxillae primus* (24) and *M. p. secundus* (25) are missing.

### Labium (Figs. 2, 3A, 4, 7, 8, 9A)

The submentum is fused to the head capsule. Its posterior margin is marked by the posterior tentorial pits. The mentum is reduced to a membrane connecting prementum and submentum. The prementum is formed as a half-round sclerite with a prominent central apodeme. This apodeme is visible externally as a deep pit. The lateral margins of the prementum are bent inwards and prolonged as lateral apodemes. The outer surface is sculptured like the rest of the head surface and is covered with many long hairs and some slender scales. The scales are located on the posterior corners of the sclerite. The prementum functions as a lid that completely covers the mouth and the ligula (the fused glossae and paraglossae), and the galeae and laciniae when they are pulled back. The palps are three-segmented and insert on a distinct palpiger. The apex of the first palpomere, the distal half of the second, and the complete apical palpomere bear some long hairs. The sensillae on the apical palpomere are concentrated in two areas. They are short in the apical area, whereas eight to ten long, apically rounded sensillae are arranged in a shallow round pit in the dorsolateral area. Dorsal and ventral surface of the ligula are covered with fine long hairs.

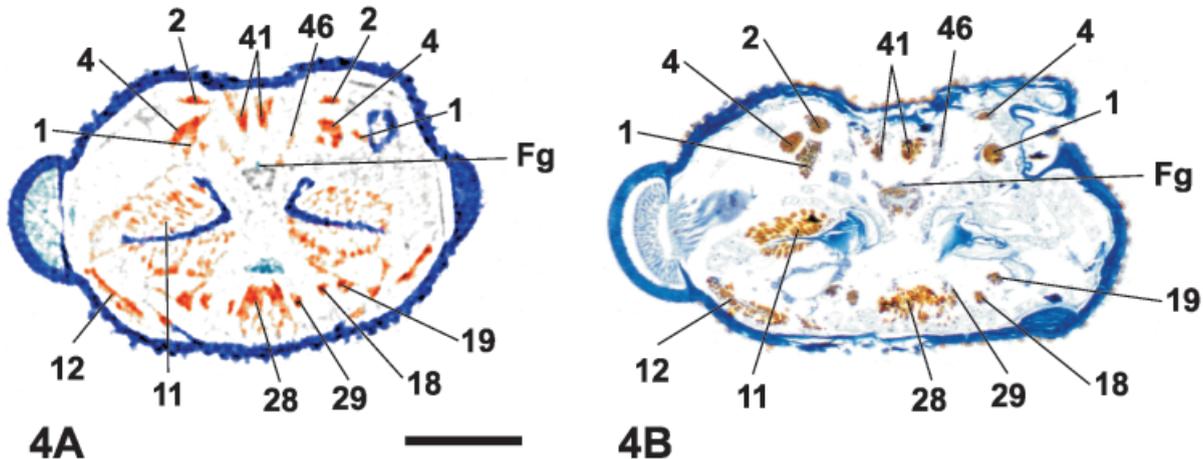


Fig. 4. Cross-sections of the head of *Priacma serrata*. **A:** False-color representation of reconstructed cross-section from X-ray tomography data. Colors are adopted from the Azan-staining used for histological sections. The section is not compressed or otherwise deformed and the tissue is not detached from the cuticle. **B:** Histological cross-section, Azan-staining. Note the compression of the section. Detachment of tissue from the cuticle (e.g., in the eye) is due to artifact. Fg, frontal ganglion. Scale bar = 0.5 mm.

*M. submentopraementalis* (28). A pair of large muscles that originates with a broad base left and right of the median line of the submentum just in front of the posterior tentorial arms. The muscles insert side by side on the apex of the central apodeme of the prementum and function as retractors of the prementum.

*M. tentoriopraementalis inferior* (29). A pair of slender muscles originating on the bases of the posterior tentorial arms. They insert ventrolaterad of the lateral apodemes of the prementum close to the palpigers. Contraction of the muscles probably moves the palps and the prementum.

*M. tentoriopraementalis superior* (30). A pair of muscles, similar in size to *M. 29*. They originate on the tentorial arms above and behind *M. 29*. The muscles pass over the apex of the central apodeme of the prementum and insert in the middle of its dorsal surface close to the base of the ligula.

*M. praementoparaglossalis/prae mentoglossalis* (31/32). In the ligula there is only one pair of muscles present. The origin lies lateral at half length on the prementum and the insertion dorsolateral in the ligula close to its apex. This muscle pair works as retractor of the ligula.

*M. praementopalpalis externus* (34). The muscle originates on the dorsolateral surface of the central apodeme of the prementum and inserts on the lateral base of the first palpomere.

The internal muscles of the labial palpus *M. palpopalpalis labii primus* (35) and *M. palpopalpalis labii secundus* (36) are both present.

### Hypopharynx (Figs. 3A, 6, 8, 9A)

The hypopharynx is not developed as a separate protruding structure but is fused with the anterior part of the labium and forms a functional unit with it.

*M. frontohypopharyngalis* (41). A pair of strong muscles that originate from a small apodeme high on the frons slightly craniad of the vertex. They insert by means of a tendon on the fulturae and function as retractor muscles of the mouth angles.

*M. tentoriohypopharyngalis*: absent.

### Cibarium (Figs. 6, 8)

The cibarium is open laterally, i.e., a closed prepharyngeal tube, formed by lateral fusion of the posterior hypopharynx and epipharynx, is absent.

*M. clypeopalatalis* (43). A pair of slender muscles originating from the epistomal area (border clypeus–frons) and inserting lateral on the dorsal wall of the cibarium, directly caudad of the epipharynx. The points of origin are slightly more separated than the insertions. The muscle pair works as dilator of the cibarium.

### Pharynx (Figs. 4, 6, 7, 8)

Fairly wide, approximately round in cross section.

The anterior pharynx has a set of longitudinal muscles and strong circular muscles, especially in the area below the frontal ganglion. Two muscles function as dilators of the anterior pharynx:

*M. frontobuccalis anterior* (45). The origin of this thin muscle lies on the clypeus-frontal border laterad of *M. clypeopalatalis*. From there it extends mediad of *M. frontohypopharyngalis* to the lateral dorsal wall of the pharynx, where it inserts closely behind the frontal ganglion.

*M. frontobuccalis posterior* (46). This muscle is distinctly larger than *M. frontobuccalis anterior*. The origin lies on the dorsal wall of the head capsule behind and slightly laterad of *M. f. anterior*. From

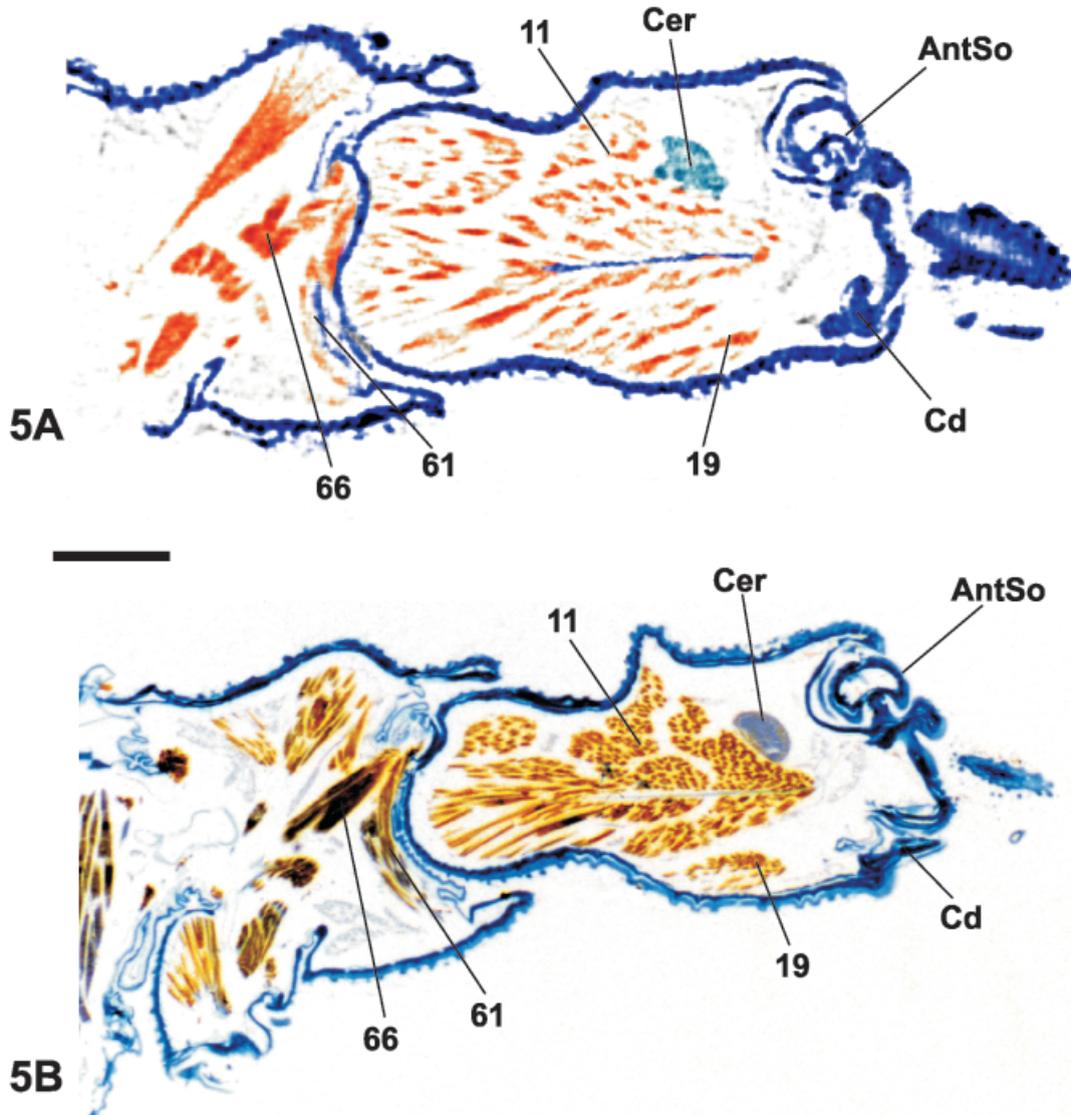


Fig. 5. Sagittal sections of head and prothorax of *Priacma serrata*. **A**: False-color representation of reconstructed section from X-ray tomography data. Colors adopted from Azan-staining used for histological sections. **B**: Histological section, Azan-staining. The different shape, if compared to **A**, results from a slightly oblique cutting plane. M. 11 detached from cuticle in the posterior part of the head. AntSo, antennal socket; Cd, cardo; Cer, cerebrum. Scale bar = 0.5 mm.

here, *M. f. posterior* extends laterad of *M. frontohypopharyngalis* to the insertion on the lateral dorsal wall of the pharynx in front of the cerebrum.

*M. verticopharyngalis* (51). The postcerebral dorsal dilator is absent.

The ventral pharyngeal dilator is composed of a series of muscles which together form a strong complex:

*M. tentoriopharyngalis* (52). A series of fairly thin muscles originates on the gular ridge posterad of the suboesophageal ganglion and from the lateral parts of the gula. They insert ventrolaterally on the posterior and anterior pharynx.

### Postoccipital region (Figs. 5, 6)

The muscles of the postoccipital and cervical area were described by Baehr (1975). Our reexamination confirms his results. Nine muscles are associated with the movements of the head.

*M. praephragmapostoccipitalis medialis* (55) = M 1 of Baehr (1975). Runs from the praephragma to the mediodorsal margin of the postocciput.

*M. pronotopostoccipitalis medialis* (57) = M 6 of Baehr (1975). Very strong muscle that extends between the pronotal disk and the membrane adjacent to the ventral margin of the postocciput.

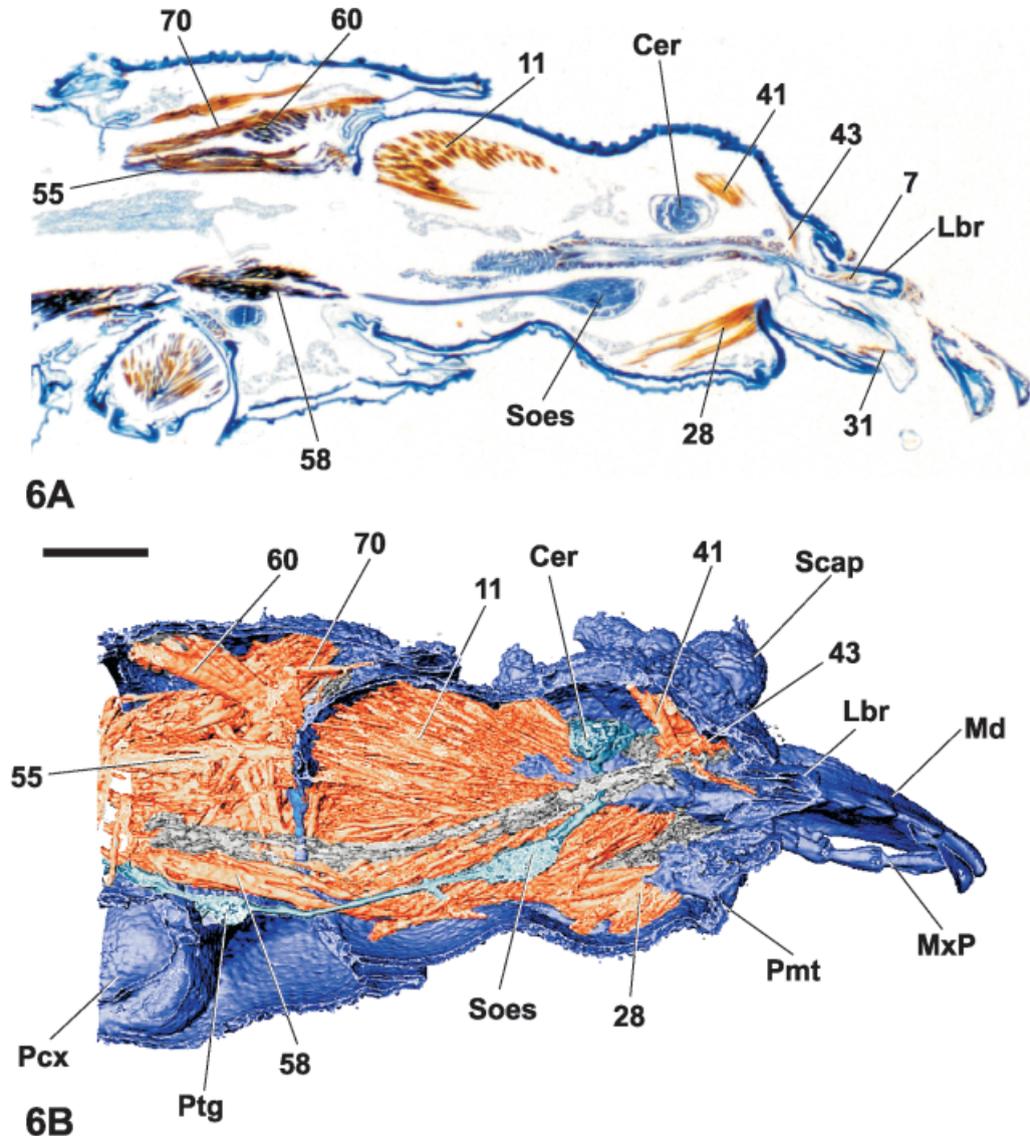


Fig. 6. Sagittal section, head and prothorax of *Priacma serrata*. **A**: Histological section, Azan-staining. M. 11 detached from cuticle. The detachment of the visible part of M. 41 from the protrusion on the upper frons is an artifact. **B**: False-color representation of 3D reconstruction from X-ray tomography data. Colors adopted from Azan-staining. All muscles are attached to the cuticle. Gray structures in the horizontal center line are fragments of the intestine, which does not absorb X-rays well enough to give a clear image. Cer, cerebrum; Lbr, labrum; Md, mandible; MxP, maxillary palp; Pcx, procoxa; Pmt, prementum; Ptg, prothoracic ganglion; Scap, scapus; Soes, subesophageal ganglion. Scale bar = 0.5 mm.

*M. profurcatentorialis* (58) = M 5 of Baehr (1975). Long muscle that originates from the mesal side of the profurca and inserts on the mesal side of the gular ridge in the posterior third of the head.

*M. profuracervicalis* (59) = M 8 of Baehr (1975). This muscle originates from the profurca laterad of M. 58 and is attached to the ventrolateral margin of the postoccipt laterad of M. 57.

*M. pronotocervicalis* (60) = M 2 of Baehr (1975). A fan-shaped muscle with the origin on the posterolateral part of the pronotum. Runs between M. 55 and M. 70 to the dorsolateral margin of the postoccipt laterad of M. 55.

*M. cervicopostoccipitalis* (61) = M 9 of Baehr (1975). The wide and strong muscle originates in the antero-lateral area of the sternum, immediately posterad of the neck-membrane. It inserts on the dorsolateral margin of the postoccipt ventrolaterad of M. 60.

*M. propleuracervicalis* (64) = M 7 of Baehr (1975). The very thin muscle originates from the lateral caudal area of the pronotum and inserts laterally on the neck-membrane.

*M. profuracervicalis* (66) = M 10 of Baehr (1975). Fairly strong muscle that originates on the profurca laterad of M. 59 and inserts on the dorsolateral margin of the postoccipt laterad of M. 61.

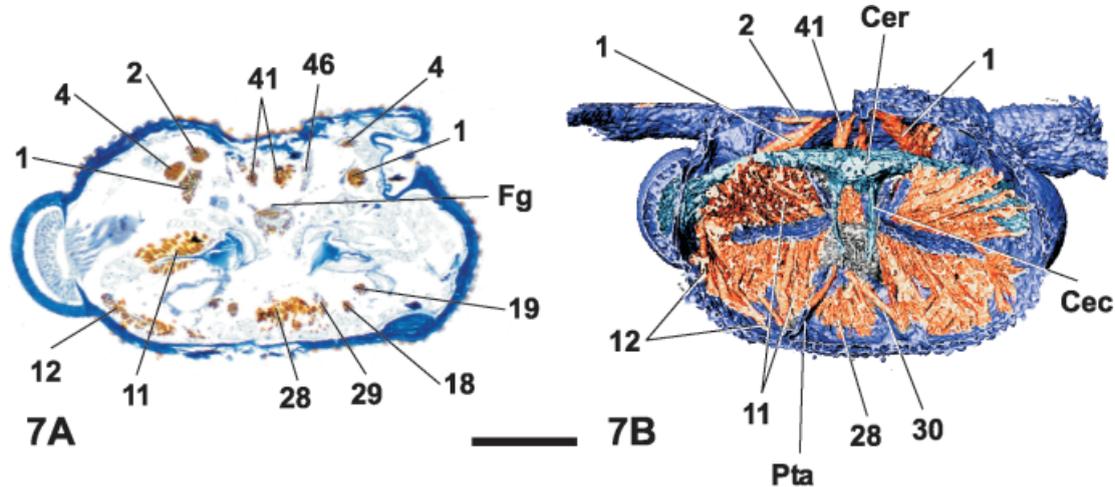


Fig. 7. Cross sections of head of *Priacma serrata*. **A:** Histological section, Azan-staining. Eye tissue detached from cuticle, section slightly compressed. **B:** False-color representation of 3D reconstruction from X-ray tomography data. Colors adopted from Azan-staining. Left half of dorsal head capsule removed. All muscles attached to their points of origin and insertion. No deformation visible. Cec, circumoesophageal connective; Cer, cerebrum; Fg, frontal ganglion; Pta, posterior tentorial arm. Scale bar = 0.5 mm.

*M. conjunctiva-antecostalis* (70) = M 3 of Baehr (1975). Originates on the prophragma laterad of M. 55 and runs laterad of M. 60 to the insertion on the neck-membrane slightly dorsolaterad of M. 55.

#### Cerebrum and Suboesophageal Ganglion (Figs. 5, 6, 8)

The cerebrum is comparatively small in relation to the head size and located between the compound eyes. The suboesophageal ganglion is fairly slender and laterally enclosed by the gular ridges.

#### Glands (Fig. 8)

A compact gland is present in the posterior labio-hypopharyngeal region.

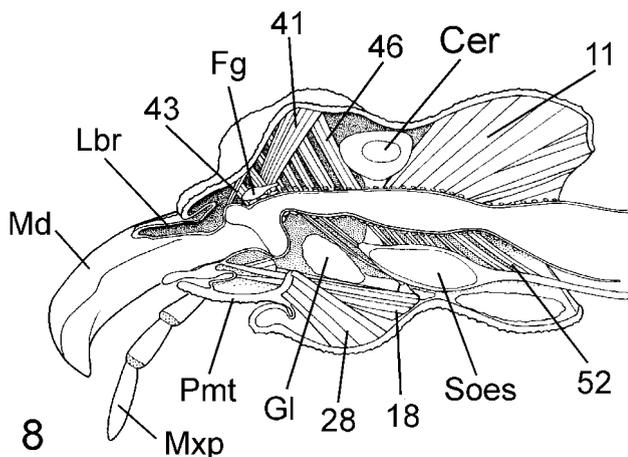


Fig. 8. Head of *Priacma serrata*, sagittal section, manual reconstruction. Cer, cerebrum; Fg, frontal ganglion; Gl, gland; Lbr, labrum; Md, mandible; Mxp, maxillary palp; Pmt, prementum; Soes, suboesophageal ganglion.

#### Results of the Phylogenetic Analysis (List of Characters, see Appendix A; Character State Matrix, see Appendix B; Fig. 10A,B)

Branch and bound search resulted in 621 trees with a minimal number of 65 steps (CI: 0.862; HI 0.138, RC 0.768). Monophyletic groups (represented in all trees) and apomorphies (unambiguous unless otherwise noted) are listed below.

Archostemata: scales present (1.1), constricted neck region (2.1; accelerated transformation), partly reduced tentorium (6.1), plate-like prementum (11.1), dorsal and ventral emarginations of head capsule (21.1), frontal suture absent in later instars (23.1), larval mandible with three apical teeth (26.1), larval ligula sclerotized and enlarged (29.1), submentum of larvae fused with mentum, constricted between maxillary grooves (30.1), abdominal segments I–III of larvae combined longer than thorax (31.1), tergal ampullae of larvae (33.1), segment X of larvae not visible externally (39.1).

Ommatidae: mandibular apex rotated, spoon-like, teeth arranged in vertical row (9.1; delayed transformation = DEL), apical palpomeres with deep cavity (12.2; DEL), mesepisternal condyle and propleural socket (17.2; DEL).

Micromalthidae + Cupedidae: abdominal sternites overlapping (19.1), stemmata reduced to one pair or absent (24.1), antennal length of larvae less than 20% of head capsule (25.1), retinaculum of larval mandible absent (27.1), mola of larval mandible quadrangular, with distinct margin (28.1), sternal asperities (34.1), larval tergum IX with sclerotized process (36.1), segment IX of larvae with ventrolateral eversible lobes (37.1), wood-associated habits of larvae (40.1).

Cupedidae: four cone-shaped protuberances on dorsal side of head (3.2), galea with narrow stalk

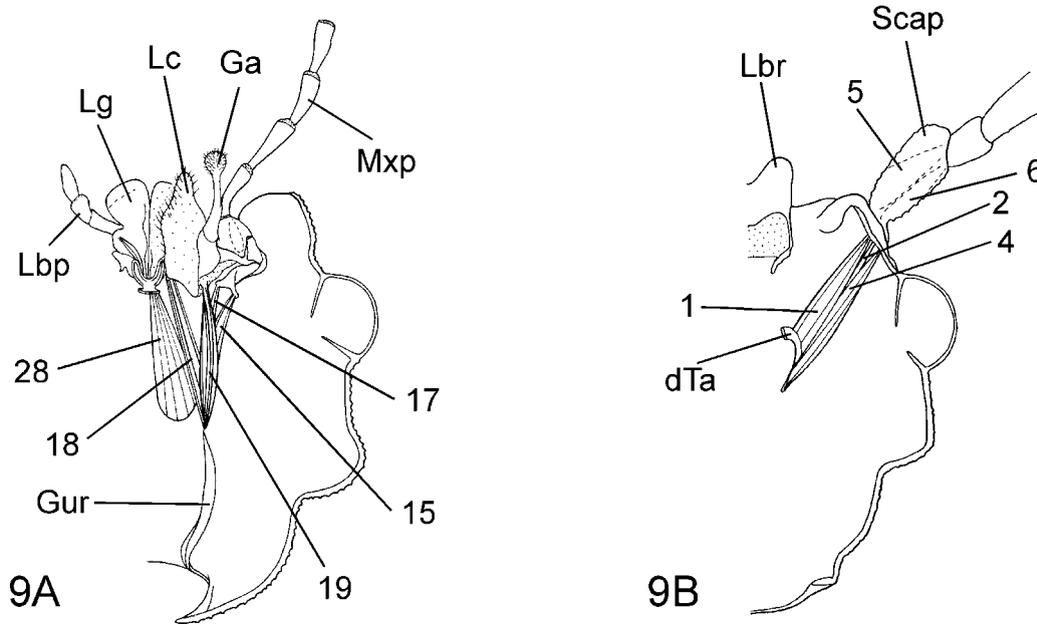


Fig. 9. Head of *Priacma serrata*, horizontal section. **A:** Ventral part, maxilla, labium, gular ridge. **B:** Dorsal part, labrum, antennal muscles. Ga, galea; Gur, gula ridge; Lbp, labial palp; Lc, lacinia; Lg, ligula; Mxp, maxillary palp.

and rounded, pubescent distal part (10.1), prosteral process reaching beyond hind margin of procoxae (15.1), propleural condyle, and mesepisternal socket (17.1), penultimate tarsomere broadened (18.1; CI: 0.500).

Cupedidae excl. *Priacma*: antennal insertion shifted dorsally (8.1), anteromedian pit of mesoventrite present (16.1).

Cupedidae excl. *Priacma* and *Paracupes*: prosteral tarsal groove (14.1).

*Adinolepis* + *Ascioplaga*: posterior protuberances partly reduced (3.1).

## DISCUSSION

### Morphology, Function, and Phylogeny (Fig. 10A,B)

The head and other body parts of adults of *Priacma serrata* differ strongly from corresponding structures of non-archostematan representatives of Coleoptera. An unusual feature among extant beetles is the tuberculate surface structure of the cuticle of all species of Cupedidae and Ommatidae (Figs. 2A,B). The cuticular surface is smooth in most adults of Adephaga, Myxophaga, and Polyphaga, and also in adults of the archostematan families Crowsoniellidae (Pace, 1975) and Micromalthidae. It is likely that this is an apomorphic condition, whereas the tuberculate surface structure is a groundplan feature of Coleoptera. Similar surface modifications are already present in early Permian stem-lineage representatives such as *Sylvacoleus* or

*Tshecardocoleus* (Tshecardocoleidae; Ponomarenko, 1969; Beutel, 1997). Secondary loss of tubercles in *Micromalthus debilis* (only known species of Micromalthidae) and *Crowsoniella relictata* (only known species of Crowsoniellidae) may be the result of miniaturization. Both species are much smaller than any species of Cupedidae and Ommatidae.

Scales and more or less strongly modified setae are another characteristic surface structure of adults of Cupedidae and Ommatidae. This may represent an apomorphic groundplan feature of the suborder even though this interpretation was not supported by the parsimony analysis (see above). Parallel evolution of these very similar structures in adults of Ommatidae and Cupedidae appears less likely than secondary absence in *Crowsoniella relictata* and *Micromalthus debilis*, which may be correlated with small size in both species (see above). Endogeous habits may have played an additional role in *Crowsoniella* and a very short life span and sporadic appearance of adults in *Micromalthus*. Scales have probably evolved with a shift from subcortical habits to a more exposed lifestyle (Lawrence, 1999). They provide a concealing color pattern or even a pattern mimicking harmful insects such as mutillid wasps (*Omma mastersii*; Lawrence, 1999). The tendency towards day-active and floricolous habits in Cupedidae is correlated with an increasing size of the compound eyes (Crowson, 1962). They are still rather small in adults of *Priacma*, but distinctly enlarged in other members of the family.

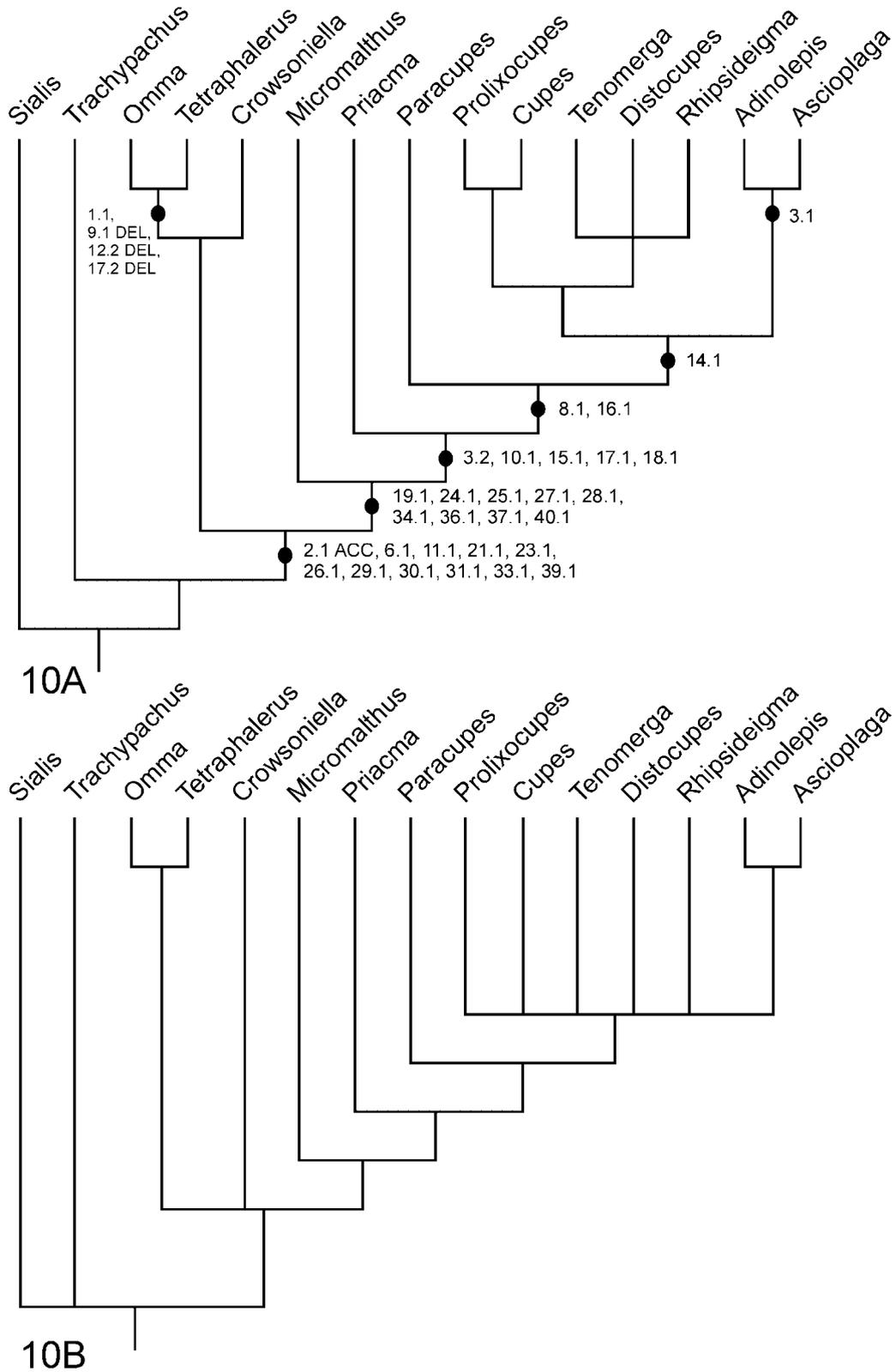


Fig. 10. Trees showing interrelationships of archostematan genera. **A:** Tree 12 of 198 minimal length cladograms. Apomorphies mapped on tree, unambiguous unless otherwise noted. ACC, accelerated transformation; DEL, delayed transformation. **B:** Strict consensus tree.

A well-defined, constricted neck region is present in all adults of Cupedidae, Ommatidae, and *Crowsoniella*. This is likely a derived condition and an autapomorphy for Archostemata (Fig. 10A: char. state 2.1). A similar condition is not found in most representatives of the other suborders of Coleoptera. This interpretation implies that the absence of a distinct neck in *Micromalthus* is a secondary feature.

Protuberances on the dorsal side of the head (Fig. 2A) are a characteristic feature of adult Cupedidae and Tetraperhalerinae. The presence of two more or less conical pairs on the posterior head region of adults of the latter subfamily is probably autapomorphic for this group. One pair posterior to the antennal base and one conical pair between the compound eyes is typical for adults of Cupedidae and likely an autapomorphy of this family (Fig. 10A: char. state 3.2). The posterior pair is completely absent in adults of *Paracupes brasiliensis* and varying degrees of reductions occur in adults of *Ascio-plaga* and *Adinolepis* (Neboiss, 1984; Fig. 10A: char. state 3.3). Protuberances are absent in adults of *Omma*, *Micromalthus*, and *Crowsoniella* and in adults of most other groups of Coleoptera.

The separation of the genae by the submentum in adults of *Priacma* is a plesiomorphic feature, which is also found in adults of most other archostematan genera and in adults of other groups of Coleoptera (Hydraenidae may be an exception; Jäch et al., 2000). Confluent genae as found in adults of *Prolixocupes* (Vulcano and Pereira, 1975) are a derived condition and probably an autapomorphy of this genus (char. state 4.1).

The connection of the posterior tentorial arms with elongate gular ridges is a condition shared with all adult beetles examined, but also with adults of Megaloptera (Röber, 1942: fig. 18). This presumably derived condition is correlated with the presence of a sclerotized gula, which is a shared derived feature of Coleoptera, Megaloptera, and Raphidioptera (e.g., Hennig, 1969; Beutel and Gorb, 2001). The reduced condition of the tentorium is probably an autapomorphy of Archostemata. A very similar condition as that found in adults of *Priacma* is also present in adults of *Omma stanleyi*. A well-developed tentorium is present in the vast majority of representatives of Adephaga, Myxophaga, and Polyphaga.

A labrum connected with the clypeal margin by a membrane is found in adults of *Priacma* and other cupedid genera. This is almost certainly a plesiomorphic feature, which is also found in adults of most other groups of Coleoptera. A labrum fused with the head capsule (*Omma*) or only indistinctly separated from the clypeus (*Tetraperhalerus*) was considered an autapomorphy of Ommatidae by Lawrence (1999). However, a completely fused labrum is also found in the miniaturized adults of *Crowsoniella* (Pace, 1975) and *Micromalthus*. A stronger degree of labral immobilization may be considered as a common ten-

dency in the *Crowsoniella*-Ommatidae lineage. The interpretation of a fully immobilized labrum as a derived groundplan feature of this possible clade (char. state 7.2) implies reversal in *Tetraperhalerus*, which appears rather unlikely.

The absence of both frontoepipharyngeal muscles is a derived condition shared by Archostemata and Adephaga (e.g., Belkaceme, 1991). Apparently the labrum cannot be moved actively in adults of *Priacma*, even though it is not fused with the clypeus. Both muscles are present in adults of Megaloptera (Röber, 1942) and the lateral muscle is preserved in adults of Polyphaga (e.g., Hydraenidae, Hydrophilidae, Meloidae; Schneider, 1981; Jäch et al., 2000; pers. obs., Beutel).

The lateral insertion of the antenna of *Priacma* is a plesiomorphic feature. The same condition is found in adults of Ommatidae, Micromalthidae and *Crowsoniella*, and also in adults of most other groups of Coleoptera. A dorsal insertion with approximate antennal bases was considered as a possible autapomorphy of Cupedidae by Lawrence (1999). However, it appears more likely that this is a shared derived feature of Cupedidae excluding *Priacma* (Fig. 10A: char. state 8.1).

The mandibles of adults of *Priacma* are similar to those of adults of other cupedid genera, even though they are longer than in most other species. The teeth are arranged longitudinally and not in a vertical row, as is the case in adults of Ommatidae and Micromalthidae. The latter condition was considered an autapomorphy of Ommatidae by Lawrence (1999). This interpretation, i.e., independent evolution in Micromalthidae, is supported by the cladistic analysis. The absence of a mola is probably a plesiomorphic feature, which is shared with adults of Adephaga and Megaloptera (Röber, 1942). Consequently, the presence of a mola should be interpreted as an apomorphic groundplan feature of a clade comprising Myxophaga and Polyphaga (Beutel and Haas, 2000).

The maxilla of adults of *Priacma* and other cupedid genera is characterized by small size, a densely pubescent lacinia, and a globulous, pubescent distal galeomere. The latter condition is considered an autapomorphy of Cupedidae (Fig. 10A: char. state 10.1; Lawrence, 1999). It is probably correlated with anthophily (Lawrence, 1999). Pollen grains in the gut of *Cupes capitatus* were reported by Crowson (1962).

The insertion and composition of the maxilla is largely in agreement with what is found in other beetles and this is also largely the case with its musculature. However, one unusual feature is the mesal origin of *M. craniolacinalis* between *M. craniocardinalis* and the posterior tentorial arms. In all other beetles examined, this muscle originates from lateral parts of the head capsule.

A conspicuous feature and a possible autapomorphy of Archostemata is the plate-like prementum with a central apodeme (Fig. 10A: char. state 11.1).

A somewhat similar condition is only found in adults of Melolonthinae (Scarabaeidae; pers. obs., Hörnschemeyer). The mentum is reduced or fused to the submentum in most species of Archostemata, but is still distinct in adults of *Tetraphalerus* (Monros and Monros, 1952: fig. 7) and *Crowsoniella* (Pace, 1975). The labial musculature of *Priacma* and *Omma* also differs clearly from what is found in other beetles. The unusually large M. submentopraementalis does not insert on a fold at the posteromedian premental margin as usual, but at the premental apodeme. Contraction of this muscle lifts the prementum, which then closes the mouth opening like a lid.

The presence of a large ligula is possibly an autapomorphy of Cupedidae (char. state 13.1). It is represented by a pair of simple lobes in *Priacma* (Fig. 9A), by slightly divided lobes in *Prolixocupes* and by three or four separate lobes in *Cupes*, *Tenomerga*, *Adinolepis*, and *Distocupes*. The relatively primitive condition of the ligula in *Priacma* (Fig. 3A) supports a basal position of the genus within Cupedidae.

An elongate prepharyngeal tube as found in adults of Adephega is absent from adults of *Priacma* and *Omma*. Only a short preoral chamber is present anterior to the anatomical mouth (Fig. 8). This is probably a plesiomorphic condition and is correlated with nonpredacious habits. The prepharyngeal musculature is less well developed than in most other beetles. It is only represented by a single pair of small muscles in *Priacma*.

The pharyngeal musculature of adults of *Priacma* differs strongly from what is found in adults of Megaloptera or other groups of Coleoptera. The postcerebral dorsal dilator (M. 51) is absent, whereas the posterior precerebral dilator (M. 46) is unusually strong and comprises a series of bundles (Figs. 4, 7, 8). The posterior ventral dilator is also composed by several subcomponents. M. 46 and M. 52 form a pumping apparatus together with the pharyngeal ring muscles. It is likely to assume that pollen grains are mixed with secretions of the labial glands in the preoral chamber, which is closed by the elevation of the premental plate (M. 28) and then pumped back by the pharyngeal pump. Labial glands as present in adults of *Priacma* are absent from most beetles examined (e.g., Belkaceme, 1991; Jäch et al., 2000). However, similar glands are described for adults of Rhysodidae.

The cervical muscles of *Priacma* are similar to those found in other beetles and insects (Larsén, 1966). They guarantee a high movability of the posteriorly constricted head, which articulates with the prothorax in a ball-and-socket manner.

Beutel and Hörnschemeyer (2001) performed an analysis with a similar set of 13 taxa and a somewhat different composition of characters. Most of their 50 characters (33) refer to larval morphology. The remaining 17 characters are from adult morphology. The result of their analysis is very similar to the results presented herein. They found that

*Omma* and *Tetraphalerus* are sister-groups and *Crowsoniella*, *Omma*, and *Tetraphalerus* together are the sister-group to Cupedidae. Within Cupedidae their interpretation is congruent with our findings, as demonstrated in Fig. 10. *Paracupes*, *Cupes*, *Adinolepis*, and *Ascioploga* were not included in their matrix.

### X-ray Tomography vs. Histology

The comparison of histological sections and X-ray tomography data demonstrates that the currently available X-ray technology is very useful for the study of insect musculature but also has some limitations. The resolution of 5.4  $\mu\text{m}$  per pixel and 10.9  $\mu\text{m}$  section-to-section distance allow a 3D reconstruction of the complete muscle system of the head of *Priacma serrata*.

The main advantages of the X-ray tomography method over standard histological serial sections are:

- 1) All organs and structures are in their natural position. There are no deformations or damages due to the cutting of the specimen (Figs. 4, 5, 6, 7).
- 2) The preparation of specimens involves only fixation and drying. Even drying is not necessary if gradual evaporation of the fixation fluid during the process can be avoided. The time needed to process the specimen into sections is greatly reduced. A typical scan for a *Priacma* specimen took approximately 7 h for the entire dataset. Including 2 days for fixation and drying of fresh specimens, the total processing time is about 3 days. The preparation of the specimens for cutting took about 2 weeks including 1 week softening in Diaphanol. It took another week to cut and mount the sections. Also, there is a high risk of producing artifacts during this preparation process. In specimens treated with Diaphanol the soft tissue is nearly always detached from the cuticle (Figs. 4B, 5B, 6A, 7A).
- 3) If a 3D-reconstruction is based on histological sections (Beutel and Haas, 1998), there is always the problem of aligning the sections correctly. In addition, the sections are almost inevitably mechanically deformed during the sectioning process. These two problems can only be handled manually and the resulting 3D reconstruction is never exactly congruent with the original specimen. In X-ray tomography, these problems do not exist.
- 4) Specimens used for X-ray tomography are still available for further studies. They are not irreversibly transformed like specimens used for sectioning.
- 5) The 3D dataset allows nearly unlimited manipulations like rotation, virtual sections in different axes, cutouts, etc. (depending on the software

used), thus allowing a very detailed study of the specimen without destroying it. These possibilities turned out to be very useful for teaching insect morphology.

At present, the X-ray technology has two major disadvantages:

- 1) Spatial resolution at 3  $\mu\text{m}$  at best is still relatively coarse. With the given resolution specimens with a minimum body size of approximately 2 mm can be studied satisfactorily as long as only the skeleton, the musculature, and the major parts of the nervous system are of interest. Musculature and nerves of the appendages are problematic even in bigger specimens. Some tissues (i.e., fat, glands, intestine, etc.) absorb X-ray radiation only in such a minor degree that no useful image of these tissues can be obtained without special staining. Unfortunately, at present there are no useful staining methods available.
- 2) Even if the structures give a strong enough signal to produce an image, it is sometimes very difficult to interpret this image correctly. All parts of the insect including the skeleton consist of organic carbon compounds. For the X-ray picture, this means that all structures have very similar or even identical absorption values. Consequently, it is not possible to distinguish different tissues in the X-ray images. This can lead to wrong interpretation of structural features (e.g., orientation of muscles). In the head of *Priacma*, *M. tentoriopraementalis superior* (30) passes over the central apodeme of the prementum between its origin on the posterior tentorial arm and its insertion. From the point where it touches the apodeme to its actual insertion the muscle is not visible as an individual structure in the X-ray images. Thus it appears as if the muscle would insert on the apex of the apodeme. Such problems can occur whenever two structures lie very closely together.

## CONCLUSIONS

*Priacma* and other representatives of Archostemata are characterized by many derived features of the head, which are partly due to a more exposed lifestyle and partly to specialized pollen-feeding habits. Archostemata, Ommatidae and Cupedidae are monophyletic. Micromalthidae is the sister-group of Cupedidae and *Priacma* the sister-group of the remaining cupetid genera. The position of Crowsoniellidae remains unclear.

There can be no doubt that classical histological methods are and will for a long time be very important for the investigation of insect morphology, but the new technique of X-ray tomography is a valuable enrichment of the tool-set of the insect morphologist. With increasing spatial resolution of the X-ray equipment and the development of staining methods

for soft tissues it will become more important for our work.

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6. Tentorium: (0) well developed, with bridge; (1) strongly reduced, bridge absent.
  7. Labrum: (0) free, connected with clypeus by membrane; (1) indistinctly separated from clypeus; (2) fused with head capsule.
  8. Location of antennal insertion on head capsule: (0) lateral; (1) dorsal.
  9. Mandibular apex: (0) unmodified, curved, teeth arranged in horizontal rows if present; (1) rotated, spoon-like, teeth arranged in vertical row; (2) atrophied.
  10. Galea: (0) basal part about as broad as proximal part; (1) divided into a narrow proximal stalk and a rounded, pubescent club (Lawrence, 1999).
  11. Prementum: (0) not enlarged, not plate-like; (1) enlarged, plate-like, with median apodeme; (2) broad at its base, with long median projection between labial palps; (3) very small.
  12. Dorsal sensorial patch on apical palpomeres: (0) absent; (1) small, shallow pit; (2) deep cavity; (3) large flat field; (4) digitiform sensilla.
  13. Ligula: (0) small and simple or absent; (1) represented by a pair of undivided lobes; (2) lobes slightly divided, (3) strongly divided (Lawrence, 1999).
  14. Prosternal grooves for tarsomeres: (0) absent; (1) present.
  15. Prosternal process: (0) not reaching beyond hind margin of procoxae; (1) reaching beyond hind margin of procoxae.
  16. Anteromedian pit of mesoventrite for reception of prosternal process: (0) absent or only very shallow concavity anteriorly delimited by rim along margin of mesoventrite; (1) present; (2) large hexagonal groove.
  17. Propleuro-mesepisternal locking mechanism: (0) absent; (1) propleural condyle and mesepisternal socket; (2) mesepisternal condyle and propleural socket.
  18. Shape of penultimate tarsomere: (0) not distinctly bilobed; (1) distinctly bilobed (Lawrence, 1999).
  19. Abdominal sterna: (0) abutting, not overlapping; (1) tegular or overlapping (Lawrence, 1999).

## APPENDIX: LIST OF CHARACTERS

### Adults

1. Cuticular scales: (0) absent; (1) present.
2. Constricted neck: (0) absent; (1) present.
3. Protuberances on dorsal side of head: (0) absent; (1) one pair posterior to antennal base; (2) one pair posterior to antennal base and one pair between eyes; (3) protuberances between eyes partly reduced, transformed into ridge or indistinct; (4) two pairs on posterior head region.
4. Genae: (0) not confluent; (1) confluent.
5. Antennal groove on head: (0) absent; (1) below compound eye; (2) above compound eye.

### Larvae

20. Head shape of later instars: (0) parallel-sided, slightly narrowing anteriorly, or evenly rounded; (1) transverse, strongly rounded laterally, greatest width near hind margin.
21. Posteromedian emarginations of head capsule: (0) absent; (1) present.
22. Endocarina: (0) absent; (1) present, undivided; (2) present, forked.
23. Frontal suture of later instars: (0) distinct; (1) indistinct or absent.
24. Stemmata: (0) more than one pair of stemmata; (1) one pair of stemmata or eyeless.

