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External and internal head anatomy of *Drusus monticola* (Trichoptera, Limnephilidae)

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Abstract: External and internal head anatomy of *Drusus monticola* (Trichoptera, Limnephilidae). Caddisflies have evolved to a staggering diversity, and their larvae inhabit a wide range of different habitats. Also, the larvae differ in their (feeding) ecology, and hydrological niche preference. Consequently, groups differ in their external morphology, a fact that allows to identify many taxa to species-level in the larval stage. However, a comparative treatise on the internal anatomy of larval Trichoptera remains to be presented. Here, we provide a detailed study on the external and internal head anatomy of *Drusus monticola*, a member of the limnephilid subfamily Drusinae.

We found 26 major muscles using µCT-scans, of which the muscles operating the mandibles were the largest. Overall, we could differentiate four main muscle groups: muscles operating the labrum, muscles operating the mandibles, muscles operating the maxillolabium and muscles operating the alimentary canal.

The situation as observed in *D. monticola* is highly similar to that of *D. trifidus*, the only other Drusinae in which cephalic anatomy is known. We propose that the configuration (muscle origins and number) observed here is characteristic for an evolutionary lineage within Drusinae in which all known members share a scraping grazer feeding ecology. Other Drusinae, including such with modified head capsules, remain to be investigated.

Zusammenfassung: Externe und interne Anatomie des Kopfes von *Drusus monticola* (Trichoptera, Limnephilidae). Köcherfliegen haben eine beeindruckende Diversität, und ihre Larven besiedeln ein breites Spektrum unterschiedlicher Habitate. Zudem unterscheiden sich diese Larven in ihrer (Ernährungs)-Ökologie und der Präferenz bestimmter hydrologischer Nischen. Folglich unterscheiden sich diese Gruppen in ihrer Morphologie, ein Umstand, durch den sie erst bestimmbar werden. Eine umfassende vergleichende Bearbeitung der internen Anatomie von Köcherfliegenlarven steht allerdings noch aus. Hier legen wir eine genaue Studie der Kopfkapselanatomie von *Drusus monticola* vor, einer Limnephilidae aus der Unterfamilie der Drusinae.

Wir konnten mittels µCT-Scans 26 Muskeln feststellen, wobei die Mandibelmuskeln bei weitem die größten sind. Insgesamt konnten wir vier Muskelgruppen differenzieren: Muskeln des Labrums, Muskeln der Mandibeln, Muskeln des Maxillolabiums und Muskeln des Verdauungstrakts.

Die Organisation, die bei *D. monticola* vorgefunden wurde, entspricht weitestgehend der, die anhand von *D. trifidus* beschrieben wurde – der einzigen anderen daraufhin erforschten Drusinae. Wir schließen daraus, dass die beobachtete Konfiguration für die evolutionäre Linie der schabenden Weidegänger innerhalb der Drusinae typisch ist. Bezüglich der Anatomie anderer Drusinae, insbesondere solcher mit abgewandelten Kopfkapseln, sollten weitere Forschungen angestellt werden.

Keywords: Drusinae, cephalic muscles, µCT, larva, caddisfly

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Introduction

Larvae of caddisfly subfamily Drusinae (Trichoptera: Limnephilidae) are highly diverse niche specialists comprising 178 extant taxa in two genera, namely *Drusus* Stephens, 1837 and *Ecclisopteryx* Kolenati, 1848 (Vitecek et al. 2017; Oláh et al. 2017). The majority of Drusinae are mostly cold-stenotopic and occur in turbulent running waters in hard-substrate channels of the Eurasian mountain systems. Diversity of Drusinae is highest in the Alps, the Balkans and the Pyrenees, with a total range of the group from the Iberian Peninsula to the Alborz Mts. in northern Iran. Speciation in Drusinae seems to have been enhanced by a combination of extrinsic (mostly climatic, and geological factors) and intrinsic (a supposedly low dispersal capacity) drivers, isolating distinct populations and inducing independent evolution. In particular, isolation of populations during historic glaciations in favorable patches of habitat could have contributed to the high extant diversity of larval morphologies in Drusinae (Malicky 1983).

In contrast to the latest proposed morphological classification of Drusinae, based on adults (Oláh et al. 2017), larval morphology and phylogenetic studies suggest the existence of three distinct, evolutionary lineages, reflecting the feeding ecology of the larvae (Pauls et al. 2008; Vitecek et al. 2015): (1) Omnivorous shredders with rounded head capsules and toothed mandibles, putatively representing the ancestral head capsule shape and feeding ecology, (2) carnivorous filter feeders that exhibit modified head capsules, and have toothed shredder-like edges to their mandibles and additional filtering spines on their legs, and (3) epilithic grazers with rounded head capsules that develop spoon-shaped mandibles without teeth. The shifts in feeding ecology of Drusinae larvae certainly contributed to their diversification by allowing for exploitation of other, less limited food sources (Pauls et al. 2008). As concerns external larval morphology, Drusinae are well-characterized and exhibit characters generally found in Limnephilidae. Seminal studies by Krafka (1923), Betten (1934) and Nielsen (1942) provide comparative assessment on external larval morphology, but unfortunately did not include internal organization of caddisfly larvae.

Detailed studies on the head anatomy of Limnephilidae larvae were conducted on *Limnephilus flavicornis* (Fabricius, 1787) (Winkler 1959; as *Limnophilus flavicornis*) and *D. trifidus* (Fotius-Jaboulet 1961) by means of dissections and self-intersecting serial semi-thin sections. More recently, internal head anatomy was described in *Rhyacophila fasciata* Hagen, 1859, Rhyacophilidae (Friedrich et al. 2015). However, a general analysis of external and internal head anatomy among caddisflies is still missing. Groups with strongly modified head capsules could potentially develop internal anatomical novelties to ensure that head function is not compromised. Alternatively, such taxa could undergo modifications or losses of certain features to allow for these changes in head capsule shape.

To study such processes, the subfamily Drusinae represents an excellent model as both regular and modified head capsules are present in this monophyletic group. Thus, internal anatomical features of species with and without modified head capsules can be scrutinized in a set of closely related taxa, without the need to integrate potentially deviating evolutionary origin. The original description of Drusinae internal head anatomy by Fotius-Jaboulet (1961) based on *D. trifidus* indicates the presence of 30 cephalic muscles. These muscles originate mostly at the head capsule, but also on the tentorium. Modifications of the head capsule as observed in other Drusinae larvae (Vitecek et al. 2015) could therefore instigate changes in points of muscle origin.

In this contribution, we describe the head anatomy of *Drusus monticola* McLachlan, 1876, using micro-computed tomography (µCT) to reconstruct head muscles. We compare our reconstructions with available data and predict high congruence of internal anatomy of *D. monticola* and *D. trifidus* larval heads.

Methods

Examined specimen, preparation and microCT imaging

The specimen used for micro-CT analysis was collected in the Schreierbach near Lunz am See, Ybbs catchment, Lower Austria (47°50'10" N; 15°04'11" E; 700 m above sea level) on the 25th of July 1992 (leg. Johann Waringer). The larva was picked from the mineral substrate using a forceps and was subsequently preserved and stored in 90 % ethanol, and identified as *Drusus monticola* McLachlan, 1876. The specimen was stained for 21 days in 1 % (w/v) phosphotungstic acid (PTA) in 70 % ethanol. After staining, the larva was washed in 70 % ethanol to remove unbound PTA from tissue. Subsequently, the larva was mounted vertically in 70 % ethanol in the tip of a plastic pipette, and sealed in with parafilm. The specimen was scanned on an XRadia MicroXCT-400 (Carl Zeiss X-ray Microscopy, Pleasanton, CA, USA) at 80kVp / 100µA using the 4X detector assembly. Projections were recorded with 15s exposure time (camera binning = 1) and an angular increment of 0.225° between projections over a 360° rotation. Tomographic slices were reconstructed with a voxel resolution of 5.73 µm (reconstruction binning = 2) using the XMReconstructer software provided with the microCT system.

Image processing, computer-based 3D reconstruction and post processing

The merged volume was exported as *.TXM file and imported into Amira 2019.1 (FEI SAS, Mérignac, France (part of Thermo Fisher Scientific™)). The image volume was filtered using a 3D bilateral filter for noise reduction. Before image segmentation, bit depth was reduced to 8 bit and the processed volume was saved in *.AM format. Image segmentation was achieved in Amira 6.5.0 (Visage Imaging, Inc., San Diego, CA, USA). Head capsule, tentoria, head muscles, central nervous system (cerebral ganglion mass, gnathal ganglion mass, frontal ganglion) and innervation patterns were manually segmented and assigned to different "materials" within the segmentation editor. Based on this manual segmentation, three-dimensional surface renderings were created using the Amira Surface Generate tool.

Results

The head of *D. monticola* is orthognathous (Fig. 1), rounded (Fig. 1), and bears 18 pairs of primary setae (indicated in Fig. 1–4 by small numbers #1–18). The head is composed of the anterior frontoclypeus (frontoclypeal apotome sensu Wiggins (1996); Fig. 1, 2 fa), the lateral parietal sclerites (referred to as 'a pair of sclerites' in (Friedrich et al. 2015); Fig. 1–4 ps), and the ventral submentum (anterior ventral apotome sensu Wiggins, 1996); Fig. 3 va). These sclerites are separated by the dorsal and ventral ecdysial lines (epi- and hypocranial sutures): the frontoclypeal suture (Fig. 2 fs) and the coronal suture (Fig. 2 cs) dorsally, and the ventral ecdysial line (hypocranial suture; Fig. 3 hs) ventrally. Proximally, these sclerites form the foramen occipitale (Fig. 3 of). Distally extend the cephalic appendages, the mouthparts comprising a sclerotized labrum (Fig. 2 lb), well-developed mandibles lacking terminal teeth (Fig. 4 m), and a maxillolabium with tiny maxillary and labial palps (Fig. 5, mp, lp) as well as a terminal silk gland opening (Fig. 3, os). The labrum is roughly semicircular in shape and bears 6 pairs of setae (# 1–6; Fig. 6) and setal brushes in each anteriolateral corner (Fig. 5 sb). The labrum is connected with the head capsule by an internal membranous fold that likely corresponds to the anteclypeus; the lateral margins of the labrum are strongly sclerotized and internally extend dorsad to form the curved tormae (Fotius-Jaboulet 1961; Fig. 6 e, t). Extending from the secondary mandibular joint, anterolateral carinae extend along the sagittal plane towards the eyes (Figs. 1, 4 c). The antennae are located medially on the carinae (Fig. 1 a). The eyes consist of six stemmata each and are positioned anterolaterally, somewhat dorsal to the dorsoventral plane (Fig. 1 e). Both frontoclypeus and parietal sclerites show multiple muscle attachment spots, each corresponding to internal muscle bundles (Fig. 7–9), and discussed in detail below. Two pairs of depressions along the frontocylpeal suture (Fig. 9 at, dt) mark the points where the internal skeleton of the head capsule, the tentorium, is attached to the cephalic sclerites (Figs. 3, 8, dotted circles, Fig. 10). The tentorium of *D. monticola* splits into an anterior tentorial arm and a dorsal tentorial arm (Fig. 10–11), roughly at a dorso-ventral plane along the secondary mandibular joint–eye axis.

The labrum has 2 pairs of intrinsic muscles, the labral compressor muscles (Fig. 11–13 Mla-ep). Extrinsic muscles of the labrum comprise the paired median labrum levator (Fig. 12–13 Mfr-la) that originates in the dorsal quarter of the frontoclypeus (i.e., dorsal of the eyes; Fig. 9, 13–14 Mfr-la) and inserts mesad of the anterior section of the labrum. The lateral labrum abductors originate in the lateral sections of the dorsal third of the frontoclypeal apotome and insert at the dorsal projections of the tormae (Fig. 9, 12–14 Mfr-ep).

The mandibles are stout, well-sclerotized subpyramidal structures with smooth, toothless cutting edges (Fig. 15 ce), a mediad setal brush (Fig. 15 sb), two primary setae (#1, 2; Fig. 15), and is articulated at the anterior secondary mandibular joint and the posterior primary mandibular joint (Fig. 15 dc). The mandibular adductor tendon *sensu* Friedrich et al. 2015 is well-developed and elongate, whereas the mandibular abductor tendon is short. The mandibles have no intrinsic musculature; extrinsic mandible muscles are the mandible adductors (Fig. 11, 13–15 Mcr-md(m), Mte-ma) and the mandible abductors (Fig. 13–15 Mcr-md(l)). These are the most prominent cephalic muscles in *D. monticola* and occupy a large part of head capsule space (Fig. 13–15). In addition, small adductor muscles originating from the tentorium insert at the anterior internal mandible wall close to the median apodeme (Fig. 11,14 Mte-ma). The mandible adductors originate in the anterodorsal and laterodorsal portions of the parietals (Fig. 9, 13), are clearly discernible as large groups of muscle attachment spots from the outside (Figs. 7–9) and insert at the mandible adductor tendon. In *D. monticola*, the mandibule adductors Mcr-md(m) are composed of two portions: an anterior portion, originating from the anterodorsal sections of the parietals, and a laterodorsal portion, originating from the laterodorsal sections of the parietals (Fig. 7–8, 11, 13–14). The mandible abductor Mcr-md(l) originates in the lateral sections of each parietal (Fig. 7–8, 14) and inserts at the mandibule abductor tendon.

The maxillolabium comprises the fused maxilla and labium (Fig. 5, 16 mb); while homology of parts of this structure is still under debate (cf Friedrich et al. 2015), it is characterized by bearing both maxillary and labial palps (Fig. 5, 16 lp, mp) as well as the terminal silk gland orifice (Fig. 16 os). Into the maxillolabium a number of muscles insert that originate from the tentorium (Fig. 14 Mte-ca, Mte-st) or apophyses formed by the occipital foramen (Fig. 11 Mcr-ds(l), Mcr-pm(a), Mcr-pm(l)). From the former group, the anterior M. tentorio-stipitalis (Fig. 14, 16 Mte-st) and the posterior M. tentorio-cardinalis (Fig. 14, 16, Mte-ca) insert in the proximal section of the stipes and the medial section of the cardo. Muscles originating from the occipital foramen apophyses comprise: (from lateral to medial) M. cranio-dististipitalis lateralis (Fig. 11, 16, Mcr-ds(l)) with two points of origin that inserts in the lateral section of the dististipes (palpiger sensu Fotius-Jaboulet 1961), the M. cranio-prementalis lateralis with one point of origin (Fig. 11 Mcr-pm(l)) that inserts in the posterior prelabial sclerite (Fig. 16 ps), and the M. cranio-praementalis anterior with two points of origin (Fig. 11, 16 Mcr-pm(a)) that inserts in tendons of the anterior prelabial sclerites (Fig. 16 ps). In addition to these extrinsic muscles, the maxillolabium bears four sets of intrinsic muscles: two sets of muscles originate from the stipes and insert (a) in the dististipes (Fig. 16, 11 Mst-ds(m)), (b) in a lateral tendon of the dististipes (Fig. 11, 16 Mst-ds(l)), and, most distally in the labium and close to the silk gland orifice, a ventral and a dorsal pair (the latter dorsal pair present as two distinct bundles) of muscles are located that originate from the posterior labial sclerites (posterior silk gland opening muscles) or the anterior labial sclerites (anterior silk gland muscles) and insert in the silk gland duct (Fig. 16, 11 Mhy-sa and Mpr-sa).

The labral epipharynx and the maxillar hypopharynx fuse at the level of the dorsal labral margin to form prepharynx that becomes the pharynx at the level of the frontal ganglion. Several sets of paired muscles operate the pharynx. From apophyses at the occipital foramen near the tentorium base several muscle pairs originate and insert at the ventral pharynx wall: the M. cranio-prepharyngealis at the border of the buccal cavity (Fig. 11

Mcr-pb), near to the maxillary glands the M. cranio-posteropharyngalis anterior (Fig. 11 Mcr-pp(a)), close to the connective the M. cranio-posteropharyngalis posterior (Fig. 11 Mcr-pp(p)), and dorsad of the connective, the system of the M. cranio-oesophagialis (Fig. 11 Mcr-oe). Further, the pharynx is operated by sets of muscles originating from the frontoclypeal apotome and the parietal sclerites: the M. clypeo-pharyngalis ,the M. fronto-pharyngalis medialis, the M. fronto-pharyngalis ventralis (Fig. 13 Mcl-ph, Mfr-ph(v), Mfr-ph(m)) and the M. parieto-pharyngalis lateralis, Fig. 13 Mpa-ph(l)); a single pair of muscles inserts in the dorsal pharynx wall dorsad of the connective (M. parieto-oesophagialis; Fig. 13–14 Mpa-oe).

The central nervous system (CNS) comprises the cerebral ganglion mass (Fig. 11) and the gnathal ganglion mass (Fig. 11) that are connected by the connectives. Further associated structures comprise the frontal ganglion and the corpora allata (located laterally at the oesophagus) (Fig. 11). Other nervous organs (e.g., the corpora cardiaca) could not be reconstructed, but nerve stalks to primary setae, eyes and antennae (Fig. 11) could be discerned.

Discussion

Comparison of *Drusus monticola* and *Drusus trifidus*

As expected, the external head capsule morphology and the internal sclerotized structures of the two members of the Drusinae grazer clade, *D. monticola* and *D. trifidus*, are highly congruent. This includes the biramal configuration of the tentorium, and the absence of a tentorial bridge. This pattern seems to be somewhat unusual, as the trichopteran groundplan was reported to include a delicate tentorial bridge, connecting the right and left tentorial arms (Friedrich et al. 2015). However, available data on Limnephilidae indicate a general absence of this structure in this family. The biramal distal portion of the tentoria is noteworthy, as this differs from the reported situation in other Limnephilidae, in particular *Limnephilus flavicornis* (Winkler 1959). Investigations on other Drusinae taxa could reveal whether this configuration is an ancestral character, or a novelty of the grazer clade.

Basically, the situation of cephalic muscles corresponds to that of *D. trifidus* (Table 1). It seems that species of the Drusinae grazer clade with rounded head capsules are highly similar concerning their cephalic muscles. From an evolutionary perspective, this finding is not surprising: any change in a working system is costly and will only manifest if benefits outweigh disadvantages. In contrast, the variation in head capsule complexity in the Drusinae carnivore clade could be interpreted as effects of other ecological constraints (Vitecek et al. 2015).

Innervation patterns

Unfortunately, the µCT-approach allowed only for a very rough assessment, but there seem to be no deviations from the gross insect bauplan (Beutel et al., 2013). All primary setae seem to be innervated, but histological sections should be used to confirm this assumption. Innervation of primary cephalic setae was long presumed from their general bauplan (Wiggins 1996), but to date there is no data based on which this situation could be demonstrated. What also remains to be clarified is whether Drusinae larvae in which

Tab. 1: List of larval head muscles found in *Drusus monticola* (this study) and *Drusus trifidus* (FOTIUS-JABOULET 1961)

Drusus monticola	**Abbreviation**	***Drusus trifidus***
M. labro-epipharyngalis	Mla-ep	cplr
M. fronto-epipharyngalis	Mfr-ep	mrlp
M. fronto-labralis	Mfr-la	mlra
M. cranio-mandibularis medialis	Mcr-md(m)	add1, add2
M. cranio-mandibularis lateralis	Mcr-md(l)	abd
M. tentorio-mandibularis	Mte-ma	add3
M. tentorio-stipitalis	Mte-st	adst
M. tentorio-cardinalis	Mte-ca	adcd
M. cranio-dististipitalis lateralis	Mcr-ds(l)	17
M. cranio-praementalis lateralis[1]	Mcr-pm(l)	Adlb2
M. cranio-praementalis anterioris	Mcr-pm(a)	rhphy
M. basistipido- dististipitalis medialis	Mst-ds(m)	16
M. basistipido-dististipitalis lateralis	Mst-ds(l)	15
M. hypopharyngo-salivaris	Mhy-sa	s1
M. praemento-salivarialis	Mpr-sa	s2
M. cranio-prepharyngealis	Mcr-pb	dvc
M. cranio-posteropharyngalis anterior	Mcr-pp(a)	dvphy
M. cranio-posteropharyngalis posterior	Mcr-pp(p)	dvpphy
M. cranio-oesophagialis	Mcr-oe	dvpphy
M. clypeo-epipharyngalis	Mcl-ph	dlcb
M. clypeo-cibarialis	Mcl-ci	dlbc
M. fronto-pharyngalis ventralis	Mfr-ph(v)	ddphy1
M. fronto-pharyngalis centralis	Mfr-ph(c)	ddphy2
M. fronto-pharyngalis medialis	Mfr-ph(m)	ddphy3
M. parieto-pharyngalis lateralis	Mpa-ph(l)	ddphy4
M. fronto-pharyngalis dorsalis	Mfr-ph	rao
M. parieto-oesophagialis	Mpa-oe	ddpphy

additional setae/spines are present (e.g. genus *Ecclisopteryx*) experienced a multiplication of setae or developed new structures unconnected with the nervous system. Further, there appear to be secondary ganglia in the vicinity of the mouthparts, but, again, different approaches should be pursued to study these structures.

Ecological implications of Drusinae head morphology

Morphological features and extracorporal structures (i.e., cases) of caddisfly larvae often represent adaptations to ecological constraints. Here, seemingly minor differences in larval morphology may be of great importance by modulating the capacity of the larvae to use certain habitat or resource types (PAULS et al. 2008; HJALMARSSON et al. 2019).

Different patterns of morphology in Drusinae larvae were previously linked to various types of feeding ecology (Pauls et al. 2008; Vitecek et al. 2015). However, the significance of Drusinae morphology for hydrological niche utilization is still unassessed. *Drusus monticola* is an example of a scraper-grazer among Drusinae species occurring in slowly flowing spring habitats. Its head capsule is evenly rounded, bears antennal carinae and lacks further modifications. Given the high congruence of internal head anatomy between *D. monticola* and *D. trifidus*, a species that colonizes similar habitats, we consider this configuration and hydrological niche as representing the baseline for the Drusinae grazer clade.

The most original Drusinae habitat is likely utilized by the two representatives of the shredder clade: *D. alpinus* and *D. franzi*. Both species occur in lenitic sections of cold spring brooks, and mostly in packets of organic matter, or under stones. Other groups, such as the Drusinae grazer and the Drusinae carnivore clade inhabit similar habitats, but occur in lotic habitats exposed to higher hydrological stress. Drusinae carnivores are characterized by modified head capsules that potentially represent integral structures of their filter-feeding apparatus (Vitecek et al. 2015). Also, among Drusinae grazers, several taxa occupy habitats with fast flow and thus high hydraulic stress. However, to date no quantitative assessment of their respective hydrological niches has been reported, and the hydraulic significance of these Drusinae heads remains unexplored.

Conclusions

We regard *D. monticola* as representing the anatomical groundplan of Drusinae due to the high congruence with *D. trifidus*, but head capsule modifications as observed in other Drusinae could be accompanied by internal anatomical adjustments of this groundplan. Also, it is possible that head anatomy of *D. alpinus* and *D. franzi* – probably exhibiting the most ancestral Drusinae characters – differs from that of this hypothetical groundplan.

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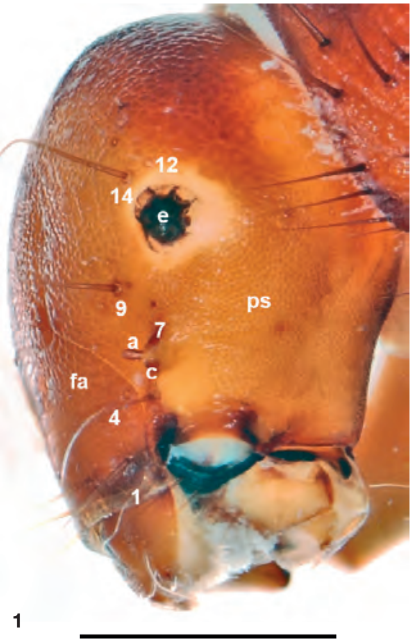

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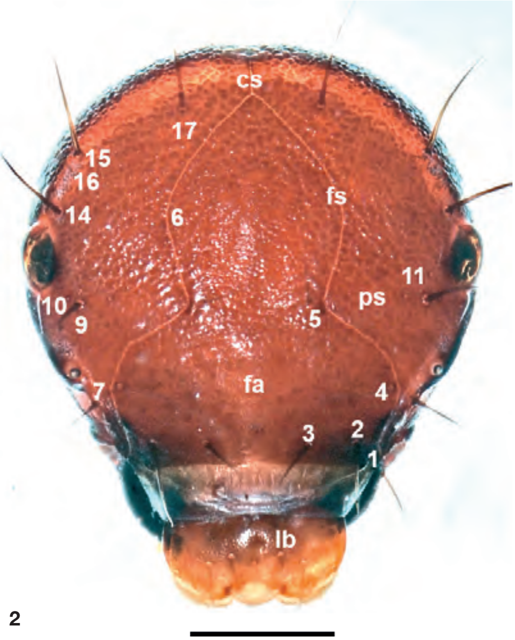

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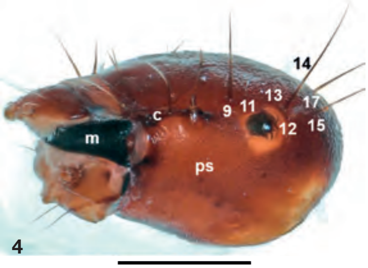

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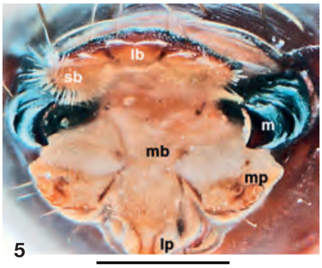

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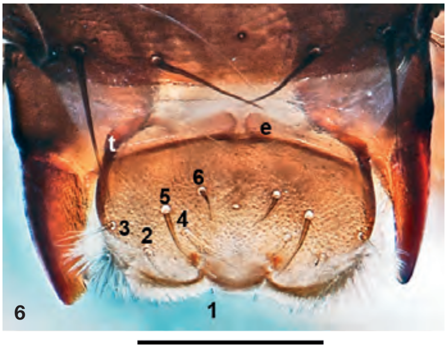
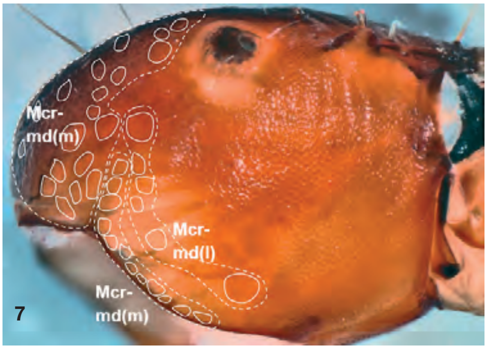
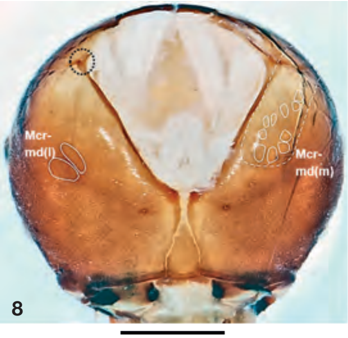
Fig. 1: *Drusus monticola*, head (5th instar larva), scale bar: 0.5 mm; lateral view (a = antenna, c = carina, s = stemmata, fa = frontoclypeal apotome, ps = parietal sclerite, numbers = setal positions). **Fig. 2:** *Drusus monticola*, head (5th instar larva), scale bar: 0.5 mm; frontal view (cs = coronal suture, fa = frontoclypeal apotome, fs = frontoclypeal suture, lb = labrum, ps = parietal sclerite, numbers = setal positions). **Fig. 3:** *Drusus monticola*, head (5th instar larva), scale bar: 0.5 mm; left lateral view (c = carina, m = mandible, ps = parietal sclerite, numbers = setal positions, dotted circle = ventral insertion of tentorium). **Fig. 4:** *Drusus monticola*, head (5th instar larva), scale bar: 0.5 mm; ventral view (hs = hypocranial suture, of = occipital foramen, os = orifice of silk gland, ps = parietal sclerite, va = ventral apotome, dotted circle = invagination groove of the right tentorium, numbers = setal positions). **Fig. 5:** *Drusus monticola*, head (5th instar larva), scale bar: 0.5 mm; anterior view (lb = labrum, lp = labial palp, m = mandible, mb = maxillolabium, p = maxillary palp, sb = setal brush of labrum).







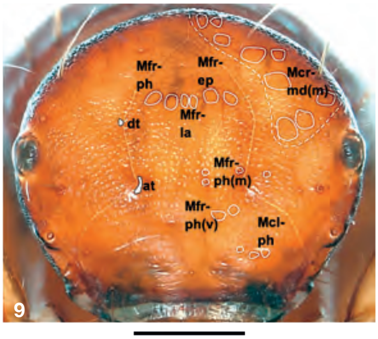
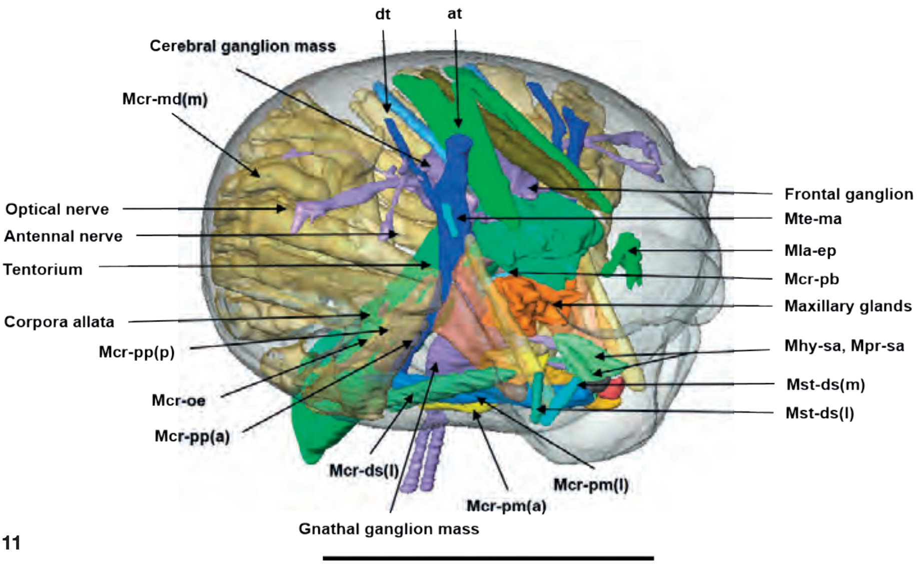


Fig. 6: *Drusus monticola* (5th instar larva), scale bar: 0.5 mm; Labrum, dorsal view (e = endolabrum, t = tormae, numbers = setal postitions). **Fig. 7:** *Drusus monticola*, head (5th instar larva), scale bar: 0.5 mm; right lateral view, muscle attachment spots created by muscle insertions (Mcr-md(m) = M. cranio-mandibularis medialis, Mcr-md(l) = M. cranio-mandibularis lateralis, arrow = ventral insertion of tentorium) **Fig. 8:** *Drusus monticola*, head (5th instar larva), scale bar: 0.5 mm; ventral view, muscle attachment spots created by muscle insertions (Mcr-md(m) = M. cranio-mandibularis medialis, Mcr-md(l) = M. cranio-mandibularis lateralis, dotted circle = ventral insertion of tentorium). **Fig. 9:** *Drusus monticola*, head (5th instar larva), scale bar: 0.5 mm; frontal view, tentorial pits and muscle attachment spots created by muscle insertions (at = anterior tentorial arm, dt = dorsal tentorial arm, Mcr-md(m) = Musculus cranio-mandibularis medialis, Mfr-ep = M. fronto-epipharyngalis, Mfr-la = M. fronto-labralis, Mfr-ph = M. fronto-pharyngalis dorsalis, Mfr-ph(m) = M. fronto-pharyngalis medialis, Mfr-ph(v) = M. fronto-pharyngalis ventralis). **Fig. 10:** *Drusus monticola*, head (5th instar larva), scale bar: 0.2 mm; right lateral view, detail of tentorium (at = anterior tentorial arm, dt = dorsal tentorial arm).



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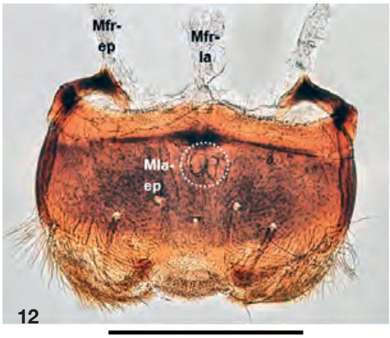

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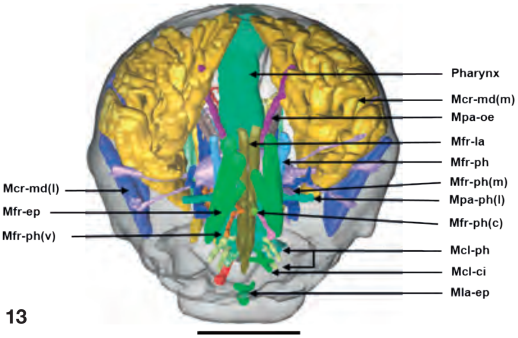

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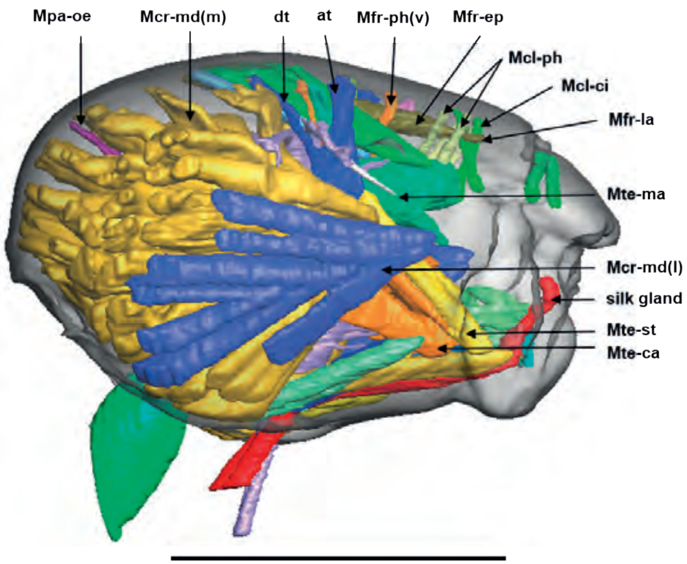
Fig. 11: Internal anatomy of *Drusus monticola* (5th instar larva), scale bar: 0.5 mm; 3D-reconstruction based on µCT data, right ventro-lateral view (Mcr-ds(l) = M. cranio-dististipitalis lateralis, Mcr-md(m) = M. cranio-mandibularis medialis, Mcr-oe = M. cranio-oesophagialis, Mcr-pb = M. cranio-prepharyngealis, Mcr-pm(a) = M. cranio-praementalis anterioris, Mcr-pm(l) = M. cranio-praementalis lateralis, Mcr-pp(a) = M. cranio-posteropharyngalis posterior, Mcr-pp(p) = M. cranio-posteropharyngalis posterior, Mhy-sa = M. hypopharyngo-salivaris , Mla-ep = M. labro-epipharyngalis, Mpr-sa = M. praemento-salivarialis , Mst-ds(m) = M. basistipido-dististipitalis medialis, Mst-ds(l) = M. basistipido- dististipitalis lateralis, Mte-ma = M. tentorio-mandibularis, at = anterior tentorial arm, dt = dorsal tentorial arm). **Fig. 12:** *Drusus monticola* (5th instar larva), scale bar: 0.5 mm;, ventral view (Mfr-ep = M. fronto-epipharyngalis, Mfr-la = M. fronto-labralis, Mla-ep = M. labro-epipharyngalis). **Fig. 13:** Internal anatomy of *Drusus monticola* (5th instar larva), scale bar: 0.5 mm; 3D-reconstruction based on µCT data, frontal view (Mcl-ci = M. clypeo-cibarialis, Mcl-ph = M. clypeo-epipharyngalis, Mcr-md(m) = M. cranio-mandibularis medialis, Mcr-md(l) = M. cranio-mandibularis lateralis, Mfr-ep = M. fronto-epipharyngalis, Mfr-la = M. fronto-labralis, Mfr-ph = M. fronto-pharyngalis dorsalis, Mfr-ph(m) = M. fronto-pharyngalis medialis, Mfr-ph(c) = M. fronto-pharyngalis centralis, Mfr-ph(v) = M. fronto-pharyngalis ventralis, Mla-ep = M. labro-epipharyngalis, Mpa-oe = M. parieto-oesophagialis, Mpa-ph(l) = M. parieto-pharyngalis lateralis).



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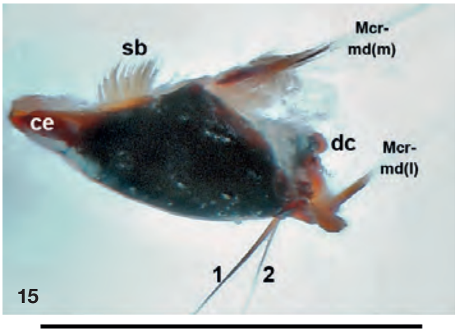

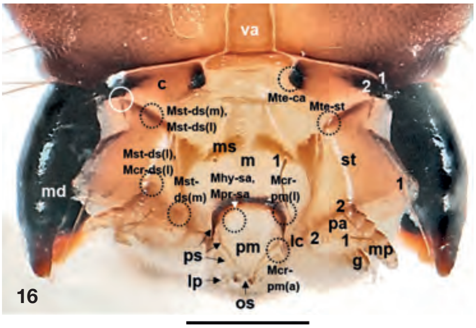

Fig. 14: Internal anatomy of *Drusus monticola* (5th instar larva), scale bar: 0.5 mm; 3D-reconstruction based on µCT data right lateral view (at = anterior tentorial arm, dt = dorsal tentorial arm, Mcl-ci = M. clypeo-cibarialis, Mcl-ph = M. clypeo-pharyngalis, Mcr-md(m) = M. cranio-mandibularis medialis, Mcr-md(l) = M. cranio-mandibularis lateralis, Mfr-ep = M. fronto-epipharyngalis, Mfr-la = M. fronto-labralis, Mfr-ph(v) = M. fronto-pharyngalis ventralis, Mpa-oe = M. parieto-oesophagialis, Mte-ca = M. tentorio-cardinalis, Mte-ma = M. tentorio-mandibularis, Mte-st = M. tentorio-stipitalis). **Fig. 15:** *Drusus monticola* (5th instar larva), scale bar: 0.5 mm; left mandible, ventral view (ce = teethless cutting edge, dc = anterior condylus, sb = setal brush, numbers = setal positions, Mcr-md(m) = M. cranio-mandibularis medialis, Mcr-md(l) = M. cranio-mandibularis lateralis). **Fig. 16:** *Drusus monticola* (5th instar larva), scale bar: 0.5 mm; maxillolabium, ventral view (c = cardo, g = galea, lc = sclerite of lacinia, lp = labial palp, m = mentum, md = mandible, mp = maxillary palp, ms = mental sclerite, os = orifice of silk gland, pa = palpiger, pm = prementum, ps = prelabial sclerites, st = stipes, va = ventral apotome, Mcr-ds(l) = M. cranio- dististipitalis lateralis, Mcr-pm(a) = M. cranio-praementalis anterioris, Mcr-pm(l) = M. tentoriopraementalis superior, Mst-ds(m) = M. basistipido- dististipitalis medialis, Mst-ds(l) = M. basistipido-dististipitalis lateralis, Mte-ca = M. tentorio-cardinalis, Mte-st = M. tentorio-stipitalis , white full circle = cardo-stipes articulation, white dotted circles = muscle insertions, numbers = setal positions).

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