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 adecticous 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 dead 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 includs 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 comples 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 prepharygeal 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. Posteriror hypopharynx laterally fused to posterior epipharynx, forming the ventral prepharygeal 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 funtional 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 quardrangular 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 muscls

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 disappearence 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 *Nevrorthus* [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 *Nevrorthus* [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,* themodifications 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

Not applicable

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	Basal part of the maxilla stylet	adductor of maxilla stylet
0mx3	Proximal part of anterior tentorial arms	Cardo	protractor of maxilla
0mx4	Proximal part of the anterior tentorial arms	Anterior part of stipes	protractor of maxilla
0mx5	Anterior tentorial arms, anterior to the 0mx4	posterior part of stipes	protractor of maxilla
0mx6	stipes	Basal edge of the maxilla stylet	adductor of lacinia
imm	Basal part (dorsal) of the maxilla stylet	Basal part (ventral) of the maxilla stylet	
Labium/3			
0la5	Posterior tentorial arms	Posterolateral 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			

Muscle Abb./No.	Origin	Insertion	Presumed function
0ci1	clypeus	Roof of the cibarium	dilatator ofcibarium
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	Bentral wall of the bucca	dilator of pharynx
prhy	Lateral region of the prementum	Anterior region of the ventral bucca	dilator of pharynx
0ph1	Posterior region of vertex	Dorsal wall the pharynx	
0ph2	Posterior tentorial arms	Ventral and lateral region of the pharynx	dilator of pharynx
0st1	Ring muscle layer that covers the entire pharynx		constrictor of the pharynx
0st2	Longitudinal muscle layer directly above musculus annularis stomodaei		constrictor of the pharynx

Table 2. Cephalic musculature of the pupae of *Chrysopa pallens* (Rambur, 1838).

Muscle Abb.	Origin	Insertion
Labrum/3		
0lb1	Lateral on the frons, below the antennal base	Mesally on the outer basal wall of the labrum
0lb2	Laterally on the frons	Tormae
0lb4	Dorsal labral wall	Ventral labral wall
Antenna/4		
0an1	Anterior tentorial arms	Anterior basal margin of the scape
0an2	Anterior tentorial arms	Posterior basal margin of the scape
0an6	Dorsal wall of the scape	Lateral wall of the pedicel
0an7	Mesal wall of the scape	Mesal edge of the pedicel
Mandible/4		
0md1	The part between occipital and compound eyes of the head capsule	Tendon that inserts at the median edge of the mandible
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 palpomere1	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		

Muscle Abb.	Origin	Insertion
0ci1	Mesally on the clypeus	Posterior region of the epipharynx, covered by 0bu1
0bu1	Anterior region of frons	Roof of the bucca
Hypopharynx/3		
0hy1	frons	Oral arms of the suspensorial sclerites
0hy2	Anterior tentorial arm	Oral arms of the suspensorial sclerites
0hy12	Anterior part of the hypopharynx	Inner wall of the ligula
Pharynx/5		
0bu2	Frons, below the antennal base	Dorsal wall of the pharynx
0bu3	Posterior part of frons, lateral region of the antennal base	Dodrsal wall of the pharynx, posterior to 0bu2
0bu5	Anterior tentorial arms, anterior to tentorial bridge	Ventral wall of the pharynx
0bu6	Anterior tentorial arms, lateral to 0bu5	Ventral wall of the pharynx
0ph2	Posterior region of the tentorial bridge	Lateral wall of the pharynx

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	Mesal basal edge of the scape	Depressor and rotator of antenna
0an6	Laterally, mesally of the dorsal wall of the scape	Lateral basal edge of the pedicel	Extensor of flagellum
0an7	Inner wall of scape	Basal edge of the pedicel	Flexor of flagellum
0an9	Ventral wall of the scape	Posteriro basal region of the pedicel	Depressor of the antenna
0an10	Dorsal wall of the scape	Anterior basal region of the pedicel	Elevator of the antenna
Mandible/5			
0md1	Posterior part between occipital and compound eye of the head capsule	Tendon that inserts at the median edge of the mandible	Adductor of mandible
0md3	Gena and postgena	Tendon that inserts at the lateral edge of the mandible	Abductor of mandible
0md4	Lateral wall of the hypopharynx	Inner median wall of the mandible	Protractor of anatomical mouth opening
0md6	Ventral side of the anterior tentorial arms	ventral basal margin of the mandible	Adductor of mandible
0md8	Anterial tentorial arms	Mediodorsal wall of the mandibular cavity	
Maxilla/13			
0mx1	Posterior part of the head capsule	Basal cardinal process	Promoter of maxilla
0mx2	Posterior part of gena	Basal part of the lacinia	Adductor of lacinia
0mx3	Ventral side of the anterior tentorial arms	Carostipital sulcus	Adductor of cardo and protractor of maxilla
0mx4	Ventral side of the anterior tentorial arms, under 0mx3	Anterior edge of the stipes	Adductor of maxilla

Muscle Abb.	Origin	Insertion	Presumed function
0mx5	Lateral part of tentorial bridge	Basally on the stipes, close to 0mx4	Adductor of stipes and protractor of maxilla
0mx6	Lateral wall of stipes, close to carostipital sulcus	Basal edge of the lacinia	Adductor of lacinia
0mx7	Mesal wall of stipes	Basal edge of the galea	Abductor of galea
0mx8	Lateral wall of the stipes, basal to the maxillary palpus	Basal edge of the first palpomere	Abductor of maxillary palp
0mx10	Stipital ridge	Distal edge of the palpomere 1	Adductor of maxillary palp
0mx12	Basal edge of palpomere 1	Basal edge of palpomere 2	Adductor of maxillary palpomere ii
0mx13	Basal edge of palpomere 1	Basal edge of palpomere 3	Abductor of maxillary palpomere iii
0mx14	Basal edge of palpomere 3	Basal edge of palpomere 4	Adductor of maxillary palpomere iv
0mx15	Basal edge of palpomere 4	Basal edge of palpomere 5	Adductor of maxillary palpomere v
Labium/6			
0la5	Posterior tentorial arms	Laterobasal edge of prementum	Adductor of praementum
0la8	Posterior part of submentum	Posterior edge of mentum	Retractor of praementum
0la13	Distally on the prementum	Distal edge of the labial palpus	Adductor of labial palpomere i
0la14	Basal edge of the prementum	Basal edge of the labial palpus	Levator of labial palp
0la16	Basal edge of palpomere 1	Basal edge of palpomere 2	Flexor of labial palpomere ii
0la17	Basal edge of palpomere 2	Basal edge of palpomere 3	Flexor of labial palpomere iii
Epipharynx/2			
0ci1	Mesally on the clypeus	Posterior region of epipharynx	Dilatator ofcibarium
0bu1	Anterior region of frons	Roof of the bucca	Dilator of buccal cavity
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

Muscle Abb.	Origin	Insertion	Presumed function
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 directly above musculus annularis stomodaei		Constrictor of the pharynx

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.	Larvae	Pupae	Adults	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 posterior	0md3	□	□	□	abductor of mandible
M. hypopharyngomandibularis	0md4	□	□	□	protractor of anatomical mouth opening,
M. tentoriomandibularis lateral inferior	0md6	□	□	□	adductor of mandible
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

Muscle name	Abb.	Larvae	Pupae	Adults	Presumed function
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
M. palpopalpalislabii primus	0la16	□	□	□	flexor of labial palpomere ii
M. palpopalpalislabii secundus	0la17	□	□	□	flexor of labial palpomere iii
Epipharynx		**2**	**2**	**2**	
M. clypeopalatalis	0ci1	□	□	□	dilatator ofcibarium
M. clypeobuccalis	0bu1	□	□	□	dilator of buccal cavity
Hypopharynx		**1**	**3**	**5**	
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**	**5**	**9**	
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 (Insecta) and the systematic position of the order, with a new nomenclature for the head muscles of Dicondylia. Systematic Entomology. 36: 241–266.

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)	Mille□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	□	□	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

Muscle name	Abb.	Present study	Kéler (1963)	Mille□1933□
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
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

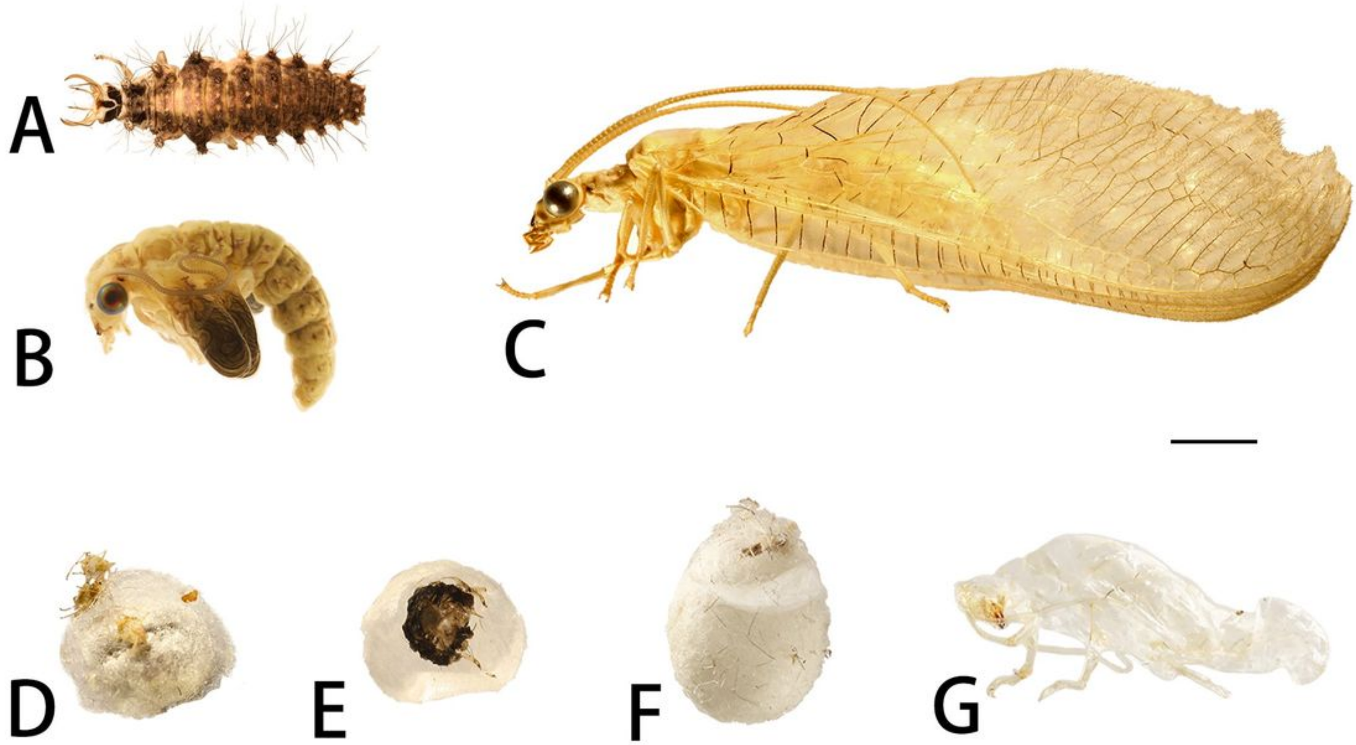

Figure 1

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.

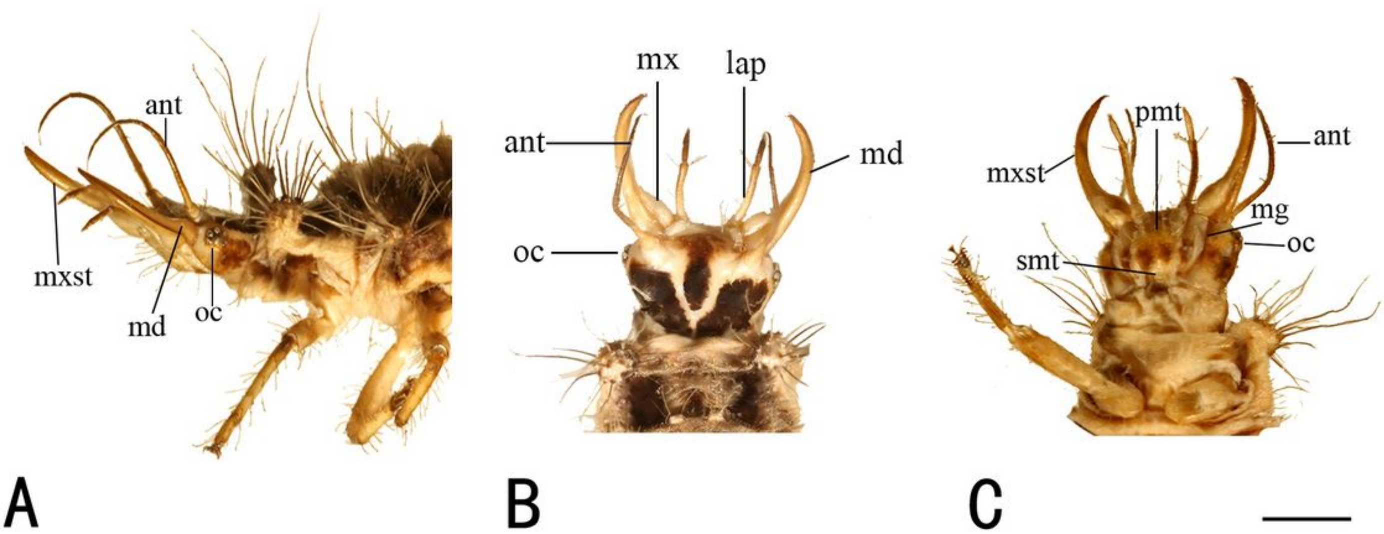

Figure 2

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.

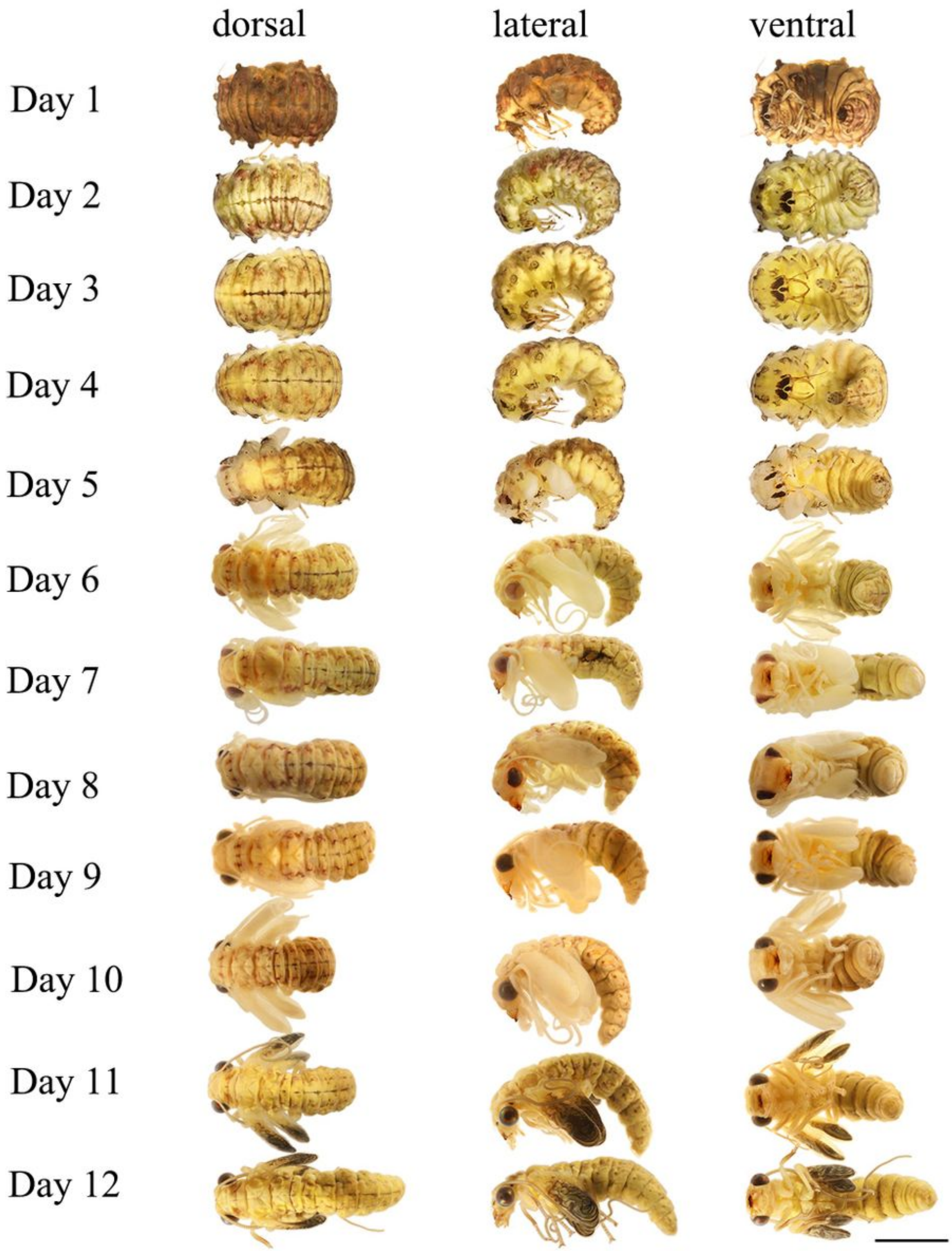

Figure 3

Chrysopa pallens (Rambur, 1838), photographs: Pupae from Day 1 to Day 12, dorsal, lateral, and ventral view. Scale bar: 2.5mm.

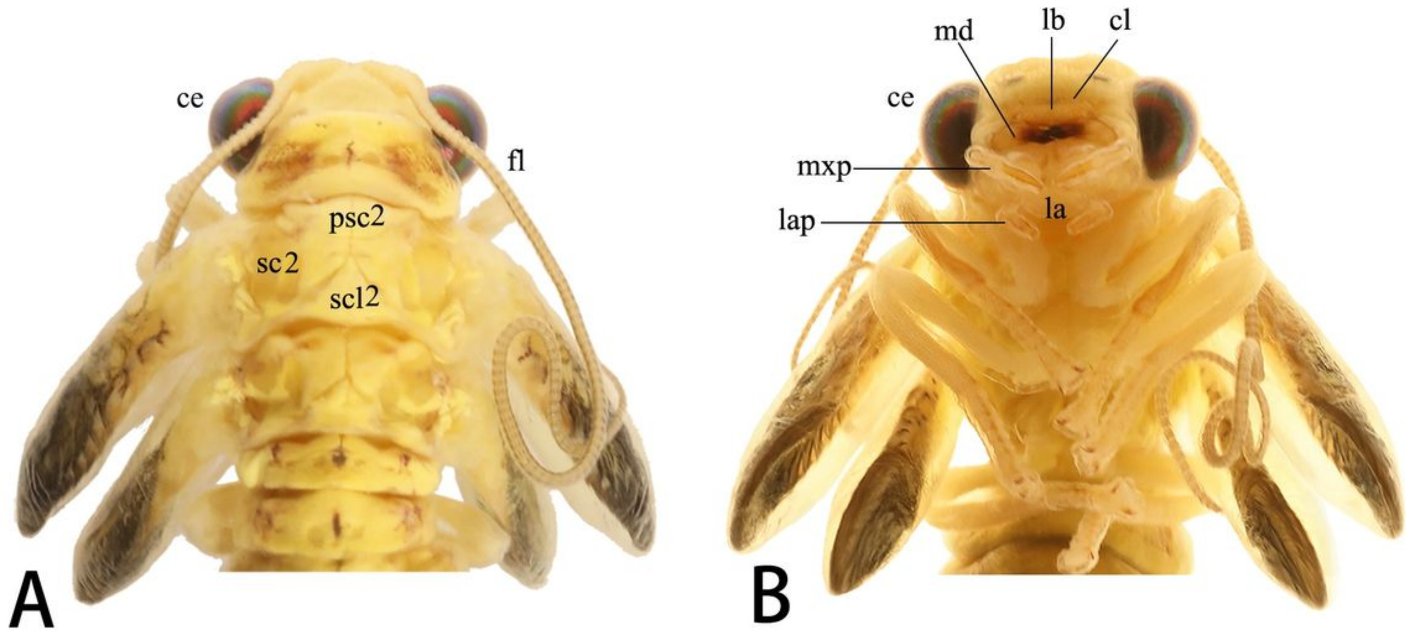

Figure 4

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.

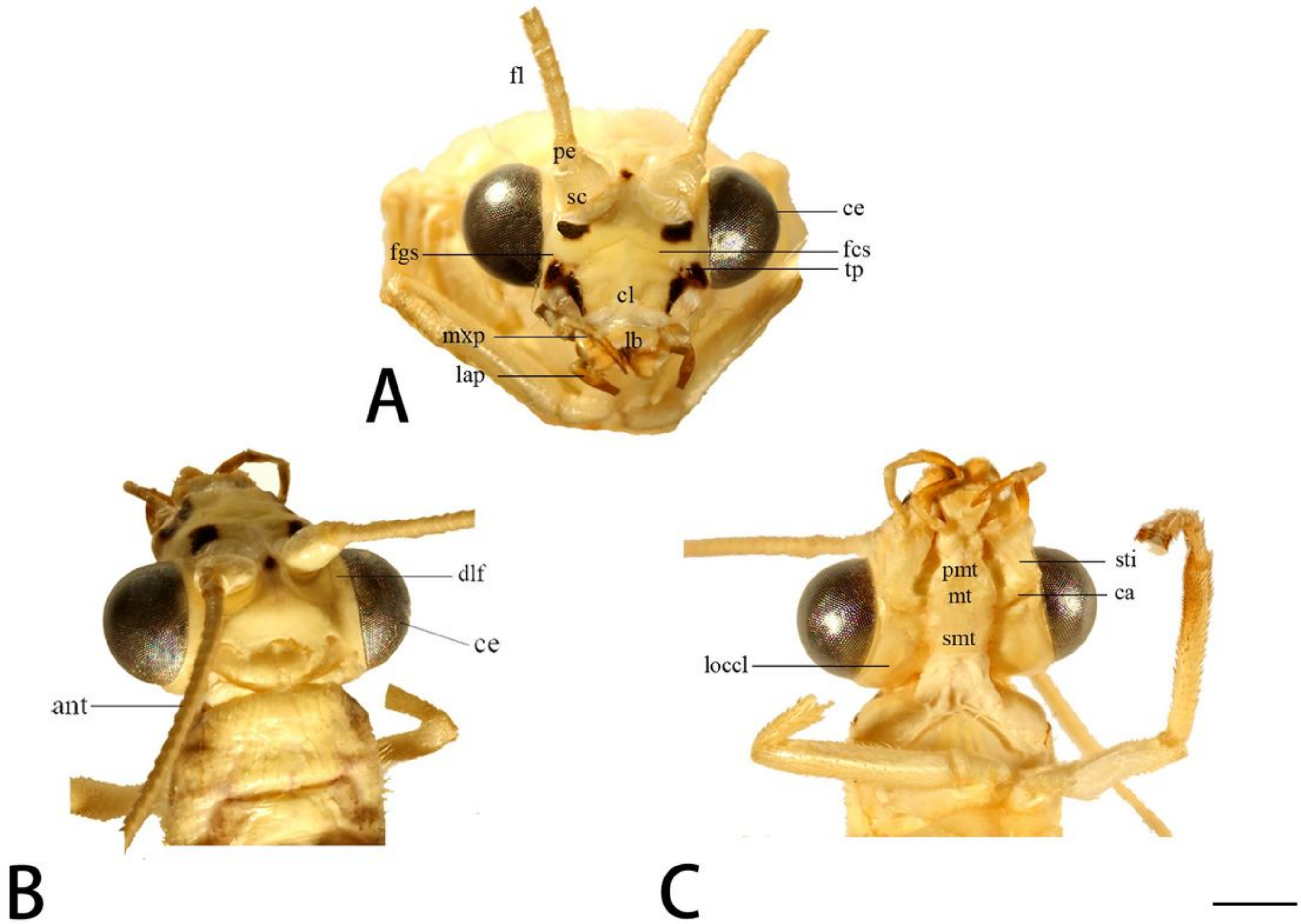

Figure 5

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.

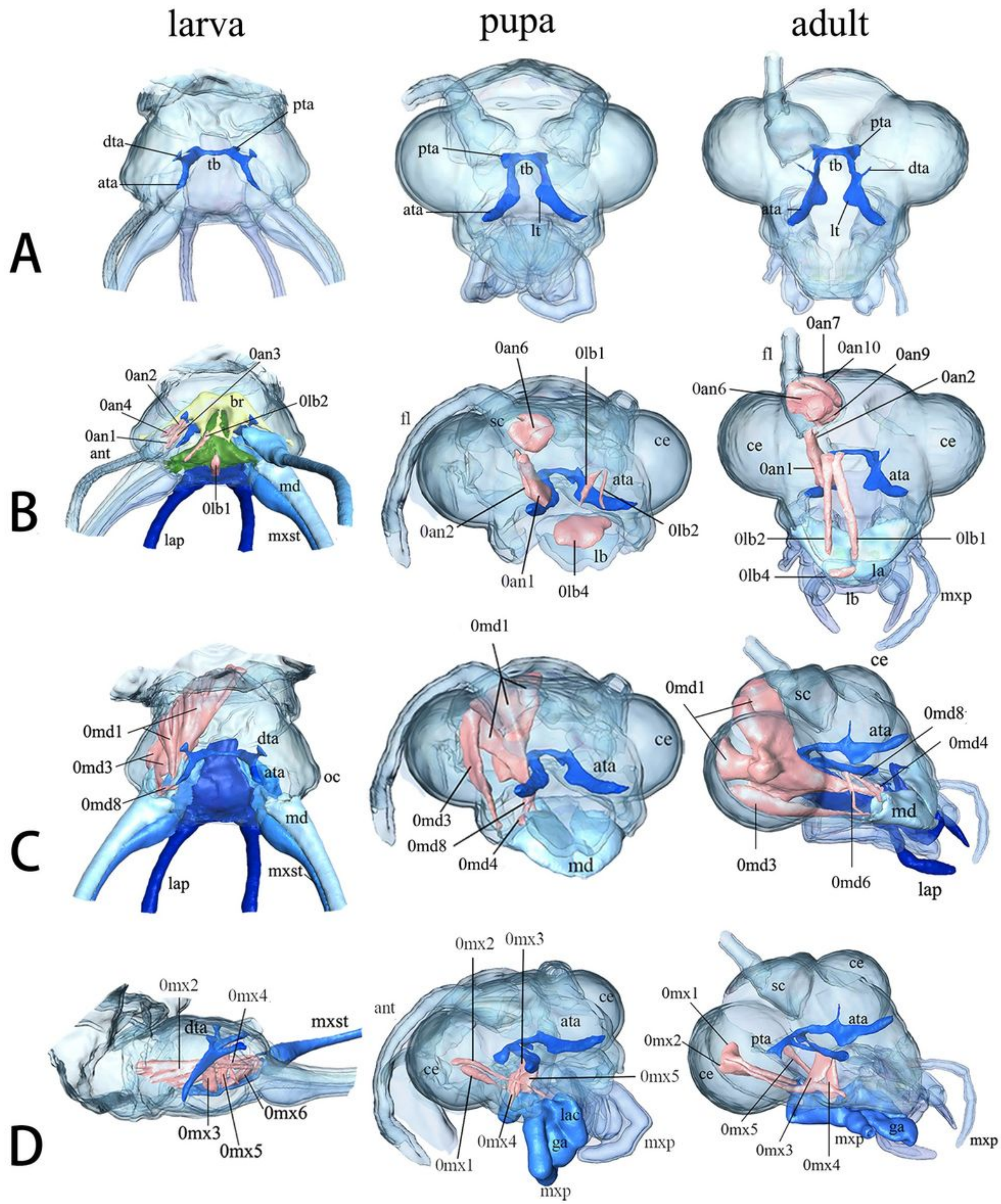

Figure 6

Chrysopa pallens (Rambur, 1838), 3D reconstructions of head internal structures of larvae, pupae (Day 11), and adults, cuticle rendered tranparent, 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.

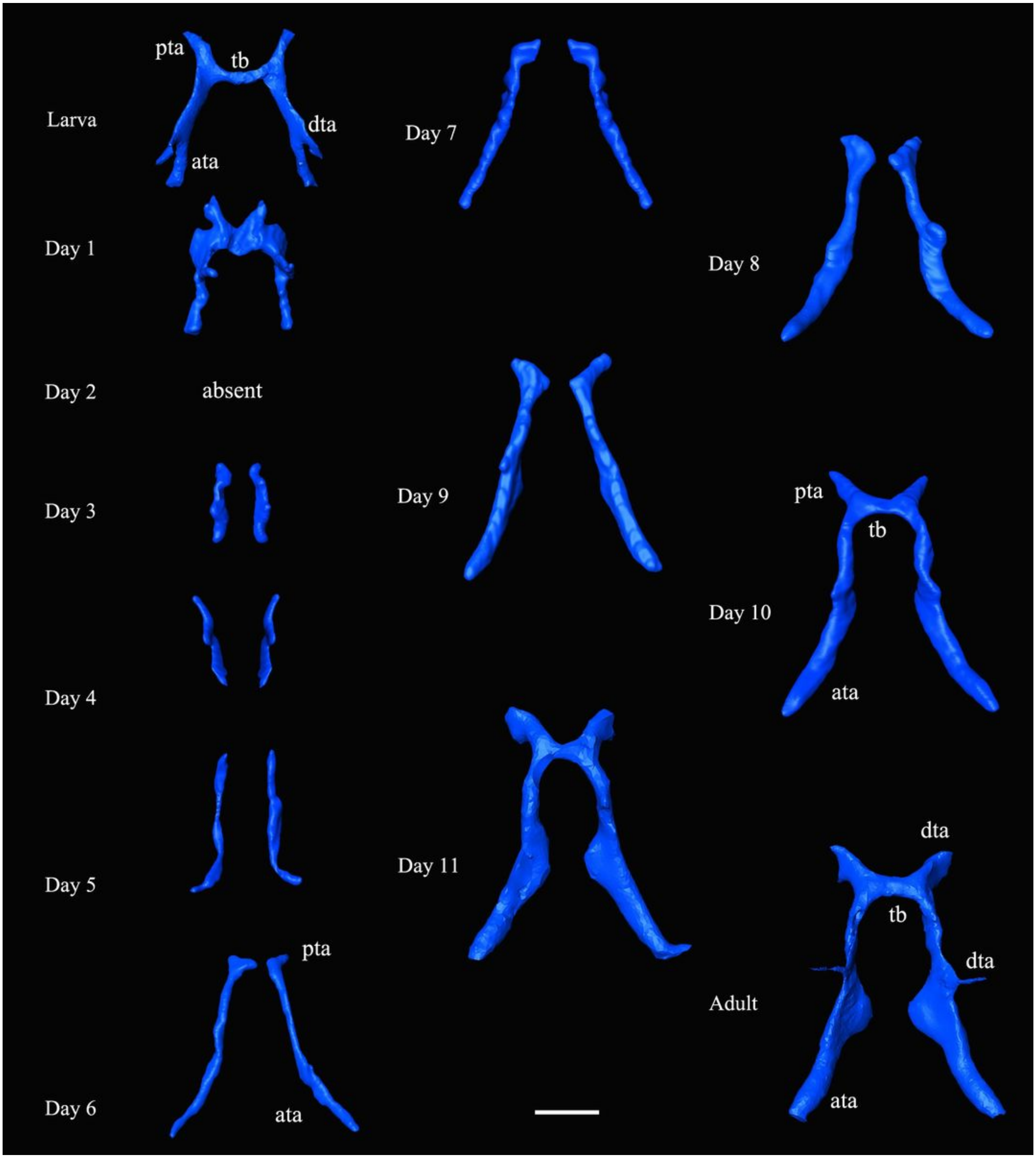

Figure 7

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.

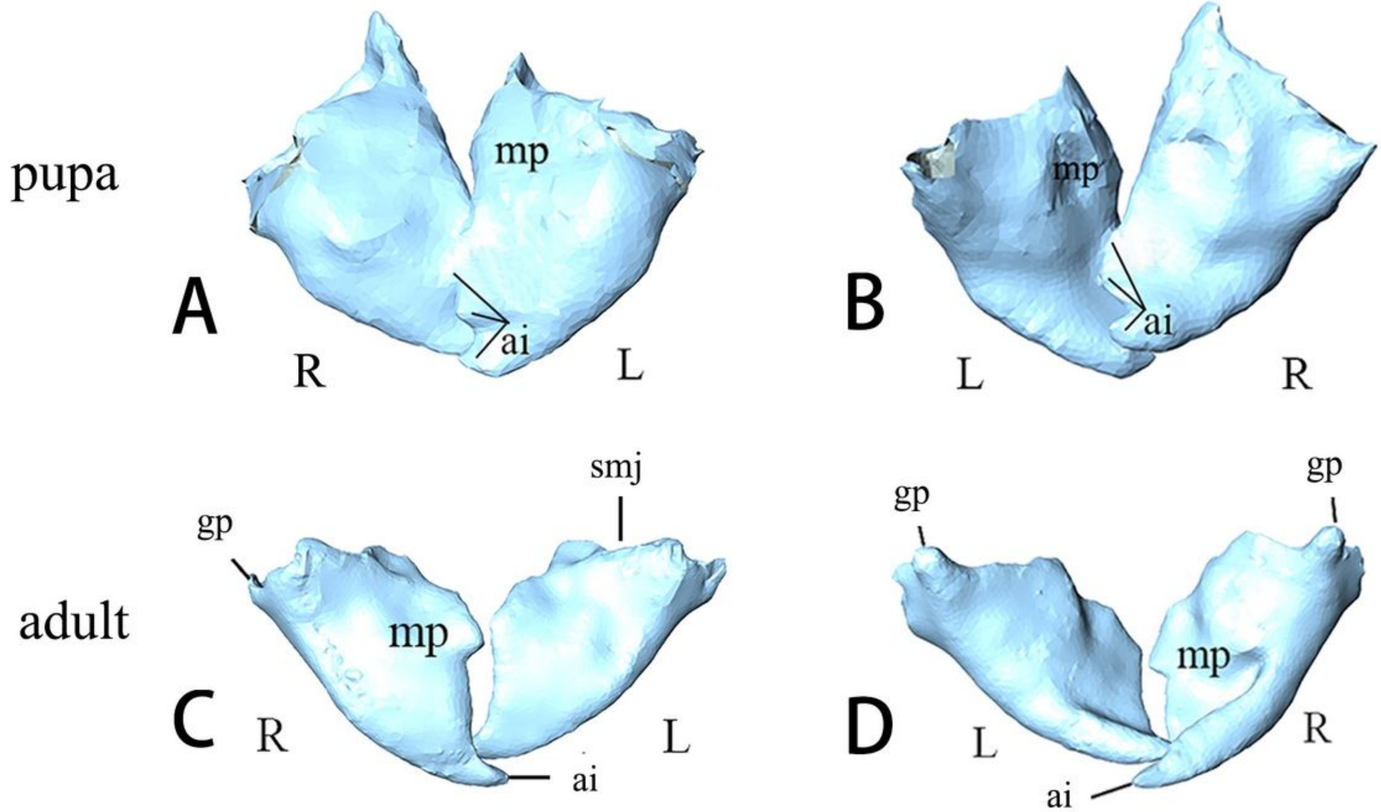

Figure 8

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 mandiblular joint); L: left; mp: molar process; R: right; smj: secondary mandibular joint.

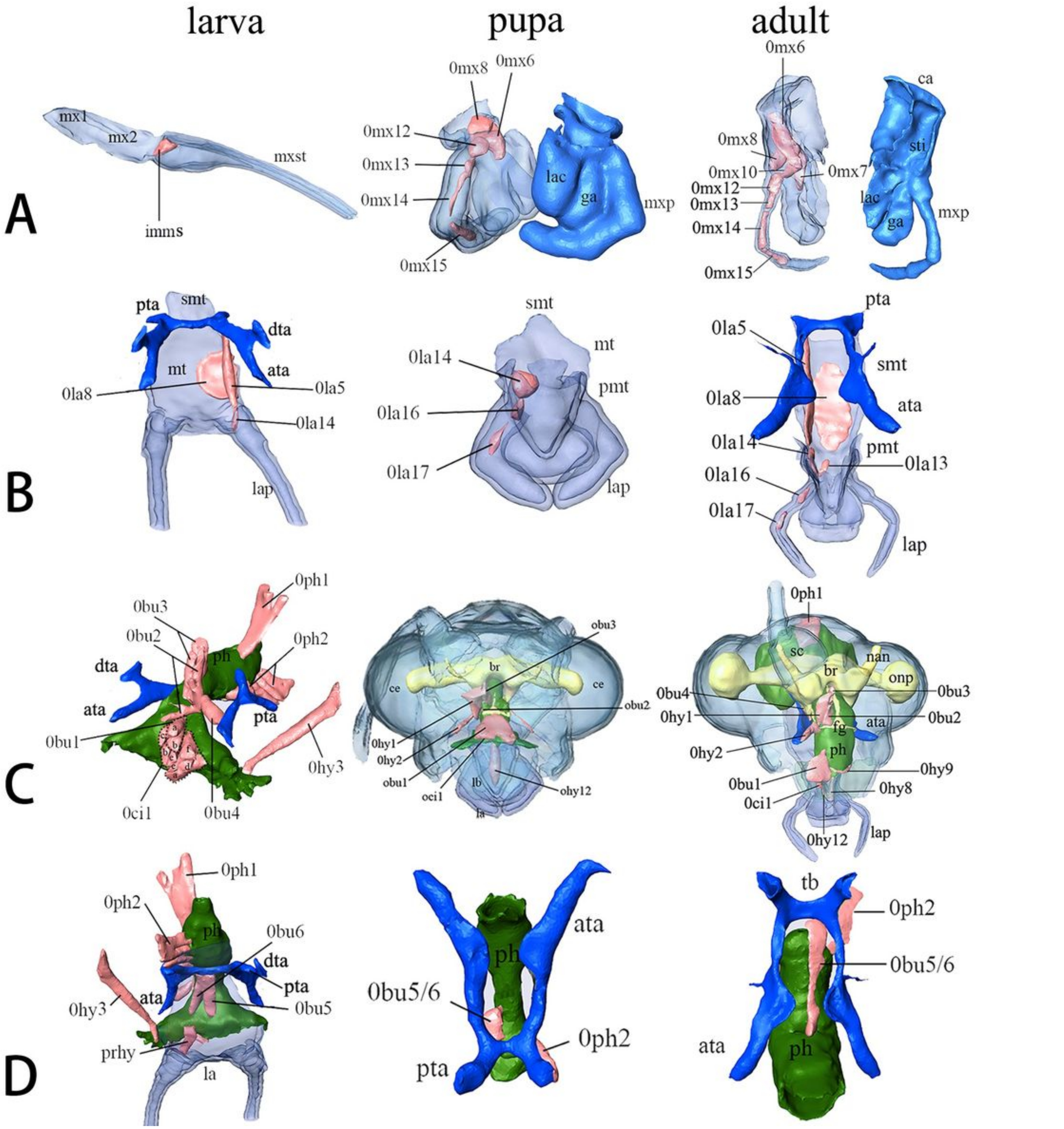

Figure 9

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：lacinia ; 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.

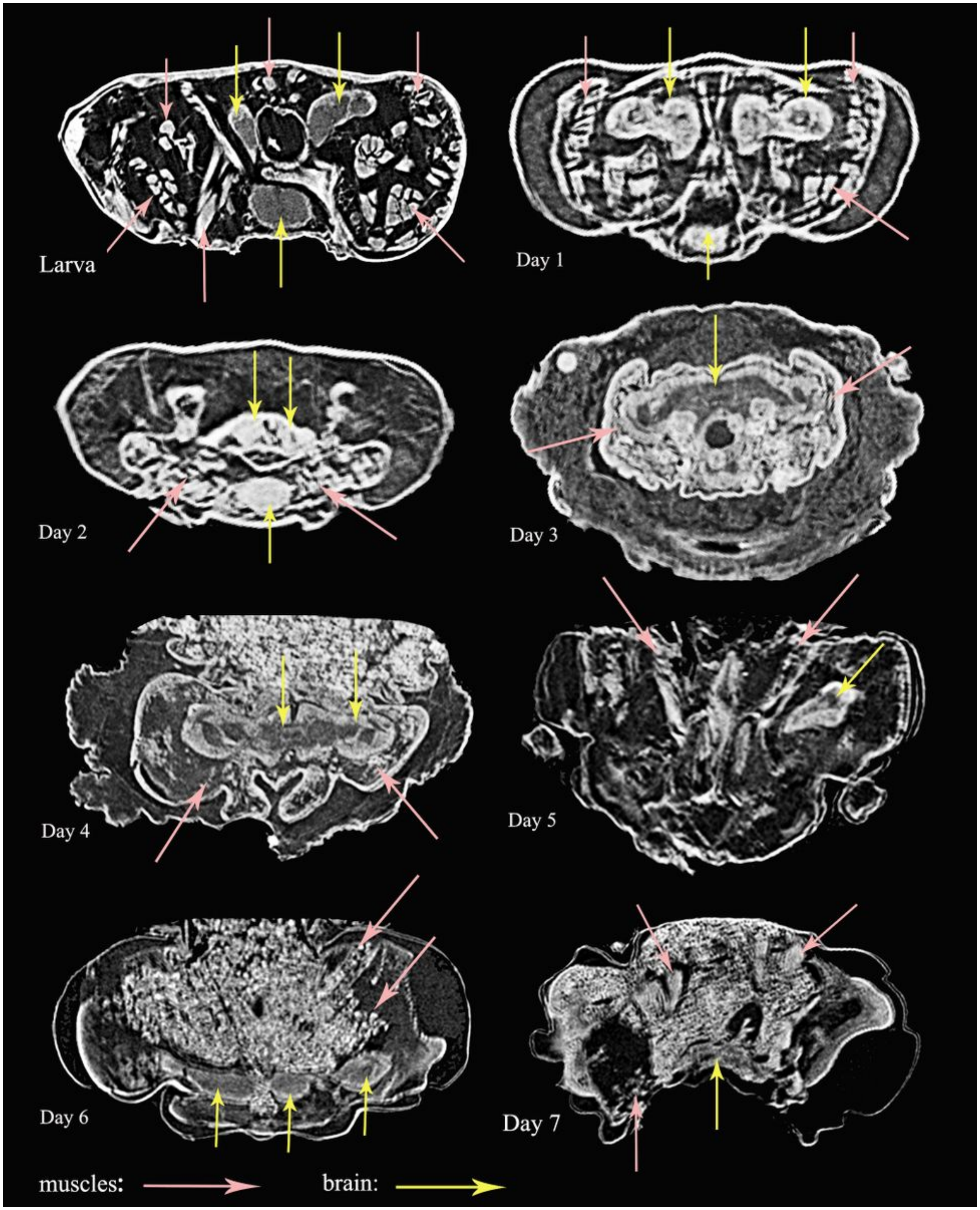

Figure 10

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.

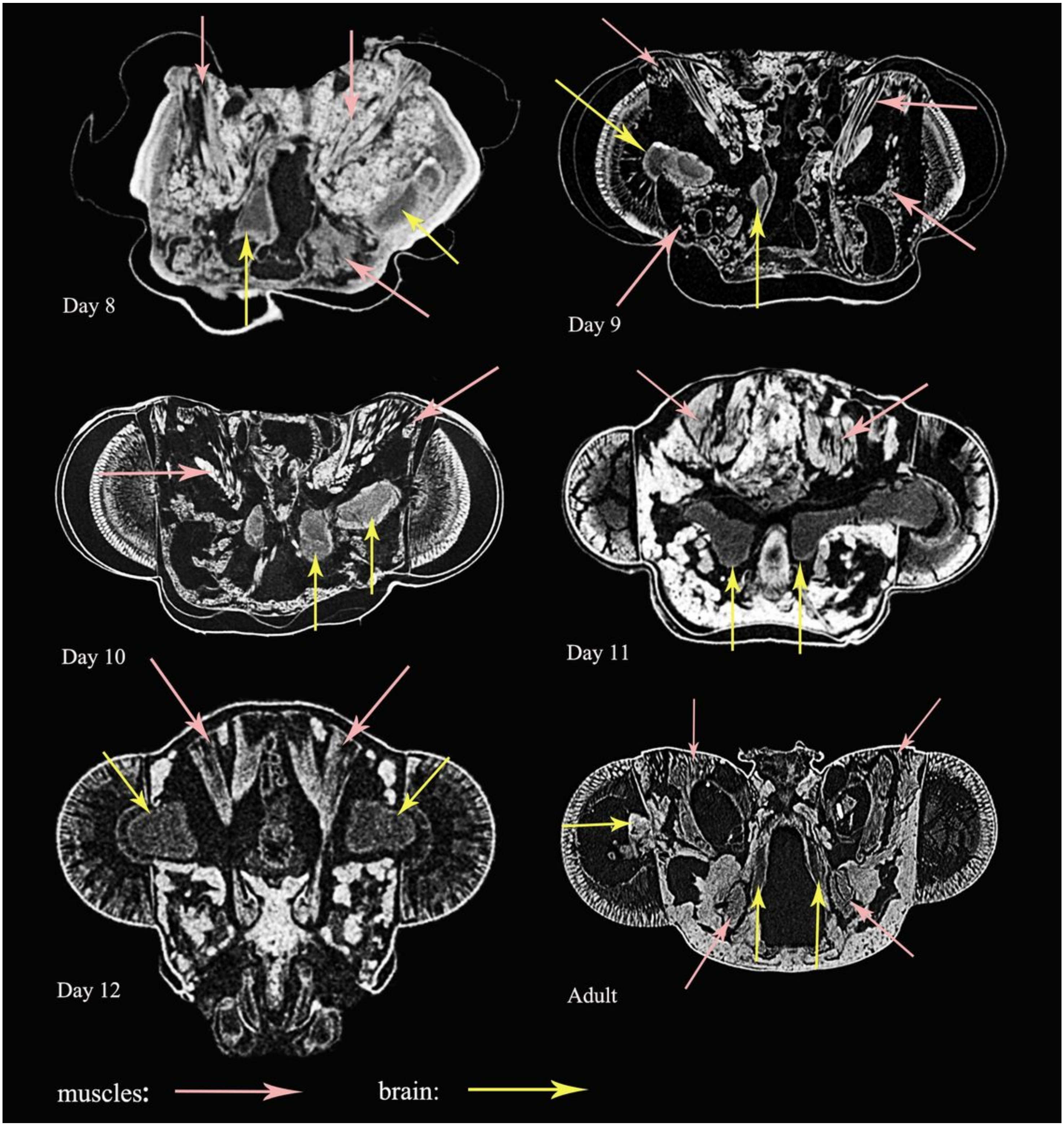

Figure 11

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.

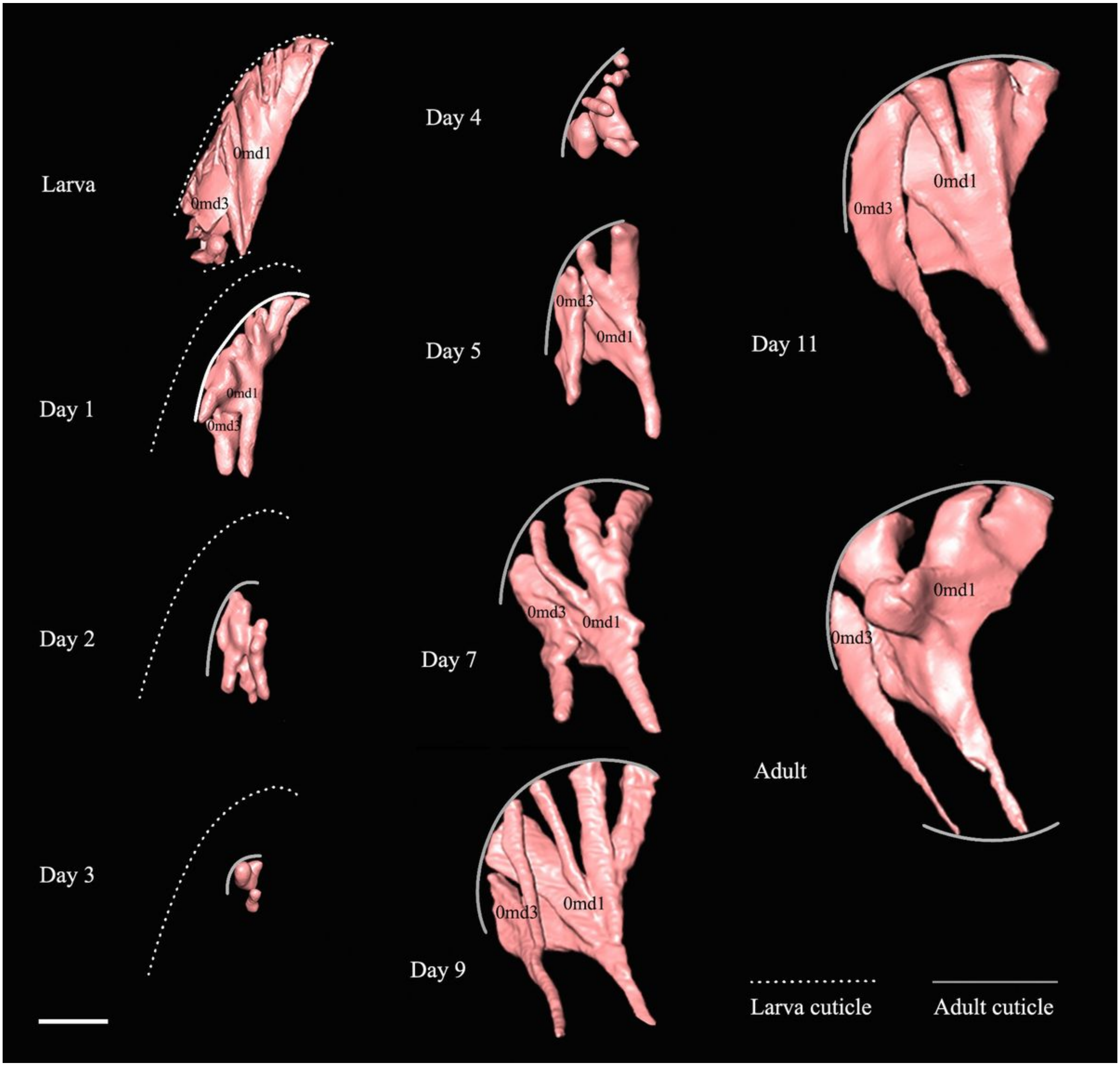

Figure 12

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.

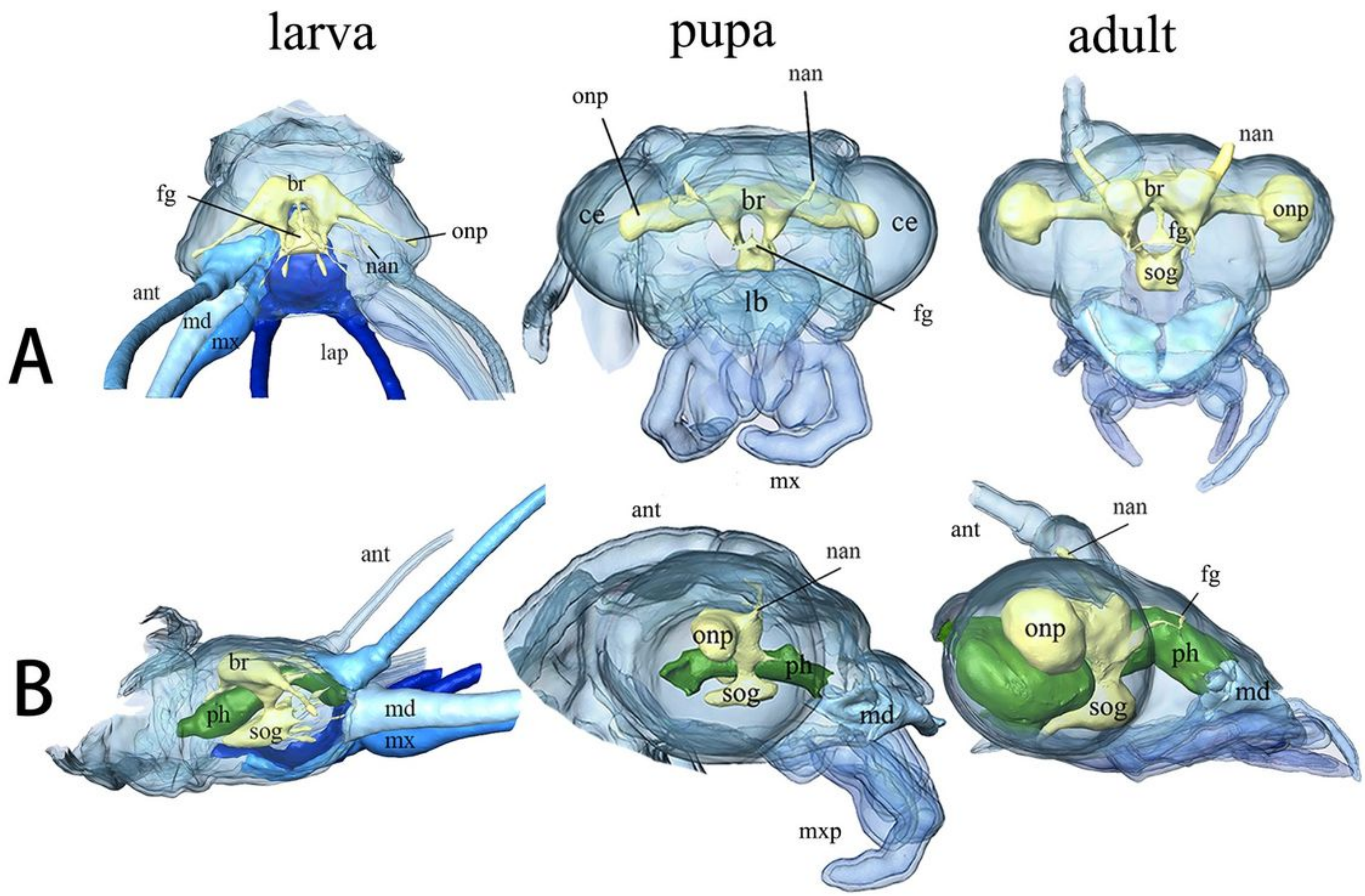

Figure 13

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.

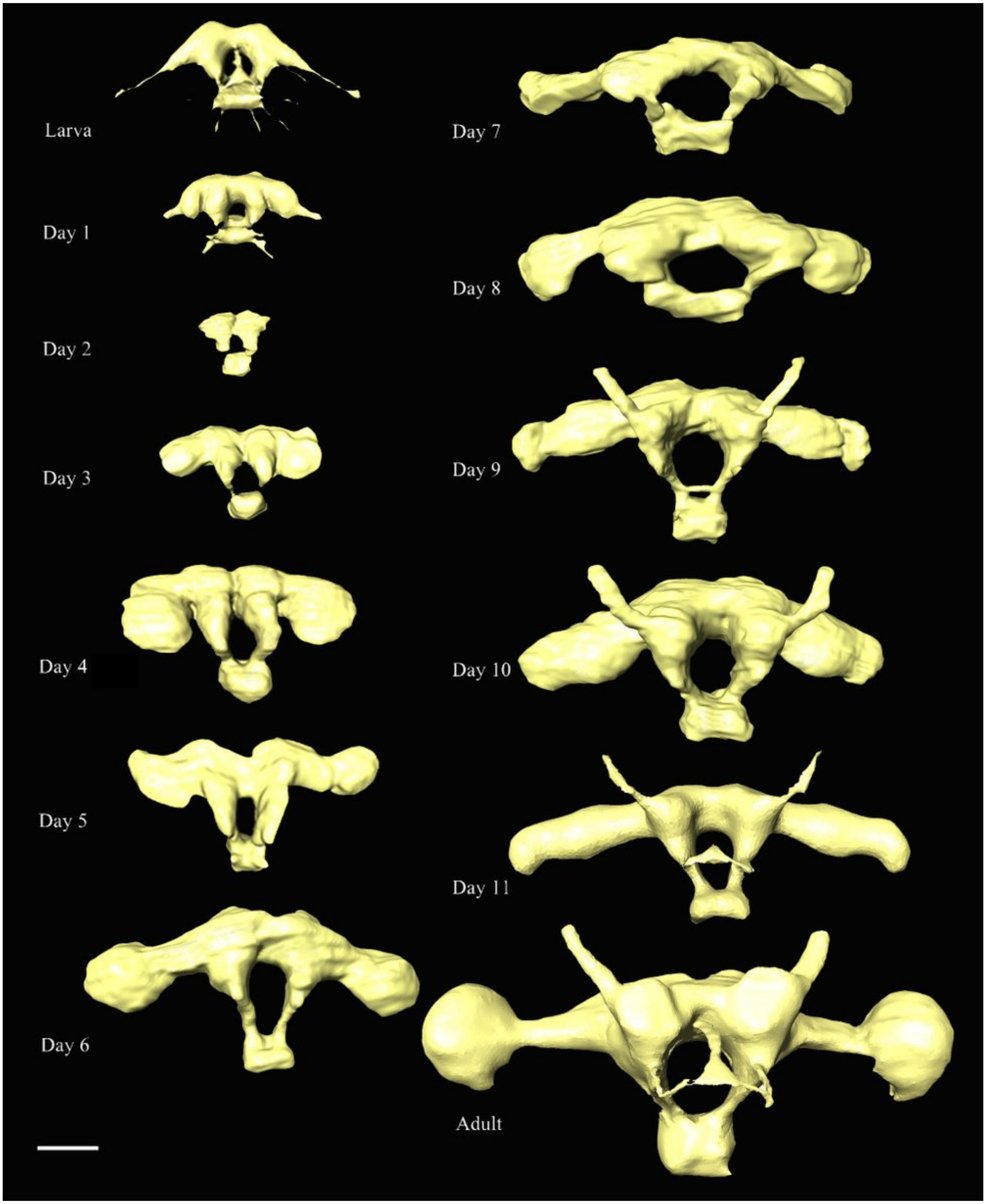

Figure 14

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

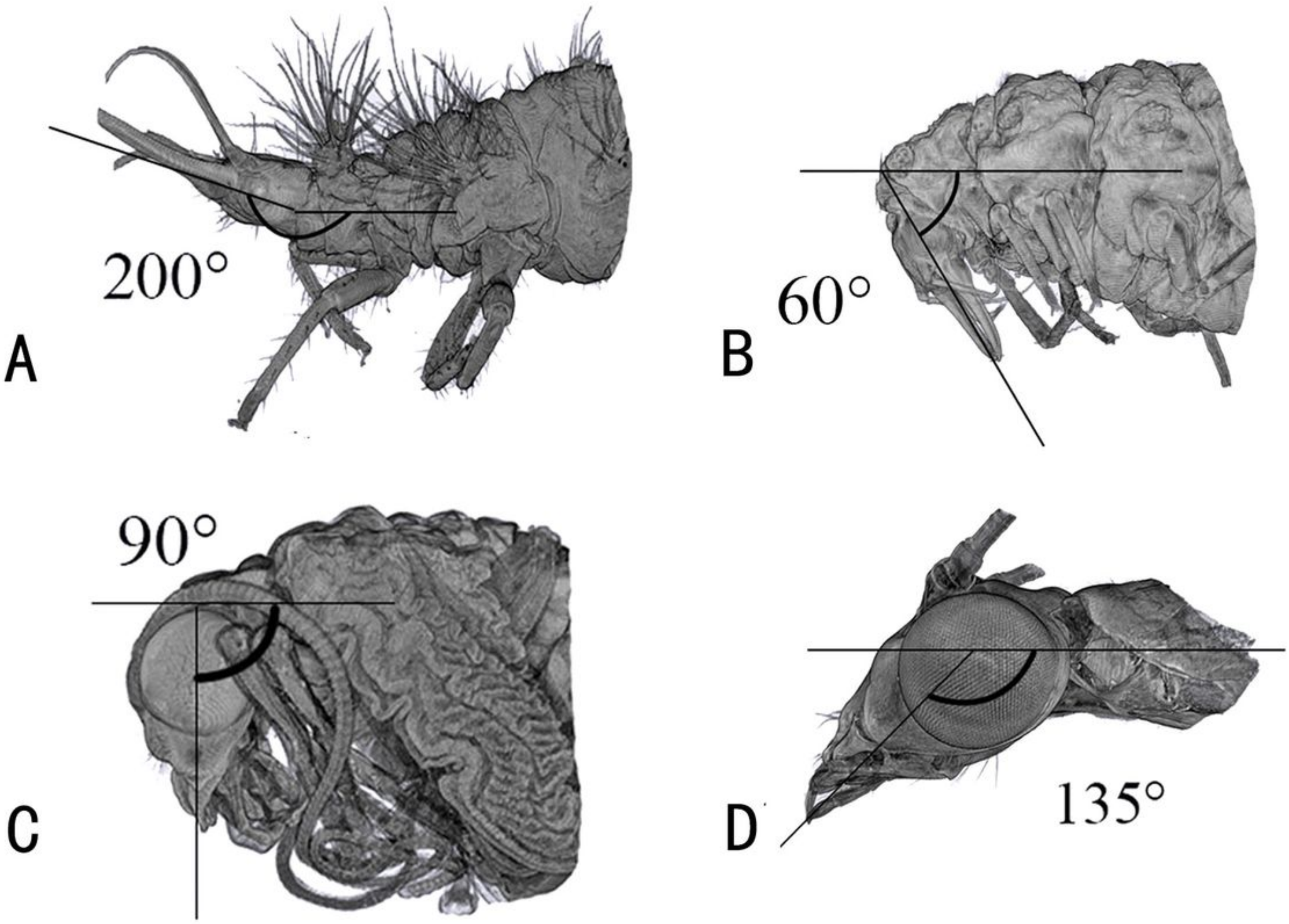

Figure 15

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).