Transformation of internal head structures during the metamorphosis of Chrysopa pallens (Neuroptera: Chrysopidae)

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Abstract

Background The metamorphosis is a complicated but very interesting process because of the highly dynamic transformation in sheath. Very few studies had coverage on the head muscles of larvae, pupae, and adults. Most of these studies were focusing on the model organisms about the rough changes of the external and internal tissues or the time of metamorphosis based on the traditional methods. In our study, the skeleto-muscular system of head, as well as the brain of Chrysopa pallens (Rambur, 1838) from larvae to adults are described in detail for the first time by the technology of micro computed tomography (µ-CT). The transformations of these systems during pupal stage are studied for the first time.

Results The morphological differences and functional adaptations between the stages are assessed. Muscles are distinctly slender in larvae than in adults with a significantly larger quantity. A larger brain with improved sensory perception is suggested to be essential for dispersal, mating and flying for adults. For the pupae, the results show that the histolysis of the muscles happens in first third of the pupal period and their reconstruction happens in the following days. The brain exists all along.

Conclusion We suggest the transformations of the skeleton occur earlier than the musculature. Most of the transformations are related to tasks they play in the developmental stages.

Background

Holometabola ( = Endopterygota) with approximately 800,000 described species comprise about two thirds of the known animals [1]. Driven by different factors, the outbursts of diversifications took place in different megadiverse subgroups, for example, the co-evolution between flight apparatus and angiosperms [1,2,3,4]. Nevertheless, the metamorphosis from larvae to pupae and from pupae to adults might be a crucial feature for the evolution of the corresponding lineage [5]. This includes the ontogenetic developments including diet, reduced intraspecific competition between juveniles and adults, et al. [1].

Most studies of the metamorphosis of holometabolous insects were focusing on the model organisms, like the fruit fly Drosophila melanogaster. Some of these studies were about the rough changes of the external and internal tissues or the time of metamorphosis based on the traditional methods [6,7],
some were about the genes expression during metamorphosis based on genome approach [8,9]. Also, the nerves of the mealworm *Tenebrio molitor* during metamorphosis [10,11] and the transformation of the abdominal muscles of the blowflies during metamorphosis (*Calliphora* [12]) were focused. However, Oertel [13] omitted the detailed information of the cephalic musculature of honeybee *Apis mellifera*. Polilov & Beutel [14,15] focused on the effects of miniaturization and phylogeny by comparing different beetles. Ge et al. [16] omitted the transformations during pupal stage of Chrysomelinae beetles. Even though there have been several cases of studies on head musculature of different species of Neuroptera reported, such as *Osmylus fulvicephalus* [17], *Coniopteryx pygmaea* [18], and *Sisyra terminalis* [19], *Nevrorthus apatelios* [20], very few detailed studies have been carried out on the metamorphosis of Neuroptera. Occasionally, these skeleton-muscular features have been used in Phylogenetic analyses [20,21,22,23,24]. Almost the same happened to Megaloptera and Raphidioptera, which belong to Neuropterida. The head muscles of *Chauliodes formosanus* and *Sialis flavilatera* of Megaloptera [25,26] and *Raphidia flavipes* of Raphidioptera [27] were described in detail based on the traditional methods. Recently, the larval head muscles of *Raphidia (Phlaeostigma) notata* were described by Beutel & Ge [28] based on the 3D reconstruction method. In most cases, the studies of the cephalic nervous system were included in the studies of the head musculature of larvae or adults. The nervous system of the pupae was rarely studied. Recently, the 4th day pupae were reconstructed in detail by Ge et al. [16]. The metamorphosis is a complicated but very interesting process because of the highly dynamic transformation in sheath. Very few studies had coverage on the head muscles of larvae, pupae, and adults. Therefore, the morphological transformations during metamorphosis are presently still very insufficiently known.

Finally, by concerning the recent studies about the morphological methods [29,30,31], the non-destructive method-computed tomography (μ-CT) and the conspicuous lack of information on metamorphosis of lacewings induced us to execute this comparative study of the head structures. The focus of this study was the detailed documentation of transformations in the head muscular system, the cephalic nervous system between different developmental stages of the green lacewings *Chrysopa pallens*. *Chrysopa pallens* (Rambur, 1838) belonging to Chrysopidae. The green lacewing is
one of the most common encountered families of insect of the order Neuroptera. They distributed in all major biogeographic regions of the world [32,33]. They are used as predacious biological control agents of insect pests such as aphids in many agricultural applications [34,35,36,37]. Within this family, members of genus *Chrysopa* and *Chrysoperla*, for instance, *Chrysopa pallens* (Rambur, 1838), and *Chrysoperla carnea* (Stephens), have been mass reared and sold by numerous commercial insectaries.

The adult cephalic and thoracic musculature of another species of *Chrysopa, Chrysopa Plorabunda,* has already examined in detail by Miller [38]. However, the study was based on traditional methods and the homology of the muscles remained ambiguous. Some muscles were apparently overlooked such as tentoriomandibularis muscles [38]. Some muscles were subsumed under one name whereas they were treated as separate units by Wipfler et al. [39]. In the present study, we documented the muscles of the head, the cephalic nervous system from larvae to adults, by micro-CT reconstruction techniques, with the complete data of pupal stage included.

**Methods**

**Examined specimens**
The *Chrysopa pallens* (Rambur, 1838) were raised in the laboratory with temperature 25°C and humidity 75%. Three samples were collected every day from the first day of pupae to the emergence.

All materials were preserved in 75% ethanol for less than 24 hours before dehydrated.

**X-ray computer tomography**
All materials used for X-ray micro-computed tomography (µ-CT) were dehydrated in pure n-propanol, then in ethanol solutions from 75% to 100% stepwise. Then they were dried at the critical point (Leica EM CPD 300). The specimens were scanned by an X-radia Micro CT–520 scanner at Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences (beam strength: 40KV, absorption contrast) and X-radia Micro CT–400 scanner at the Institute of Zoology, Chinese Academy of Sciences (beam strength: 60KV, absorption contrast).

**Three-dimensional reconstruction (3D)**
The muscles and the brains of the larvae, pupae and adults were reconstructed and smooth with Amira 5.4 based on the obtained image stacks from micro-CT. Final figures were prepared with Adobe
Photoshop (CS6).

Photography
Habit photos of the larvae, pupae and adults were taken by a 5D mark III Canon camera connected to a ZEISS Stemi 2000-c stereoscope (Carl Zeiss, Jena, Germany). Final figures were prepared by Adobe Photoshop (CS6).

Terminology
The terms used for the head muscles followed the terminology of Wipfler et al. [39].

Results
The head skeleto-muscular system and the cephalic nervous system of the third instar larvae, the pupae (from Day 1 to Day 12), and the adults of *Chrysopa pallens* (Rambur, 1838) are described.

Skeleto-muscular system
The external structures of head are described. During the pupal stage, from Day 1 to Day 4, the skeletal system is almost same to what is in the 3rd instar larvae, thus only the latter is described in detail. In Day 5, the larval cuticle cracked, and the newly present structures keep themselves in the following 7 days, so only the pupae of the 11th day, which is well developed, is described in detail. Muscles in pupal stage are described in this section, too. The description of head muscles is in tables 1-3.

General appearance
*Third instar larvae (Figs 1A, 2).* Body of mature, living third instar larva fusiform and humped. Length ~7.00 mm and height ~1.30 mm. Cuticle light brown with dark brown markings dorsolaterally. Spinules and long microsetae present dorsally. All setae smooth, dark brown to light brown. Flat head 1.00 mm in length and 0.70 mm in width. Thorax unsclerotized with rows of short, acute setae. Legs slender and well developed, inserted on semimembranous ventrolateral articulatory areas posteriorly. Lateral tubercles broadly cylindrical dorsolaterally and tapering distally with elongated setae. Long setae all tapering and hooking at tips. Tubercles and long setae carry the debris for camouflage.

*Pupae from Day 1 to Day 4 (Fig 3).* Pupae immobile adecticus exarate type. Cuticle light brown-yellow. Body C-shape with 0.50 mm in length and 0.30 mm in width. Head bends inward, morphologically almost same to larvae. Segments of thorax and abdomen similar in shape. Lateral
tubercles smaller and long setae disappeared. Cocoon 0.40 mm in length and 0.30 mm in width, with
death aphids covering on cocoon (Fig 1D).

_Pupae from Day 5 to Day 10 (Figs 3, 4)._ Color and body shape stay same. In Day 5, larval cuticle
cracked and wings present. Larval cuticle gathers under abdomen in cocoon (Fig 1E). Pharate adult
6.00 mm in length and 2.50 mm in height. Head 0.15 mm wide and 0.12 mm long. Compound eyes,
basal antenna, labrum, and mandible similar to adults. Color of labrum and mandibles turn red to
crimson from Day 6 to Day 10. Compound eyes red to metallic black-red. Maxillary palps, labial palps,
and curly antenna present with milky color. Frontoclypeal sulcus present. Wings become larger in
size. Prothorax, mesothorax, metathorax, and legs similar to adults in shape. Short setae present on
frons.

_Pupae Day 11 (Figs 1B, 3, 4)._ Pharate adults develop well within pupal sheath, less sclerotized than
adults. Wings brown to dark from base to distal margin.

_Pupae Day 12 (Fig 3)._ Pupae break out from cocoon (Fig 1F). After 3 hours, they emerge (Fig 1G).

_Adults (Figs 1C, 5)._ All structures develop well, pale yellow. Adults 12.00 mm in length and 4.00 mm
in height. Head 0.20 mm in width and 0.15 mm in length.

**Head capsule.**

_Third instar larvae (Fig 2)._ Head Prognathous, roughly triangular, round posteriorly. Dorsum cream to
light yellow with dark brown markings. Frontal markings confluent mesally, elongate. Epicranial
markings paired, V-shape, not confluent mesally, extending to cervical margin. Eyes with six
stemmata. Clypeus and labrum unmarked, fused to frons. Membranous connection between labrum
and clypeus completely reduced. Frontoclypeal sulcus absent. Anterior margin of head oblique in
lateral view. Front region V-shape posteriorly and parallel-side anteriorly. Mandible amber, dark
apically. Ventral maxilla smooth. Labium light brown. Gula absent.

_Pupae Day 11 (Figs 1B, 3, 4)._ Head hypognathous, nearly triangular in frontal view, yellow to pale
brown from vertex to mouthparts. Posterior vertex slightly concave. Compound eyes hemispherical,
metallic black, occupying half of head width. Ocellus absent. Antennas locate between compound
eyes. Antennomeres extremely elongated, covering on sides of body. Clypeus broad. An indistinct
suture present between clypeus and labrum. Lateral gena strongly round. Ventrally, labium connects with maxilla.

Adults (Figs 1C, 5). Same shape and color to Day 11 pupae. Posterior vertex concave. Compound eyes large and metallic black, composed by numerous small and hexagonal ommatidia. Ocellus absent. Scapus swollen in antennal socket. Antenna filiform and almost as long as body length. Head nearly wedge-shaped in lateral view, gradually narrowing to mouthparts. Ecdysial line vestigial. Frontoclypeal sulcus and frontogenal suture present. Dorsolateral longitudinal furrow extends from dorsolateral margin of hind head capsule to mandible articulation. Lateral occipital lobes slightly exposed and hemispherical. Frontogenal suture connects anterior antennal fossa with dark anterior tentorial pits. Subgenal suture above mandible articulation vestigial. Lateral clypeus round. Anterior clypeus concave slightly with convex median line.

Tentorium.

Third instar larvae (Figs 6A-larvae, 7). Tentorium fully sclerotized, tubular, solid throughout, connecting anterior tentorial pits at posterolateral clypeal margin with posterior tentorial pits at the foramen magnum. Tentorial bridge (tb) connects posterior tentorial arms (pta). Anterior tentorial arms (ata) diverge slightly. Dorsal tentorial arms (dta) well developed, attaching to head capsule directly.

Pupae from Day 1 to Day 2 (Fig 7). In Day 1, ata, pta, and tb still exist, but tentorium dramatically compressed. In Day 2, tentorium disappeared.

Pupae from Day 3 to Day 10 (Fig 7). From Day 3, new tentorium present, including two separated arms. Boundary of ata and pta indistinct before Day 6. In Day 10, tb present.

Pupae Day 11 (Figs 6A-pupae, 7). Tentorium sclerotized and hollow. Laminatentorium (lt) present which serves as attachment area of muscles (0an1, 0mx3, 0mx4, and 0mx5). Ata slender and diverge anteriorly.

Adults (Fig 6A-adult, 7). Tentorium fully sclerotized, connecting larger anterior pits at posterolateral clypeal margin with posterior pits below occipital. Dta present but very thin. Lt protruding, serving as the attachment of 0an1, 0mx3, and 0mx4.

Labrum.
Third instar larvae (Fig 2). Labrum fused to clypeus but recognized by slightly convex structure.

Musculature: in Fig 6B-larvae.

Pupae Day 11 (Fig 4). Labrum dark brown and clypeus brown. Anterior labrum margin slightly convex. Anterolateral edges round. Musculature: in Fig 6B-pupae.

Adults (Fig 5). Labrum short, moving freely by labrum muscles. Anterior margin slightly convex. Two short tormae present on posterolateral labrum. Musculature: in Fig 6B-adults.

Antenna.

Third instar larvae (Fig 2). Antenna glabrous and multisegmented in a slightly elevated socket. Basal segment globular and tapering distally. Pseudosegments cylindrical and separated indistinctly. Apical antennomere slender. Musculature: in Fig 6B-larvae.

Pupae Day 11 (Figs 3, 4). Antennae filiform and multisegmented, composed by scapus, pedicellus and flagellomeres. Flagellomeres extremely elongate, about 1.5 times as long as pupae length, covering sides of thorax. Scapus proximally wide and narrow distally. Pedicellus near cylindrical with almost identical diameter and length. Musculature: in Fig 6B-pupae.

Adults (Fig 5). Antenna filiform, about 1/3 as long as fore wing. Same location to pupae. Socket indistinct. Short setae present around each flagellomeres. Musculature: in Fig 6B-adults.

Mandible.

Third instar larvae (Fig 2). Mandibles strongly elongate, slender with apical parts, slightly upturned, longer than labial palps, closely connected with elongate maxilla. Sucking channel enclosed by mandible and maxilla. Basal mandible wide. Apical mandibular stylet curved mesad and apically pointed. Mola, prostheca and subapical teeth absent. Mandibular surface smooth. Musculature: in Fig 6C-larvae.

Pupae Day 11 (Figs 4, 8). Mandibles roughly triangular and not quite symmetric. Joints not clear. Upper surface convex and ventral concave. Both left and right mandibles possess three apical incisors. Molar process presents in middle region of mesal edge. Ventromesally, left molar concave to fit with convex right one. Musculature: in Fig 6C-pupae.

Adults (Figs 5, 8). Mandibles heavily sclerotized. Primary mandibular joint is a globular protrusion,
articulated with shallow emargination of head capsule. Secondary mandibular joint formed by a cavity of mandible and a corresponding protrusion of head capsule. Left and right mandibles moderately asymmetric. Each has an apical incisor. Dorsal side slightly convex and ventral side moderately concave. Cutting edge nearly straight on left mandible but curved on right. Small triangular Molar process present in middle region of mesal edge. It is more distinct on right than on left. Musculature: in Fig 6C-adults.

Maxilla.

Third instar larvae (Figs 2, 9A-larvae). Maxilla composed of a proximal element, an intermediate part and an elongate distal maxillary stylet. Proximal element small, round laterally, oblique anteriorly. Intermediate piece larger, round laterally. A seta inserted in median region. Maxillary stylet elongates, similar to mandible in shape, forming the ventral part of sucking jaw. Apical part enfolds mandible. Musculature: in Figs 6D-larvae, 9A-larvae.

Pupae Day 11 (Figs 4, 9A-pupae). Maxilla posterior to mandible. Cardo roughly quadrangular, broad. Stipes in similar shape with cardo and narrowing distally. 5-segmented maxilla palpus insert on stipes distolaterally. Palpomere 1 shorter and broader. Palpomere 2 longer than 1 but wide distally. Three distal palpomeres slender. Palpomere 5 with a spindle-shaped apex. Proximal lacinia fused to dosal stipes. Distal part slightly sickle-shaped. Galea slender proximally and wide distally, inserting between palp and lacinia. Musculature: in Figs 6D-pupae, 9A-pupae.

Adults (Figs 5, 9A-adults). Maxilla connects with submentum by membrane. Cardo roughly triangular. Stipes narrower and longer than cardo, forming an acute angle laterally at base. Palp inserts in lateral stipes. Palpomere 1 much broader than other segments. Distal three palpomeres elongate and slender. Lacinia basally fused to dorsal stipes. Distal part slightly curved and sickle-shaped. Galea includes slender basigalea and broader distigalea. Musculature: in Figs 6D-adults, 9A-adults.

Labium.

Third instar larvae (Figs 2, 9B-larvae). Labium composed by submentum, mentum, and prementum, forming a complexes with anterior hypopharynx. Submentum narrow and rectangular, laterally connecting with cardo. Anterior edge separated from mentum by distinct convex. Anterior mentum
flat, wide, round anterolaterally. Two pairs of setae insert at anterior mentum. Prementum small and medially divided by a cleft. Glossae, paraglossae, and ligula absent. 3-segmented palp (lap) distinctly elongate. Basal segment cylindrical. Segment 2 extremely elongate, about ten times as long as wide and slightly wide distally. Palpomere 3 slender, with same length to palpomere 1. Musculature: in Fig 9B-larvae.

**Pupae Day 11 (Figs 4, 9B-pupae).** Submentum short and narrow, separated by mentum by suddenly wide anterior margin. Mentum flat and slightly swollen. Prementum carries ligula with 3-segmented palp. Palpomere 3 longer than palpomere 1 and 2. Ligula diamond-shaped and sclerotized. Musculature: in Fig 9B-pupae.

**Adults (Figs 5, 9B-adults).** Elemental composition stays same to Day 11 pupae. Submentum edge not clear, recognized by muscles attachment. 3-segmented palps develop well. Ligula large and sclerotized with paired paraglossae. Musculature: in Fig 9B-adults.

## Epipharynx

**Third instar larvae.** Epipharynx, ventral surface of anterior clypeolabrum, sclerotized and slightly convex. Posterior membranous epipharynx fused to anterior pharynx and posterior hypopharynx laterally, forming the dorsal part of the closed prepharyngeal tube. Musculature: in Fig 9C-larvae.

**Pupae Day 11.** Anterior epipharynx membranous, covering basal mandible. Posterior epipharynx and hypopharynx fused to anterior pharynx margin, forming anterior pharynx. Musculature: in Fig 9C-pupae.

**Adults.** Same to mature Pupae. Musculature: in Fig 9C-adults.

## Hypopharynx and salivarium.

**Third instar larvae.** Anterior hypopharynx closely connected with anterior labium. Weak sclerotized above prementum and mentum. Posterior hypopharynx laterally fused to posterior epipharynx, forming the ventral prepharyngeal tube. Salivarium absent. Musculature: in Fig 9D-larvae.

**Pupae Day 11.** Hypopharynx not fully developed, fused to ventral pharynx. Salivarium and salivary duct not well developed. Musculature: in Fig 9C-pupae.

**Adults.** Hypopharynx forms a structural and functional unit with anterior labium. Anterior part extends
to ligula. Dorsolaterally, oral arms slender and run along hypopharynx. Hypopharyngeal suspensorial sclerites forms lateral short branch, closely connected with ventral ridge of prementum. Salivary duct broad and quadrangular in cross section above submentum and mentum. Musculature: in Fig 9C-adults.

**Pharynx.**

*Third instar larvae (Figs 9C-larvae, 9D-larvae).* Anterior precerebral pharynx V-shape. Following region approximately quadrangular in cross section with indistinct longitudinal folds for muscles attachment. Protocerebrum pharynx gradually narrow distally and irregular in cross section. Musculature: in Figs 9C-larvae, 9D-larvae.

*Pupae Day 11 (Figs 9C-pupae, 9D-pupae).* Pharynx narrow especially beneath brain. Precerebral pharynx slightly wide anteriorly. Cross section nearly oval. Pharynx wall thick and longitudinal folds indistinct. Postcerebral pharynx narrow. Musculature: in Figs 9C-pupae, 9D-pupae.

*Adults (Figs 9C-adults, 9D-adults).* Anterior precerebral pharynx wide and nearly round in cross section. Pharynx wall thin and no distinct longitudinal folds. Postcerebral pharynx suddenly wide with thick wall. Longitudinal folds present. Musculature: in Figs 9C-adults, 9D-adults.

**Pupal muscIs**

The transformation of head muscles from Day 1 to Day 12 are illustrated in Figs 10, 11. Taking an example, the mandible muscles 0md1 and 0md3 are reconstructed in detail in Fig 12. In Day 1, muscles compressed by inner cuticle. In Day 2, inner cuticle strongly compressed, and most muscles disintegrated. New skeletal structures begin to construct. In Day 3, remaining muscle tissues disintegrate continuously. In Day 4, new muscle granules present. In Day 5, muscle fibers present. More and more muscle fibers and bundles present in following days. In Day 12, almost all muscles present in bundle form.

**Cephalic nervous system**

The main elements of the central nervous system are brain and subesophageal ganglion. The latter is the first ganglion of ventral nerve cord. The two with the frontal ganglion are the main elements of the cephalic nervous system.
Cerebrum, suboesophageal complex, and frontal ganglion

Third instar larvae (Fig 13-larvae). Size of brain and suboesophageal ganglion (sog) about 20% that of entire head capsule. Brain composed by protocerebrum, deutocerebrum, and tritocerebrum. Protocerebrum dumbbell-shaped and optical nerves extremely slender with very slightly round lobe. Two thin antennal nerves originate from slightly protruding region of deutocerebrum. Frontal connectives originate from tritocerebrum and circumoesophageal connectives continuous with tritocerebrum. Sog ovoid-shaped below tb. All slender nerves of labium, maxilla, and mandible originate from sog. Frontal ganglion triangular, connecting with the protocerebrum and tritocerebrum by three curved frontal connectives.

Pupae Day 11 (Fig 13-pupae). Volume of brain and suboesophageal complex small, occupying about 12.5% that of head capsule. Protocerebrum unrepresentative dumbbell-shaped. Optical nerves cylindrical with slightly round lobe. Antennal nervus slender and bending upwards. Tritocerebrum bears circumoesophageal connectives. Suboesophageal complex nearly oval. Front ganglion triangular and connected by two curved frontal connectives.

Adults (Fig 13-adults). Volume of brain and suboesophageal complex occupies about 33.3% that of head capsule. Protocerebrum dumbbell-shaped with two large optic neuropils. Suboesophageal complex oval. Triangular frontal ganglion connected by three nerves like larvae.

Pupal brains
Transformation of brains from Day 1 to Day 11 is illustrated in Figs 10, 11, 14. In Day 1, brain becomes small and simple. Antennal nerves, optical neuropils, and mouthparts nerves strongly short. Frontal ganglion disintegrated. In Day 2, brain strongly compressed and suboesophageal ganglion separated from brain due to disappearance of circumoesophageal connectives. From Day 3, brain stops compression but becomes more and more larger in following days. In Day 9, slender antennal nerves present. In Day 11, frontal ganglion present.

Discussion
Functional adaptations in larvae and adults
The morphology of the larvae and adults differ significantly. Nearly all character systems would be affected during the process of metamorphosis. The homologization of the muscles between all stages is concluded in tables 4. Tables 5 show the homological patterns of muscles reported previously. The specialization of larvae and adults, which could lead to the possibility of living in different habitats and feeding on various food substrates, results in a reduced intraspecific competition between the stages. This was addressed as one factor contributing to the unparalleled evolutionary success of Holometabola [1,4]. It is also conceivable that a division of ecological selection between developmental stages may bring about the selections for reduced equipment in larvae, which do not need elaborate sense organs like the sensillum on antennae and the compound eyes of adults. Besides, the organs for dispersal over long distances are absent in larva. The main target for larvae is to be feed and accumulate energy-rich substances in fat body. Whereas, the principal functions of the adults are dispersal and reproduction. The present study reveals how these divergent functions affect the metamorphosis of different structural elements.

The most conspicuous change is the orientation of the head: it is prognathous in larvae but orthognathous in adults. Functionally, this may relate to the height of the body. The larval legs are relatively short. Thus, the body would almost touch the ground and the preys would be almost as tall as the larvae. The prognathous head can help the sucking channel piercing the upper part of the prey. However, the adult’s legs are longer than larval legs and the mouthparts are relatively higher than larvae. The orthognathous mouthparts are more conducive for capturing and getting the small preys. The downward orientation of the mouthparts from larvae to adults also closely relates to the head morphology between different stages, such as the concave submentum, the broader vertex of the adults. Additionally, the wedge-shaped capsule of the adult is in favor of the development of the mouthparts and the cephalic nervous system, as well as the development of the strong muscles, such as 0an1 and 0an2. For most holometabolous insects, the upward or downward orientation from larvae to adults may possibly depend on their styles of mouthparts in larvae, such as the upward orientation in Coleoptera [16].

The necessity to find a potential mating partner requires a far more complicated sensory system in
the adults than is present in larvae: instead of simple stemmata, the complex compound eyes are present. This change in the visual system also requires strong modifications in the brain, notably in the optic lobes which greatly increase in size. Similarly, the antennae are greatly elongated in adults. The antennal nerves also become larger in size than previous stages. To ensure controlling the movements of adult antennae, a more complex muscle system is required. Three extrinsic and four intrinsic muscles are present in antennae of adults, whereas only four small extrinsic muscles and no intrinsic muscle present in that of larvae. It is conceivable that the intrinsic muscles could have a more effective control towards the flight mechanism than the extrinsic muscles. Additionally, from larvae to adults, the segment number of the labial palp is the same but two more intrinsic muscles and one more extrinsic muscle are presented in adults. The Maxillary palps is absent in larvae, but the five-segment palp, four more intrinsic, and two more extrinsic muscles are presented in adults. In addition to the modifications mentioned above, a muscle intrinsic muscle of maxillary stylet (imms) connecting the dorsal wall and ventral wall of stylet is presented in larvae but absent in adults. The muscle is also reported in the larvae of *Nevorthus* [40]. Functionally, it probably controls the movement of stylet. The volume of the sucking channel is springy thanks to the muscular contraction, which assists in sucking the fluids.

In the pharynx musculature, ten muscle bundles are presented in larvae and nine are presented in adults. The only one missing is the muscle M. prelabiohypopharyngeal muscle (prhy) which has unclear homology. It is closely associated with the solid food that the adults feeding on. The similar muscle is also presented in the larvae of *Nevorthus* [40]. Functionally, it might have something to do with stabilizing the structure of labium and anterior pharynx in larvae. However, this muscle may limit the movement of labium in adults. It is reasonable that the muscle prhy is absent in adults, whose labium needs a more flexible movement.

**Transformation in Pupae**

In general, the mature pupae resemble the adults in almost all skeletal elements except for the absence of the dta and the curly elongated antenna. Aside from these skeletal features, there is another major transformation taking place in the orientation of the head. The larvae are clearly
prognathous with an angle of approximately 200° between longitudinal body axis and longitudinal axis of the mouthparts (Fig 15). The adults are orthognathous with an angle of 135°. From the Figure 3, we found that the pupae have the angle 60° from Day 1 to Day 4 but have the angle 90° from Day 5 to Day 11. Comparing these angles from larvae to adults, it is easy to assume that the orientation of the mouthparts is a sudden shift rather than a continuous shift. The sudden shift from 200° to 60° may take place during pupating and with the similar angle in the following four days. After the larval cuticle being cracked in Day 5, the angle is 90° and become a little bigger after the cocoon breaks. The angle becomes 135° once the emergence happens. It was suggested in Ge et al. [16] that “anterior orientation of the mouthparts is a continuous shift rather than a sudden reorientation and takes place more or less continuously during the six days of pupal metamorphosis.” Considering their data only contains one day as the sample of pupae stage, it is hard to compare if the two studies are talking about the one or two phenomena.

Based on the results, we found that the construction of the new cuticle and the histolysis of the internal structures (such as muscles and tentorium) almost happen in the first third of the period. At the beginning of the pupal stage, the muscle fibers can be recognized easily but smaller than larvae. With the presence of the new cuticle and the histolysis of the larval muscles, some new muscle granules are presented in Day 4. However, the great increase of the muscle granules happens in Day 6. We guess the most muscle bundles would take shape in the next day. In fact, the data of Day 7 prove it. This is consistent with the study of honeybee whose muscle bundles are presented after 150 hours of pupation [13]. However, the musculature of the mature pupae appears frayed or do not attach to the skeletons, such as M. craniomandibularis internus (0md1), P6 and P7 that only one end attached to the sclerites. This phenomenon also exists in Coleoptera [16].

We also found that the modifications of skeleton happen earlier than the internal soft parts during metamorphosis. Even though the compound eyes are already presented in the pharate adults, the optic lobes of the brain are still undeveloped in the last day. In addition, the results show that the brain and the suboesophageal ganglion are not disintegrated during the pupal stage. Indirectly, the importance of central nervous system in development and metamorphosis during the life history is
verified. Concerning the beginning time of the modification, the musculature lags behind the nervous system. We thus conclude that in *Chrysopa*, the modifications of the skeleton begins earlier then the nervous system and the musculature. All these systems would develop well in the end of pupal stage or after the emergence. It is consistent with the research of Oertel [13] about the transformation of the honeybee, as well as the study of leaf beetle [16]. However, due to the diversity of events occurring throughout the Holometabola, the observations in only several species is insufficient and certainly this interpretation is preliminary.

Conclusions
Our study showed that muscles are distinctly slender in larvae than in adults with a significantly larger quantity. Most of these transformations are related to tasks they play in the developmental stages. A larger brain with improved sensory perception is suggested to be essential for dispersal, mating and flying for adults. For the pupae, the results showed that the histolysis of the muscles happens in first third of the pupal period and their reconstruction happens in the following days. The brain exists all along. Insect metamorphosis is arguably among the most complex processes in animal life. It almost covers the knowledge from morphology, neurology, and developmental biology [7,9,41,42,43,44]. It is apparent that more detailed comparative studies involving representatives of all principal endopterygote groups are required. Future studies involving broader taxon sampling with the advanced methods, may lead to a better understanding of this remarkable phenomenon, which apparently played an important role in insect evolution [4].

Abbreviations
tb: Tentorial bridge; pta: posterior tentorial arms; ata: Anterior tentorial arms; dta: Dorsal tentorial arms; lt: Laminatentorium; sog: suboesophageal ganglion; fg: ganglion frontale

Declarations
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Authors’ contributions
CJZ and DY conceived the study; MQW collected the sample; CJZ and CXG analysed the data; CJZ, YA, RM, XYL, CFT, KYZ and ML participated in fundamental discussion about the interpretation of results; CJZ wrote the manuscript. All the authors have read and approved the manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

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Consent for publication

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Competing interests

Not applicable

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Tables
Table 1. Cephalic musculature of the larvae of *Chrysopa pallens* (Rambur, 1838).

| Muscle Abb./No. | Origin | Insertion | Presumed function |
|-----------------|--------|-----------|------------------|
| Labrum/2        |        |           |                  |
| 0lb1            | Mesally on the frons | Mesally on the basal wall of the labrum | levator of labrum |
| 0lb2            | Laterally on the frons | tormae | levator of labrum |
| Antenna/4       |        |           |                  |
| 0an1            | dorsal tentorial arms | Anterior antennal base (ventral) | depressor and flexor of antenna |
| 0an2            | dorsal tentorial arms | Posterior antennal base (dorsal) | levator of antenna |
| 0an3            | dorsal tentorial arms | Lateral antennal basal margin |                  |
| 0an4            | dorsal tentorial arms | Mesal antennal basal margin |                  |
| Mandible/3      |        |           |                  |
| 0md1            | Posterior, lateral, and dorsal parts of the head capsule | Tendon that inserts at the median edge of the mandible | adductor of mandible |
| 0md3            | Lateral, ventral, and dorsal parts of the head capsule | Tendon that inserts at the lateral edge of the mandible | abductor of mandible |
| 0md8            | anterior tentorial arms | Mediodorsal wall of the mandibular cavity | adductor of mandible |
| Maxilla/6       |        |           |                  |
0mx2 Ventrolateral, anterolateral parts of the head capsule

0mx3 Proximal part of anterior tentorial arms

0mx4 Proximal part of the anterior tentorial arms

0mx5 Anterior tentorial arms, anterior to the 0mx4

0mx6 stipes Basal edge of the maxilla stylet

imm Basal part (dorsal) of the maxilla stylet

Labium/3

0la5 Posterior tentorial arms Posterior lateral part of the prementum adductor of praementum

0la8 Posterior region of the mentum Posterior edge of the prementum retractor of praementum

0la14 Anterior edge of the prementum Basal part of the labial palp levator of labial palp

Epipharynx/2

0ci1 clypeus Roof of the cibarium dilatator of cibarium

0bu1 Postclypeus Roof of the bucca dilator of buccal cavity

Hypopharynx/1

0hy3 Posterolateral part of the head capsule Anterolateral part of hypopharynx levator of the hypopharynx

Pharynx/10

0bu2 Middle region of the frons Dorsal buccal wall dilator of pharynx

0bu3 Frons, posterior to the 0bu2 Dorsal buccal wall dilator of pharynx

0bu4 anterior tentorial arms Lateral wall of the bucca dilator of pharynx

0bu5 Tentorial bridge Ventral wall of the bucca dilator of pharynx

0bu6 Tentorial bridge Ventral wall of the bucca dilator of pharynx
| prhy | Lateral region of the prementum | Anterior region of the ventral bucca | Dorsal wall the pharynx | Dorsal wall the pharynx | Ventral and lateral region of the pharynx | Dorsal wall of the scape | Lateral wall of the pedicel | Mesal wall of the scape | Mesal edge of the pedicel | The part between occipital and compound eyes of the head capsule | Tendon that inserts at the median edge of the mandible |
|------|----------------------------------|-------------------------------------|-------------------------|-------------------------|------------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------------------------------|--------------------------------------------------|
| 0ph1 | Posterior region of vertex       | Dorsal wall the pharynx             |                         |                         | Dorsal wall the pharynx                 |                         |                         |                         |                         |                                                 |                                                 |
| 0ph2 | Posterior tentorial arms         | Ventral and lateral region of the pharynx |                         |                         | Dorsal wall of the scape | Mesal wall of the scape | Mesal edge of the pedicel | The part between occipital and compound eyes of the head capsule | Tendon that inserts at the median edge of the mandible |
| 0st1 | Ring muscle layer that covers the entire pharynx | Constrictor of the pharynx |                         |                         | Dorsal wall of the scape | Mesal wall of the scape | Mesal edge of the pedicel | The part between occipital and compound eyes of the head capsule | Tendon that inserts at the median edge of the mandible |
| 0st2 | Longitudinal muscle layer directly above musculus annularis stomodaei | Constrictor of the pharynx |                         |                         | Dorsal wall of the scape | Mesal wall of the scape | Mesal edge of the pedicel | The part between occipital and compound eyes of the head capsule | Tendon that inserts at the median edge of the mandible |

Table 2. Cephalic musculature of the pupae of *Chrysopa pallens* (Rambur, 1838).
| Code  | Description                                                                 | Location                                                                                     |
|-------|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| 0md3  | Posterior part of head capsule, lateral region of the 0md1                    | Tendon that inserts at the lateral edge of the mandible                                     |
| 0md4  | Lateral part of the hypopharynx                                              | Inner side of the median edge of the mandible                                               |
| 0md8  | Anterior tentorial arms                                                      | Mediodorsal wall of the mandibular cavity                                                   |
| Maxilla/11 |                                                                                   |                                                                                             |
| 0mx1  | Posterior part of the gena, close to the 0md1                                | Basal region of the cardo                                                                   |
| 0mx2  | Post part of the gena                                                        | Basal region of the lacinia                                                                 |
| 0mx3  | Ventral side of the anterior tentorial arms                                  | Cardo                                                                                       |
| 0mx4  | Ventral side of the anterior tentorial arms                                  | Anterior edge of the stipes                                                                |
| 0mx5  | Ventral side of the anterior tentorial arms                                  | Anterior edge of the stipes, close to the 0mx4                                             |
| 0mx6  | Stipital base                                                                | Basal edge of the lacinia                                                                  |
| 0mx8  | Inner wall of the stipes                                                     | Distal basal edge of the maxillary cardo                                                    |
| 0mx12 | Basal edge of palpomere 1                                                    | Basal edge of palpomere 2                                                                  |
| 0mx13 | Basal edge of palpomere 1                                                    | Basal edge of palpomere 3                                                                  |
| 0mx14 | Basal edge of palpomere 3                                                    | Basal edge of palpomere 4                                                                  |
| 0mx15 | Basal edge of palpomere 4                                                    | Basal edge of palpomere 5                                                                  |
| Labium/3 |                                                                                   |                                                                                             |
| 0la14 | Basal edge of the prementum                                                  | Basal edge of the labial palpus                                                             |
| 0la16 | Inner wall of the palpomere 1                                                | Basal edge of the palpomere 2                                                               |
| 0la17 | Inner wall of the palpomere 2                                                | Basal edge of the palpomere 3                                                               |
| Epipharynx/2 |                                                                                   |                                                                                             |
| 0ci1  | Mesally on the clypeus                                                       | Posterior region of the epipharynx, covered by 0bu1                                         |
| 0bu1  | Anterior region of frons                                                     | Roof of the bucca                                                                           |
| Hypopharynx/3 |                                                                                   |                                                                                             |
Table 3. Cephalic musculature of the adults of *Chrysopa pallens* (Rambur, 1838).

| Muscle Abb. | Origin | Insertion | Presumed function |
|-------------|--------|-----------|------------------|
| **Labrum/3** |        |           |                  |
| 0lb1        | frons  | Mesally on the outer basal wall of the labrum | Levator of labrum |
| 0lb2        | frons  | tormae    | Levator of labrum |
| 0lb4        | Dorsal part of the labrum | Ventral part of the labrum | Compressor of labrum |
| **Antenna/7** |        |           |                  |
| 0an1        | Anterior tentorial arms | Anterior basal edge of the scape | Depressor and flexor of antenna |
| 0an2        | Anterior tentorial arms, posterior to 0an1 | Posterior basal edge of the scape | Levator of antenna |
| 0an4        | Dorsal tentorial arms | Medial basal edge of the scape | Depressor and rotator of antenna |
| 0an6        | Laterally, mesally of the | Lateral basal edge of the pedicel | Extensor of flagellum |
| Term       | Description                                                                 |
|------------|-----------------------------------------------------------------------------|
| 0an7       | Inner wall of scape                                                          |
| 0an9       | Ventral wall of the scape                                                    |
| 0an10      | Dorsal wall of the scape                                                     |
| Mandible/5 | Posterior part between occipital and compound eye of the head capsule       |
| 0md1       | Gena and postgena                                                           |
| 0md3       | Lateral wall of the hypopharynx                                              |
| 0md4       | Ventral side of the anterior tentorial arms                                  |
| 0md6       | Anterio tentorial arms                                                       |
| Maxilla/13 | Posterior part of the head capsule                                           |
| 0mx1       | Posterior part of gena                                                       |
| 0mx2       | Ventral side of the anterior tentorial arms, under 0mx3                      |
| 0mx3       | Lateral part of tentorial                                                   |
| 0an7 Basal edge of the pedicel Flexor of flagellum |                      |
| 0an9 Posterio basal region of the pedicel Depressor of the antenna | |
| 0an10 Anterior basal region of the pedicel Elevator of the antenna |
| 0md1 Tendon that inserts at the median edge of the mandible Adductor of mandible |
| 0md3 Tendon that inserts at the lateral edge of the mandible Abductor of mandible |
| 0md4 Inner median wall of the mandible Protractor of anatomical mouth opening |
| 0md6 ventral basal margin of the mandible Adductor of mandible |
| 0md8 Mediodorsal wall of the mandibular cavity |
| 0mx1 Basal cardinal process Promoter of maxilla |
| 0mx2 Basal part of the lacinia Adductor of lacinia |
| 0mx3 Carostipital sulcus Adductor of cardo and protractor of maxilla |
| 0mx4 Anterior edge of the stipes Adductor of maxilla |
| 0mx5 Basally on the stipes, close to Adductor of stipes and protractor of |

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bridge

0mx6 Lateral wall of stipes, close to carostipital sulcus

0mx7 Mesal wall of stipes

0mx8 Lateral wall of the stipes, basal to the maxillary palp

0mx10 Stipital ridge

0mx12 Basal edge of pal pomere 1

0mx13 Basal edge of pal pomere 1

0mx14 Basal edge of pal pomere 3

0mx15 Basal edge of pal pomere 4

Labium/6

0la5 Posterior tentorial arms

0la8 Posterior part of submentum

0la13 Distally on the prementum

0la14 Basal edge of the prementum

0la16 Basal edge of pal pomere 1

0la17 Basal edge of pal pomere
| Hypopharynx/5 |  |
|---|---|---|---|
| 0hy1 | frons | Oral arms of the suspensorial sclerites | Levator and dilator of anatomical mouth |
| 0hy2 | Anterior tentorial arm | Oral arms of the suspensorial sclerites | Dilator of anatomical mouth |
| 0hy8 | Basal part of prementum | Lateral wall of salivarium | Dilator of salivarium |
| 0hy9 | Oral arm of suspensorial sclerite | Oral arm of the suspensorial sclerites on the other side | Connecting the anterior oral arms |
| 0hy12 | Anterior region of hypopharynx | Dorsolateral wall of salivarium | Dilator of salivarium |

| Pharynx/9 |  |
|---|---|---|---|
| 0bu2 | Frons, below the antennal base | Dorsal wall of pharynx | Dilator of pharynx |
| 0bu3 | Posterior region of frons, lateral to the antennal base | Dorsolateral wall of pharynx | Dilator of pharynx |
| 0bu4 | Anterior tentorial arms | Lateral wall of bucca | Dilator of pharynx |
| 0bu5 | Tentorial bridge | Ventral wall of pharynx | Dilator of pharynx |
| 0bu6 | Tentorial bridge | Ventral wall of pharynx | Dilator of pharynx |
| 0ph1 | Vertex | Dorsal wall of the pharynx | Dilator of pharynx |
| 0ph2 | Posterior tentorial arms | Lateral wall of pharynx | Dilator of pharynx |
| 0st1 | Ring muscle layer that covers the entire pharynx |  | Constrictor of the pharynx |
| 0st2 | Longitudinal muscle layer |  | Constrictor of the pharynx |
directly above musculus
annularis stomodaei

Table 4. Homologization of the cephalic musculature of *Chrysopa pallens* (Rambur, 1838) from larvae to adults with the terminology of Wipfler et al. (2011).

| Muscle name                        | Abb. | Larva | Pupa | Adult | Presumed function                                      |
|-----------------------------------|------|-------|------|-------|--------------------------------------------------------|
| Labrum                            |      | 2     | 3    | 3     |                                                        |
| M. frontolabralis                 | 0lb1 |       |      |       | levator of labrum                                       |
| M. frontoepipharyngalis           | 0lb2 |       |      |       | levator of labrum                                       |
| M. labralis transversalis         | 0lb4 |       |      |       | compressor of labrum                                    |
| Antenna                           |      | 4     | 4    | 7     |                                                        |
| M. tentorioscapalis anterior      | 0an1 |       |      |       | depressor and flexor of antenna                         |
| M. tentorioscapalis posterior     | 0an2 |       |      |       | levator of antenna                                      |
| M. tentorioscapalis lateralis     | 0an3 |       |      |       | depressor and rotator of antenna                        |
| M. tentorioscapalis medialis      | 0an4 |       |      |       | depressor and rotator of antenna                        |
| M. scapopedicellaris lateralis    | 0an6 |       |      |       | extensor of flagellum                                  |
| M. scapopedicellaris medialis     | 0an7 |       |      |       | flexor of flagellum                                     |
| M. scapopedicellaris posterior    | 0an9 |       |      |       | depressor of the antenna                               |
| M. scapopedicellaris anterior     | 0an10|       |      |       | elevator of the antenna                                |
| Mandible                          |      | 3     | 4    | 5     |                                                        |
| M. craniomandibularis internus    | 0md1 |       |      |       | adductor of mandible                                   |
| M. craniomandibularis externus    | 0md3 |       |      |       | abductor of mandible                                   |
| M. hypopharyngomandibularis       | 0md4 |       |      |       | protractor of anatomical mouth opening,                 |
| M. tentoriomandibularis lateral inferior | 0md6 |       |      |       | adductor of mandible                                   |
| Muscle Name | Abbreviation | Function |
|-------------|--------------|----------|
| M. tentoriomandibularis medialis inferior | 0md8 | adductor of mandible |
| Maxilla | 5+1 11 13 | |
| M. craniocardinalis | 0mx1 | promoter of maxilla |
| M. craniolacinialis | 0mx2 | adductor of lacinia |
| M. tentoriocardinalis | 0mx3 | adductor of cardo and protractor of maxilla |
| M. tentoriostipitalis anterior | 0mx4 | adductor of maxilla |
| M. tentoriostipitalis posterior | 0mx5 | adductor of stipes and protractor of maxilla |
| M. stipitolacinialis | 0mx6 | adductor of lacinia |
| M. stipitogalealis | 0mx7 | abductor of galea |
| M. stipitopalpalis externus | 0mx8 | abductor of maxillary palp |
| M. stipitopalpalis internus | 0mx10 | adductor of maxillary palp |
| M. palpopalpalismaxillae primus | 0mx12 | adductor of maxillary palpomere ii |
| M. palpopalpalismaxillae secundus | 0mx13 | abductor of maxillary palpomere iii |
| M. palpopalpalismaxillae tertius | 0mx14 | adductor of maxillary palpomere iv |
| M. palpopalpalismaxillae quartus | 0mx15 | adductor of maxillary palpomere v |
| M. intrinsic muscle of maxillary stylet | imm | |
| Labium | 3 3 6 | |
| M. tentoriopraementalis | 0la5 | adductor of praementum |
| M. submentopraementalis | 0la8 | retractor of praementum |
| M. praementopalpalis internus | 0la13 | adductor of labial palpomere i |
| M. praementopalpalis externus | 0la14 | levator of labial palp |
| Muscle Name                      | Code | Function                              |
|---------------------------------|------|---------------------------------------|
| M. palpopalpalislabii primus    | 0la16| flexor of labial palpomere ii         |
| M. palpopalpalislabii secundus  | 0la17| flexor of labial palpomere iii        |
| Epipharynx                      | 2    |                                       |
| M. clypeopalatalis              | 0ci1 | dilatator of cibarium                 |
| M. clypeobuccalis               | 0bu1 | dilator of buccal cavity              |
| Hypopharynx                     | 1    |                                       |
| M. frontooralis                 | 0hy1 |                                       |
| M. tentoriooralis               | 0hy2 |                                       |
| M. craniohypopharyngalis        | 0hy3 |                                       |
| M. praementosalivaris posterior | 0hy8 |                                       |
| M. oralis transversalis         | 0hy9 |                                       |
| M. hypopharyngosalivaris        | 0hy12| dilator of salivarium                 |
| Pharynx                         | 9+1  |                                       |
| M. frontobuccalis anterior      | 0bu2 | dilator of pharynx                    |
| M. frontobuccalis posterior     | 0bu3 | dilator of pharynx                    |
| M. tentoriobuccalis lateralis posterior | 0bu4 |                                   |
| M. tentoriobuccalis anterior    | 0bu5 | dilator of pharynx                    |
| M. tentoriobuccalis posterior   | 0bu6 | dilator of pharynx                    |
| M. prelabiohypopharyngeal muscle| prhy | dilator of pharynx                    |
| M. verticopharyngealis         | 0ph1 | dilator of pharynx                    |
| M. tentoriopharyngealis        | 0ph2 | dilator of pharynx                    |
| M. annularis stomodaei          | 0st1 | constrictor of the pharynx            |
| M. longitudinalis stomodaei     | 0st2 | constrictor of the pharynx            |

“+” = present, “−” = absent

Reference

Wipfler B., Machida R., Mueller B., Beutel RG. 2011. On the head morphology of Grylloblattodea
Table 5. Presumed homologies of the cephalic muscles of *chrysopa pallens* (Rambur, 1838) with muscles reported in Kéler (1963) and Miller (1933).

| Muscle name                      | Abb. | Present study | Kéler (1963) | Miller (1933) |
|---------------------------------|------|---------------|--------------|---------------|
| **Labrum**                      |      |               |              |               |
| M. frontolabralis               | 0lb1 | M8            | 1            |               |
| M. frontoepipharyngalis         | 0lb2 | M9            | 2            |               |
| M. labralis transversalis       | 0lb4 |               | 3            |               |
| **Antenna**                     |      |               |              |               |
| M. tentorioscapalis anterior    | 0an1 | M1            | 27           |               |
| M. tentorioscapalis posterior   | 0an2 | M2            | 28           |               |
| M. tentorioscapalis lateralis   | 0an3 | M3            |              |               |
| M. tentorioscapalis medialis    | 0an4 | M4            |              |               |
| M. scapopedicellaris lateralis  | 0an6 | M5            | 29           |               |
| M. scapopedicellaris medialis   | 0an7 | M6            | 30           |               |
| M. scapopedicellaris posterior  | 0an9 | M8            | 32           |               |
| M. scapopedicellaris anterior   | 0an10|               | 31           |               |
| **Mandible**                    |      |               |              |               |
| M. craniomandibularis internus  | 0md1 | M11           | 5-1          |               |
| M. craniomandibularis externus posterior | 0md3 | M12 | 4          |
| M. hypopharyngomandibularis     | 0md4 | M13           | 5-2          |               |
| M. tentoriomandibularis lateral inferior | 0md6 |      |            |               |
| M. tentoriomandibularis medialis inferior | 0md8 |      |            |               |
| **Maxilla**                     |      |               |              |               |
| M. craniocardinalis             | 0mx1 | M15           | 6            |               |
M. craniolacinialis 0mx2 M19 10
M. tentoriocardinalis 0mx3 M17 7b
M. tentoriostipitalis anterior 0mx4 M18 8
M. tentoriostipitalis posterior 0mx5 9
M. stipitolacinialis 0mx6 M20 13
M. stipitogalealis 0mx7 M21 14
M. stipitopalpalis externus 0mx8 M22 11
M. stipitopalpalis internus 0mx10 M23 12
M. palpopalpalismaxillae primus 0mx12 M24 15
M. palpopalpalismaxillae secundus 0mx13 M25 16
M. palpopalpalismaxillae tertius 0mx14 M26 17
M. palpopalpalismaxillae quartus 0mx15 M27 18
Labium
M. tentoriopraementalis 0la5 M29 23/24
M. submentopraementalis 0la8 M28 22
M. praementopalpalis internus 0la13 M33 20
M. praementopalpalis externus 0la14 M34 19
M. palpopalpalislabii primus 0la16 M35 25
M. palpopalpalislabii secundus 0la17 M36 26
Epipharynx
M. clypeopalatalis 0ci1 M43
M. clypeobuccalis 0bu1 M44 38
Hypopharynx
M. frontooralis 0hy1 M41a 38
M. tentoriooralis 0hy2 M41b 42
M. tentoriohypopharyngealis 0hy3 M42 36
| Muscle Name                          | Code   | Reference Number | Page |
|-------------------------------------|--------|------------------|------|
| M. praementosalivaris posterior     | 0hy8   | M39              | 33   |
| M. oralis transversalis             | 0hy9   | M67              |      |
| M. hypopharyngosalivaris            | 0hy12  | M37              | 34   |
| Pharynx                             |        |                  |      |
| M. frontobuccalis anterior          | 0bu2   | M45              | 39   |
| M. frontobuccalis posterior         | 0bu3   | M46              | 40   |
| M. tentoriobuccalis lateralis posterior | 0bu4 | M49              | 43   |
| M. tentoriobuccalis anterior        | 0bu5   | M48              | 44   |
| M. tentoriobuccalis posterior       | 0bu6   | M50              | 45   |
| M. verticopharyngealis              | 0ph1   | M51              | 41   |
| M. tentoriopharyngealis             | 0ph2   | M52              | 46   |

“+” = present “-” = absent

References

Kéler SV. 1963. Entomologisches Wörterbuch. AkademieVerlag, Berlin.

Miller FW. 1933. Musculature of the lacewing (*Chrysopaflorabunda*) (Neuroptera). Journal of Morphology. 55: 29-52.

Figures
Figure 1

Chrysopa pallens (Rambur, 1838), photographs: larvae, dorsal view (A); pupae (Day11, pupal sheath with pharate adult inside), lateral view (B); adults (♂), lateral view (C); cocoon of pupal stage (D); cocoon of Day 6-11, inside view (E); cocoon of Day 12 (F); cuticle of Day 12 pupa (G). Scale bar: 2mm.
Chrysopa pallens (Rambur, 1838), photographs: head and thorax of larvae, lateral view (A); same, dorsal view (B); same, ventral view (C). Scale bar: 0.5mm. Abbreviations: ant: antenna; lap: labial palp; LT: lateral tubercles; md: mandible; mg: maxillary groove; mx: maxilla; mxst: maxillary stylet; oc: ocellus; pmt: prementum; Sc1-Sc3: first-third primary sclerites; smt: submentum; T1-T3: prothorax, mesothorax, metathorax.
Chrysopa pallens (Rambur, 1838), photographs: Pupae from Day 1 to Day 12, dorsal, lateral, and ventral view. Scale bar: 2.5mm.
Chrysopa pallens (Rambur, 1838), photographs: head and thorax of pupae Day 11, dorsal view (A); same, ventral view (B). Scale bar: 0.6mm. Abbreviations: ce: compound eye; cl: clypeus; fl: flagellomeres; la: labium; lap: labial palp; lb: labrum; md: mandible; psc2: mesothoracic prescutum; sc2: mesothoracic scutum; scl2: mesothoracic scutellum.
Figure 5

Chrysopa pallens (Rambur, 1838), photographs: adults head, frontal view (A); head and thorax of adults, dorsal view (B); same, ventral view (C). Scale bar: 0.5mm. Abbreviations: ant: antenna; ca: cardo; ce: compound eye; cl: clypeus; dlf: dorsolateral longitudinal furrow; fcs: frontoclypeal sulcus; fgs: frontogenal suture; fl: flagellomeres; la: labium; lap: labial palp; lb: labrum; loccl: lateral occipital lobes; mt: mentum; mxp: maxillary palp; pe: pedicellus; pmt: prementum; sc: scapus; smt: submentum; sti: stipes; tp: tentorial pits.
Figure 6

Chrysopa pallens (Rambur, 1838), 3D reconstructions of head internal structures of larvae, pupae (Day 11), and adults, cuticle rendered transparent, muscles in light pink, brain in yellow, and pharynx in green: tentorium, dorsal view (A); half of labrum and antennal musculature, frontal view (B); half of mandible musculature, frontal view (C); half of
maxillary musculature, lateral view (D). Abbreviations: ata: anterior tentorial arm; br: brain; ce: compound eye; dta: dorsal tentorial arm; fl: flagellomeres; ga: galea; la: labium; lac: lacinia; lap: labial palp; lb: labrum; lt: laminatentorium; md: mandible; mxst: maxillary stylet; mxp: maxillary palp; oc: ocellus; pta: posterior tentorial arms; sc: scapus; tb: tentorial bridge.
Figure 7

Chrysopa pallens (Rambur, 1838), 3D reconstructions of tentorium from larvae to adults.

Scale bar: 0.2mm. Abbreviations: ata: anterior tentorial arm; dta: dorsal tentorial arm; pta: posterior tentorial arms; tb: tentorial bridge.
Figure 8

Chrysopa pallens (Rambur, 1838), 3D reconstructions of mandibles: mandible of pupae, dorsal view (A); same, ventral view (B); mandible of adults, dorsal view (C); same, ventral view (D). Abbreviations: ai: apical incisor; gp: globular protrusion (primary mandibular joint); L: left; mp: molar process; R: right; smj: secondary mandibular joint.
Chrysopa pallens (Rambur, 1838), 3D reconstructions of head internal structures from larvae to adults: maxilla musculature, dorsal view (A); labium musculature, dorsal view (B); musculature of epipharynx, pharynx, and hypopharynx, dorsal view (C); musculature of hypopharynx and pharynx, ventral view (D). Abbreviations: ata: anterior tentorial arm; ant: antenna; br: brain; ca: cardo; ce: compound eye; dta: dorsal tentorial arm; fg: ganglion frontale; ga: galea; la: labium; lac: lacina; lb: labrum; imms, intrinsic muscle of maxillary
stylet; lap: labial palp; md: mandible; mt: mentum; mx1: proximal maxillary element; mx2: intermediate maxillary element; mxp: maxillary palp; mxst: maxillary stylet; nan: antennal nerve; onp: optic neuropils; ph: pharynx; pmt: prementum; prhy, prelabiohypopharyngeal muscle; pta: posterior tentorial arms; sc: scapus; smt: submentum; sog: suboesophageal ganglion; sti: stipes; tb: tentorial bridge.
Figure 10

Chrysopa pallens (Rambur, 1838), cross sections from micro-CT of head from larvae to pupae (Day 1-Day 7). Muscle fibers in pink arrow, nerves in yellow arrow.
Figure 11

*Chrysopa pallens* (Rambur, 1838), cross sections from micro-CT of head from pupae (Day 8-Day 12) to adults. Muscle fibers in pink arrow, nerves in yellow arrow.
Figure 12

Chrysopa pallens (Rambur, 1838), 3D reconstructions of mandible muscles (0md1, 0md3) from larvae to adults. Muscles in pink. The larval cuticle is represented by dotted line and adult cuticle is represented by solid line. Scale bar: 0.15mm.
Figure 13

Chrysopa pallens (Rambur, 1838), 3D reconstructions of cephalic nervous system from larvae to adults: brain and suboesophageal ganglion, dorsal view (A); same, lateral view (B).

Abbreviations: ant: antenna; br: brain; ce: compound eye; fg: ganglion frontale; lb: labrum; lap: labial palp; md: mandible; mx: maxilla; mxp: maxillary palp; nan: antennal nerve; onp: optic neuropils; ph: pharynx; sog: suboesophageal ganglion.
Figure 14

Chrysopa pallens (Rambur, 1838), 3D reconstructions of brain from larvae to adults in front view. Scale bar: 0.2mm.
Figure 15

Chrysopa pallens (Rambur, 1838), angles between the longitudinal body axis and the longitudinal axis of the mouthparts, 3D reconstructions: larvae, lateral view (A); pupae Day 1, lateral view (B); pupae Day 11, lateral view (C); adults, lateral view (D).