The Larval Head Anatomy of Rhyacophila (Rhyacophilidae) with Discussion on Mouthpart Homology and the…

5 authors, including:

Frank Friedrich
University of Hamburg
54 PUBLICATIONS **1,017** CITATIONS

SEE PROFILE

Felix Beckmann
Helmholtz-Zentrum Geesthacht
359 PUBLICATIONS **4,452** CITATIONS

SEE PROFILE

Fabian Wilde
Helmholtz-Zentrum Geesthacht
38 PUBLICATIONS **114** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Project   1000 Insect Transcriptome Evolution (1KITE) View project

The Larval Head Anatomy of *Rhyacophila* (Rhyacophilidae) with Discussion on Mouthpart Homology and the Groundplan of Trichoptera

Frank Friedrich,[1]* Johannes Schulz,[1,2] Martin Kubiak,[1,2] Felix Beckmann,[3] and Fabian Wilde[3]

[1]*Biozentrum Grindel, Universität Hamburg, Hamburg 20146, Germany*
[2]*Centrum für Naturkunde, Universität Hamburg, Hamburg 20146, Germany*
[3]*Institut Für Materialforschung, Helmholtz-Zentrum Geesthacht, Außenstelle am DESY, Hamburg 22607, Germany*

ABSTRACT The external and internal features of the larval head of *Rhyacophila fasciata* (Trichoptera: Rhyacophilidae) were described in detail. Anatomical examinations were carried out using a multimethod approach including histology, scanning electron microscopy, confocal laser-scanning microscopy, microcomputed tomography, and computer-based three-dimensional reconstructions. Additionally, the information on the larval head of *Limnephilus flavicornis* (Limnephilidae) and *Hydropsyche angustipennis* (Hydropsychidae) available in the literature were reinvestigated. These anatomical data were used to address major questions of homology and terminology, that is, the ventral closure of the head capsule, the sclerites, and appendages of labium and maxilla and their muscles. These topics were discussed by summarizing the main hypotheses present in the literature and a critical inclusion of new findings. Consequently, the inner lobe of the maxilla very likely represents the galea. The distal maxillary sclerite (palpifer) is an anatomical composite formation at least including dististipes and lacinia. Based on these homology hypotheses several potential groundplan features of the larval head of Trichoptera were reconstructed. The head of *Rhyacophila* shows several presumably plesiomorphic features as for instance the prognath orientation of the mouthparts, the well-developed hypocranial bridge, the triangular submentum and eyes composed of seven stemmata. Derived features of *Rhyacophila* are the reduced antennae, the anterior directing of three stemmata and the shift of the tentorio-stipital muscle to the mentum. J. Morphol. 276:1505–1524, 2015. © 2015 Wiley Periodicals, Inc.

KEY WORDS: skeleton; musculature; morphology; glands; homology; Spicipalpia

INTRODUCTION

With about 14,500 extant species Trichoptera is one of the medium-sized orders of holometabolous insects (Morse, 1997: Trichoptera World Checklist). The primary limnic larvae are important indicators for the quality of freshwater. All life stages are also important components in the limnic food web (Malicky, 1973). Trichopteran larvae are best known for building cases in different shapes and out of various materials. However, there are predatory caddisflies without larval case, as for instance the representatives of Rhyacophilidae and Hydrobiosidae (Malicky, 1973). This (primary) lack likely represents the groundplan condition in Trichoptera (Malm et al., 2013).

While the monophyly of Trichoptera and a sistergroup relationship to Lepidoptera (= Amphiesmenoptera) is traditionally well-supported (see e.g., Hennig, 1969; Kristensen, 1997; Beutel et al., 2011), the intraordinal relationships are still controversially discussed (e.g., Ross, 1967; Weaver and Morse, 1986; Wiggins and Wichard, 1989; Frania and Wiggins, 1997; Kjer et al., 2001, 2002; Malm et al., 2013). Whereas the suborders Annulipalpia and Integripalpia are very likely monophyletic, the positioning of four families traditionally assigned to the suborder Spicipalpia is still unresolved (see e.g., Holzenthal et al., 2007). Most resent investigations, mainly based on molecular data, do not support the monophyly of Spicipalpia. The families Glossosomatidae, Hydroptilidae, Hydrobiosidae, and Rhyacophilidae are discussed as paraphyletic basal branches of Trichoptera (e.g., Malm et al., 2013), as paraphyletic sistergroups of Annulipalpia (e.g., Schmid, 1989) or Integripalpia (e.g., Kjer et al., 2001, 2002) and as polyphyletic with respect to both suborders (e.g., Frania and Wiggins, 1997). Without any doubt, spicipalpians played a crucial role in the early evolution and diversification of Trichoptera.

Studies on other holometabolous orders demonstrate the usefulness of detailed, comparable anatomical data for the reconstruction of the phylogeny and evolutionary morphology (e.g., Beutel et al., 2011; Friedrich et al., 2013). Considering the unresolved relationships at the base of the trichopteran tree of life and also within Annulipalpia and Integripalpia (see e.g., Holzenthal et al., 2007) the present knowledge on the anatomy of all life stages is very poor. Comparative studies of external larval features were carried out for instance by Krafka (1923), Betten (1934) and Nielsen (1942). Unfortunately, only very few contributions consider the internal anatomy in detail. Highly detailed descriptions of the skeleto-muscular system of the larval head were provided by Fotius-Jaboulet (1961) for *Drusus trifidus* (Integripalpia, Limnephilidae) and Badcock (1961) for *Hydropsyche* spp. (Annulipalpia, Hydropsychidae). Fragmentary data are available for *Limnephilus flavicornis* (Integripalpia, Limnephilidae; Winkler, 1959). Except for some scattered information on representatives of Rhyacophilidae by Hinton (1958) and Badcock (1961) no detailed treatment of the larval head anatomy of spicipalpian caddisflies is existent.

Here, we present the first detailed, comprehensive description of the larval head anatomy of a spicipalpian representative using a broad array of traditional and modern techniques (microcomputed tomography [μCT], histology, CLSM [confocal laser scanning microscopy], SEM [scanning electron microscopy]). As Rhyacophilidae were addressed as potential sistergroup of all remaining trichopterans (Malm et al., 2013) and are "by many regarded as the most primitive of recent Trichoptera" (Hinton, 1958: 191), *Rhyacophila fasciata* is chosen for detailed investigation. Additionally, the descriptions by Winkler (1959) and Badcock (1961) are reinvestigated using SR-μCT data of *L. flavicornis* (Integripalpia) and *Hydropsyche pellucidula* (Annulipalpia). The data presented here are compared with the information available in the literature for other trichopteran, basal lepidopteran, and mecopteran taxa. The homology of sclerites and muscles is discussed and the trichopteran groundplan is reconstructed for selected features.

MATERIALS AND METHODS
Taxa Examined

The investigations were carried out on the last (i.e., the fifth) instar of *R. fasciata* Hagen, 1859. The specimens used for the preparation of serial sections were collected in the Saale river in Jena (Thuringia, Germany) in May 2006. Further investigations were based on specimens collected in the vicinity of Besdorf (Schleswig-Holstein, Germany) in May 2012. For the reinvestigation of the descriptions by Winkler (1959) and Badcock (1961) SR-μCT data were used. Specimens of the last instar of *L. flavicornis* (Fabricius, 1787) were collected in August 2011 in the near of the Biological Station in Pevestorf (Lower Saxony, Germany). Final instars of *Hydropsyche angustipennis* (Curtis, 1834) were collected in April 2011 in the river Tanger near Weißenwarte (Saxony-Anhalt, Germany).

The species were identified using the key for Central European caddisfly larvae by Waringer and Graf (2011). Seventy percent ethanol was used as primary fixation agent. Postfixation with FAE (formaldehyde + acetic acid + ethanol; 3:1:6) was applied to the material used for serial sectioning and SR-μCT.

Methods

Confocal laser scanning microscopy. Six *Rhyacophila* specimens were investigated using CLSM in order to visualize the grade of sclerotization of the skeleton (see e.g., Deans et al., 2012; Michels and Gorb, 2012; Friedrich et al., 2014). The heads were placed in drops of glycerin between two cover glasses separated by glass spacers. The autofluorescence of the cuticle induced at 488 nm laser light was carried out using a Leica TCS-SPE confocal laser scanning microscope. The emitted light was recorded in two separate channels (500–575 nm, 580–695 nm) and colored green and red, respectively. The overlay of both channels in volume renderings or maximum projections resulted in brown coloration of sclerotized structures, whereas nonsclerotized (membranous) parts of the exoskeleton remain green. A third, blue channel was recorded for some specimens using ultraviolet laser light (induction: 405 nm; emission spectrum: 420–480 nm). Volume renderings were rendered with Bitplane Imaris 5.7 software.

Scanning electron microscopy. Six specimens were dehydrated in an ethanol series and critical point dried (Balzers CPD 020). Prior to drying the samples were cleaned using an alcoholic solution of 3% Triton and ultrasonic sound. The heads were glued on metal pins and placed on a specimen holder after Pohl (2010). After coating with platinum (Polaron SC7650 Sputter Coater) pictures were taken using a LEO 1525 at 10 keV. To increase the depth of field, several images were combined from partially focused stacks using Helicon Focus Lite 5.3 software.

Histology. Two specimens (head and prothorax) were embedded in Araldite, cut with a Microm microtome (HM360) and diamond knife at 1.5 μm (cross- and longitudinal sections). Sections were stained with toluidine blue and pyronin G and finally sealed with Pertex® and cover glass. Both histological section series were a loan from the Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum of the FSU Jena.

A semiautomatic slide scanner (Leica DM6000 operated by MetaMorph software) was used for the digitalization of the section series. Every third section was selected and aligned with VSG Amira 5.4 software.

Synchrotron radiation-based microcomputed tomography. The SR-μCT scans were performed at the German Electron Synchrotron Facility (DESY, Hamburg; project numbers I-20100317, I-20120676). Low photon energy beam (8kV) and absorption contrast were used to produce data with high density resolution (see Beckmann et al., 2006, 2008; Friedrich and Beutel, 2008). One specimen per species was investigated. The specimens of *R. fasciata* and *H. angustipennis* were scanned at beamline BW2 of DORIS ring. The data set of *L. flavicornis* was created at beamline P05 of PETRA III ring.

The specimens were divided between the first and second thoracic segment. Head and prothorax were dehydrated in an ethanol series and critical point dried (Balzers CPD 020). After mounting with two-component adhesive on a metal rod, the sample was scanned in 180° rotation (0.25° steps). The resulting image stacks have a resolution (voxelsize) of 2.31 μm (*Rhyacophila* and *Hydropsyche*) and 2.53 μm (*Limnephilus*).

Computer-based 3D-reconstruction. The larval head anatomy of *R. fasciata* was 3D-reconstructed using VSG Amira 5.4 software. The reconstruction was based on the SR-μCT-data, which is free of spatial artifacts and allowed the automatic (threshold-based) segmentation of sclerotized parts. Soft

tissues (muscles, nervous system, gut, glands) were manually outlined using a Wacom Cintiq 21UX interactive pen display. The obtained segmented image stacks were used for the automatic creation of surface objects of all discrete structures in Bitplane Imaris 5.7 software. The resulting surface files were converted into object files (obj) using Transform2 64 bit software (freeware, Heiko Stark, Jena, Germany; URL: http://starkrats.de). The files were assembled and finally processed in Autodesk® Maya 2014 software. During the process, minor modeling artifacts were removed without changes in the general shape and arrangement of the structures. Surface renderings were also created with Maya software.

The surface model assembled in Maya was also used for the creation of the interactive 3D-pdf in Adobe® Acrobat® 9 Pro.

Postprocessing. Images were adjusted (contrast, color) using Adobe® Photoshop® CS5 software. Drawings were created and image tables assembled with Adobe® Illustrator® CS5.

Terminology. The skeletal terminology was mainly adopted from Beutel et al. (2013), using Badcock (1961) and Hinton (1958) for Trichoptera-specific terms. Setae and sensory pits are termed with an extended nomenclature primarily based on Williams and Wiggins (1981). Muscles are named by their areas of origin and insertion, an approach similar to the nomenclature used in the Lepidoptera Handbook of Zoology (Hasenfuss and Kristensen, 2003) and as introduced by Kubiak et al. (in press) for adult trichopterans. A detailed homologization with the nomenclatures used by von Kéler (1963) and Wipfler et al. (2011) is given in Table 1.

Names for traditional trichopteran suborders Annulipalpia, Integripalpia, and Spicipalpia are used in the sense of Wiggins and Wichard (1989). Due to the likely paraphyly of the last group (see above), the four families (Rhyacophilidae, Hydrobiosidae, Hydroptilidae, and Glossosomatidae) are neutrally termed as spicipalpians. Annulipalpia comprise Hydropsychoidea and Philopotamoidea. All remaining families are assigned to Integripalpia. The terminology of Hennig (1953) was used for supraordinal taxa. Consequently, the taxon comprising Antliophora and Amphiesmenoptera is termed Mecopterida ("Panorpoidea" of Hinton, 1958).

The terms *basal* and *derived* are not used for a comparison of sister taxa, which are of same age and evolved from the same LCA (last common ancestor). In the context of this work, *basal* is used for taxa splitting of close to the root of the tree and which are delimited by a series of nodes from *derived* taxa nested in the distal parts of the tree (e.g., Rhyacophilidae and Limnephilidae). The discrimination of *basal* and *derived* taxa is based on the phylogenetic relationships ("tree") of the group.

RESULTS
General Appearance

The used specimens of the fifth instar larva of *R. fasciata* are in average 15.7 mm long. The postcephalic body is slightly compressed dorsoventrally (campodeoid type) and composed of three thoracic and nine abdominal segments. The abdomen is not covered by a case but bears lateral bundles of tubular gills. Large parts of the body are weakly sclerotized. Besides the head, the pronotum, the thoracic pleurites and legs, and the anal claws are well sclerotized and pigmented.

Head Capsule

The prognathous head is dorsoventrally compressed and ca. 1.5 times longer than wide. Its smooth surface is ochre to brownish colored. Long and shorter sensory trichoid sensilla are distributed over the head capsule and located on the proximal areas of the mouthparts (Fig. 1). The head capsule forms a simple tube which is subdivided in only two basic sclerites by very few sutures. On the dorsal side the median coronal suture, originating on the dorsal side of the occipital foramen, splits into the frontal sutures after one third of the head length (Fig. 1A). The frontal sutures run laterad toward the eyes but converge again at the anterior tentorial pits. In front of the pits, the suture continues as fronto-genal suture toward the base of the mandibles. The sclerite delimited by both sutures is the frontoclypeus. A frontoclypeal (= epistomal) ridge is not developed. The clypeal area is subdivided by an intraclypeal cross-ridge. The short, weakly sclerotized anteclypeus is movably connected with the labrum anteriorly (see below) and the larger, thick-walled postclypeus is posteriorly continuous with the frons (Figs 1 and 2D).

The rest of the head capsule is not further subdivided but formed by a pair of sclerites meeting each other in the mid line of the ventral side. A gula is absent. The ventral closure of the occipital foramen is formed by the very long hypostomal bridge (Fig. 1B). The large foramen occipitale forms a triangular opening of the posteroventral part of the head capsule. It is enforced by an internal ridge and laterally constricted forming deep hutches, which function as main insertion sites of the prothoracic head moving muscles. Ventrally of these sites, the wall of the foramen occipitale is enforced by a distinct posteroventral part of the head capsule. At the ventromedian corner of the foramen, the common tendon of ventral thoracic head depressor muscles is attached. In fact, the resilin-rich tendon is not directly attached to the head capsule but to a tiny triangular sclerite termed protogula by Badcock (1961). The sclerite is located at the posterior end of the hypocranial suture. At the anterior end of the ridge the head capsule encloses the triangular submentum (see below). The head capsule bears 23 pairs of setae (Fig. 1: hc1-23) and 18 pairs of sensory pits (Fig. 1: phc1-18).

The larval eyes are located anterolaterally on the head capsule behind the antenna (Figs 1, 2D, and 3A). The crystalline cones of seven stemmata are circularly arranged around a dark brown pigment spot, which is distinctly silhouetted against the light colored head capsule (Fig. 2D). The three anteriormost stemmata are anteriorly directed and closely adjacent each other (diameter between 23 and 30 μm). The larger, posterior four stemmata (diameter: 39–50 μm) are laterally directed and more separated (Fig. 2D). The externally bulged or specifically thickened lenses are not developed, that is, the surface of the corneal cells is not discriminable from the surrounding cuticle of the head capsule. The crystalline cone of each stemma is composed of three cells (Fig. 4C: cc).

TABLE 1. Summary and homologization of the larval head muscles of Trichoptera, Lepidoptera (Agathiphaga), and Mecoptera (Nannochorista)

Name (in this study)	This study Rhyacophila	Badcock (1961) Hydropsyche	Fotius-Jaboulet (1961) Drusus	Winkler (1959) Limnephilus	Kristensen (1984) Agathiphaga	Beutel et al. (2009) Nannochorista	von Kéler (1963)	Wipfler et al. (2011)
M. fronto-labralis	fr-lb	a.l.m.	mlra	8	O	MM. 8 + 9	8	0lb1
M. fronto-epipharyngalis	fr-eph	l.l.m., p.l.m.	mlrp	9	2		9	0lb2
M. labro-epipharyngalis	lb-eph	acl.m.?	cplr	7	O	M. 7	7	0lb5
M. clypeo-epipharyngalis	cl-eph	cb.m.	dlcb	44	12	M. 43	43	0ci1
M. clypeo-cibarialis	cl-ci	cb.m.	dllbc	44? 45?	13	M.44	43, 44?	0bu1
M. fronto-oralis anterior	fr-oa(a)	X*	ddphy1	X*	16??	M. 49?	41?	0hy2
M. fronto-oralis posterior	fr-oa(p)	f.m.hp.	rao	43	16??	M. 41	41	0hy1
M. tentorio-hypopharyngalis	O	d.m.pm.?/O*	rhphy?	42?/O*	14?	M. 42	42	0hy3
M. fronto-pharyngalis anterior	fr-ph(a)	X*	ddphy2	45?	15	M. 45	45	0bu2
M. fronto-pharyngalis posterior	fr-ph(p)	pc.p.m.	ddphy3	46	18	M. 46	46	0bu3
M.fronto-pharyngalis lateralis	fr-ph(l)	l.pc.p.m.	ddphy4	45?	17	M. 47	47?	0hy2?
M. tentorio-cibarialis	te-ci	X*	dvc	48	14?	M. 48	48	0bu5
M. tentorio-pharyngalis anterior	te-ph(a)		dvphy	50	19	M. 50	50	0bu6
M. tentorio-pharyngalis posterior	te-ph(p)	X*	dvpphy	52	20, 21	M. 52	52, 54?	0ph2
M. cranio-pharyngalis dorsalis	cr-ph(d)	X*	ddpphy	51	O	M. 51	51	0ph1
M. cranio-pharyngalis ventralis	cr-ph(v)	X*	dvpphy	X*	22, 23	M. 54	54, 52?	0ph2?
M. cranio-pharyngalis posterior	O	O	O	O	O	M. 53	51? 53?	0ph3
M. tentorio-scapalis anterior	O	O	O	O	O	MM. 1/2/3/4	1	0an1
M. tentorio-scapalis posterior	O	O	O	O	O	O	2	0an2
M. tentorio-scapalis lateralis	O	O	O	O	O	O	3	0an3
M. tentorio-scapalis medialis	O	O	O	O	O	O	4	0an4
M. scapo-pedicellaris lateralis	O	O	O	O	O	O	5	0an6
M. scapo-pedicellaris medialis	O	O	O	O	O	O	6	0an7
M. cranio-mandibularis medialis	cr-md(m)	X*	add1, 2	11	3	M. 11	11	0md1
M. cranio-mandibularis lateralis	cr-md(l)	X*	abd	12	4	M. 12	12	0md3
M. tentorio-mandibularis	te-md	X*	add3	X*	5	M. 13	14	0md6
M. hypopharyngo-mandibularis	O	O	O	O	O	O	13	0md4
M. tentorio-cardinalis	te-cd	X* (t.ad.c.)	adcd	17	6	M. 17	17	0mx3
M. tentorio-mentalis	te-mt	t.ad.s.	adst	18	7	M. 18	18	0mx4
M. cranio-cardinalis	O	O	O	O	O	O	15	0mx1
M. cranio-dististipitalis medialis	cr-dst(m)	c.f.la.	rhphy	29	O	M. 19	19	0mx2
M. cranio-dististipitalis lateralis	cr-dst(l)	c.f.m.p.	17	19	8	M. 19a	?	?
M. basistipito-dististipitalis lateralis	bst-dst(l)	l.m.p.	15	X*	O	M. 22	22	0mx8
M. basistipito-dististipitalis medialis	bst-dst(m)	d.m.p.	16	20	O	M. 23	23	0mx10
M. stipito-lacinialis	O	O	O	O	O	O	20	0mx6
M. stipito-galealis	O	O	O	O	O	O	21	0mx7
M. palpo-palpalis maxillae primus	O	O	O	O	O	O	24	0mx12
M. palpo-palpalis maxillae secundus	O	O	O	O	O	O	25	0mx13
M. palpo-palpalis maxillae tertius	O	O	O	O	O	O	26	0mx14
M. palpo-palpalis maxillae quartus	O	O	O	O	O	O	27	0mx15
M. hypopharyngo-salivarialis	hy-sa	m.s.p.	s1	37	10	M. 37	37	0hy12
M. intrasalivarialis	O	O	O	O	O	O	37?	?
M. praemento-salivarialis	prm-sa	X*	s2	X*	11	O	38?, 39?	0hy7/0hy8?
M. cranio-praementalis medialis	O	O	O	O*	O	O	30	0la6
M. tentorio-praementalis lateralis	te-prm(l)	l.m.pm.	adlb2	30	9	O	29	0la5

Table 1. (continued).

Name (in this study)	This study *Rhyacophila*	Badcock (1961) *Hydropsyche*	Fotius-Jaboulet (1961) *Drusus*	Winkler (1959) *Limnephilus*	Kristensen (1984) *Agathiphaga*	Beutel et al. (2009) *Nannochorista*	von Kéler (1963)	Wipfler et al. (2011)
M. submento-praementalis	O	O	O	O	O	O	28	0la8
M. praemento-paraglossalis	O	O	O	O	O	O	31	0la11
M. praemento-glossalis	O	O	O	O	O	O	32	0la12
M. praemento-palpalis medialis	O	O	O	O	O	O	33	0la13
M. praemento-palpalis lateralis	O	O	O	O	O	O	34	0la14
M. palpo-palpalis labii primus	O	O	O	O	O	O	35	0la16
M. palpo-palpalis labii secundus	O	O	O	O	O	O	36	0la17

X, present; O, absent;?, homology or presence uncertain; *, addition or correction by own investigations.

Tentorium

The tentorium is complete but most parts are thin, slender, and with a very narrow lumen. The posterior tentorial arms are comparatively thick-walled and strongly sclerotized (Figs 3B and 5B). They end in slit-like posterior tentorial grooves located directly in front of the foramen occipitale and lateral of the postgular sclerite (Fig. 1B). A thin, strongly arched tentorial bridge connects the posterior tentorial arms (Fig. 4A,B). It runs anterodorsal directed above the anterior part of the suboesophageal ganglion, immediately posterior to the circumoesophageal connectives and ventral of the tritocerebral commissure (Figs 4A,B and 5). The anterior tentorial arms are three times longer than the posterior arms, slightly arched, and connect the base of the tentorial bridge with the dorsal head capsule (Fig. 5B). The small kidney-shaped anterior tentorial pits are located on the frontal suture (Fig. 1A: atp). The mid part of the anterior arms is thin-walled and laterally compressed (Fig. 6C). It forms a moderately sized, ventrad laminatentorium (Fig. 5B). The dorsal tentorial arms are very thin (Fig. 6C: dta). They originate from the anterior arms at level of the frontal ganglion and are attached to the head capsule by a broad array of collagen fibers.

Labrum

The lobe-like, semicircular labrum is movably connected with the anteclypeus by an internal membranous fold (Figs 2D and 5B). The anterior rim of the labrum bears rows of short microtrichia. Its apex is membranous and unpigmented (Fig. 2D). Six pairs of long trichoid setae (Fig. 1A: L1–6) are dorsally arranged along the anterior and lateral margins of the labrum. Two pairs of sensory pits are closely adjacent (Fig. 1A: Pl2, 3), while a third one is located centrally (Pl1). The posterolateral corners contact the lateral edges of the epipharynx and form short, strong processes, the tormae.

Musculature. M. labro-epipharyngalis (lb-eph), three sequent bundles, O (= origin): proximal part of labrum, I (= insertion): mesally on the posterior and central part of the epipharynx. M. fronto-labralis (fr-lb), well developed, O: paramedially on the frons, directly above the brain, I: posterior margin of the labrum, close to the midline; M. fronto-epipharyngalis (fr-eph), moderately sized, slightly tapering, O: frons, laterad fr-lb, I: tormae (Figs 3A, 5, and 6).

Antenna

The extremely minute antenna is located between the stemmata and the mandibular base (Figs 1A, 2D, and 7E). The antennal foramen has a diameter of only 30 μm. The single segment is weakly sclerotized and bears two large sensilla basiconica and a single central trichoid sensillum (Fig. 7F).
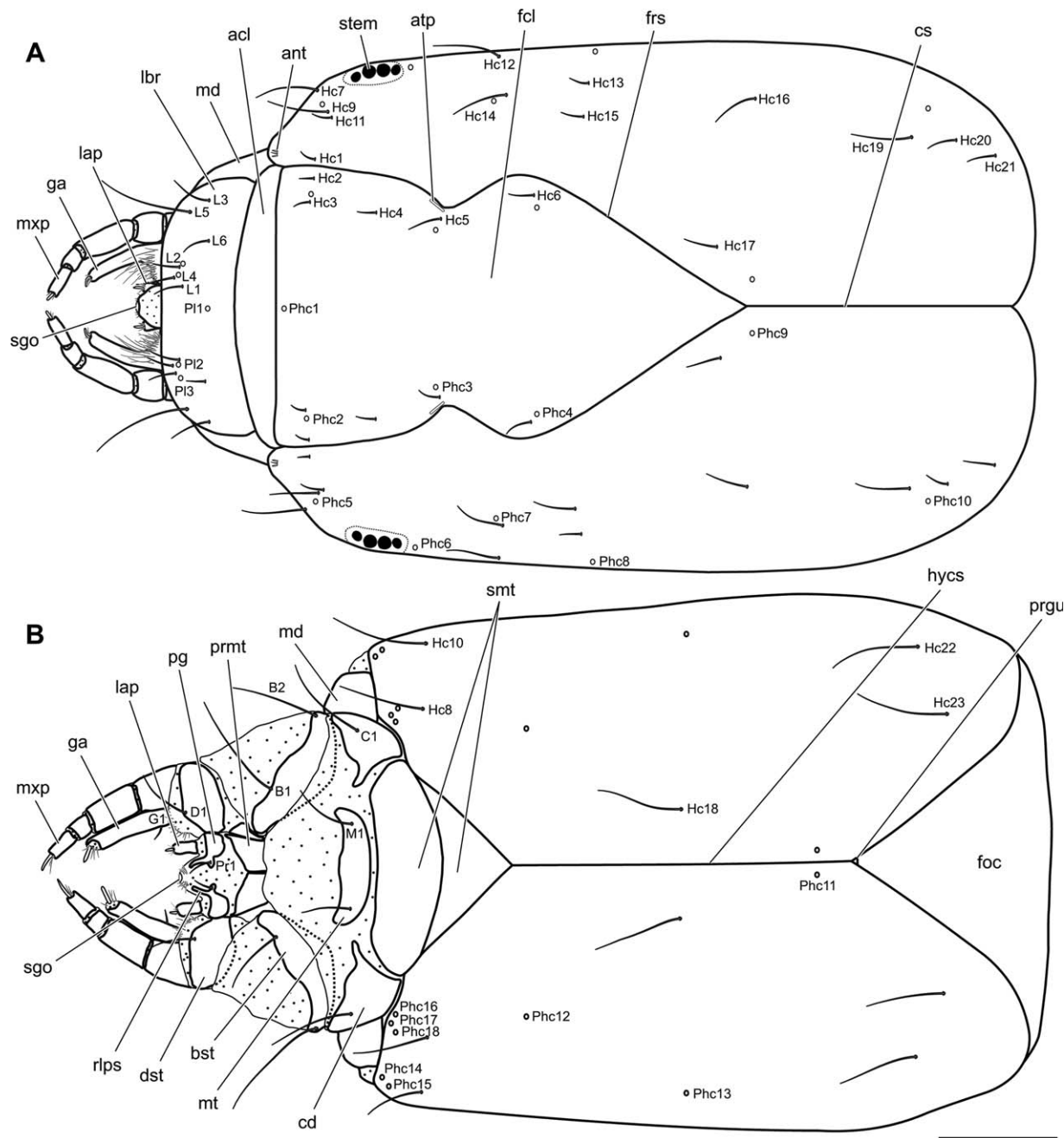
Fig. 1. Head of *Rhyacophila fasciata*. (**A**) Dorsal view. (**B**) Ventral view. acl, anteclypeus; ant, antenna; atp, anterior tentorial pit; B1 + 2, setae of basistipes; bst, basistipes; C1, seta of cardo; cd, cardo; cs, coronal suture; D1, seta of dististipes; dst, dististipes; fcl, frontopostclypeus; foc, occipital foramen; frs, frontal suture; G1, seta of galea; ga, galea; Hc1-23, setae of head capsule; hycs, hypocranial suture; L1-23, setae of labrum; lap, labial palp; lbr, labrum; M1, seta of mentum; md, mandible; mt, mental sclerite; mxp, maxillary palp; pg, palpiger; Phc1-18, sensory pits of head capsule; Pl1-3, sensory pits of labrum; Pr1, seta of prementum; prgu, protogula; prmt, prementum; rlps, rod-like premental sclerite; sgo, orifice of the spinning glands; smt, submentum; ste, stemma. Scale bar: 250 μm.

Musculature. Extrinsic and intrinsic muscles absent.

Mandible

The mandibles are strongly sclerotized and pigmented. They are triangular shaped and bear apically a single long incisivus (Fig. 2C). The inner side of the incisivi and the sharp cutting edges of the mesal face of the mandible are the most hardened parts of the mandible. The proximal end of the cutting edge is marked with a short, blunt process. The mandibles show a distinct asymmetry: in the left-side mandible the mesal side of the incisivus shows a deep, V-shaped furrow guiding the tip of the right mandible during movement
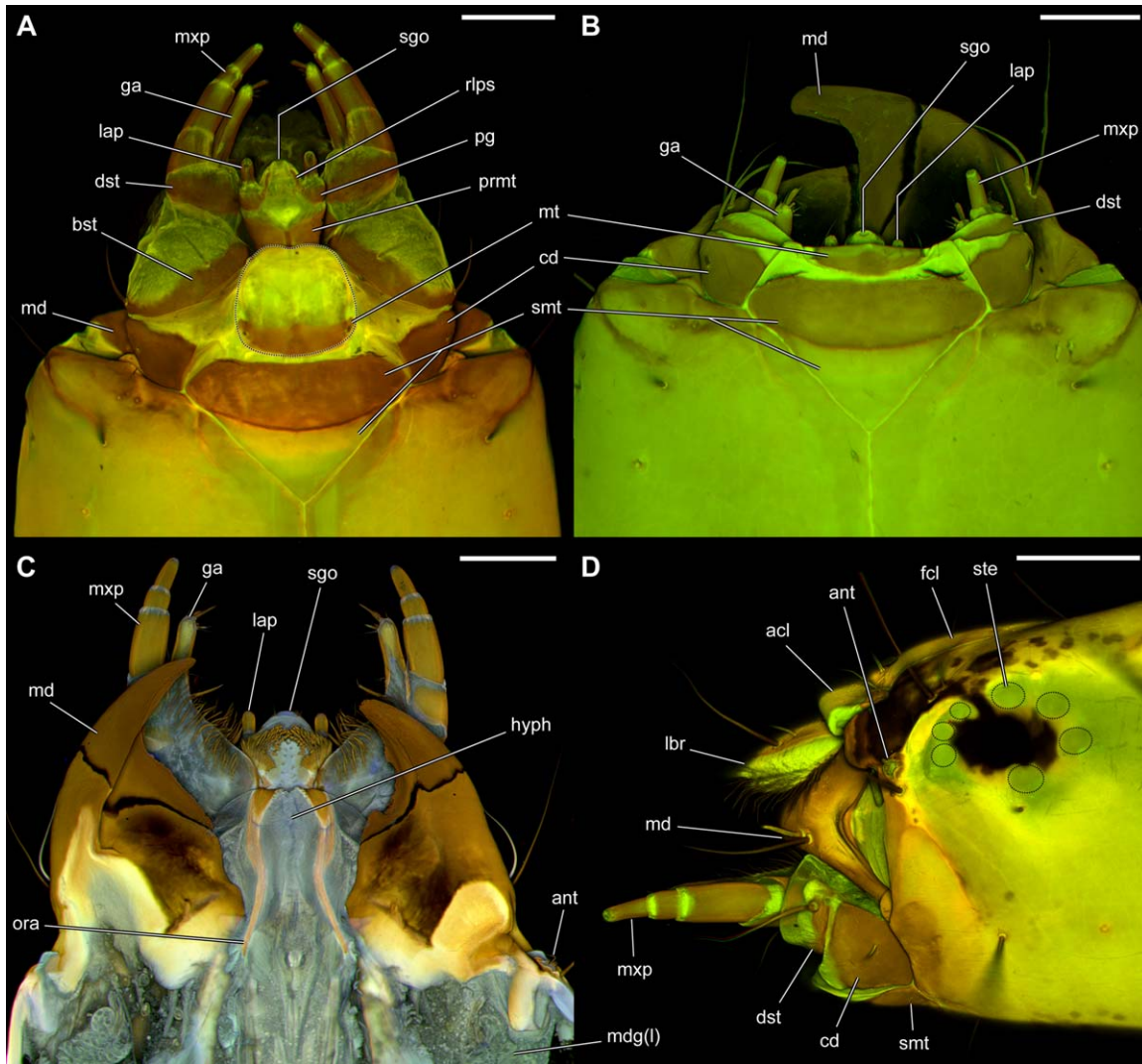
Fig. 2. Mouthparts of *Rhyacophila fasciata*, CLSM images. (**A**) Ventral view, maxillolabium extended (mental area marked by dotted line). (**B**) Ventral view, maxillolabium retracted. (**C**) Dorsal view on hypopharynx and maxillolabium (specimen horizontally bisected). (**D**) Lateral view, maxillolabium retracted (crystalline cones of stemmata marked by dotted circles). acl, anteclypeus; ant, antenna; bst, basistipes; cd, cardo; dst, dististipes; fcl, frontopostclypeus; ga, galea; hyph, hypopharynx; lap, labial palp; lbr, labrum; md, mandible; mdg(l), lateral mandibular gland; mt, mental sclerite; mxp, maxillary palp; ora, oral arm of hypopharynx; pg, palpiger; prmt, prementum; rlps, rod-like premental sclerite; sgo, orifice of the spinning glands; smt, submentum; ste, stemma. Scale bars: 200 µm.

(Fig. 2C). In both mandibles, the apical half is discriminated from the proximal part by a distinct circular line (Fig. 2B,C). The mandibular base is connected to the head capsule by two joints (Figs 2D and 7E): dorsally articulates a process of the head capsule with a fossa of the mandible. The ventral mandibular joint is formed by a mandibular joint ball and a corresponding cavity of the head capsule. The base of the elongated tendon of the large mandibular adductor muscle is strongly sclerotized and thickened. Two prominent setae are located laterally on the mandibular base (Figs 2B–D and 3B).

Glands. Two paired glands are associated with the mandible (Figs 2C, 3B, 5, and 6A,B). The medial gland is located at the base of the mandible

and fills substantial parts of the sclerites lumen (Fig. 6A: mdg(m)). The central, tubular duct opens into the space between mandible and maxilla. The lateral gland is about twice the size of its medial counterpart. It fills the haemolymph space between the mandibular tendons (Figs 3B and 6A,B: mdg(l)). Its long duct leads into the space between the dorsal side of the mandible and the base of the labrum.

Musculature. M. cranio-mandibularis medialis (cr-md(m)), by far the largest head muscle, composed of several bundles with a common strong tendon, O: almost the complete posterior two third of the head capsule (except for the attachment area of cr-md(l)), I: mesally on the mandibular base by means of a very strong adductor tendon; M.
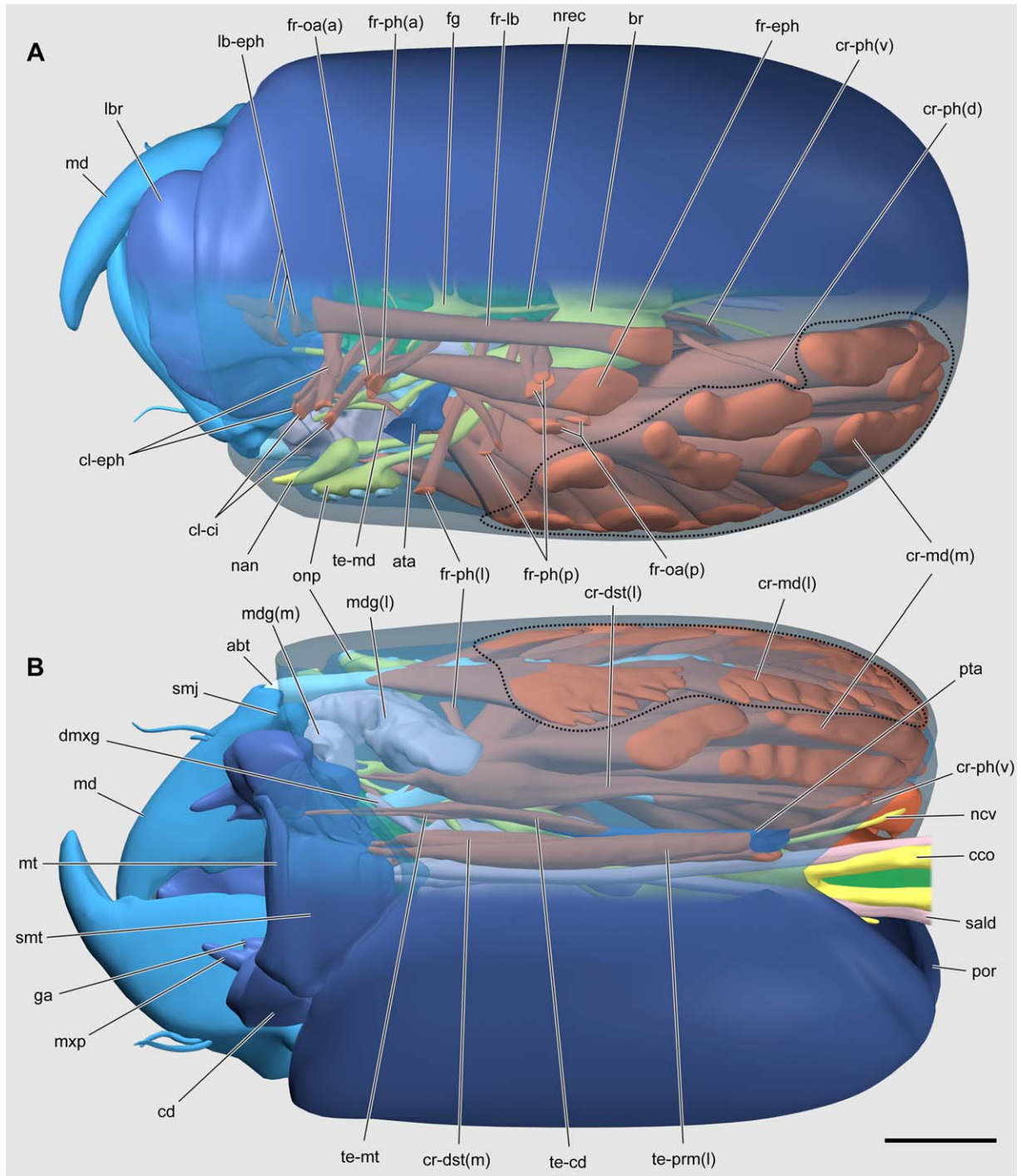
Fig. 3. Head anatomy of *Rhyacophila fasciata*, 3D-reconstruction based on SR-µCT data. (**A**) Dorsal view. (**B**) Ventral view. abt, mandibular abductor tendon; ata, anterior tentorial arm; br, brain; cco, cervical connectives; cd, cardo; cl-ci, M. clypeo-cibarialis; cl-eph, M. clypeo-epipharyngalis; cr-dst(l)/(m), M. cranio-dististipitalis lateralis/medialis; cr-md(l)/(m), M. cranio-mandibularis lateralis/medialis; cr-ph(d)/(v), M. cranio-pharyngalis dorsalis/ventralis; dmxg, duct of the maxillary gland; fg, frontal ganglion; fr-eph, M. fronto-epipharyngalis; fr-lb, M. fronto-labralis; fr-oa(a)/(p), M. fronto-oralis anterior/posterior; fr-ph(a)/(l)/(p), M. fronto-pharyngalis anterior/lateralis/posterior; ga, galea; lb-eph, M. labro-epipharyngalis; lbr, labrum; md, mandible; mdg(l)/(m), lateral/medial mandibular gland; mt, mentum; mxp, maxillary palp; nan, nervus antennalis; ncv, nervus cervicalis; nrec, nervus recurrens; onp, optic neuropil; por, postoccipital ridge; pta, posterior tentorial arm; te-cd, M. tentorio-cardinalis; te-md, M. tentorio-mandibularis; te-mt, M. tentorio-mentalis; te-prm(l), M. tentorio-praementalis lateralis; sald, salivary duct; smj, secondary mandibular joint; smt, submentum. Scale bar: 250 µm. An interactive view is available in the electronic version of the article.
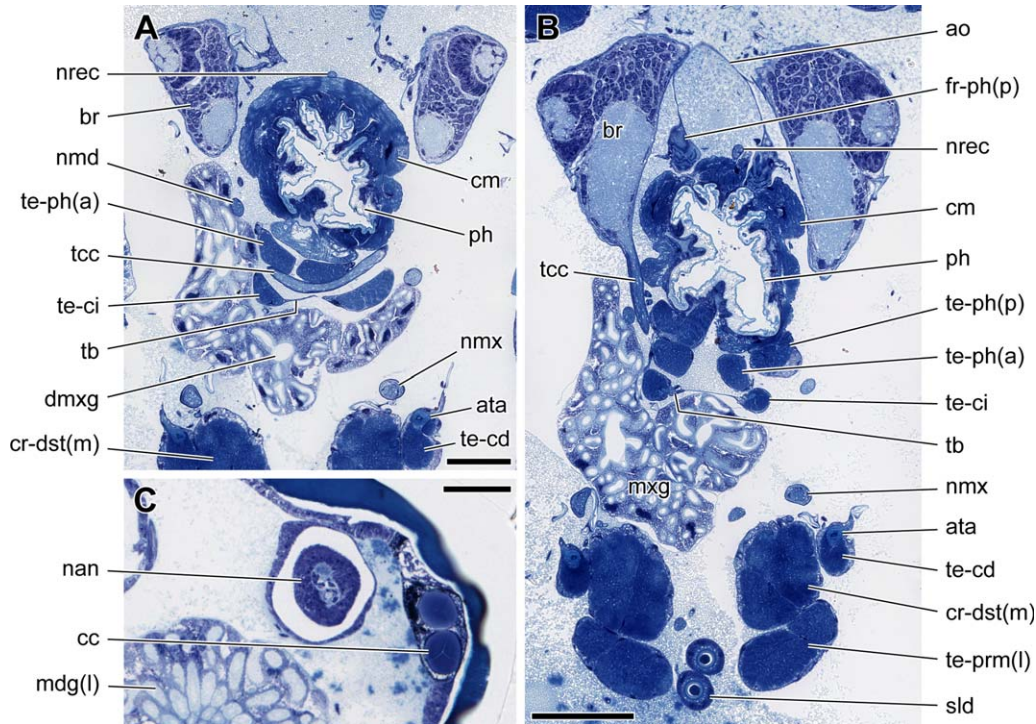
Fig. 4. Head of *Rhyacophila fasciata*, histological details. ao, aorta; ata, anterior tentorial arm; br, brain; cc, crystalline cone of stemma; cm, circular musculature of the gut; cr-dst(m), M. cranio-dististipitalis medialis; dmxg, duct of the maxillary gland; fr-ph(p), M. fronto-pharyngalis posterior; mdg(l), lateral mandibular gland; mxg, maxillary gland; nan, nervus antennalis; nmd, nervus mandibularis; nmx, nervus maxillaris; nrec, nervus recurrens; ph, pharynx; tb, tentorial bridge; tcc, tritocerebral commissure; te-cd, M. tentorio-cardinalis; te-ci, M. tentorio-cibarialis; te-ph(a)/(p), M. tentorio-pharyngalis anterior/posterior; te-prm(l), M. tentorio-praementalis lateralis; sld, salivary duct. Scale bars: A, B 100 μm, C 50 μm.

cranio-mandibularis lateralis (cr-md(l)) feather-shaped, much smaller then its antagonist, O: lateral side of the head capsule (between the dorsal and ventral bundles of cr-md(l)), I: lateralmost part of the mandibular base by the abductor tendon; M. hypopharyngo-mandibularis (hy-md), absent; M. tentorio-mandibularis (te-md), extremely thin, composed of two muscle fibers accompanied by the mandibular nerve, O: anterior tentorial arm, near the head capsule, I: dorsally on inner mandibular surface (Figs 3, 5, and 6B–D).

Maxillolabium

The proximal elements of maxilla and labium are closely adjacent and form a functional unit, that is, the maxillolabium (Fig. 1A). However, the sclerites of the mouthparts are well discriminable (Fig. 2A,B).

The maxilla is composed of cardo, stipes, maxillary palp, and a single maxillary lobe (see Discussion for homology debate). The well-sclerotized cardo is broader than long and articulates posteriorly with the head capsule (Fig. 2A,B). Its mesal margin is anteromesally pointed, membranous connected with the submentum and slightly overlapped by it (Fig. 2A). Cardo and stipes are movably connected. The membranous area between both sclerites increases from lateral to mesal (Fig. 2A,B). The stipes is divided into a proximal basistipes and a distal dististipes, bearing two and one pair of setae, respectively, (Fig. 1B: B1,2 & D1). The posteromesal half of the large basistipes is well sclerotized whereas the rest is membranous and covered with short microtrichia (Fig. 2A). The dististipes is about one third of the size of the previous element and bears the maxillary palp and the inner lobe. The inner half of the anterior margin is covered with rows of medium-sized hair-like microtrichia (Fig. 2C). Similar microtrichia are also located on the mesal margin and on the dorsal side (facing the mandibles). The maxillary lobe, addressed as galea by most workers, is inserted on the anterior margin of the dististipes, immediately mesal of the base of the palpus (Figs 1 and 2A–C). The pipe-like basal segment is about five-times longer than wide and well sclerotized. Its apex is equipped with mixture of slender and spine-like microtrichia and blunt trichoid sensilla of various lengths (Fig. 7B). Centrally in the mostly membranous tip of the galea is a well sclerotized, segment-like tube located. Terminally, it bears two long sensilla trichodea. The four-segmented maxillary palp is approximately two times thicker and one fourth longer than the galea (Figs 1 and 2). All palpomeres are rounded and tube-like. The
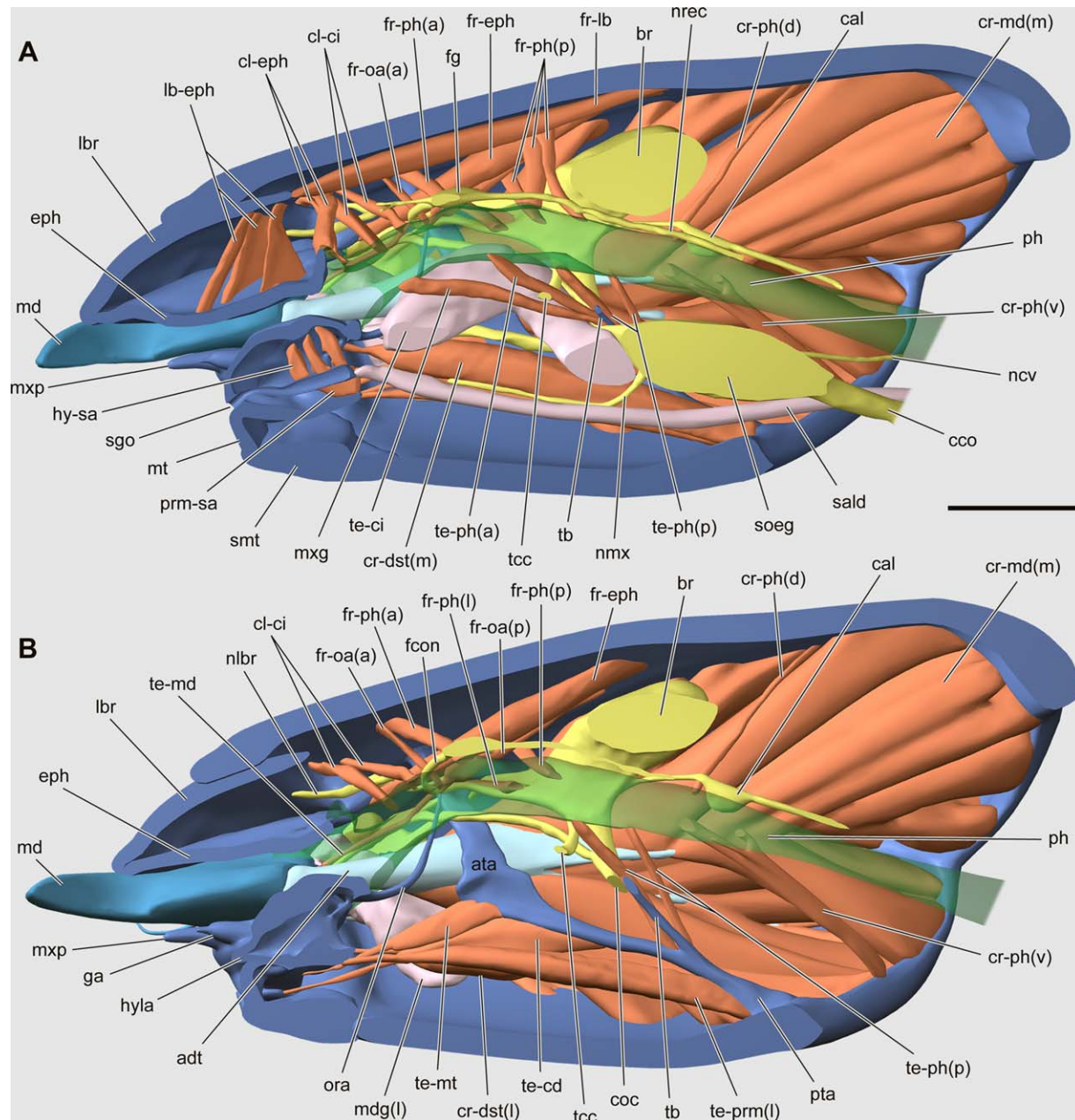
Fig. 5. Head anatomy of *Rhyacophila fasciata*, 3D-reconstruction based on SR-μCT data. (**A**) Sagittal section. (**B**) Parasagittal section, (inner muscle layer and suboesophageal ganglion removed). adt, mandibular adductor tendon; ata, anterior tentorial arm; br, brain; cal, corpora alata; cco, cervical connectives; cl-ci, M. clypeo-cibarialis; cl-eph, M. clypeo-epipharyngalis; coc, circumoesophageal commissure; cr-dst(l)/(m), M. cranio-dististipitalis lateralis/medialis; cr-md(m), M. cranio-mandibularis medialis; cr-ph(d)/(v), M. cranio-pharyngalis dorsalis/ventralis; eph, epipharynx; fcon, frontal connective; fg, frontal ganglion; fr-eph, M. fronto-epipharyngalis; fr-lb, M. fronto-labralis; fr-oa(a)/(p), M. fronto-oralis anterior/posterior; fr-ph(a)/(l)/(p), M. fronto-pharyngalis anterior/lateralis/posterior; ga, galea; hyla, hypopharyngo-labial lobe; hy-sa, M. hypopharyngo-salivarialis; lb-eph, M. labro-epipharyngalis; lbr, labrum; md, mandible; mdg(l), lateral mandibular gland; mt, mentum; mxg, maxillary gland; mxp, maxillary palp; ncv, nervus cervicalis; nlbr, nervus labralis; nmx, nervus maxillaris; nrec, nervus recurrens; ora, oral arm of hypopharynx; ph, pharynx; prm-sa, M. praemento-salivarialis; pta, posterior tentorial arm; tb, tentorial bridge; tcc, tritocerebral commissure; te-cd, M. tentorio-cardinalis; te-ci, M. tentorio-cibarialis; te-md, M. tentorio-mandibularis; te-mt, M. tentorio-mentalis; te-ph(a)/(p), M. tentorio-pharyngalis anterior/posterior; te-prm(l), M. tentorio-praementalis lateralis; sald, salivary duct; sgo, orifice of the spinning glands; soeg, suboesophageal ganglion; smt, submentum. Scale bar: 250 μm.

sclerites have a smooth surface and are delimited by short membranous areas. The diameter of the palpomeres is reduced from proximal to distal (Fig. 2A). The first and the second palpomere are almost equally thick, but the second is about twice as long. Palpomere 3 is the shortest one and as
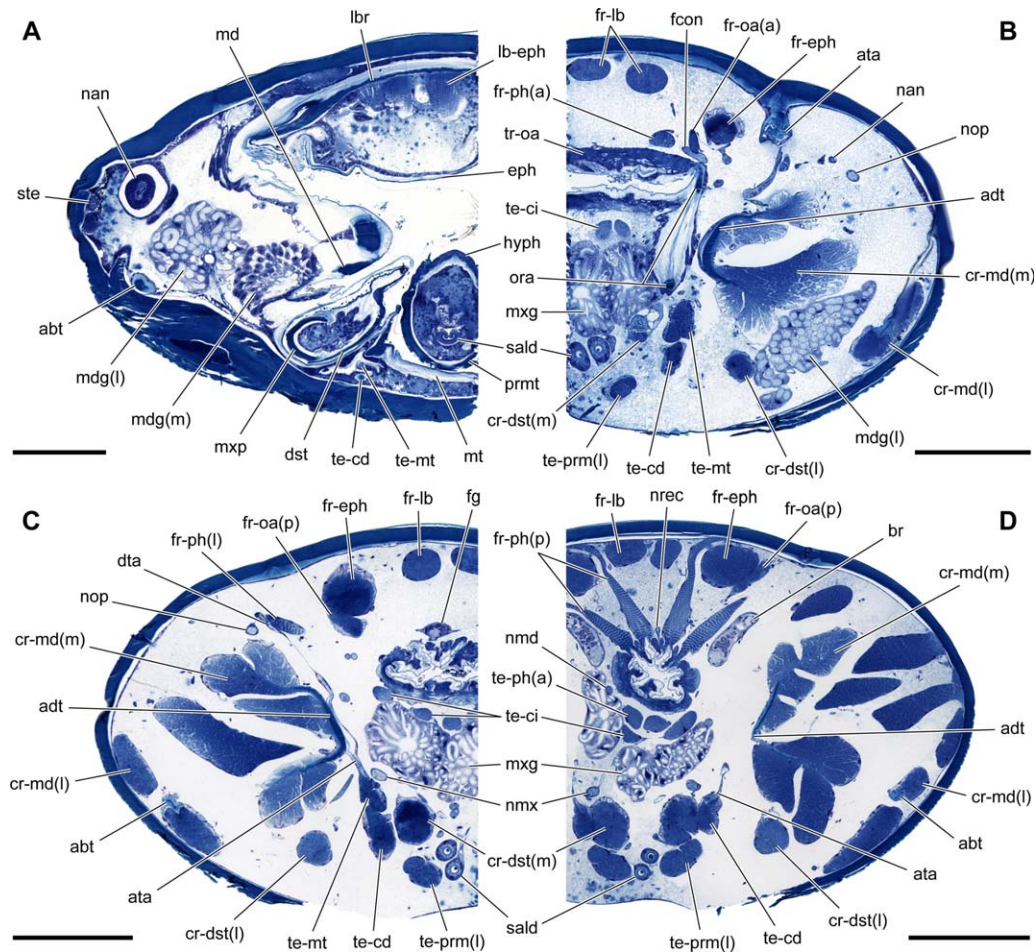
Fig. 6. Head of *Rhyacophila fasciata*, histological cross-sections. abt, mandibular abductor tendon; adt, mandibular adductor tendon; ata, anterior tentorial arm; br, brain; cr-dst(l)/(m), M. cranio-dististipitalis lateralis/medialis; cr-md(l)/(m), M. cranio-mandibularis lateralis/medialis; dst, dististipes; dta, dorsal tentorial arm; eph, epipharynx; fcon, frontal connective; fg, frontal ganglion; fr-eph, M. fronto-epipharyngalis; fr-lb, M. fronto-labralis; fr-oa(a)/(p), M. fronto-oralis anterior/posterior; fr-ph(a)/(l)/(p), M. fronto-pharyngalis anterior/lateralis/posterior; hyph, hypopharynx; lb-eph, M. labro-epipharyngalis; lbr, labrum; md, mandible; mdg(l)/(m), lateral/medial mandibular gland; mt, mentum; mxg, maxillary gland; mxp, maxillary palp; nan, nervus antennalis; nmd, nervus mandibularis; nmx, nervus maxillaris; nop, nervus opticus; nrec, nervus recurrens; ora, oral arm of hypopharynx; prmt, prementum; te-cd, M. tentorio-cardinalis; te-ci, M. tentorio-cibarialis; te-mt, M. tentorio-mentalis; te-ph(a), M. tentorio-pharyngalis anterior; te-prm(l), M. tentorio-praementalis lateralis; tr-oa, M. transoralis; sald, salivary duct; ste, stemma. Scale bars: 200 μm.

long as wide. The fourth segment is more than two times longer than wide. Its tip bears several sensilla basiconica (Fig. 7D).

Glands. One pair of large maxillary glands is located below the precerebral gut and the muscles associated with it (Figs 3A, 4A,B, and 6B-D: mxg). The central duct runs anterolaterad and opens into the cavity at the base of the mandible (Figs 3B and 4A: dmxg).

The labium is composed of prementum and postmentum, the latter is divided into the submentum and the mental area (Figs 1B and 2A,B). The triangular posterior half of the submentum is enclosed by the head capsule, whereas the trapezoid anterior half is located between the cardines. The firm connection between submentum and head capsule impedes significant movement of the sclerite. Anterior of the submentum a membranous region repre-

sents the mental area (Fig. 2A: dotted circle). Posteriorly an unpaired, half-moon-shaped sclerite, the mental sclerite, is developed, which bears a pair of setae (Figs 1B and 2A). The lateral margins of the mental area are continuous with the membranous regions of the maxilla. The labial retractor muscles mainly affect the mental area: the mental sclerite can be folded inwards up to 60° (compare Fig. 2A,B). The membranous anterior part can be strongly bent posterad, resulting in a parallel position to the submentum and the (also retracted) prementum (Figs 5 and 6A). The base of the prementum is reinforced by a clasp-like sclerite, which is basally overlapped by the mental area even in the extended position (Figs 1B and 2A). It is about twice as wide as long. The widely membranous region in front of this unpaired premental sclerite bears the labial palps and the median
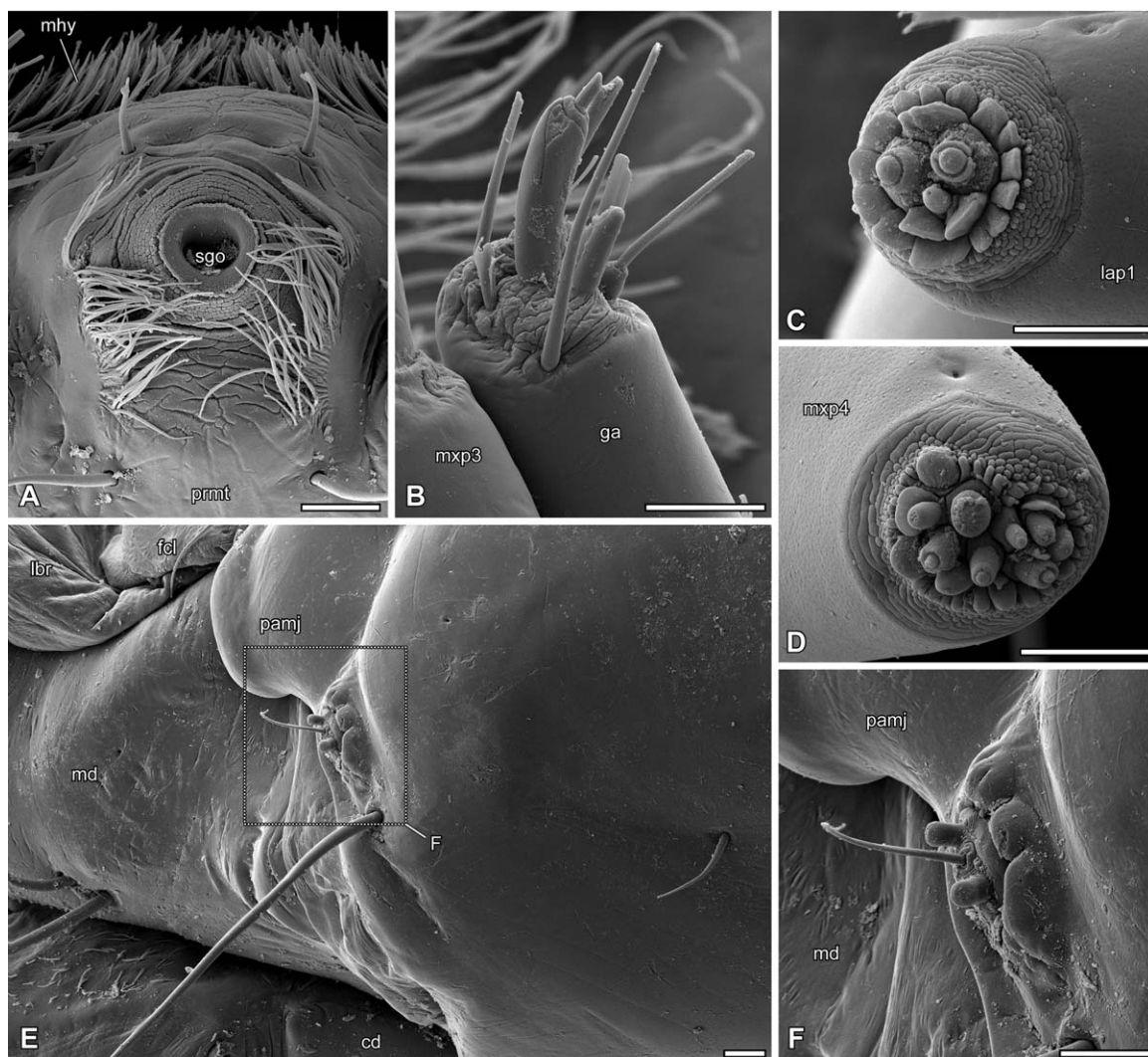
Fig. 7. External cephalic features of *Rhyacophila fasciata*, SEM images. (**A**) Labio-hypopharyngeal lobe, anterior view. (**B**) Distal element of inner maxillary appendage (galea), ventral view. (**C**) Tip of labial palp. (**D**) Tip of maxillary palp. (**E**) Lateral aspect of the head areas around the mandibular base. (**F**) Antenna, lateral view. cd, cardo; fcl, frontopostclypeus; ga, galea; lap1, first labial palpomere; lbr, labrum; md, mandible; mhy, hypopharyngeal microtrichia; mxp3/4, third/fourth maxillary palpomere; pamj, process of the anterior mandibular joint; prmt, prementum; sgo, orifice of the spinning glands. Scale bars: 20 μm.

premento-hypopharyngeal lobe. Paraglossae and glossae are absent. The bases of the one-segmented labial palps are ventrolaterally surrounded by a pair of distinct premental sclerites, that is, the palpigers. (Fig. 2A) The tip of the single, tube-like palpomere is equipped with three sensilla basiconica (Fig. 7C). Between the labial palps arise the cone-shaped premento-hypopharyngeal lobe, which is built by the distal part of the prementum ventrally and the hypopharynx dorsally (Fig. 2A,C). The circular opening of the spinning glands is located at its apex (Figs 2A,C, and 7A: sgo). The lobe is almost completely membranous but stabilized by a pair of rod-like ventrolateral sclerites articulating with the palpiger (Fig. 2A). At this point the proximal part of each sclerite bears a stiff sensory seta (Fig. 1B: Pr1). The premento-hypopharyngeal lobe

is laterally equipped with a row of long microtrichia (Fig. 7A). Also the dorsal aspect of the lobe is smooth except for a pair of sensory setae (Fig. 2C).

Maxillary musculature. M. cranio-cardinalis (cr-cd), absent; M. cranio-dististipitalis lateralis (cr-dst(l)), slender, two moderately sized bundles, O: laterally on postoccipital ridge and adjacent area of head capsule, I: posterolaterally on dististipes by a short tendon (together with bst-dst(l)); M. cranio-dististipitalis medialis (cr-dst(m)), strong, composed of two parallel bundles, O: behind te-prm(l) on mesal face of posterior tentorial arms and adjacent area of head capsule, I: with long tendons on posteromesal margin of dististipes (i.e., the vestiges of lacinia); M. tentorio-cardinalis (te-cd), fan-shaped, O: ventrally on large area of the anterior tentorial arm (posterolaterally

te-mt), I: mesally directed process of the cardo by a tendon; M. basistipito-dististipitalis lateralis (bst-dst(l)), moderately sized, O: posterior margin of dististipes, I: posterolateral edge of dististipes (together with cr-dst); M. basistipito-dististipitalis medialis (bst-dst(m)), thinner than the previous muscle, O: posterior margin of dististipes (immediately lateral of bst-dst(l)), I: ventromesally on dististipes. Muscles directly associated with the galea or the maxillary palps are absent (Figs 3B, 4A,B, 5, and 6).

Labial musculature. M. tentorio-mentalis (te-mt), three times thinner than te-cd, O: anterior tentorial arm in front of te-cd, I: lateralmost edge of mental sclerite with a long tendon; M. tentorio-praementalis lateralis (te-prm(l)), well-developed, two bundles, O: proximal part of anterior tentorial arm; I: posterolaterally on prementum; M. cranio/tentorio-praementalis medialis (te-prm(m)), absent; Mm. praemento-palpales et palpo-palpales labii are absent (Figs 3B, 4B, 5, and 6).

Hypopharynx

The anterior part of the hypopharynx forms the dorsal face of the premento-hypopharyngeal lobe (Fig. 2C). Behind this smooth, membranous region, the hypopharynx bears a dense vestiture of different types of microtrichia (Fig. 2C). It is laterally bordered by a row of sickle-shaped microtrichia, which are mesally directed and lie almost parallel to the surface of the hypopharynx. Between these rows, a triangular field of stout cone-shaped microtrichia is located. The posterior half of the hypopharynx is enforced by a pair of elongate sclerites (Fig. 2C). Posterolaterally, each hypopharyngeal sclerite bears a long dorsad arm bend around the lateral angles of the anatomical mouth opening (Fig. 5B: ora). These heavily sclerotized rods (= oral arms) stabilize the cibarium (see below).

Musculature. M. fronto-oralis anterior (fr-oa(a)), O: anterior frontal area ((anteromesal of fr-ph(a)), I: angle of the anatomical mouth, close to the apex of the oral arm (together with fr-oa(p)); M. fronto-oralis posterior (fr-oa(p)), O: posterior frontal area (laterally to fr-eph), I: angle of the anatomical mouth, immediately in front of the oral arm tip (together with fr-oa(a)); M. tentorio-hypopharyngalis (te-hy), absent; M. transoralis (tr-oa), well-developed, O: tip of oral arm, I: oral arm of contralateral side (Figs 3A, 5, and 6B–D).

Epipharynx and Cibarium

The epipharynx forming the ventral wall of the labrum is slightly sclerotized. Its mesal area becomes longitudinally bulged in posterior direction, whereas the lateral parts are rather straight, altogether reflecting the shape of the upper side of the mandibles (Fig. 5A).

The posterior half of the epipharynx is laterally continuous with the hypopharynx, forming a non-sclerotized, tube-like cibarium (Fig. 6B). The border between the anterior epipharynx and the epipharyngeal roof of the cibarium is demarcated by a prominent membranous cross-fold (Fig. 5A). The cibarium (= prepharynx), that is, the fused part of epi- and hypopharynx in front of the frontal ganglion, is dorsally equipped with three semicircular cross-muscle bundles. Its floor (= hypopharyngeal side) is supported by a single cross-muscle interconnecting the oral arms.

Musculature. M. clypeo-epipharyngalis (cl-eph), two bundles with slightly different attachment sites, O: anterior region of frontoclypeus, I: posterior margin of epipharyngeal sclerite and membranous region behind (in front of cross-fold); M. clypeo-cibarialis (cl-ci), two serial bundles, O: frontoclypeus, immediately behind cl-eph, I: roof of cibarium (between dorsal cross-muscle bundles); M. tentorio-cibarialis (te-ci), two bundles with discrete insertion sites, O: mesally on the tentorial bridge (close to te-ph(a)), I: mesally (mesal bundle) and laterally (lateral bundle) on the floor of the cibarium (at level of the frontal ganglion) (Figs 3, 4A,B, 5, and 6B-D).

Pharynx

The pharynx forms a simple tube of almost constant diameter (Fig. 5). Its endothelium is strongly folded in the precerebral part (Fig. 4A,B). After passage through the brain, the pharyngeal endothelium forms four longitudinal folds, resulting in an X-shaped lumen. The precerebral pharynx (between frontal ganglion and brain) is completely covered by a strong musculature of circular fibers, whereas only few longitudinal fibers are present in the folds on the dorsal side (Figs 4A and 6D). At the beginning of the brain, the ring muscle fibers get less and thinner (Fig. 4B). Longitudinal muscle fibers are lacking in the postcerebral pharynx.

Musculature of the precerebral pharynx. M. fronto-pharyngalis anterior (fr-ph(a)), O: frontoclypeus (posterolateral of fr-oa(a)), anterior of the level of the anterior tentorial pits, I: dorsal face of pharynx, directly besides the frontal ganglion and posterior to the frontal connective; M. frontopharyngalis posterior (fr-ph(p)), two vertical and one horizontal bundle, O: two bundles at the posteriorly (directly in front of the brain), one bundle posterolaterally (posterad anterior tentorial pits) on the frontoclypeus, I: dorsally and dorsolaterally on the pharynx (in front and below the brain); M. fronto-pharyngalis lateralis (fr-ph(l)), O: head capsule, lateral of anterior tentorial pit, I: centrally at the lateral face of the precerebral pharynx; M. tentorio-pharyngalis anterior (te-ph(a)), O: laterally on the tentorial bridge, I:

mesally on the anteriormost part of the pharynx, opposite to the dorsal dilators of the precerebral pharynx (Figs 3, 4A,B, 5, and 6A-D).

Musculature of the postcerebral pharynx. M. cranio-pharyngalis dorsalis (cr-ph(d)), one slender bundle, O: paramedian on vertex, anteromesally of cr-md(m), I: dorsolaterally on postcerebral pharynx; M. cranio-pharyngalis ventralis (cr-ph(v)), two slender bundles, O: ventrolaterally on postoccipital ridge (mesally of cr-st), I: ventrolaterally on the posterior end of the pharynx; M. tentorio-pharyngalis posterior (te-ph(p)), two slender bundles, O: tentorium, around the base of the tentorial bridge, I: ventrolateral edge of the pharynx behind and directly between the circumoesophageal commissures (Figs 3 and 5).

Salivarium

The salivary (= labial) glands of *Rhyacophila* are modified to produce silk. The long, tubular glands are located in the thorax and the abdomen. They enter the head and run ventromedial into the premento-hypopharyngeal lobe (Fig. 5A). The ducts unite immediately in front of the sclerotized salivarium. Its mid-part is U-shaped in cross-section and composed of a thick, strongly sclerotized floor, and a thinner roof (Fig. 6A: sald). The posterior angles of the floor are extended as moderately sized dorsad arms, which form lever moved by the posteriormost bundle of the hypopharyngo-salivarial muscle. Anteriorly, distally of the strong dorsoventral muscles, the salivarium has a circular wall. The round orifice of the salivary (spinning) tube is located on the apex of the premento-hypopharyngeal lobe (Fig. 5A).

Musculature. M. hypopharyngo-salivarialis (hy-sa), three compact serial bundles, O: broadly on the hypopharyngeal sclerite in front of the oral arms, I: medially on the roof of the salivarium, the posteriormost bundle on the hind margin of the salivarium; M. praemento-salivarialis (prm-sa): strong, compact, O: proximal margin of the clasp-like premental sclerite, I: lateral and posterodorsal areas of the salivary floor (including salivary arms) (Figs 5A and 6A).

Central Nervous System

The small central nervous system (= supra- and suboesophageal ganglion) is centrally located in the head (Fig. 5B). The brain (= supraoesophageal ganglion) is about one quarter as wide as the head capsule and composed of two weakly separated hemispheres. Proto-, deuto- and tritocerebrum are inseparably fused and only identifiable by the outgoing nerves. The anterolateral corners of the brain give rise to the optic and antennal nerves. The proximal parts of both nerve tracts run parallel and closely adjacent anterolaterad. The strong optical nerve merges into the optic neuropils of

the stemmata (Fig. 3A). The proximal half of the antennal nerve is thinner than the optical nerve but strongly expands distally (Figs 3A and 4C). Finally two very thin nerves enter the antenna. Below the origin of the former nerves, the labral nerves emerge from the tritocerebral part of the brain. Their basal parts release the thin, mesally directed frontal connectives, which curve anteriorly around the tendons of the fronto-oral muscles before entering the frontal ganglion. The labral nerves continue anterad, splitting into several branches and innervate the labral and epipharyngeal muscles. The rhomboid frontal ganglion is located on the dorsal side of the prepharynx (Fig. 5) demarcating the anatomical mouth, that is, the border between prepharynx and anterior (or precerebral) pharynx. It releases the unpaired nervi procurrens and recurrens anterad and posterad, respectively. Very thin lateral branches innervate the cibarial muscles closely beside the frontal ganglion. The comparatively thin circumoesophageal connectives connect the brain with the suboesophageal ganglion. They are about twice as thick as the mandibular nerves. The dorsal ends of the connectives are interconnected by a curved tritocerebral commissure (Figs 4A,B and 5: tcc). It separates the courses of M. tentorio-cibarialis and M. tentorio-pharyngalis anterior (Figs 4A and 5A). The mandibular nerves arise anteriorly from the central part of the circumoesophageal connectives and run straight toward the mandibles. The equally thick maxillary nerves leave the anterior face of the suboesophageal ganglion. Behind the former arise the comparatively thin labial nerves. Very thin, paired nerve branches arising laterally from the suboesophageal ganglion innervate the dorsal and lateral cervical muscles of the head. The cervical connectives are well developed.

Circulatory System

The thin cephalic aorta enters the head along the dorsal side of the pharynx (Fig. 4B: ao). Its diameter distinctly increases between the circumoesophageal commissures. Directly, in front of the brain the aorta runs dorsad and opens freely into the cephalic cavity. There are no dilator muscles or specific pulsatile organs directly associated with the aorta.

Tracheal System

Each of the two tracheal stem pairs split into two branches before entering the head capsule. The dorsal branches supply the dorsal and lateral areas of the head, for instance the strong mandibular muscles. The ventral branches accompany the gut laterally, run around the brain anterad supplying the anterior half of the head capsule and the central nervous system.

Fat Body

Aggregations of fat cells fill large parts of the haemolymph spaces, especially of the anterior half of the head capsule.

DISCUSSIONS
Terminology and Interpretation of Anatomical Structures

The homology of several sclerites of the larval head in Trichoptera was discussed controversially in the first half of the last century (see Hinton, 1958 and Badcock, 1961). However, while some aspects are certainly clear now, others are still not resolved. In the following paragraphs, the interpretation of anatomical key structures described for *Rhyacophila* will be discussed.

Frontal and clypeal regions. The region of the head capsule enclosed by the paired frontal sutures was termed as clypeus by several authors in the first half of the 20th century (e.g., Cook, 1944) and rarely also interpreted as frons (e.g., Betten, 1934). However, there is no doubt that this region is composed of both frontal and clypeal elements and, therefore, has to be termed frontoclypeus (e.g., Malicky, 1973; see also discussion in Badcock, 1961). Considering the flexible intraclypeal ridge separating a membranous anteclypeus (present in Rhyacophilidae, other spicipalpian taxa, and Annulipalpia; e.g., Badcock, 1961) the well-sclerotized part represents a frontopostclypeus in these taxa.

The lack of the epistomal ridge impedes the delimitation of the original regions based on sclerites. As described by Snodgrass (1947) and later affirmed by Badcock (1961), the muscles attached to the gut and the frontoclypeal area indicate the approximate position of the border (i.e., of the epistomal ridge). Muscles inserting dorsally on the pharynx between frontal ganglion and the brain always originate from the frons whereas the prepharyngeal muscles attached in front of the frontal ganglion always arise from the clypeus (Snodgrass, 1947: p.11 ff). However, in *Rhyacophila* the lateral fronto-pharyngeal muscle pair originates distinctly outside (laterally) of the "frontal" sutures. This inconsistency was also detected by Hinton (1958) and Badcock (1961) in hydropsychid species and led the latter author to the assumption that the frontal area is not restricted to the delimited median region but might be also extended laterally. This explanation seems to be reasonable but a shift of the muscle insertion site to the genal region cannot be excluded.

Labium and hypopharynx. The labium of trichopteran larvae is primarily subdivided in a proximal postmentum and a distal prementum. The postmentum is divided into mentum and submentum, the latter was often misinterpreted as gula. While the submentum is always well sclerotized the mental area comprises more or less extensive membranous parts. Nevertheless, it is almost always identifiable by its pair of mental setae (Hinton, 1958). The proximal part of the labium, that is, the premental area, is dorsally fused with the hypopharynx forming a (pre)labiohypopharyngeal lobe which bears the unpaired opening of the silk ducts. The number and shape of premental sclerites are often species-specific. Likewise, the sclerotized areas of the mentum can be paired (e.g., Glossosomatidae, *Hydropsyche*; Badcock, 1961) or unpaired (e.g., *R. fasciata*). Submentum and mentum lack muscle insertions, as it is generally the case in mecopterid larvae (Hinton, 1958). There are only very few exceptions: The results of this study and information given by Hinton (1958: p. 193) show that the insertion site of the tentorio-stipital muscle is secondarily shifted to the lateral margin of the mentum in Rhyacophilidae and Polycentropodidae. This rather unconventional shift is possible due to the strong interconnection of labium and maxilla in Trichoptera. The prementum is equipped with a single extrinsic muscle composed of two bundles which originate at the posteriormost part of the tentorium and/or the adjacent part of the head capsule. Based on its insertion site, this muscle has to be regarded as homologous to the lateral retractor of the prementum (M. 29 of von Kéler, 1963). The second, "dorsal" extrinsic muscle of the prementum described by Badcock (1961: d.m.pm) for *Hydropsyche* is likely homologous to the tentoriohypopharyngeal muscle (Table 1; M. 42 of von Kéler, 1963). However, the presence of this muscle could not be confirmed by own investigations on *H. angustipennis*. It is also lacking in *Rhyacophila* and *Limnephilus*. The "rétracteurs de l'hypopharynx" is described as attached to a hypopharyngeal sclerite by Fotius-Jaboulet (1961: rhphy). Considering the simultaneous lack of the medial cranio-dististipital muscle, (which is present in all species investigated here) and the identical position of both muscles in cross-sections (compare Fig. 6B–D and Fotius-Jaboulet, 1961: fig. 34) it is plausible to assume that both muscles are homologous and derived from the primary extensor of the lacinia (see below). The fusion of premental and hypopharyngeal regions and the close association of labium and maxilla might facilitate the shift of insertion sites (see also above: tentorio-stipital muscle).

The labial palps are the sole appendages of the prementum in trichopteran larvae. Discrete paraglossae and glossae are always lacking. The palps are one- or two-segmented (e.g., Hinton, 1958; Badcock, 1961). Some authors misinterpreted the palpiger, in fact a more or less distinct sclerite of the prementum, as the basal palpomere. However,

today the premental origin of this paired sclerite is widely accepted (see e.g., Hinton, 1958; Badcock, 1961).

Maxilla. The homology of the maxillary sclerites and the corresponding muscles is discussed controversially over decades. Fusion or secondary subdivision of sclerites as well as the loss of a series of maxillary muscles strongly complicates a clear assignment of structures. The major controversies are about the true nature of the sclerite often described as "palpifer" and the derivation of the inner maxillary appendage (lacinia vs. galea). Both points were investigated and discussed in detail by Hinton (1958) and Badcock (1961), resulting in widely conflicting hypotheses. In the following, these hypotheses will be evaluated implementing new results on *Rhyacophila* and data from the literature not considered in the former contributions (e.g., Winkler, 1959; Fotius-Jaboulet, 1961).

The inner lobe of the maxilla, located mesally of the palpus, was regarded to represent the lacinia (Krafka, 1923; Das, 1937; Badcock, 1961), the galea (Crampton, 1921; Nielsen, 1942; Winkler, 1959; Fotius-Jaboulet, 1961;) or a fused product of both structures (Betten, 1934). The cylindrical shape of the lobe and its association with sensory sensilla in *Rhyacophila* and other trichopterans are usually attributes of the galea in more generalized endopterygote insects (e.g., Kramer, 1955; Badcock, 1961; Beutel and Friedrich, 2008). The lack of any muscles usually in association with this appendage in generalized insects impedes the designation of its components in Trichoptera. However, the presence of a mesal cranio-dististipital muscle (cr-dst[m]) facilitates the interpretation. As its homology with the primitive cranial flexor of the lacinia is rather certain (e.g., Hinton, 1958; Kristensen, 1984) the (at least partial) inclusion of the lacinia into the dististipes has to be favored (see also below). The incorporation of a lacinial part into the mesal appendage in Trichoptera is not supported by anatomical data.

Hinton (1958: 188ff) proposed a transversely divided stipes, that is, two separate stipital sclerites, as a groundplan feature of Mecopterida. In Trichoptera the proximal basistipes is connected to the cardo and was often regarded as the whole stipes (e.g., Winkler, 1959), whereas the distal dististipes, usually bearing the maxillary palp and the mesal appendage (= galea), was addressed as palpifer (e.g., Crampton, 1921; Fotius-Jaboulet, 1961) or lacinia (Krafka, 1923: fig. 4). In the definition given by Snodgrass (1935), a palpifer is a specific part of the stipes bearing the maxillary palp. As in Trichoptera, the sclerite in question bears also the inner maxillary appendage, this term is not applicable. The muscles attached to the distal stipital sclerite were termed "cranial flexor/extensor of the dististipes" and "stipital flexor/extensor of the dis-

tistipes" by Hinton (1958), implying a series of shifts of insertions. As pointed out by Badcock (1961), the cranial flexor of Hinton likely represents the flexor of the lacinia in other endopterygote insects, a homology also favored by Hinton (1958: p. 191). Studies on hydropsychids and other trichopterans led Badcock (1961: 227ff) to the assumption that Hinton's dististipes represents a fusion of the proximal (5th) maxillary palpomere and the base of the inner lobe (Badcock's lacinia). Indeed, Hinton already homologized his intrastipital muscles with the levator and the depressor of the maxillary palp (1958: 192ff). His denial of a papal component of the sclerite requires the shift of the insertion sites of these muscles, a factor already criticized by Kristensen (1984: 78). The inclusion of the basal (5th) palpomere into the dististipes renders such shifts unnecessary (see Badcock, 1961; Kristensen, 1984). Considering the anatomical conditions found in *Rhyacophila* and other trichopterans it appears reasonable that the mesal part of the dististipes is formed by the vestiges of the lacinia (see above). Despite all typically stipital muscles are attached to the basistipes, the stipital derivation of parts of the dististipes is evident by the position of the maxillary appendages. In summary, the dististipes has to be seen as a "morphologically composite formation" as correctly stated by Kristensen (1984). Due to the lack of any associated muscles it cannot be evidently determined if also proximal parts of the galea (lacinia of Badcock, 1961) are implemented. However, the comparative analyses presented by Badcock (1961) apparently support also this.

The complex anatomy of maxillary, labial, and hypopharyngeal components, which can be partly fused, is a strong handicap for the interspecific homologization of the muscles attached to these structures (see also Table 1). This resulted in several potential misinterpretations in descriptions of the head muscles in trichopteran larvae (Winkler, 1959; Badcock, 1961; Fotius-Jaboulet, 1961). The muscles in question comprise the lateral and mesal basistipito-dististipital muscles [bst-dst(m), bst-dst(l); flexor and extensor of Hinton (1958)] and the lateral and mesal cranio-dististipital muscles [cr-dst(m), cr-dst(l); flexor and extensor of Hinton (1958)]. The origin and insertion sites of the interstipital muscles allow for a rather certain homologization with the stipito-palpal muscles in generalized insects. This was also recommended by most authors (Hinton, 1958; Badcock, 1961; Fotius-Jaboulet, 1961; Kristensen, 1984). Own reinvestigations of *Limnephilus* revealed that Winkler (1959: muscle 20) misinterpreted the inner muscle [bst-dst(m)] as the stipito-lacinial muscle and overlooked its lateral counterpart.

The mesal cranio-dististipital muscle was already homologized with the cranio-lacinial

muscle of generalized insects by Hinton (1958: 191). Even if the lacinia is not retained as a separate element, the remnants of this structure, as well as the insertion sites of potentially lacinial muscles, have to be located mesally of the base of the maxillary palp. This applies to the cranial flexor of the lacinia described for Hydropsychidae by Badcock (1961: c.f.la.) and the muscle 29 of Winkler (1959) in *Limnephilus* (which he interpreted as a tentorio-premental muscle). If the muscle Fotius-Jaboulet (1961) described as "rétracteurs de l'hypopharynx" (rhphy) is really attached to a hypopharyngeal sclerite or to the closely adjacent mesal region of the dististipes (as it is the case in *Limnephilus*; pers. obs.) could not be reinvestigated. Detailed, comparative investigations on additional integripalpian taxa are strongly needed to identify potential shifts of the insertion site.

The most intriguing muscle of the trichopteran maxilla is the lateral cranio-dististipital muscle (extensor sensu Hinton, 1958). The potential homology of this muscle is discussed in detail by Fotius-Jaboulet (1961: 28ff). As pointed out by Kristensen (1984) the most reasonable interpretation is that it represents a former tentorio-stipital muscle, which shifted its origin toward the head capsule. This unique configuration led Kristensen (1981) to consider it as a potential autapomorphy of Mecopterida.

Ventral closure of the head capsule. There are two major types of the ventral closure of the occipital foramen developed within Trichoptera. In Rhyacophilidae and the vast majority of the other prognathous taxa (as Hydropsychidae and Glossosomatidae), both sides of the head capsule meet each other in the ventromedian line forming a distinct hypostomal bridge (sensu Beutel et al., 2013). Similar conditions are also present in some hypognathous forms as for instance *Limnephilus* and Sericostomatidae. Another type is present in most hypognathous forms, were the postgenae are distinctly separated by the proximal part of the labium, that is, the submentum, which was often misinterpreted as gula (e.g., Krafka, 1923; Nielsen, 1942; Winkler, 1959; Korboot, 1964). Both kinds of ventral closure are always located in front of the posterior tentorial grooves, whereas a true gula is defined as located behind these grooves (Snodgrass, 1935; Hinton, 1958; von Kéler, 1963; see also Badcock, 1961).

The ventromedian angle of the occipital foramen of Rhyacophilidae, Hydropsychidae, Hydroptilidae, and Glossosomatidae bears a minute sclerite termed protogula by Badcock (1961). It represents very likely a secondary sclerotization of the cervical membrane caused by the forces of the attached prothoracic muscles. We agree with Badcock (1961) that this structure is certainly not homologous with the gula of other insects.

Groundplan of the Larval Head in Trichoptera

Rhyacophilidae were often regarded as basal representatives of Trichoptera, considering their morphological features as plesiomorphic for the order (e.g., Ross, 1964; Klemm, 1966). Generally, the anatomy of individuals (as representatives of species) is a mosaic of plesiomorphic and apomorphic features. Consequently, it is not accurate to assume that a species (or other taxon) represents the complete groundplan of a superordinate taxon (e.g., an order). However, it has been demonstrated that "basal" representatives of an insect taxon usually retain a higher number of groundplan conditions of the superordinate taxon (plesiomorphies of the basal taxon) than more derived taxa (e.g., Friedrich et al., 2009; Beutel et al., 2009, 2011; Blanke et al., 2013).

As the basal branching pattern of the trichopteran phylogeny is not resolved yet (e.g., Kjer et al., 2002; Holzenthal et al., 2007; Malm et al., 2013), the diversity of morphological features is often difficult to interpret (see also Kubiak et al., in press). Today, a basal position of the spicipalpian families (e.g., Wiggins and Wichard, 1989; Malm et al., 2013) or spicipalpians and Annulipalpia (e.g., Frania and Wiggins, 1997; Kjer et al., 2001, 2002; Holzenthal et al., 2007) appears to be the best supported scenarios. Therefore, the (partial) reconstruction of the groundplan of the larval head in Trichoptera presented in the following is the result of an informal character analysis based on the branching patterns proposed by Holzenthal et al. (2007) and Malm et al. (2013).

In the trichopteran groundplan, the larval head is very likely prognathous and distinctly longer than wide as it is also the case in basal lepidopteran (Kristensen, 1984) and mecopteran groups (Beutel et al., 2009). The ventral closure is formed by a hypocranial bridge, which is anteriorly bordered by a primarily triangular submentum. Heads of this type are found in Rhyacophilidae, other spicipalpians and annulipalpians (see e.g., Lepneva, 1970). A more or less strong elongation of the submentum occurred independently in several lineages (e.g., Hydropsychidae, Goeridae, Phryganeidae). The trichopteran groundplan retains several apomorphies of Mecopterida: a closely associated maxillo-labial complex (Hasenfuss and Kristensen, 2003), the subdivision of the labium, labial palps without directly associated muscles, the presence of well-developed postmental areas (also without associated muscles) and the subdivision of the stipes into basi- and dististipes. In contrast to antliophoran taxa basistipes and cardo are distinct sclerites (Hinton, 1958). Furthermore, two-segmented labial palps as present in Mecoptera (Beutel et al., 2009) and most lepidopterans (Hasenfuss and Kristensen, 2003) are

very likely a groundplan feature of Mecopterida (Hinton, 1958). It is plausible to assume that the loss of labial palpomeres and the shortening of the palp occurred several times independently within Trichoptera. With only few exceptions, the set of maxillary muscles present in *Rhyacophila* is close to the groundplan of Mecopterida proposed by Hinton (1958). The shift of insertion of the tentorio-stipital muscle to the lateral mental rim is clearly a derived feature of Rhyacophilidae. A similar modification is only known from Polycentropodidae (Hinton, 1958).

A distinct delimitation of membranous anteclypeus and well-sclerotized frontopostclypeus can also be assigned to the trichopteran groundplan as it is present in spicipalpian and annulipalpian taxa. It is plausible to assume that the antenna in the groundplan of Trichoptera is one-segmented, located very close to the anterior margin of the head capsule (e.g., Frania and Wiggins, 1997) and lacks extrinsic and intrinsic muscles. Further characteristics are controversially discussed (e.g., Ross, 1967; Denis, 1984; Frania and Wiggins, 1997). Two large basiconic sensilla are likely a groundplan feature (Frania and Wiggins, 1997: 12). As correctly concluded by Frania and Wiggins (1997), the short antenna of *Rhyacophila* represents not the "primitive type" in Trichoptera (as proposed by Nielsen, 1942), but is very likely a result of secondary reduction. On the other hand, the simple, tube-like silk press associated with two dilator muscles present in *Rhyacophila* can be assigned to the trichopteran groundplan as it is similarly developed in basal lepidopterans (Kristensen, 1984; see also Frania and Wiggins, 1997).

The tentorium of most spicipalpian and annulipalpian groups (except e.g., Glossosomatidae) is very slender. A very thin tentorial bridge and also thin dorsal tentorial arms are usually present and very likely part of the trichopteran groundplan (Hinton, 1958). The tentorial bridge is secondarily lacking in some hydropsychid and sericostomatid species (Badcock, 1961). The comparatively stout tentorial bridge in *Agapetus* (Glossosomatidae; Badcock, 1961) represents very likely a secondary enforcement as effect of miniaturization.

The number of stemmata varies strongly in Trichoptera. The highest number of seven separate stemmata is present in Rhyacophilidae (Fig. 2D) and Hydropsychidae and represents the trichopteran groundplan condition (Paulus and Schmidt, 1978). Among Lepidoptera, only Heterobathmiidae have seven stemmata while in the majority of other families the maximum number is six (Hasenfuss and Kristensen, 2003; Paulus and Schmidt, 1978; Paulus, 1986). Paulus and Schmidt (1978) showed that each stemma is composed of three crystal cone cells in the groundplan of Amphiesmenoptera (apomorphy) and Trichoptera (plesiomorphy). At the first view the number of stemmata is six in all integripalpian species investigated so far (Paulus and Schmidt, 1978). Ultrastructural investigations led Paulus and Schmidt (1978) to the conclusion that the first and second stemmata are fused in these species (double stemma composed of six cone cells) and represent a potential autapomorphy of Integripalpia. There is some evidence for a trend of reducing the number of stemmata within Annulipalpia (only two are present in Philopotamidae; Paulus and Schmidt, 1978; Frania and Wiggins, 1997). The number of stemmata is still unknown for a series of trichopteran families. Frania and Wiggins (1997: 12) mentioned the difficulties of precisely counting the number in unstained specimens. Among others, this is caused by the lack of specific corneal lenses which could be easily observed by SEM or light microscopy (see Paulus and Schmidt, 1978). Ultrastructural studies on the cellular composition of the stemmata as carried out by Paulus and Schmidt (1978) are necessary but rather time-consuming. A fast and simple way of counting the number of stemmata is the application of confocal laser scanning microscopy as presented here (Fig. 2D). The stemmata (i.e., their crystal cones) are well-visible in the image stacks. Even the number of cone cells can be visualized at higher magnification.

The results and interpretations presented here show the high number of plesiomorphic character conditions present in Rhyacophilidae. Furthermore, the groundplan of Trichoptera can be reconstructed for several larval head characters with good certainty. The reconstruction of the evolutionary history of trichopteran lineages (e.g., Annulipalpia, Integripalpia) is still strongly impeded by the lack of anatomical data for many families and the unresolved phylogeny of the order. We are confident that the application of modern techniques in insect morphology (e.g., μCT, CLSM) and the sophisticated molecular techniques (e.g., NGS; see e.g., www.1kite.org) will help to overcome these drawbacks.

ACKNOWLEDGMENTS

LITERATURE CITED

Badcock RM. 1961. The morphology of some parts of the head and maxillo-labium in larval Trichoptera, with special

reference to the Hydropsychidae. Trans Roy Entomol Soc Lond 113:217–248.

Beckmann F, Donath T, Fischer J, Dose T, Lippmann T, Lottermoser L, Martins RV, Schreyer A. 2006. New developments for synchrotron-radiation-based microtomography at DESY. Proc SPIE Int Soc Opt Eng 6318:1–10.

Beckmann F, Herzen J, Haibel A, Müller B, Schreyer A. 2008. High density resolution in synchrotron-radiation-based attenuation-contrast microtomography. Proc SPIE 7078:D-1–13.

Betten C. 1934. The caddis flies or Trichoptera of New York State. N.Y. State Mus Bull 292:1–116.

Beutel RG, Friedrich F. 2008. Comparative study of larval head structures of Megaloptera (Hexapoda). Eur J Entomol 105: 917–938.

Beutel RG, Kristensen NP, Pohl H. 2009. Resolving insect phylogeny: The significance of cephalic structures of the Nannomecoptera in understanding endopterygote relationships. Arthropod Struct Dev 38:427–460.

Beutel RG, Friedrich F, Hörnschemeyer T, Pohl H, Hünefeld F, Beckmann F, Meier R, Misof B, Whiting MF, Vilhelmsen L. 2011. Morphological and molecular evidence converge upon a robust phylogeny of the megadiverse Holometabola. Cladistics 27:341–355.

Beutel RG, Friedrich F, Yang XK, Ge S. 2013. Insect Morphology and Phylogeny. Berlin: De Gruyter. 516 p.

Blanke A, Beckmann F, Misof B. 2013. The head anatomy of *Epiophlebia superstes* (Odonata: Epiophlebiidae). Org Divers Evol 13:55–66.

Cook EF. 1944. The morphology and musculature of the labrum and clypeus of insects. Microentomology 9:1–35.

Crampton GC. 1921. The sclerites of the head, and the mouthparts of certain immature and adult insects. Ann Entomol Soc Am 14:65–110.

Das GM. 1937. The musculature of the mouth-parts of insect larvae. Q J Microsc Sci 80:39–80.

Deans AR, Mikó I, Wipfler B, Friedrich F. 2012. Evolutionary phenomics and the emerging enlightenment of arthropod systematics. Invertebrate Syst 26:323–330.

Fotius-Jaboulet M-C. 1961. Squelette et Musculature Cephaliques de la Larva de *Drusus trifidus* Mac Lachan (Trichoptera—Limnophilidae). Trav Lab Zool Stn Aquic Grimaldi Dijon 40:154.

Frania HE, Wiggins GB. 1997. Analysis of morphological and behavioural evidence for the phylogeny and higher classification of Trichoptera (Insecta). Roy Ontario Mus Life Sci Contrib 160:1–67.

Friedrich F, Beutel RG. 2008. Micro-computer tomography and a renaissance of insect morphology. Proc SPIE 7078:1–6.

Friedrich F, Farrell BD, Beutel RG. 2009. The thoracic morphology of Archostemata and the relationships of the extant suborders of Coleoptera (Hexapoda). Cladistics 25:1–37.

Friedrich F, Pohl H, Beckmann F, Beutel RG. 2013. The head of *Merope tuber* (Meropeidae) and the phylogeny of Mecoptera (Hexapoda). Arthropod Struct Dev 42:69–88.

Friedrich F, Matsumura Y, Pohl H, Bai M, Hörnschemeyer T, Beutel RG. 2014. Insect morphology in the age of phylogenomics: Innovative techniques and its future role in systematics. Entomol Sci 17:1–24.

Hasenfuss I, Kristensen NP. 2003. Skeleton and muscles: Immatures. In: Kristensen NP, editor. Handbuch der Zoologie. Band IV: Arthropoda: Insecta. Teilband 36: Lepidoptera, Moths and Butterflies. Volume 2: Morphology, Physiology, and Development. Berlin: De Guyter. pp 133–164.

Hennig W. 1953. Kritische Bemerkungen zum phylogenetischen System der Insekten. Beitr Entomol 3:1–85.

Hennig W. 1969. Die Stammesgeschichte der Insekten. Frankfurt A.M.: Waldemar Kramer Verlag.

Hinton HE. 1958. The phylogeny of the panorpoid orders. Annu Rev Entomol 3:118–206.

Holzenthal RW, Blahnik RJ, Prather AL, Kjer KM. 2007. Order Trichoptera Kirby, 1813 (Insecta), Caddisflies. Zootaxa 1668: 639–698.

Kjer KM, Blahnik RJ, Holzenthal RW. 2001. Phylogeny of Trichoptera (Caddisflies): Characterization of signal and noise within multiple datasets. Syst Biol 50:781–816.

Kjer KM, Blahnik RJ, Holzenthal RW. 2002. Phylogeny of caddisflies (Insecta, Trichoptera). Zool Scr 31:83–91.

Klemm N. 1966. Die morphologie des Kopfes von *Rhyacophila* pict. (Trichoptera). Zool Jahrb/Abt Anat Ontol Tiere 83:1–51.

Korboot K. 1964. Comparative studies of the external and internal anatomy of three species of caddis flies (Trichoptera). Univ Queensland Papers Dept Entomol 2:3–44.

Krafka J. 1923. Morphology of the head of trichopterous larvæ as a basis for the revision of the family relationships. J New York Entomol Soc 31:31–52.

Kramer S. 1955. The musculature of the head of the *Corydalus* larva (Neuroptera, Sialidae). J Morphol 96:1–30.

Kristensen NP. 1981. Phylogeny of insect orders. Annu Rev Entomol 26:135–157.

Kristensen NP. 1984. The larval head of *Agathiphaga* (Lepidoptera, Agathiphagidae) and the lepidopteran ground plan. Syst Entomol 9:63–81.

Kristensen NP. 1997. Early evolution of the Trichoptera + Lepidoptera lineage: Phylogeny and the ecological scenario. Mém. Mus. nat. Hist. nat. 173:253–271.

Kubiak M, Beckmann F, Friedrich F. The adult head of the annulipalpian caddisfly *Philopotamus ludificatus* McLachlan, 1878 (Insecta: Trichoptera: Philopotamidae), mouthpart homology and phylogenetic implications. Arthropod Syst Phyl (in press).

Lepneva SG. 1970. Larvae and pupae of Annulipalpia. Jerusalem: Published for the Smithsonian Institution and the National Science Foundation, Washington D.C. by the Israel Program for Scientific Translations.

Malicky H. 1973. Morphologie. In: Malicky H, editor. Part 29 Trichoptera (Köcherfliegen). Berlin: de Gryter. pp 32–49.

Malm T, Johanson KA, Wahlberg N. 2013. The evolutionary history of Trichoptera (Insecta): A case of successful adaptation to life in freshwater. Syst Entomol 38:459–473.

Michels J, Gorb SN. 2012. Detailed three-dimensional visualization of resilin in the exoskeleton of arthropods using confocal laser scanning microscopy. J Microsc 245:1–16.

Morse JC. 1997. Checklist of world Trichoptera. In: Holzenthal RW, Flint OS Jr., editors. Proceedings of the 8th International Symposium on Trichoptera. Ohio Biol Survey, Columbus, Ohio. pp 339–342.

Nielsen A. 1942. Über die Entwicklung und Biologie der Trichopteren mit besonderer Berücksichtigung der Quelltrichopteren Himmerlands. Arch Hydrobiol 17:255–631.

Paulus HF. 1986. Comparative morphology of the larval eyes of Neuropteroidea. In: Gepp J, Aspöck H, Hölzel H, editors. Recent Research in Neuropterology. Graz. pp 157–164.

Paulus HF, Schmidt M. 1978. Evolutionswege zurn Larvalauge der Insekten: Die Stemmata der Trichoptera und Lepidoptera. Z Zool Syst Evol-Forsch 16:188–216.

Pohl H. 2010. A scanning electron microscopy specimen holder for viewing different angles of a single specimen. Microsc Res Tech 73:1073–1076.

Ross HH. 1964. Evolution of the caddisworm cases and nets. Am Zool 4:209–220.

Ross HH. 1967. The evolution and past dispersal of the Trichoptera. Annu Rev Entomol 12:169–206.

Schmid F. 1989. Les Hydrobiosides (Trichoptera, Annulipalpia). Bull Inst R Sci Nat Belg Ent 1–154.

Snodgrass RE. 1935. Principles of Insect Morphology. New York, London: McGraw-Hill Book Company.

Snodgrass RE. 1947. The insect cranium and the "epicranial suture". Smiths Misc Coll 107:1–52.

von Kéler S. 1963. Entomologisches Wörterbuch mit besonderer Berücksichtigung der morphologischen Terminologie. Berlin: Akademie-Verlag. 761 p.

Waringer J, Graf W. 2011. Atlas der Mitteleuropäischen Köcherfliegenlarven. Dinkelscherben: Erik Mauch Verlag.

Weaver JS, Morse JC. 1986. Evolution of feeding and case-making behavior in Trichoptera. J North Am Benthol Soc 5: 150–158.

Wiggins GB, Wichard W. 1989. Phylogeny of pupation in Trichoptera, with proposals on the origin and higher classification of the order. J North Am Benthol Soc 8:260–276.

Williams N, Wiggins GB. 1981. A proposed setal nomenclature and homology for larval Trichoptera. Proceedings 3rd International Symposium on Trichoptera, Series Entomologica, Vol. 20. pp 421–429.

Winkler D. 1959. Die Muskulatur der Larve von *Limnophilus flavicornis* Fabr. (Trichopt., Limnophilidae). Deut Entomol Z 6:112–128.

Wipfler B, Machida R, Müller B, Beutel RG. 2011. On the head morphology of Grylloblattodea (Insecta) and the systematic position of the order, with a new nomenclature for the head muscles of Dicondylia. Syst Entomol 36:241–266.