

From swimming towards sessility in two metamorphoses – the drastic changes in structure and function of the nervous system of the bay barnacle *Amphibalanus improvisus* (Crustacea, Thecostraca, Cirripedia) during development

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Abstract

Knowledge about the development of the nervous system in cirripeds is limited, particularly with regard to the changes that take place during the two metamorphoses their larvae undergo. This study delivers the first detailed description of the development of the nervous system in a cirriped species, *Amphibalanus improvisus* by using immunohistochemical labeling against acetylated alpha-tubulin, and confocal laser scanning microscopy. The development of the nervous system in the naupliar stages corresponds largely to that in other crustaceans. As development progresses, the protocerebral sensory organs differentiate and the intersegmental nerves forming the complex peripheral nervous system appear, innervating the sensory structures of the cephalic shield. During metamorphosis into a cypris the lateral sides of the cephalic shield fold down into a bilateral carapace, which leads to a reorganization of the peripheral nervous system. The syncerebrum of the cypris exhibits the highest degree of complexity of all developmental stages, innervating the frontal filaments, nauplius eye, compound eyes and the antennules. During settlement, when the second metamorphosis occur, the closely associated frontal filaments and

compound eyes are shed together with the cuticle of the carapace and the antennules. In adults, the syncerebral structures are reduced while the ventral nerve cord and the peripheral nervous system increase in complexity. The peripheral nervous system plays an important role in processing sensory input and also in settlement. In summary, through the larval development we observed a structural and thus also functional increase of complexity in favor of the peripheral nervous system and the ventral nerve cord.

Keywords

Crustacea – Cirripedia – nervous system – development – metamorphosis – CLSM – immunohistology

Introduction

The bay barnacle *Amphibalanus improvisus* (Darwin, 1854) is the most common brackish-water cirriped (Lang, 1979), inhabiting low and subtidal areas in estuaries all over the world (Dineen & Hines, 1992). As in most other Thoracica, the life cycle of *A. improvisus* encompasses six naupliar stages followed by a highly specialized cypris larva and subsequent metamorphosis into a sessile juvenile (Jones & Crisp, 1954; Glenner & Høeg, 1993; Høeg & Møller, 2006; Høeg et al., 2009). The complex development of cirripeds is accompanied by a reduction, transformation and specialization of sensory organs and the associated structures of the central nervous system (Doochin, 1951; Walley, 1969; Høeg, 1987; Høeg & Lützen, 1995; Høeg et al., 2012; Maruzzo et al., 2012; Noever et al., 2016). There have been several studies to date on the nervous system of cirriped nauplii (Walley, 1969; Semmler et al., 2008; Ponomarenko, 2014) and cypris larvae (Gallus et al., 2005, 2009, 2012; Harrison & Sandeman, 1999). Among the sensory organs found in the cypris and nauplius larvae of barnacles are the pair of frontal filaments studied by Kauri (1962, 1966), Walker (1974) and Obukhova et al. (2015, 2016). Frontal filaments are also found in various crustacean groups including Branchiopoda (Fritsch & Richter, 2010), Remipedia (Fanenbruck et al., 2004), Copepoda (Elofsson, 1971) and several Ostracoda (Andersson, 1977).

Fritsch et al. (2013) distinguished the filamentous external frontal filament from the underlying frontal filament organ. In crustaceans, there are different names for this latter organ (e.g., Bellonci organ, x-organ; see Fritsch et al., 2013) but the homology of these structures is generally accepted. Their function is still under debate, but Walker (1974) considered the frontal filaments to have either a mechanosensory or chemosensory function. The other major sensory organs are the nauplius eye, which becomes transformed and reorganized in the cypris stage into the ocelli of the adults (Takenaka et al., 1993), and the compound eyes, which only start to develop in the late nauplius, are fully developed in the cypris larva and become completely reduced during metamorphosis into the juvenile (Kauri, 1962; Walker & Lee, 1976). Other external sensory structures such as the lattice organs, sensory setae, fronto-lateral horn pores and various features of the antennules (Rybakov et al., 2003; Glenner & Høeg, 1995; Walker & Lee, 1976; Walker, 1985; Blomsterberg et al., 2004; Brickner & Høeg, 2010) allow *A. improvisus* larvae to settle on a wide variety of substrates (Lagerström et al., 2003).

The sessility of adult Thecostraca is associated with a drastic reduction of nervous structures compared with their own vagile larvae (Høeg et al., 2009). Although some studies focused on the adult nervous system have shown the changes that take place

in the proto- and tritocerebrum and their respective sensory organs and appendages (compound eyes and antenna) (Gwilliam & Cole, 1979; Webster, 1998; Callaway & Stuart, 1999), knowledge about the development of the nervous system in cirripeds is limited. It is still not clear how larval morphological features differentiate into the highly derived morphology of adults, and our understanding of the peripheral nervous system in cirripeds and crustaceans in general is particularly inadequate.

In the present study, we aimed to describe the development of the cirripedian nervous system in detail from early nauplius stages to the young adult. By combining immunohistochemical labeling with confocal laser scanning microscopy and 3D reconstruction, we are able to visualize the nervous system three-dimensionally. We provide insights into the development of the central nervous system in nauplius larvae, and into the changes associated with the metamorphoses into cypris larvae and later adults, whose nervous system is simultaneously reduced and modified. In addition, we describe the remarkable processes of reduction and interconnection that take place in the peripheral nervous system throughout larval development. This study is intended to complement earlier developmental studies on neuroanatomy in Malacostraca (Harzsch, 2003, Vilpoux et al., 2006; Ungerer et al., 2011), Branchiopoda (Fritsch & Richter, 2012; Fritsch et al., 2013; Frase & Richter, 2016), Cephalocarida (Stegner & Richter, 2011, 2015), Remipedia (Fanenbruck et al., 2004; von Reumont et al., 2012) and Oligostraca (Brenneis & Richter, 2010) and thereby further our understanding of crustacean evolution.

Material and methods

All larval stages of *A. improvisus* were sampled between early May and the end of June, 2016,

from the harbor in Rostock. Larval specimens were sampled using a plankton net of 100 μm mesh. We kept all larval stages of *A. improvisus* in a small aquarium filled with water at room temperature taken from the Warnow River near the sampling point. All stages were fixed in 4% paraformaldehyde (PFA, 16% stock solution, Electron Microscopy Sciences) solution in 0.1 M PBS (pH 7.4) at room temperature before being preserved in 100% methanol.

To determine species, adults from the quay wall (where these barnacles appear in high densities) and specimens that had developed from larvae into adults in the aquarium were identified using Luther (1987). In addition, the mitochondrial cytochrome C oxidase subunit I (COI) of 20 larvae each in the nauplius and cypris stages was sequenced using the “universal” DNA primers LCO 1490 and HCO 2198. DNA extraction was performed using the inuPREP Forensic Kit (Analytik Jena). All examined specimens proved to belong to the species *A. improvisus*.

Immunolabelling and mounting

Immunohistochemical labelling was performed as described by Fritsch & Richter (2010). Before antibody staining, larvae and adults were exposed to several short pulses in a bath ultrasonicator (Elmasonic One) to facilitate permeation. Further, specimens were washed several times in 0.1 mol PBT (phosphat buffered saline (PBS) with 0.3% Triton X-100, 1.5% dimethyl sulfoxid, 0.5% bovine serum albumin) and pre-incubated in PBT containing normal goat serum (NGS). The primary antibody monoclonal mouse anti-acetylated alpha-tubulin (clone 6 – 11 B-1, Sigma T6793, dilution 1:100) in PBT+NGS was applied for two days. Subsequently, specimens were rinsed in PBT and incubated with a secondary fluorochrome-conjugated antibody (goat anti-mouse Cy3, Jackson ImmunoResearch 155-165-003, dilution 1:200) in PBT+NGS for two days. Once antibody staining was complete, the

TABLE 1 Overview of examined specimens and stages of stained *Amphibalanus improvisus*

Stage	NI	NI I	NI II	NI III	NI IV	NI V	NI VI	Cypris	Juvenile
Scanned and analysed specimens	2	5	18	12	7	71	33	11	

specimens were incubated in SYTOX Green (Molecular Probes, S-7020, dilution 1:600 in 0.1 mol PBS) for 30–90 min to stain the cell nuclei. Finally, the specimens were washed several times in PBT and mounted in Rapi Clear 1.47 (SunJin Lab Co.). Additionally, before mounting, cypris larvae were “glued” onto cover slips in a dorso-ventral orientation using Mowiol 4-88 (Carl Roth GmbH + Co. KG).

By labelling the cytoskeletal protein alpha-tubulin, we were able to document the entire neuritic part of the nervous system.

Microscopy and 3D-reconstruction

Labelled specimens were analyzed using a Leica DMI6000 CFS microscope equipped with a Leica TCS SP5 II confocal laser scanning unit. Image stacks of optical sections were recorded at a step size of 0.3–2 μm . The resulting images were processed using the software IMARIS 7.0 (Bitplane, Switzerland). In some cases, the “contour surface” tool was used to artificially highlight specific structures, or to mask structures that obscured the view of the nervous system. All figure plates were edited in the graphics software CorelDRAW version 13 (Corel).

Identification of developmental stages

We separated the nauplius stages by examining cLSM-generated auto-fluorescence pictures of the cuticle and compared the larval features with various keys for stage determination (e.g., Arnsberg, 2001; Jones & Crisp, 1954; Lang, 1979; Conway, 2012; Semmler et al., 2009).

Orientation and terminology

The orientation and terminology used to describe nervous system structures is broadly

based on Richter et al. (2010) and specifically on Frase & Richter (2016). Table 1 gives an overview of the number of animals examined for each stage.

Results

Nauplius

From the earliest larval stage of *A. improvisus* the naupliar nervous system (i.e., the nervous system of the naupliar region) consists of a proto- (pc: fig. 1A), deuto- (dc: fig. 1A) and tritocerebrum (tc: fig. 1A), and a mandibular neuromere (md nm: fig. 1A) connected to the other parts via circumesophageal connectives. Lateral to the protocerebrum, the paired frontal filament nerves (ff n: fig. 1A) extend in an antero-lateral direction into the paired frontal filament organs (ff o: fig. 1C) before proceeding further into the paired frontal filaments (ff: fig. 1A, C). The three cups of the nauplius eye are present between the frontal filament organs. A primordial deutocerebrum is situated posterior to the protocerebrum, only detectable by the root of the pair of antennular nerves (an1 n: fig. 1A, B, C, D). Further posteriorly, the tritocerebral nerves extend into the paired antennae (an2 n: fig. 1A, B, C). The tritocerebral ganglia are connected via the prominent tritocerebral commissure (tc c: fig. 1A). The ventrally located labral commissure originates slightly anterior to these ganglia. Posterior to the labral commissure is the mandibular neuromere. The prominent mandibular nerves emerge bilaterally next to the mandibular ganglia (md n: fig. 1A, B, C), which are connected by the mandibular commissure (md c: figs. 1A, 2D). Stage VI is the last nauplius stage and shows the highest degree

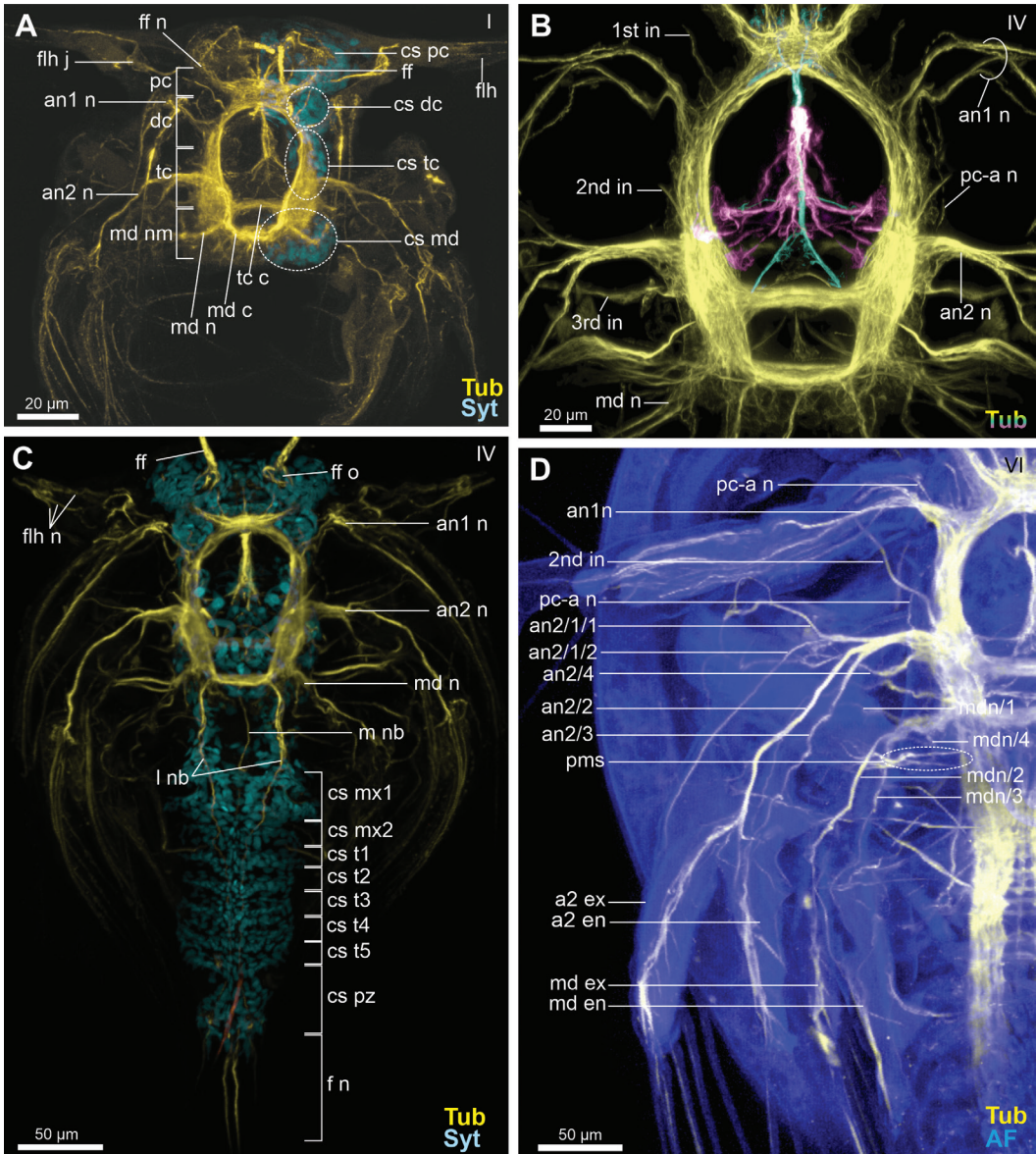


FIGURE 1 Nervous system of nauplius larval stages I, IV and VI of *Amphibalanus improvisus*, showing syncerebrum, ventral nerve cord and appendages; with alpha-tubulin labeled in yellow, magenta and green, DNA in cyan, and autofluorescence of the cuticle in blue. A: Overview of nervous system of nauplius stage I, ventral view, showing main distribution of anatomically left cell somata (within dotted circles and ovals). B: Ventral view close-up of nervous system of nauplius stage IV, with origin and pathway of inferior ventricular nerve highlighted in green, stomatogastric nerve and associated structures in magenta. C: Overview of nervous system of nauplius stage IV, ventral view. D: Ventral view close-up of innervation pattern of antennules, antennae and mandibles in nauplius stage VI, with dotted ellipses demarcating innervation of mastigatory spines.

Abbreviations: a2 en, nerves of antennal endopod; a2 ex, nerves of antennal exopod; an1 n, antennular nerve; an2 n, antennal nerve; an2/..., branches of antennal nerve; cs dc, cell soma of deutocerebrum; cs md, cell soma of mandible; cs mx1, cell soma of maxillula; cs mx2, cell soma of maxilla; cs pc, cell soma of protocerebrum; cs pz, cell soma of proliferation zone; cs t, cell soma of thoracopods; cs tc, cell soma of tritocerebrum; dc, deutocerebrum; f n, furcal nerves; ff n, frontal filament nerve; ff o,

of complexity. The anlagen of the compound eyes appear in stage VI (ce: fig. 2C).

The postnaupliar nervous system (i.e., the nervous system of the postnaupliar region, pn ns: fig. 2A) is simple in stage I. The primordial ventral nerve cord (vnc) is formed by two longitudinal neurite bundles (l nb: figs. 1C, 2A), which are connected behind the mandibular commissure with the axons of two large terminal neurons (t n: fig. 2A) at the end of the trunk. Each of these pioneer neurons sends a single nerve to the tip of the furcal spines (furcal nerves, f n: figs. 1C, 2C, D, 8). A median neurite bundle (m nb: fig. 1C) originates at the mandibular commissure and proceeds posteriorly, ending between the terminal neurons at the base of the furcal spines.

During stages III to VI, the complexity of the ventral nerve cord increases.

The first commissures to develop in stage IV are those of the maxillular segment. The anterior commissure (mx1 a c: fig. 2D) connects the anterior branches of the maxillular nerves on each side, while the posterior commissure (mx1 p c: fig. 2D) connects the posterior branches (maxillula nerves mx1 n: fig. 2D).

Between stages IV and V there is significant progress in the development of the postnaupliar nervous system. Intersegmental nerves originate laterally from the paired longitudinal neurite bundles. The thoracopodal nerves that innervate the incipient thoracic appendage formed inside have their origin ventrally. Commissures develop along an anterior-posterior gradient. In later development, in addition to the terminal pioneer neurons present in stage I, two further pair of nerves

are present at the posterior end of the trunk (trunk nerves, tr n: fig. 2C).

The ventral nerve cord with all its corresponding appendage nerves is fully developed in stage VI (see figs. 2D, 8) and displays an orthogonal ladder-like structure. In early stage VI a commissure appears directly posterior to the mandibular commissure (posterior mandibular commissure, p md c: fig. 2D). The median neurite bundle (m nb: fig. 2D) interconnects the commissures from the anterior mandibular to the posterior commissure of the 6th thoracomere, but not the single commissure of the naupliar furcal spines (furcal commissure, f c: fig. 2D). The six paired thoracopods are innervated by one nerve for each thoracopodal anlage (thoracopodal nerve, tp n: fig. 2D), which bifurcates into the exopod and the endopod. More proximally, this nerve sends two projections in a dorsal direction, the anterior-most one proceeding into the respective anterior intersegmental nerve (6th–12th in: fig. 2D).

The differentiation of the peripheral nervous system starts in stage I with three pairs of intersegmental nerves. The 1st intersegmental nerve (1st in: figs. 1B, 2A) originates between the frontal filament nerve and the antennular nerve, proceeding antero-dorsally into the fronto-lateral horn junction (flh j: figs. 1A, 2B) before ending in the tip of the fronto-lateral horn (flh: figs. 1A, 8). The 2nd pair of intersegmental nerves (2nd in: figs. 1B, 2A, 8) emerges from the circumesophageal connectives slightly anterior to the antennular nerve and ultimately also enters the fronto-lateral horn. The 3rd intersegmental nerve (3rd in: figs. 1B, 2A)

FIGURE 1 frontal filament organ; ff, frontal filament; flh j, fronto-lateral horn junction; flh n, fronto-lateral horn nerves; flh, fronto-lateral horn; in, intersegmental nerves; l nb, longitudinal neurite bundle; m nb, median neurite bundle; md c, mandibular commissure; md en, nerves of mandibular endopod; md ex, nerves of mandibular exopod; md n, mandibular nerve; md nm, mandibular neuromere; mdn/., branches of mandibular nerve; pc, protocerebrum; pc-a n, protocerebrum-accompanying nerve; pms, premastigatory spines; pn ns, postnaupliar nervous system; tc c, tritocerebral commissure; tc, tritocerebrum.

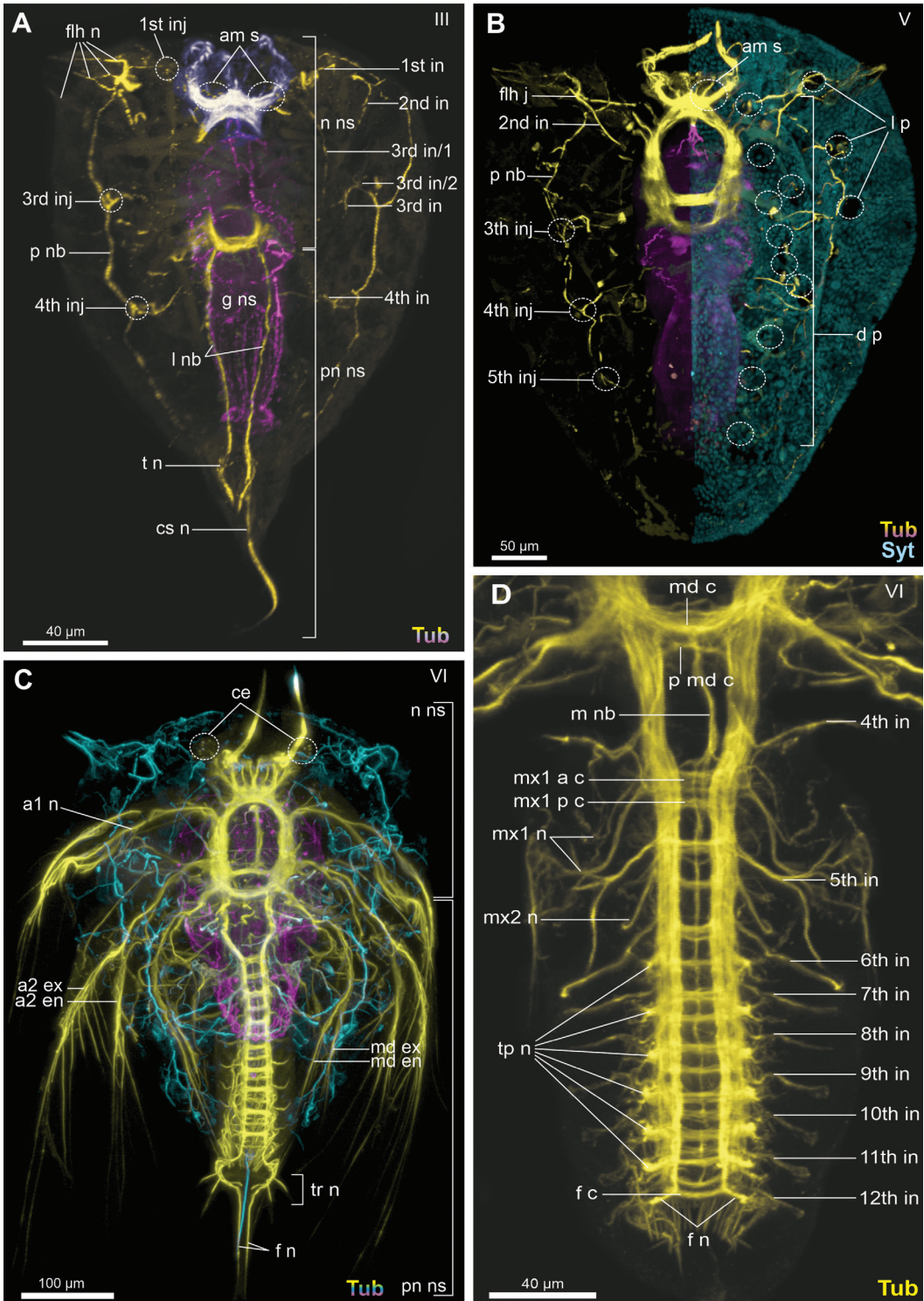


FIGURE 2 Nervous system of nauplius larval stages III-VI of *Amphibalanus improvisus*, showing ventral nerve cord and peripheral nervous system; with alpha-tubulin labeled in yellow, blue, green and magenta, and DNA in cyan. A: Overview of distribution of

originates between the antennal nerve and the nerve of the mandible. It proceeds in a dorso-lateral direction and bifurcates into two branches. The dorsal branch (3rd in/1: fig. 2A) ends in two paired antero-median setae (am s: fig. 2A, B) situated on the cephalic shield. The ventral branch (3rd in/2: fig. 2A) connects with a pair of lateral peripheral neurite bundles (p nb: figs. 2A, B, 3, 8), which in later stages connects all the intersegmental nerves from the 1st to the 5th with the exception of the 2nd. From stage III on, the peripheral neurite bundle is connected to the 1st, 3rd and 4th intersegmental nerves (1st, 3rd, 4th intersegmental junction, 1st, 3rd, 4th inj: figs. 2A, 3). The 2nd connection is formed by a laterally projecting neurite bundle that originates from the antero-median setae ("2nd Inj": fig. 3). From stage IV onwards a 5th intersegmental nerve (5th in: figs. 2D, 3) leaves the ventral nerve cord posterior to the maxillular commissures and connects to the peripheral neurite bundle. In stage VI, seven additional intersegmental nerves (6th in –12th in: fig. 2D) appear between the maxillary segmental nerve and the furcal commissure. The 5th intersegmental nerve (5th in: figs. 2D, 3), however, remains the most posterior to connect with the peripheral neurite bundle.

From stage IV onwards the protocerebrum-accompanying nerve (pc-a n: fig. 1B) originates near the base of the antennal nerve (an2 n: figs. 1B, 8) and bifurcates immediately. The anterior branch runs along the somata cluster of the deutocerebrum and bifurcates again. The antero-lateral branch joins the fronto-lateral horn junction, while the anterior branch laterally proceeds along the somata cluster of what will later be the compound eye and ends ventrally of the median eye cup of the nauplius eye. In stage VI, the protocerebrum-accompanying nerve (pc-a n: fig. 1D) runs parallel to the circumesophageal connectives into the nerve root of the antennules (an1 n: figs. 1D, 8).

Anteriorly, at the lateral margin of the cephalic shield, three pairs of lateral pores (1 p: fig. 2B) are recognizable. The dorso-median part of the cephalic shield bears ten pairs of dorsal pores (d p: fig. 2B). All pores are situated in nearly parallel longitudinal rows, each in a cuticular pit, and connect with the peripheral neurite bundle.

The paired peripheral neurite bundle forms a highly ramified and strongly interconnected network of nerves that pervade the whole cephalic shield (see figs. 2C, 3). Anterior to the first intersegmental junction (1st inj: fig. 3) the

FIGURE 2 peripheral nervous system of nauplius stage III, dorsal view, with protocerebrum, frontal filaments and nauplius eye region highlighted in blue, and gastric nervous system in magenta. B. Overview of distribution of peripheral nervous system of nauplius stage V, dorsal view, with stomatogastric nervous system highlighted in magenta; also showing cell somata in anatomical right side of cephalic shield. C: Overview of nervous system nauplius stage VI, ventral view, with peripheral nervous system highlighted in cyan and stomatogastric nervous system in magenta. D: Close-up in dorsal view of ventral nerve cord of nauplius stage VI.

Abbreviations: a2 en, nerves of antennal endopod; a2 ex, nerves of antennal exopod; am s, antero-median setae; an1 n, antennular nerve; ce, compound eye; cs n, caudal spine nerve; d p, dorsal pores; f c, furcal commissure; f n, furcal nerves; flh n, fronto-lateral horn nerves; g ns, gastric nervous system; in, intersegmental nerves; in/., branches of in; inj, intersegmental junctions; l nb, longitudinal neurite bundle; l sf, lateral pores; m nb, median neurite bundle; md c, mandibular commissure; md en, nerves of mandibular endopod; md ex, nerves of mandibular exopod; mx1 a c, maxillular anterior commissure; mx1 p c, maxillular posterior commissure; mx1 n, maxillular nerves; mx2 n, maxillary nerves; n ns, naupliar nervous system; p md c, posterior mandibular commissure; p nb, peripheral neurite bundle; pn ns, postnaupliar nervous system; t n, terminal neurons; tp n, thoracopodal nerves; tr n, trunk nerves.

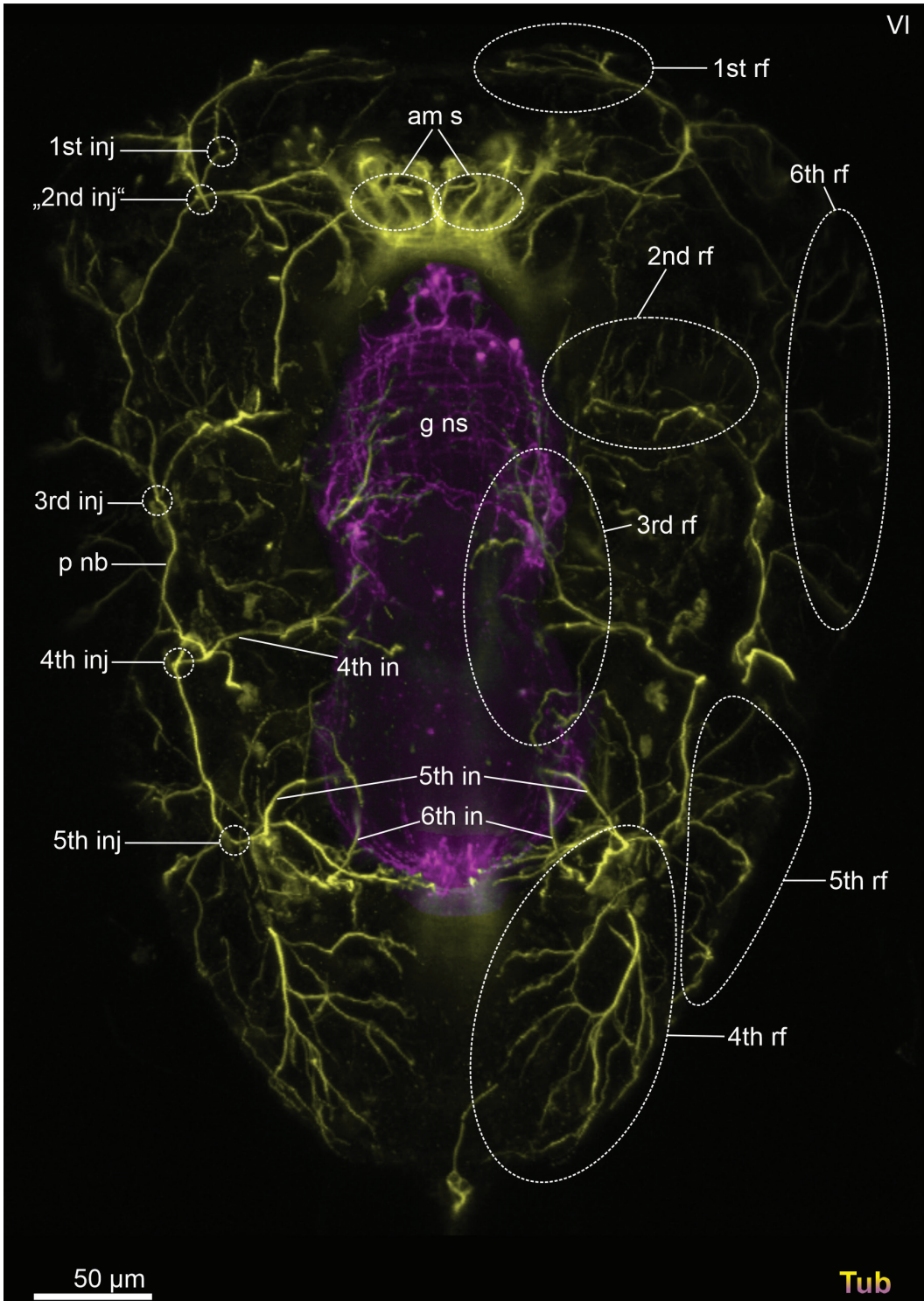


FIGURE 3 Nervous system of nauplius larval stage VI of *Amphibalanus improvisus*, showing peripheral nervous system, dorsal view, with stomatogastric nervous system highlighted in magenta; dotted ovals on anatomical right half demarcating the locations and borders of the different ramification fields.

anterior-most 1st ramification field (1st rf: figs. 3, 8) is identifiable. The 2nd ramification field (2nd rf: fig. 3) originates from the dorsal branch of the 3rd intersegmental nerve, while the median 3rd ramification field (3rd rf: fig. 3) originates from the 4th intersegmental junction (4th inj: fig. 3). Further posterior, the peripheral neurite bundle gives rise to the 4th ramification field (4th rf: figs. 3, 8) and the postero-lateral 5th ramification field (5th rf: fig. 3). More anteriorly, five small neurite bundles project from the peripheral neurite bundle (p nb: figs. 3, 8) to the lateral margin of the cephalic shield, forming the laterally arranged 6th ramification field (6th rf: fig. 3).

Cypris

In the cypris stage the protocerebrum (pc: fig. 4A) is larger and more complex than in the last naupliar stage. The structure of the cups of the nauplius eye has not changed (see fig. 4A). A globular proximal and a small distal compound eye neuropil are present laterally of the paired frontal filament nerves (vn: fig. 4A). The compound eyes themselves are located ventrally. The nerve of the antennule (an1 n: figs. 4D, 9) is now accompanied by several axons from a newly developed deutocerebral medio-lateral lobe (ml cs dc: fig. 4A). Directly posterior to the nerve root of the antennule, a second nerve root (secondary deutocerebral nerve) appears. It consists mainly of axons originating from somata of the deutocerebral medio-lateral lobe and gives rise to three nerves. The anterior-most (anterior secondary deutocerebral nerve: sec dc n/1: figs. 4D, 9) proceeds in a ventral direction and follows the antennular nerve. The second, thinner nerve (protocerebrum-accompanying nerve: pc-a n: figs. 4A, 9) projects in a dorsal direction, connects with the 1st intersegmen-

tal nerve (1st in: fig. 9) and runs along the protocerebrum anteriorly. The third, posterior-most nerve (posterior secondary deutocerebral nerve: sec dc n/2: figs. 4A, 9) proceeds in a posterior direction and apparently ends dorso-laterally to the circumesophageal connectives. The axons of the secondary deutocerebral cell cluster connect to the now more complex deutocerebral neuropil (dc np: fig. 4A) before proceeding into its anterior and posterior nerves. A small tract (asterisks in fig. 4A) from this neuropil runs into the protocerebrum without connecting with any prominent substructure there. The nerves of the antenna (an2 n: figs. 4D, 5, 9) are highly reduced and end blindly in the ventral trunk. The appendage itself has been lost entirely.

The ventral nerve cord (vnc: fig. 4B) is highly condensed in cypris larvae and is much more complex than in nauplius larvae. The more anterior ganglia of the nervous system are confluent. Although the nerves of the next two pairs of appendages, the maxillula (mx1 n: figs. 4C, 9) and maxilla (mx2 n: figs. 4C, 9), are located ventrally in the cephalic cavity (see fig. 4D), the respective ganglia are situated in the thoracic cavity. Two nerves (mx1 n, mx2 n) project from each hemiganglion in an anterior direction, one into the maxillula, the other into the maxilla. The six pairs of thoracopodal nerves (tp n1-6: fig. 4D) extend ventro-laterally from the ventral nerve cord and split before turning ventrally with each nerve sending two thin neurite bundles into the dorsal periphery. The furca (f: figs. 4B, D, 9) is innervated by at least four nerves coming from the posterior end of the ventral nerve cord and projecting into the furcal tips and setae.

As a result of numerous modifications that take place during this stage of development, including the downfolding of the cephalic

FIGURE 3 *Abbreviations:* am s, antero-median seta; g ns, gastric nervous system; in, intersegmental nerves; inj, intersegmental junctions; p nb, peripheral neurite bundle; rf, ramification field.

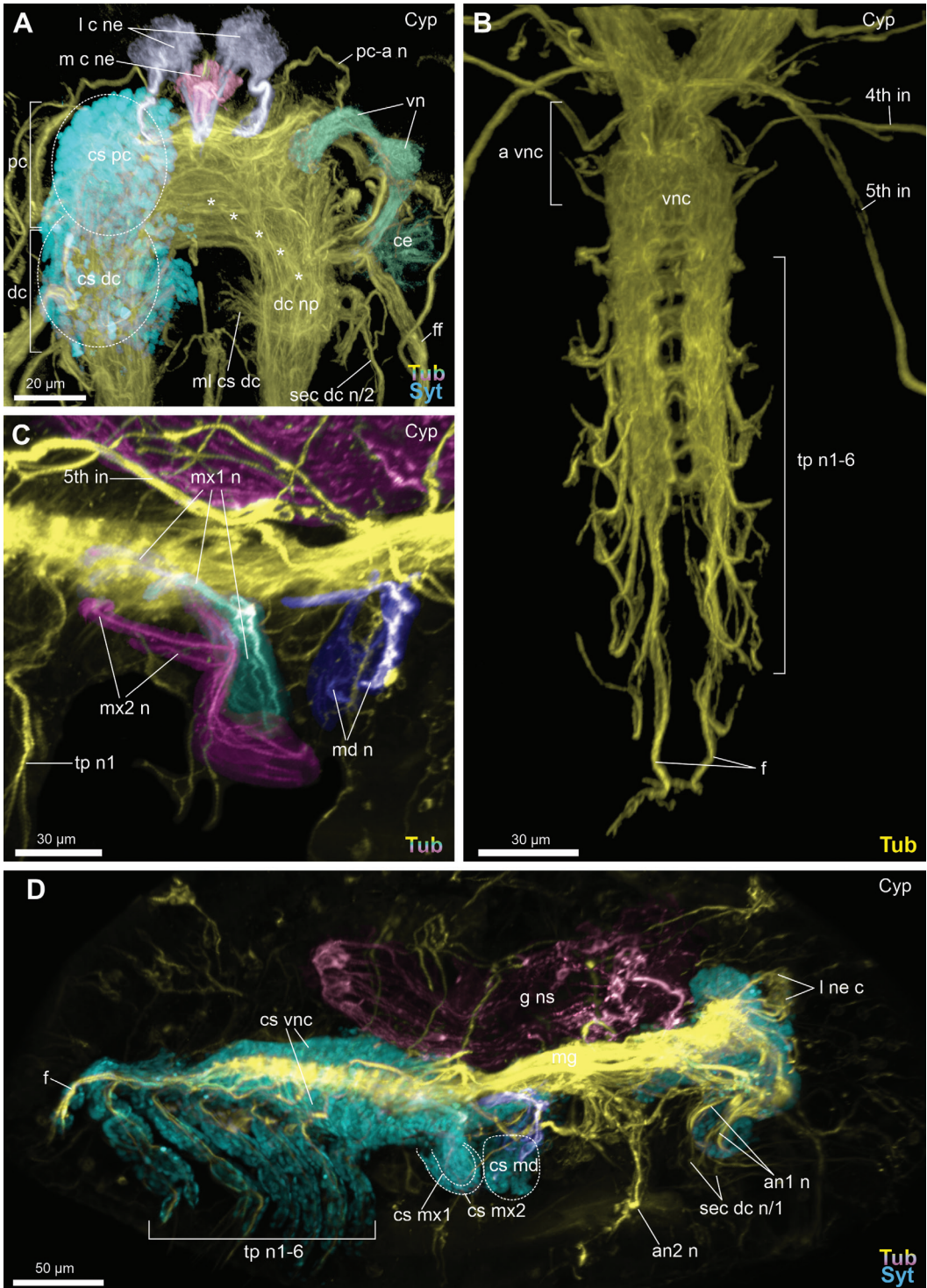


FIGURE 4 Nervous system of cypris larva of *Amphibalanus improvisus*, showing central nervous system; with alpha-tubulin labeled in yellow, blue, green and magenta, and DNA in cyan. A: Close-up in dorsal view of proto- and deutocerebral region, with lateral cups of nauplius eye highlighted in blue, median cup

shield into a bilateral carapace (because of the absence of a hinge, the carapace cannot be considered as bivalved) (cara: fig. 6B, C), the framework of the peripheral nervous system changes its position relative to that of the central nervous system during this stage of development. The most pronounced nerves of the peripheral nervous system are now the 4th (4th in: figs. 4B, 5, 9) and 5th intersegmental nerves (5th in: figs. 4B, 5, 9). Both follow a pattern comparable to that followed in the nauplius stages by the peripheral neurite bundle, which is no longer recognizable. The position of the ramification fields with respect to one another is not unlike that observed in stage VI nauplius larvae.

The 4th and 5th intersegmental nerves leave the condensed anterior portion of the ventral nerve cord (a vnc: fig. 4B). The 4th intersegmental nerve bifurcates laterally into an anterior (4th in/1: fig. 5) and posterior branch (4th in/2: fig. 5), from which most parts of the peripheral nervous system are derived.

The anterior branch projects in an antero-ventral direction, sending five nerves to the ventral margin of the bilateral carapace, where they ramify to form the 6th ramification field (6th rf: fig. 5). The anterior branch of the 4th intersegmental nerve is connected to the 3rd intersegmental nerve (3rd in: figs. 5, 9) via a

long nerve (4th in/1/2: fig. 5, in nauplius larvae 3rd in/1 and 3rd in/2, see fig. 2A), which proceeds in a dorsal direction close to the lateral margin of the midgut. This nerve gives rise to the 2nd ramification field (2nd rf: figs. 5, 9), as in stage VI nauplius larvae. Further anterior, dorsally to the 2nd intersegmental nerve (2nd in: figs. 5, 9), the dorsal projection of the anterior branch of the 4th intersegmental nerve (4th in/1/1/2: fig. 5) joins the 1st ramification field (1st rf: figs. 5, 9). The 2nd intersegmental nerve proceeds in an anterior direction and bifurcates before ramifying into the 1st ramification field at the level of the compound eye (ce: fig. 5). The ventral branch of the 2nd intersegmental nerve projects ventrally and proceeds along a pair of pits, previously the fronto-lateral horns (fronto-lateral horn pits, flh p: figs. 5, 6B). The 1st intersegmental nerve (1st in: fig. 9) joins the protocerebrum-accompanying nerve (pc-a n: figs. 4A, 9) and finally ramifies in the 1st ramification field.

The posterior branch of the 4th intersegmental nerve (4th in/2: fig. 5) proceeds laterally in a posterior direction. A neurite bundle (4th in/2/2: fig. 5) that projects dorsally from the 4th intersegmental nerve forms the 3rd ramification field (3rd rf: figs. 5, 9), as in stage VI nauplius larvae. Further posteriorly, the 4th

FIGURE 4 in magenta and compound-eye-related structures in green; with dotted circles demarcating principal areas of left half containing cell somata and asterisks (*) marking projection of route of a small tract. B: Close-up in dorsal view of ventral nerve cord. C: Close-up in lateral view of middle portion of the central nervous system, with anterior to right, mandible highlighted in blue, maxillula in green, and maxilla and gastric nervous system in magenta. D: Overview of the cypris larval nervous system in lateral view, with anterior right, mandible highlighted in blue, and stomatogastric nervous system in magenta, dotted lines demarcating the position of the cell soma of mandible, maxillula and maxilla. *Abbreviations:* a vnc, anterior portion of ventral nerve cord; an1 n, antennular nerve; an2 n, antennal nerve; ce, compound eye; cs dc, cell soma of deutocerebrum; cs mx1, cell soma of maxillula; cs mx2, cell soma of maxilla; cs pc, cell soma of protocerebrum; cs vnc, cell soma of ventral nerve cord; dc, deutocerebrum, dc np, deutocerebral neuropil; f, furca; ff, frontal filament; g ns, gastric nervous system; in, intersegmental nerves; l c ne, lateral cups of nauplius eye; m c ne, median cup of nauplius eye; md n, mandibular nerve; ml cs dc, axons of deutocerebral medio-lateral cell soma; mx1 n, maxillular nerves; mx2 n, maxillary nerves; pc, protocerebrum; pc-a n, protocerebrum-accompanying nerve; sec dc n/..., branches of secondary deutocerebral nerve; t n, terminal nerves; t nc, terminal neuronal cell; tp n, thoracopodal nerve; vn, visual neuropil.

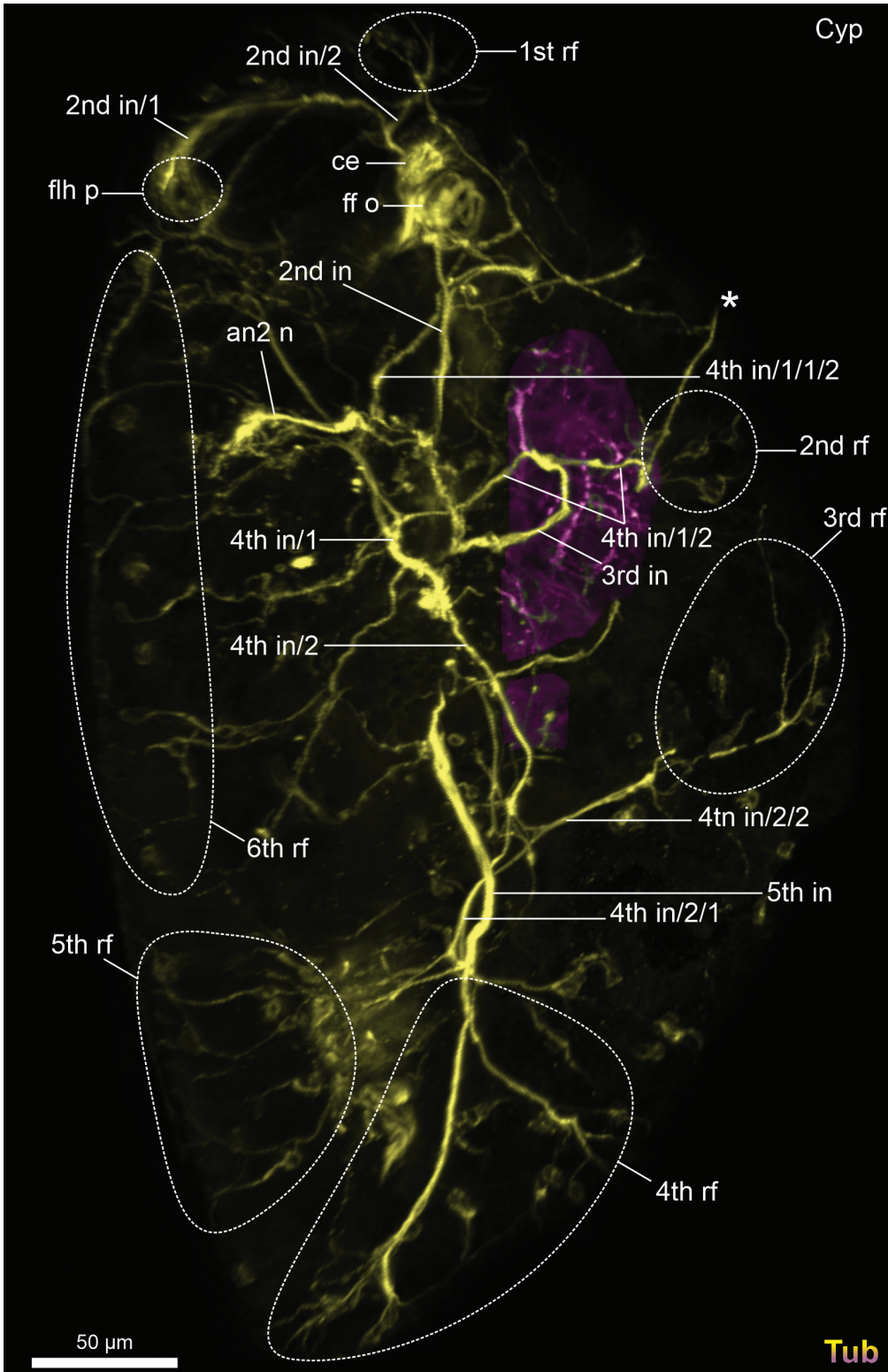


FIGURE 5 Nervous system of cypris larva of *Amphibalanus improvisus*, showing peripheral nervous system in lateral view, with anterior up and dorsal right, with dotted areas demarcating different ramification

intersegmental nerve (4th in/2/1: fig. 5) fuses with the 5th intersegmental nerve (5th in: fig. 5). In close proximity to this junction the ventral projections of both nerves form the 5th ramification field (5th rf: fig. 5) at the postero-ventral margin of the carapace. The 5th intersegmental nerve projects further in a posterior direction and ends at the posterior tip of the carapace by ramifying into several neurites, which form the 4th ramification field (4th rf: figs. 5, 9).

Late cypris

The visual neuropils and the frontal filament nerve start to degenerate. Directly before the cypris settles, the paired vascular frontal filament organs (ff o: fig. 6A) and the associated frontal filaments (ff: fig. 6A) are connected by a fine neurite bundle to the distal portion of the antennules (an1 n: fig. 6A, B). The reduced compound eyes (ce: fig. 6A) are situated in close proximity to the 1st ramification field (1st rf: fig. 6A). The protocerebrum starts to degenerate, too, while the median (m c ne: fig. 6A, 6C) and the two lateral cups (l c ne: fig. 6A) of the nauplius eye drift apart in an anterior direction. The cypris uses the adhesive discs (ad: fig. 6B) at the tips of the antennules to attach itself to the chosen substrate. The ventral nerve cord and associated structures bend 90° in a dorsal direction while the peripheral nervous system and the carapace remain in the original position (see fig. 6B). The six pairs of thoracopods become elongated and differentiate into the cirri. The protocerebrum-accompanying nerve (pc-a n: fig. 6A) has now joined the antennular nerve. It acts as a pioneer, guiding the 2nd intersegmental nerve and the posterior branch of the secondary deutocerebral nerve (sec dc n/2: fig. 6A) along

the pathway of the antennular nerve (an1 n: fig. 6A) in an anterior direction. At the distal ends of the nerves in the ramification fields, sensilla-like structures appear, later innervating the “cuticular hairs” (*sensu* Glenner & Høeg, 1993, cuticular hair nerves, ch n: fig. 6A).

Adult

As it settles, the juvenile barnacle turns from its ventral to its dorsal side as it lies on the substrate. Meanwhile, the cypris carapace (cara: fig. 6B, C) is shed.

Following this second metamorphosis into the juvenile barnacle, the protocerebrum (pc: fig. 7A) becomes highly reduced. Its anterior portion now consists of the rudimentary nauplius eye neuropil (ne np: fig. 7A). The two lateral nerves of the lateral cups of the nauplius eye (l c ne: fig. 10) enter the protocerebrum laterally. The posterior portion of the protocerebrum has a commissure-like appearance and connects to the deutocerebrum (dc: figs. 7A, C, 10) and the circumesophageal connectives. The deutocerebrum is the most prominent part of the adult syncerebrum. The antennular nerve (an1 n: figs. 7C, 10) projects in an anterior direction before describing a 180° curve in a dorsal direction and ramifying at the point of attachment. The posterior secondary deutocerebral nerve (sec dc n/2: figs. 7C, 10) is now part of the peripheral nervous system. The tritocerebrum is fully reduced with no sensory input: no remnants of the antennal nerve remain. The circumesophageal connectives connect the proto- and deutocerebrum with the highly condensed anterior portion of the ventral nerve cord.

The ventral nerve cord consists of an anteriorly strongly condensed portion (a vnc: fig. 7B) comprising the neuromeres of the mandibles,

FIGURE 5 fields and gastric nervous system highlighted in magenta. The asterisk marks the nerve of the former antero-median seta

Abbreviations: am s, antero-median seta; an2 n, antennal nerve; ce, compound eye; ff o, frontal filament organ; flh p, fronto-lateral horn pits; in, intersegmental nerves; in/., branches of in; rf, ramification field.

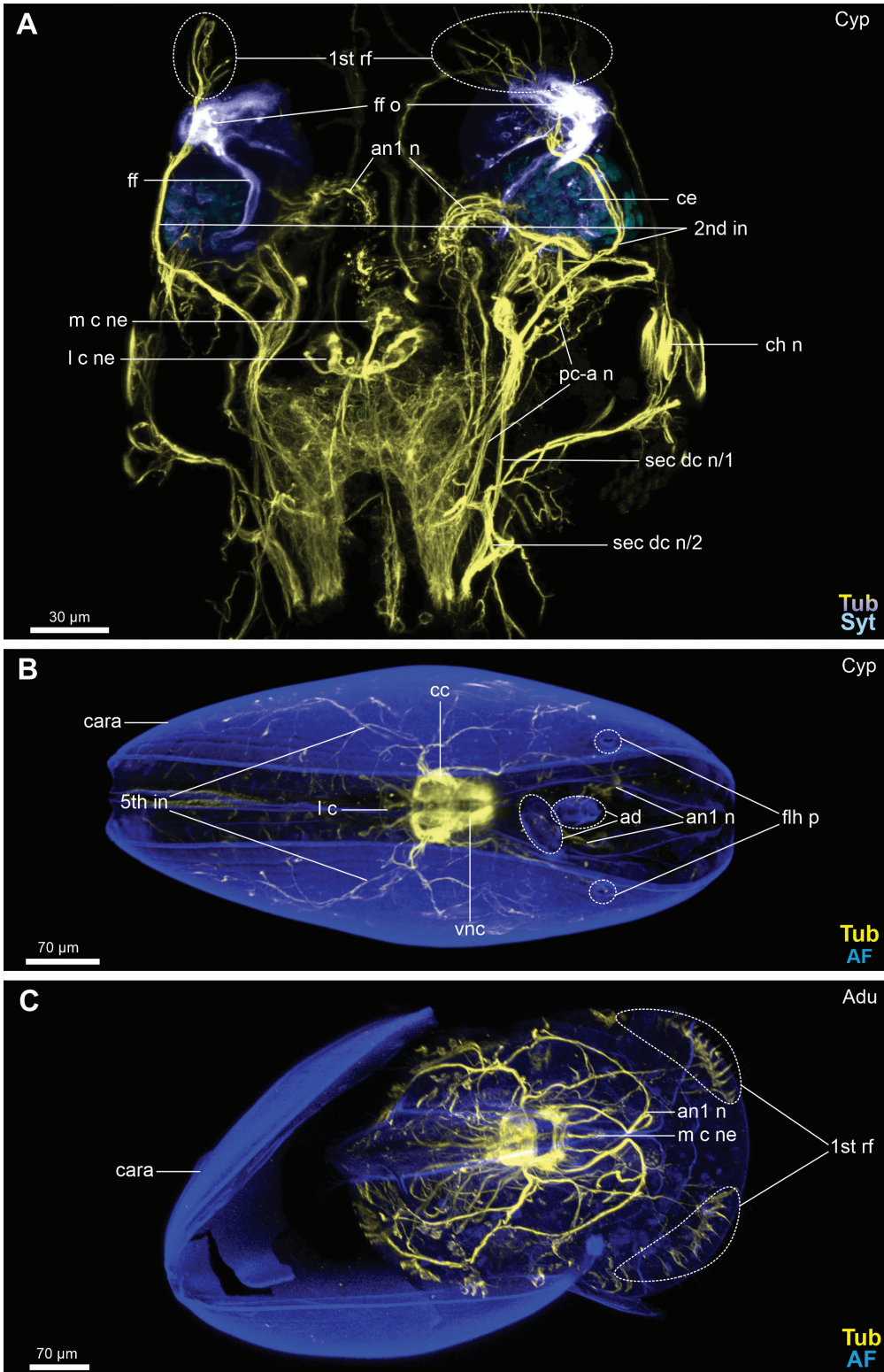


FIGURE 6 Nervous system of late cypris stage and juvenile settled barnacle of *Amphibalanus improvisus* during the second metamorphosis, with alpha-tubulin labeled in yellow and blue, DNA in cyan, Downloaded from Brill.com11/03/2020 03:23:21AM via free access

maxillulae and maxillae, and a posteriorly less condensed portion consisting of the neuromeres of the six pairs of thoracopods. The mandibular nerve (md n: fig. 7B) leaves the ventral nerve cord directly posterior to the labral commissure. It proceeds in a postero-ventral direction, sending two thin lateral neurites into the 4th intersegmental nerve (4th in: figs. 7B, 10). The mandibular nerve branches several times, with the median branch ultimately innervating the mandible and a ventro-lateral branch projecting into two sensilla. The maxillular nerve (mx1 n: fig. 7B) proceeds in a postero-ventral direction, ramifying into several neurites. The maxillary nerve (mx2 n: fig. 7B) emerges more postero-medially and exhibits the same branching pattern.

In the posterior, less condensed part of the ventral nerve cord, commissures interconnect the hemiganglia. All thoracopodal nerves bifurcate distally and several thin nerves arise, innervating the distal setae of the cirri. The furca is now reduced, as are the associated commissure and the 12th intersegmental nerve.

During metamorphosis from cypris to adult, the barnacle turns onto its back as it lies on the substrate. The peripheral nervous system undergoes several modifications as the metamorphosing barnacles turns onto its back, but the relative orientation of the nervous structures to each other remains fairly constant. The 2nd intersegmental nerve (2nd in: fig. 7C,

10) leads into the 1st ramification field (1st rf: figs. 7C, 10). The posterior secondary deutocerebral nerve (sec dc n/2: figs. 7C, 10) proceeds in a postero-lateral direction through the whole mantle. It ramifies into the 2nd ramification field (2nd rf: figs. 7C, 10) before joining the dorsal branch of the 5th intersegmental nerve. The 4th intersegmental nerve (4th in/1/1/2: fig. 7C) joins the 2nd intersegmental nerve and projects into the 1st ramification field. No remnants of the fronto-lateral horn pits are left. Ventral branches (4th in/1/1/1 and 4th in/1/1/2: fig. 7C) of the 4th intersegmental nerve ramify into the 6th ramification field (6th rf: fig. 7C). The dorsal neurite bundle (4th in/1/2), which previously connected the 4th intersegmental nerve with the 2nd ramification field, is now fully reduced. The 5th intersegmental nerve trifurcates while proceeding in a posterior direction. The dorsal branch forms the 3rd ramification field (3rd rf: figs. 7C, 10), while the median branch forms the 4th ramification field (4th rf: figs. 7C, 10) and the ventral branch the 5th ramification field (5th rf: fig. 7C). Each ramification field innervates several “cuticular hairs” (ch: fig. 7C) at the margin of the mantle, which in turn is attached to the substrate. All six remaining intersegmental nerves (the 6th to 11th intersegmental nerves, 6th-11th in: fig. 7B) are interconnected by a pair of inner (i l nb: figs. 7B, 10) and outer lateral neurite bundles (o l nb: figs. 7B, 10).

FIGURE 6 autofluorescence of the cuticle in blue, and ommatidia of the compound eyes in blue. A: Close-up in dorsal view of late developmental stage of cypris larva, showing the degenerated proto- and deutocerebrum and the re-oriented nauplius eye cups, and with degenerated frontal filaments, frontal filament organs and compound eyes highlighted in blue. B: Ventral view of a cypris during settlement, with anterior to right, showing adhesive discs at distal ends of antennules (dotted ovals), fronto-lateral horn pits (dotted circles) and 90° dorsal bend of ventral nerve cord (handstand). C: Just-settled juvenile barnacle in ventral view, with anterior to right, showing freshly shed carapace at posterior end and with dotted crescents demarcating 1st ramification field.

Abbreviations: ad, adhesive discs; an n, antennular nerve; cara, carapax; cc, circumesophageal connectives; ce, compound eye; ch n, cuticular hair nerves; ff o, frontal filament organ; ff, frontal filament; flh p, fronto-lateral horn pit; in, intersegmental nerves; l c, labral commissure; l c ne, lateral cups of nauplius eye; m c ne, median cup of nauplius eye; pc-a n, protocerebrum-accompanying nerve; rf, ramification field; sec dc n/..., branches of secondary deutocerebral nerve; vnc, ventral nerve cord.

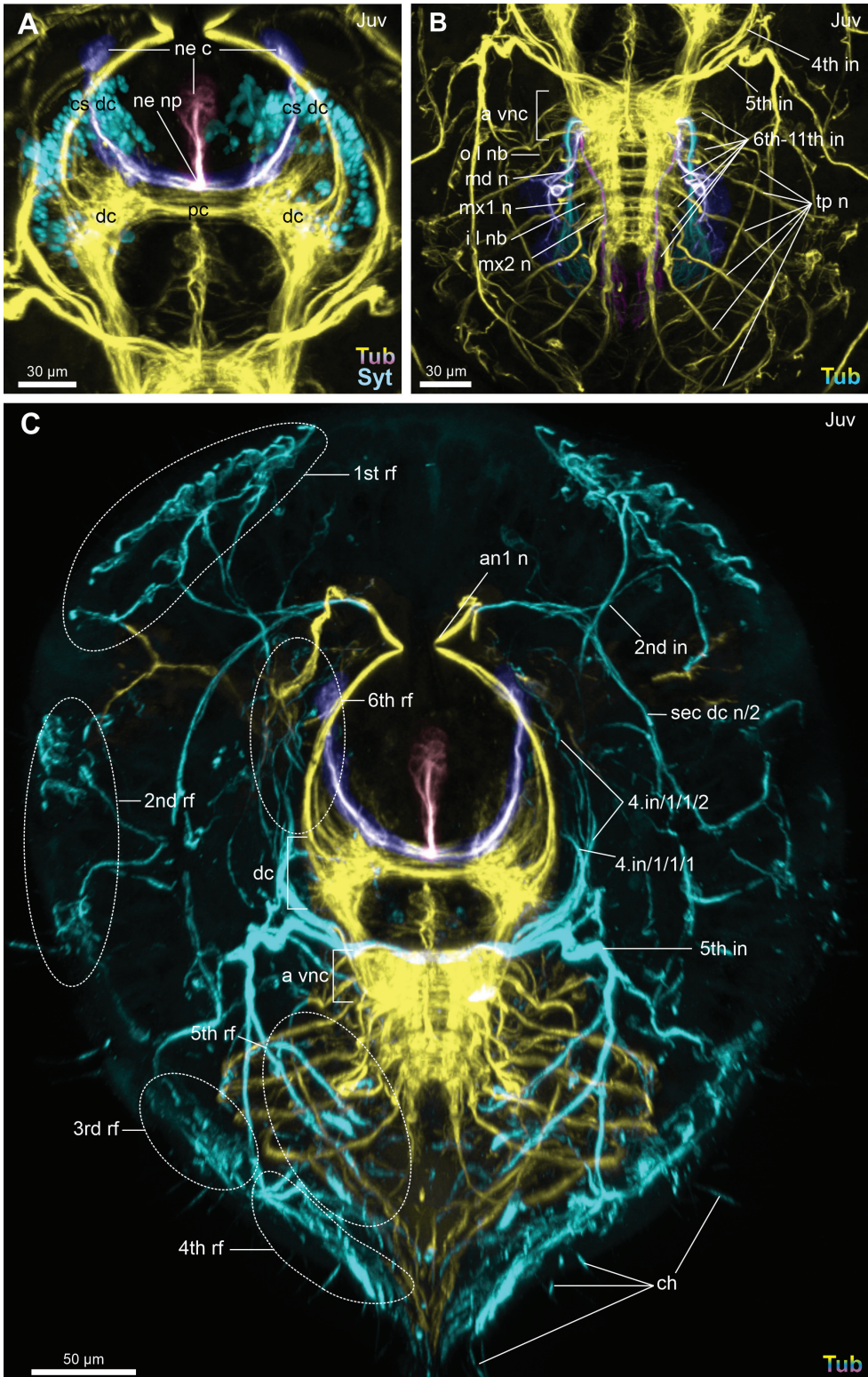


FIGURE 7 Nervous system of juvenile settled barnacle of *Amphibalanus improvisus*, with alpha-tubulin labeled in yellow, blue, cyan and magenta. A: Close-up in ventral view of cerebral region, with lateral cups of
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nauplius larva VI

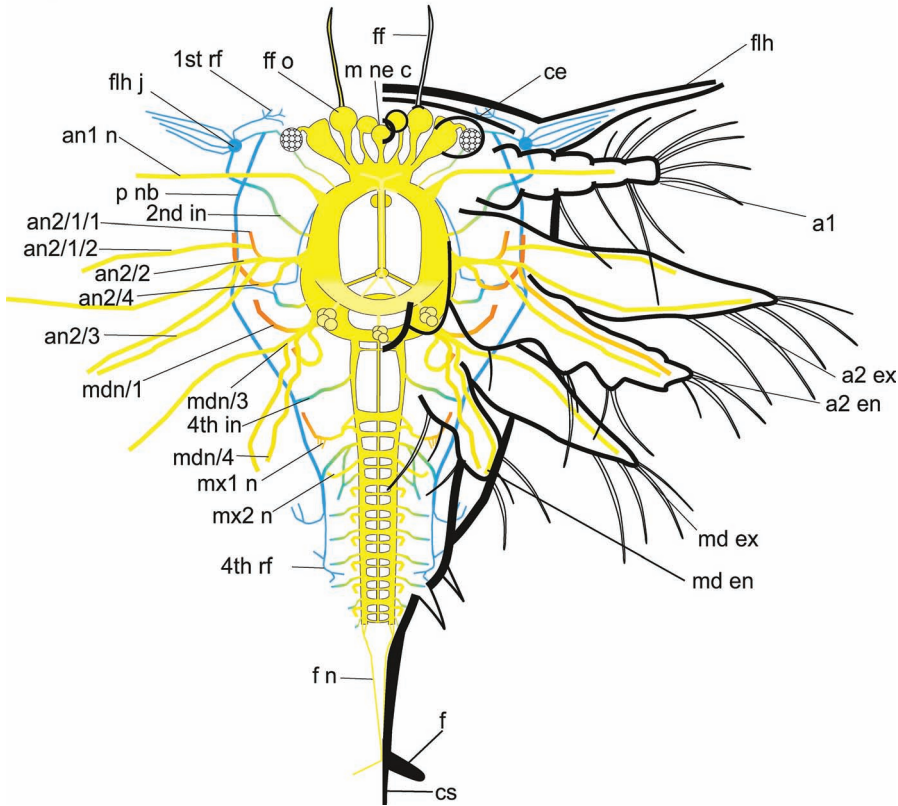


FIGURE 8 Schematic drawing of the nervous system of nauplius larval stage VI of *Amphibalanus improvisus* in ventral view, with the cuticle of anatomical right side of body omitted, showing an overview of the alpha-tubulin-immunoreactive parts of the nervous system. The central nervous system and nerves of the appendages are colored in yellow, shifting to orange in the dorsal direction and to bright yellow in the ventral direction. The coloring of the peripheral nervous system ranges from green proximally to blue distally.

Abbreviations: a1, antennule; a2 en, antennal endopod; a2 ex, antennal exopod; an1 n, antennular nerve; an2 n, antennal nerve; an2/..., branches of antennal nerve; ce, compound eye; cs, caudal spine; ff o, frontal filament organ; ff, frontal filament; flh, fronto-lateral horn; flh j, fronto-lateral horn junction; f n, furcal nerve; in, intersegmental nerves; m c ne, median cup of nauplius eye; md en, mandibular endopod; md ex, mandibular exopod; mdn/..., branches of mandibular nerve; mx1 n, maxillular nerves; mx2 n, maxillary nerves; p nb, peripheral neurite bundle; rf, ramification field; t n, terminal nerve.

FIGURE 7 nauplius eye highlighted in blue, median cup in magenta. B: Close-up in ventral view of ventral nerve cord, with mandibular nerves highlighted in blue, maxillular nerves in cyan and maxillary nerves in magenta. C: Overview of entire nervous system, ventral view, with median cup of nauplius eye highlighted in magenta, lateral cups in blue, and peripheral nervous system in cyan; with dotted circles demarcating areas innervated by different ramification fields on anatomical right side.

Abbreviations: a vnc, anterior portion of ventral nerve cord; an1 n, antennular nerve; ch, cuticular hairs; cs dc, cell soma deutocerebrum; dc, deutocerebrum; i l nb, inner lateral neurite bundle; in, intersegmental nerves; in/..., branches of in; md n, mandibular nerve; mx1 n, maxillular nerves; mx2 n, maxillary nerves; ne c, nauplius eye cups; ne np, nauplius eye neuropil; o l nb, outer lateral neurite bundle; pc, protocerebrum; rf, ramification field; sec dc n/..., branches of secondary deutocerebral nerve; tp n, thoracopodal nerve.

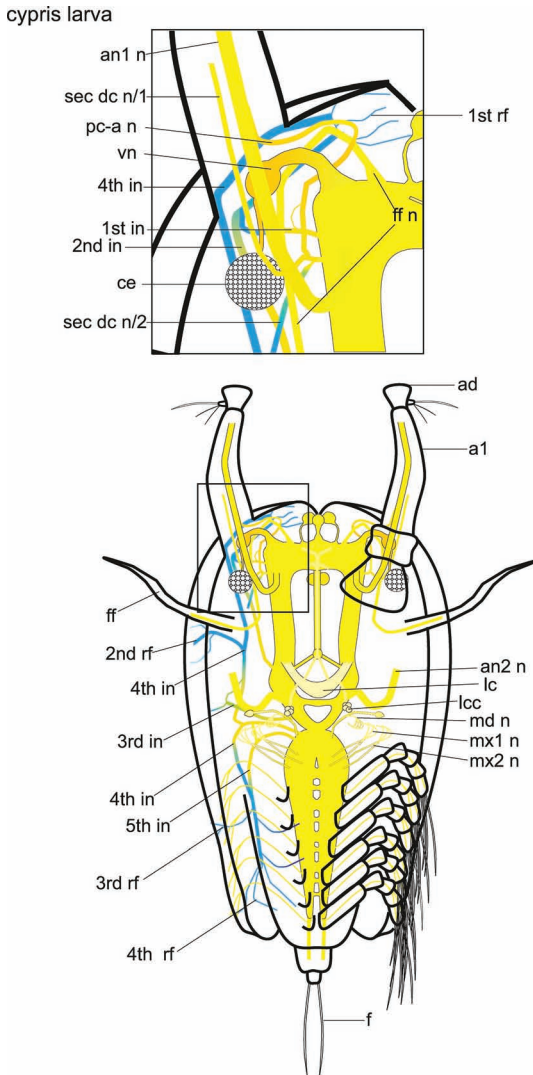


FIGURE 9 Schematic drawing of the nervous system of the cypris larva of *Amphibalanus improvisus* in ventral view, with incomplete indications of the anatomical right-side appendages, showing an overview of the alpha-tubulin-immunoreactive parts of the nervous system. The central nervous system and nerves of the appendages are colored in yellow, shifting to orange in the dorsal direction and to bright yellow in the ventral direction. The coloring of the peripheral nervous system (shown only for the anatomical right side) ranges from green proximally to blue distally.

Abbreviations: a1, antennule; ad, adhesive disks; an1 n, antennular nerve; cc, circumesophageal connectives; ce, compound eye; ff n, frontal filament nerve; ff, frontal filament; in, intersegmental nerves; lc, labral commissure; lcc, labral cell cluster; md n, mandibular nerves; mx1 n, maxillary nerves; mx2 n, maxillary nerves; pc-a n, protocerebrum-accompanying nerve; vn, visual neuropil; rf/., ramification field; sec dc n/., branches of secondary deutocerebral nerve; tp n, thoracopodal nerve; vn, visual neuropil.

Discussion

The primordial proto-, deuto- and tritocerebrum, the mandibular neuromere and the 1st–3rd intersegmental nerves are present in the nauplius larva of *A. improvisus* after hatching, all connected by circumesophageal connectives. This pattern corresponds to the early developmental stages of other crustaceans

(Vilpoux et al., 2006; Fritsch & Richter, 2010; Frase & Richter, 2016; Richter et al., 2016). The developing postnaupliar nervous system connects the terminal pioneer neurons with the mandibular commissure. The protocerebral sensory organs such as the nauplius eye and frontal filament organ are already differentiated in the early stages, while compound eyes develop in the late nauplius larvae. The

juvenile adult

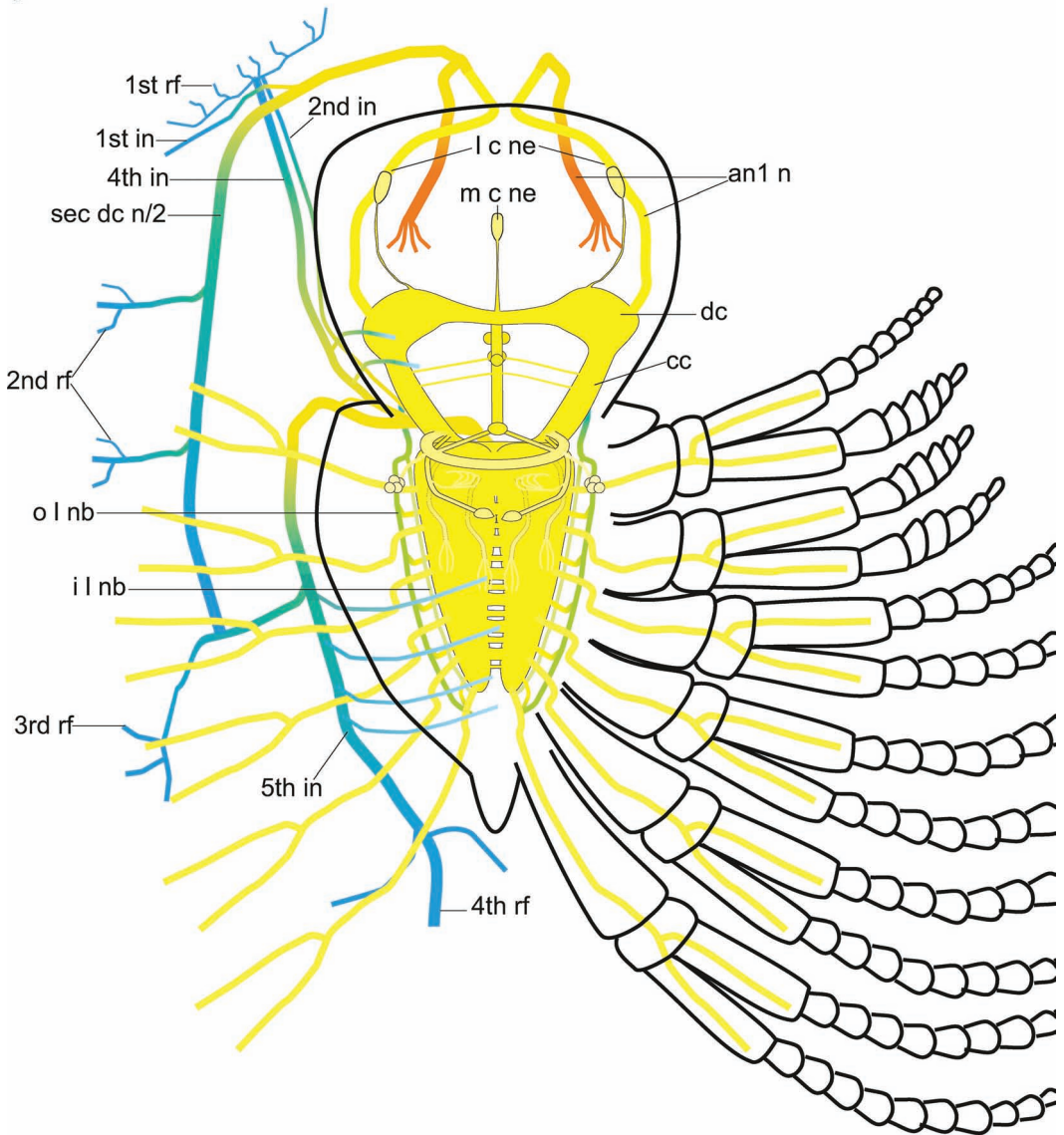


FIGURE 10 Schematic drawing of the nervous system of the settled juvenile barnacle stage of *Amphibalanus improvisus* in ventral view, with the cuticle of the anatomical right-side thoracic appendages omitted, showing an overview of the alpha-tubulin-immunoreactive parts of the nervous system. The central nervous system and nerves of the appendages are colored in yellow, shifting to orange in the dorsal direction and to bright yellow in the ventral direction. The coloring of the peripheral nervous system (shown only for the anatomical right side) ranges from green proximally to blue distally. **Abbreviations:** an1 n, antennular nerve; cc, circumesophageal connectives; dc, deutocerebrum; i l nb, inner lateral neurite bundle; in, intersegmental nerves; l c, labral commissure; l c ne, lateral cups of nauplius eye; m c ne, median cup of nauplius eye; o l nb, outer lateral neurite bundle; rf, ramification field; sec dc n/.., branches of secondary deutocerebral nerve.

development of the ventral nerve cord follows an anterior-posterior gradient. Between every two successive segments an intersegmental nerve is present, while a lateral longitudinal neurite bundle connects to the peripheral nervous system and innervates sensory structures. After the first metamorphosis, the syncerebrum of the cypris larva exhibits the highest degree of complexity of all the developmental stages. During the first metamorphosis the lateral sides of the cephalic shield fold down into a bilateral carapace, with consequent effects on the configuration of the peripheral nervous system. After the second metamorphosis, the cerebral structures become reduced while the ventral nerve cord and the peripheral nervous system increase in complexity. The peripheral nervous system undergoes another reorganization during the second metamorphosis.

The brain

The protocerebral architecture of the early nauplius larva is simple in *A. improvisus* (see also Semmler et al., 2008) and other balanomorphan cirripeds (Ponomarenko, 2014). The anterior portion of the protocerebrum mainly comprises the nauplius eye neuropil and the origin of the frontal filament nerves. Posteriorly, the protocerebrum is commissure-like in appearance. During naupliar development the protocerebrum enlarges, mainly as a result of the development of the compound eyes from stage V onwards. A very similar morphological pattern of early nervous system development is found in other crustacean larvae. For example, in Branchiopoda such as Anostraca (Frase & Richter, 2016; Harzsch & Glötzner, 2002), Notostraca (Fritsch & Richter, 2010) and Laevicaudata (Fritsch et al., 2013), and also in Cephalocarida (Stegner & Richter, 2015) and Decapoda (Vilpoux et al., 2006; Jirikowski et al., 2015), a syncerebrum comprising a proto-

deuto- and tritocerebrum is the first structure of the nervous system to be present.

After the first metamorphosis into a cypris larva, the protocerebrum of *A. improvisus* increases significantly in size, probably due to its function as a sensory integration structure (Gwilliam & Cole, 1979). It is associated with the nauplius eye, the compound eye and the frontal filaments (Walley, 1969) and is more complex in the cypris larva than in any other stage. However, the protocerebrum does not exhibit any components of a central complex, a cluster of highly structured midline neuropils consisting of an unpaired central body, an unpaired protocerebral bridge and paired lateral accessory lobes (Richter et al., 2010; Stegner et al., 2014). This agrees with the findings of studies conducted using TEM sections in the cypris of *Amphibalanus amphitrite* (formerly *Balanus*), which also failed to detect a central complex (Harrison & Sandeman, 1999) even though the cypris larvae of Cirripedia exhibit a wide range of complex behaviors during settlement and have nauplius and compound eyes (Harrison & Sandeman, 1999; Lagerström & Høeg, 2002).

Reduction processes in the late cypris and during the second metamorphosis cause the protocerebrum of adult *A. improvisus* to become vestigial (Gwilliam & Cole, 1979; Webster, 1998; Callaway & Stuart, 1999). Except for the nauplius eye, the sensory organs present in the cypris larva are now reduced, rendering the protocerebrum virtually redundant as a sensory integration structure (Gwilliam & Cole, 1979).

The deutocerebrum in the naupliar stages is relatively small compared to the protocerebrum and is only distinguishable through the antennular nerve root and a small cluster of cell somata surrounding it. In the cypris larva an additional cluster of cell somata located medio-laterally to the neuropil is present,

sending axons into the posterior portion of the deutocerebrum. These axons have numerous connections to the surrounding neuropil. The relative location of the additional deutocerebral cell cluster, its involvement in forming the now more complex deutocerebral neuropil and its projection of axons into the antennules correspond to the gross morphological arrangement of olfactory systems in malacostracans (Schachtner et al., 2005).

In the cypris larva of *A. amphitrite* a paired circular deutocerebral neuropil was described by Harrison & Sandeman (1999), which the authors consider a candidate for an olfactory lobe. We were unable to find this neuropil in the cypris larva of *A. improvisus*. The characteristic glomeruli found in the olfactory lobes in other crustaceans/tetraconates (see Schachtner et al., 2005) are absent in *A. improvisus* as well as in *A. amphitrite* (Harrison & Sandeman, 1999). Thus there is no morphological evidence of an olfactory lobe in the deutocerebrum of *A. improvisus*.

Nevertheless, the deutocerebrum is the only part of the syncerebrum that is not reduced during the second metamorphosis. The major reason seems to be the strong interconnection of the posterior secondary deutocerebral nerve (sec dc n/2) to the adult peripheral nervous system and its possible rich sensory input.

The antennal nerves of the tritocerebrum in cypris larvae are situated medially in the ventral portion of the larval trunk. Contradicting the observations of Harrison & Sandeman (1999) and Gallus et al. (2005) for the cypris larva of *A. amphitrite*, both the antennal nerve and the tritocerebrum are present in the cypris larva of *A. improvisus*. After the second metamorphosis there is no evidence of a tritocerebrum, which ties in with the complete reduction of the antennal nerve (Gwilliam & Cole, 1979).

The nervous system of the postnaupliar region

In early nauplius larvae the mandibular commissure connects to the ventral nerve cord, which consists of a pair of longitudinal neurite bundles and a median neurite bundle. Semmler et al. (2008) found the same pattern in the early naupliar stages of *A. improvisus*, as did Ponomarenko (2014) in *E. modestus*. The exact moment at which the mandibular commissure and the terminal cells begin to connect, as described for other crustacean species (Vilpoux et al., 2006; Fischer & Scholtz, 2010; Fritsch & Richter, 2012; Frase & Richter, 2016), however, remains unclear. The ventral nerve cord of stage VI nauplius larvae exhibits a classic rope-ladder-like appearance (*sensu* Richter et al., 2010) typical of crustaceans (Harzsch et al., 2012). Each of the six thoracic ganglia consists of two bilaterally arranged hemiganglia which are connected by an anterior and a posterior commissure, as in the early developmental stages of other crustaceans (Vilpoux et al., 2006; Brenneis & Richter, 2010; Fritsch & Richter, 2010; Stemme et al., 2013). However, the furcal commissure found in the stage VI nauplius of *A. improvisus* seems to exist only at this stage. Due to the condensation of the ventral nerve cord in the cypris larva we cannot determine with certainty when this commissure is reduced, but it is certainly no longer observable after settlement in juvenile bay barnacles.

In adults, the ventral nerve cord becomes even more condensed until it forms one large ganglion (Gwilliam & Cole, 1979; Webster, 1998; Callaway & Stuart, 1999). This condensed neuropil is the origin of the maxillary, maxillary and mandibular nerves and of the ventral branches of the 4th and 5th intersegmental nerves which proceed into the region of the two movable parts of the shell, the scutum and the tergum. The six pairs of cirral nerves also

originate from the less condensed posterior portion of the ventral nerve cord, which implies that this one structure is responsible for all motor control. Furthermore, the sensory input of the sensilla-like “cuticular hairs” of the mantle (see below) is received by the branches of the intersegmental nerves that proceed into the anterior portion of the ventral nerve cord (see also Gwilliam & Cole, 1979).

All in all, we can conclude that a shift takes place away from the protocerebrum and towards the ventral nerve cord as a center for sensory integration (Gwilliam & Cole, 1979) and motor control. This switch from the protocerebrum to a more posterior central nervous system structure for sensory integration, here the deutocerebrum, seems to be unique within crustaceans and must be explained by their adaptation to a sessile way of life.

The peripheral nervous system

Three intersegmental nerves which run between the proto- and deutocerebrum, the deutocerebrum, and the tritocerebrum, and the tritocerebrum and the mandibular neuromere respectively are present in stage I nauplius larvae (as also observed for *E. modestus* by Ponomarenko, 2014). During subsequent development additional intersegmental nerves originate from the ventral nerve cord from anterior to posterior until in naupliar stage VI a total of 12 pairs of intersegmental nerves are present, one pair arising between every two successive segments. In Mystacocarida, Remipedia, Anostraca and Notostraca (Brenneis & Richter, 2010; Fanenbruck et al., 2004; Frase & Richter, 2016; Fritsch & Richter, 2010) the first/anterior-most intersegmental nerve originates between the deutocerebrum, and the 1st intersegmental nerve present in *A. improvisus* is missing. In the cephalocaridan *Hutchinsoniella macracantha* and the copepods *Calanus finmarchicus* and *Pseudocalanus* sp., however,

intersegmental nerves are found between all portions of the syncerebrum (Stegner & Richter, 2011; Frase & Richter, 2020), making it plausible that this pattern is ancestral.

During larval development the 1st, 3rd, 4th and 5th intersegmental nerves are connected by a paired peripheral neurite bundle. In late naupliar larval development, several pores and four setae (probably with a mechano- and/or chemosensory function) are connected to the lateral peripheral neurite bundle. In the cypris larva, the peripheral nervous scaffold pervades the whole animal. The innervation of the external sensory structures of cypris larvae examined by Glenner & Høeg (1995) and Walker & Lee (1976), for example, is directly associated with this complex network of neurite bundles. It seems obvious to assume that the sensory structures of the cypris larva emerge from the antero-median setae and dorsal pores of nauplius stage VI, especially in the light of the transformation of the cephalic shield into a bilateral carapace. The likely derivation of the first two pairs of lattice organs in the cypris from these naupliar setae (Rybakov et al., 2003) suggests their innervation by the 3rd intersegmental nerves (fig. 2A: 3rd in/1). However, pores and setae are scattered all over the carapace of the cypris larva of *A. improvisus* (Jensen et al., 1994) and we are unable to determine conclusively which, if any, lattice organs or other sensory organs are innervated by the ramification fields revealed in our cLSM images. Nonetheless, the innervation pattern of the 4th ramification field covers the area of distribution of the 3rd – 5th pairs of lattice organs.

During the second metamorphosis the dorso-medial 2nd and 3rd ramification fields shift in a lateral direction. The positions of the ramification fields in young adults of *A. improvisus* correspond to those of the yet-to-develop calcified plates of the shell. The 1st

ramification fields correspond to the positions of the left and right halves of the unpaired anterior plate, the rostrum. The 2nd ramification fields correspond to the paired lateralialia, the 3rd ramification field to the paired carinolateralialia and the 4th ramification fields to the left and right halves of the unpaired posterior carina. The two ventral ramification fields (5 and 6) correspond to the movable shell parts, the scutum and tergum. These correspondences arise because each part of the mantle that produces the shell has “cuticular hairs” (*sensu* Glenner & Høeg, 1993), which are innervated by the ramification fields. The anlagen of these nerves are already observable in late cypris larvae (see cuticular hair nerve: ch n; fig. 6A). Glenner & Høeg (1993) hypothesized that one to two rows of “cuticular hairs” (see their fig. 2A-D) may, via muscular contraction, act as a restraining cord during the positioning of the mantle on the substrate. Due to the basal innervation of these “cuticular hairs” they might be better considered as sensilla with a potential mechanosensory function. The association between the cuticular hairs/sensilla and the deutocerebrum might be an indication of an additional chemosensory function.

Darwin (1854) suggested the terms great splanchnic nerve and suprasplanchnic nerve for the two largest peripheral nerves in adult barnacles, and other authors adopted them (Gwilliam & Cole, 1979; Webster, 1998; Callaway & Stuart, 1999). After comparing descriptions and drawings we conclude that the great splanchnic nerve corresponds to the 5th intersegmental nerve and the suprasplanchnic nerve to the posterior secondary deutocerebral nerve in *A. improvisus*.

The intersegmental nerves of the ventral nerve cord in adult bay barnacles are connected by a pair of inner and outer lateral neurite bundles. The outer lateral neurite bundle is connected to the median neurite bundle of

the ventral nerve cord (not shown). The inner lateral neurite bundle might correspond to the lateral longitudinal neurite bundle observed in mystacocaridans (Brenneis & Richter, 2010), which interconnects the intersegmental nerves of all the thoracic appendages without connecting to the median neurite bundle. In the branchiopods of the taxa Cyclotherida and Spinicaudata (Fritsch & Richter, 2012) an additional lateral neurite bundle is present, and in *Branchinella* sp. (Anostraca) two additional bundles are found (Frase & Richter, 2016). The additional lateral neurite bundles found in Cyclotherida, Spinicaudata and *Branchinella* sp. are potentially homologous to the lateral neurite bundles of *A. improvisus*, despite the lack of a median unpaired neurite bundle of the ventral nerve cord in all Branchiopoda (Frase & Richter, 2016) examined so far.

Metamorphosis

Indirect development with metamorphosis is common to most of the marine invertebrate taxa whose lifecycles include planktonic larvae and benthic adults (Jägersten, 1972; Gebauer et al., 2003; Wolfe, 2017). With regard to the nervous system, metamorphosis in crustaceans is less dramatic than in other invertebrate taxa (Richter et al., 2016).

There is agreement about defining metamorphosis as a sudden drastic change in morphology during postembryonic development which coincides with a change in habitat, lifestyle and mode of feeding or resource requirements (Bishop et al., 2006; Passano, 1961; Werner, 1988). These changes in morphology include: “(1) regression of embryonic and larval features, (2) transformation of larval into adult structures; and (3) de novo development of structures for the adult” (Fritsch, 1990, p. 1011). As the developmental transformations from the sixth nauplius stage

to the cypris larva and from the cypris larva to the sessile adult fulfill all the criteria, we postulate that the postembryonic development of *A. improvisus* is characterized by two true metamorphoses.

In the following, we list the morphological changes that occur with each metamorphosis in *A. improvisus*. Numbers correspond to the three major types of changes mentioned above (1-3, Fritzsche, 1990). Changes in habitat and lifestyle are listed under (4):

1st metamorphosis

- (1) Loss of the antennae and transformation of the antero-median setae into lattice organs.
- (2) Elongation and re-orientation of the frontal filaments, transformation of the cephalic shield into a bilateral carapace, of the fronto-lateral horns into fronto-lateral horn pits, of six pairs of non-functional anlagen of thoracic appendages into swimming legs, of the natatory/masticatory mandible into an "non-functional" mandible, of the ladder-like ventral nerve cord into a highly condensed ventral nerve cord.
- (3) Development of compound eyes, adhesive discs on antennules, and an additional deutocerebral lobe with two associated nerves.
- (4) Change from pelagic to pelago-benthic substrate-associated, from feeding to non-feeding.

2nd metamorphosis

- (1) Reduction of the antennal nerve, proto- and tritocerebrum, compound eyes, frontal filaments and frontal filament organs.
- (2) Transformation of the carapace into the mantle, of the swimming legs into feeding cirri, of a nauplius eye with three connected cups into a nauplius eye with three separate cups.

- (3) Development of a complex neuropil representing the proto- and deutocerebrum and the highly condensed anterior portion of the ventral nerve cord, development of outer and inner lateral neurite bundles (see fig. 12).
- (4) Change from pelago-benthic substrate-associated to sessile on substrate, from non-feeding to feeding.

Authors including Høeg & Møller (2006) and Pechenik et al. (1993) have also proposed that on the grounds of the sudden change in swimming behavior, the molt between the last nauplius stage and the cypris larva is a true metamorphosis. We agree with the observation of Høeg & Møller (2006) that the change in internal morphology in the first metamorphosis is more gradual than that in the external morphology and were able to show that the same applies to the second moult (see fig. 6). Changes in the internal structures, e.g., the development of the anlagen of the compound eye in late nauplius larvae or the reduction of the protocerebrum and the degeneration of the frontal filaments and compound eyes in late cypris larvae begin earlier than the subsequent molt with its drastic and sudden changes in external morphology. Basal Thecostraca such as some ascothoracidans only exhibit minor changes in morphology from the vagile cypris larva to the sessile adult, which possibly reflects the condition in the ur-cirriped (Høeg et al., 2015), which is thought to have resembled a cypris larva and not undergone subsequent metamorphosis.

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References

- Andersson, A. (1977) The organ of Bellonci in ostracodes: An ultrastructural study of the rod-shaped, or frontal, organ. *Acta Zool.*, 58, 197–204.
- Arnsberg, A. (2001) Arthropoda, Cirripedia: The barnacles. In: A. Shanks (Ed) *An Identification Guide to the Larval Marine Invertebrates of the Pacific Northwest*, pp.155–175. Oregon State University Press, Corvallis, Oregon.
- Bishop, C.D., Huggett, M.J., Heyland, A., Hodin, J. & Brandhorst, B.P. (2006) Interspecific variation in metamorphic competence in marine invertebrates: The significance for comparative investigations into the timing of metamorphosis. *Integr. Comp. Biol.*, 46, 662–682.
- Blomsterberg, M., Høeg, J.T., Jeffries, W.B. & Lagersson, N.C. (2004) Antennular sensory organs in cyprids of Octolasmis and Lepas (Crustacea: Thecostraca: Cirripedia: Thoracica): A scanning electron microscopic study. *J. Morphol.*, 260, 141–153.
- Brenneis, G. & Richter, S. (2010) Architecture of the nervous system in Mystacocarida (Arthropoda, Crustacea) – an immunohistochemical study and 3D reconstruction. *J. Morphol.*, 189, 169–189.
- Brickner, I. & Høeg, J.T. (2010) Antennular specialization in cyprids of coral-associated barnacles. *J. Exp. Mar. Biol. Ecol.*, 392, 115–124.
- Callaway, J.C. & Stuart, A.E. (1999) The distribution of histamine and serotonin in the barnacle's nervous system. *Microsc. Res. Tech.*, 104, 94–104.
- Conway, D.V.P. (2012) Marine zooplankton of southern Britain. Part 2: Arachnida, Pycnogonida, Cladocera, Facetotecta, Cirripedia and Copepoda. *Mar. Biol. Assoc. U. K. Occ. Publ.*, 26, 1–163.
- Darwin, C.R. (1854) *A Monograph on the Sub-class Cirripedia: With Figures of All the Species: The Balanidae (or Sessile Cirripedes); the Verrucidae, etc, etc, etc.* Ray Society, London.
- Dineen, J.F. & Hines, A.H. (1992) Interactive effects of salinity and adult extract upon settlement of the estuarine barnacle *Balanus improvisus* (Darwin, 1854). *J. Exp. Mar. Biol. Ecol.*, 156, 239–252.
- Doochin, H.D. (1951) Morphology of *Balanus improvisus* Darwin and *Balanus amphrite niveus* Darwin during initial attachment and metamorphosis. *Bull. Mar. Sci.*, 15–39.
- Elofsson, R. (1971) The ultrastructure of chemoreceptor organs in the head of copepod crustaceans. *Acta Zool.*, 52, 299–315.
- Fanenbruck, M., Harzsch, S. & Wägele, J.W. (2004) The brain of the Remipedia (Crustacea) and an alternative hypothesis on their phylogenetic relationships. *Proc. Natl. Acad. Sci. USA*, 101, 3868–3873.
- Fischer, A.H.L. & Scholtz, G. (2010) Axogenesis in the stomatopod crustacean *Gonodactylaceus falcatus* (Malacostraca). *Invertebr. Biol.*, 129, 59–76.
- Frase, T. & Richter, S. (2016) Nervous system development in the fairy shrimp *Branchinella* sp. (Crustacea: Branchiopoda: Anostraca): Insights into the development and evolution of the branchiopod brain and its sensory organs. *J. Morphol.*, 277, 1423–1446.
- Frase, T. & Richter, S. (2020) The brain and the corresponding sense organs in calanoid copepods – Evidence of vestiges of compound eyes. *Arthropod Struct. Dev.*, 54, 100902.
- Fritsch, M., Kaji, T., Olesen, J. & Richter, S. (2013) The development of the nervous system in *Laevicaudata* (Crustacea, Branchiopoda): Insights into the evolution and homologies of branchiopod limbs and “frontal organs”. *Zoomorphology*, 132, 163–181.
- Fritsch, M. & Richter, S. (2010) The formation of the nervous system during larval development in *Triops cancriformis* (Bosc) (Crustacea, Branchiopoda): An immunohistochemical survey. *J. Morphol.*, 271, 1457–1481.
- Fritsch, M. & Richter, S. (2012) Nervous system development in *Spinicaudata* and *Cyclestherida* (Crustacea, Branchiopoda) – comparing two

- different modes of indirect development by using an event pairing approach. *J. Morphol.*, 273, 672–695.
- Fritzsche, B. (1990) The evolution of metamorphosis in amphibians. *J. Neurobiol.*, 21, 1011–1021.
- Gallus, L., Ramoino, P., Faimali, M., Piazza, V., Maura, G., Marcoli, M., Ferrando, S., Giroi, L. & Tagliaferro, G. (2005) Presence and distribution of serotonin immunoreactivity in the cyprids of the barnacle *Balanus amphitrite*. *Eur. J. Histochem.*, 49, 341–348.
- Gallus, L., Ferrando, S., Bottaro, M., Diaspro, A., Giroi, L., Faimali, M., Ramoino, P. & Tagliaferro, G. (2009) Presence and distribution of FMRamide-like immunoreactivity in the cyprid of the barnacle *Balanus amphitrite* (Cirripedia, Crustacea). *Microsc. Res. Tech.*, 72, 101–109.
- Gallus, L., Ferrando, S., Gambardella, C., Amaroli, A., Faimali, M., Piazza, V. & Masini, M.A. (2012) G-protein alpha subunits distribution in the cyprid of *Balanus amphitrite* (=Amphibalanus amphitrite) (Cirripedia, Crustacea). *Microsc. Res. Tech.*, 75, 1613–1622.
- Gebauer, P., Paschke, K. & Anger, K. (2003) Delayed metamorphosis in decapod crustaceans: evidence and consequences. Retraso de la metamorfosis en crustáceos decápodos: evidencias y consecuencias. *Rev. Chil. Hist. Nat.*, 76, 169–175.
- Glenner, H. & Høeg, J.T. (1993) Scanning electron microscopy of metamorphosis in four species of barnacles (Cirripedia Thoracica Balanomorpha). *Mar. Biol.*, 117, 431–439.
- Glenner, H. & Høeg, J.T. (1995) Scanning electron microscopy of cyprid larvae of *Balanus amphitrite* (Cirripedia: Thoracica: Balanomorpha). *J. Crustac. Biol.*, 15, 523–536.
- Gwilliam, G.F. & Cole, E.S. (1979) The morphology of the central nervous system of the barnacle *Semibalanus cariosus* (Pallas). *J. Morphol.*, 159, 297–310.
- Harrison, P.J.H. & Sandeman, D.C. (1999) Morphology of the nervous system of the barnacle cypris larva (*Balanus amphitrite* Darwin) revealed by light and electron microscopy. *Biol. Bull.*, 197, 144–158.
- Harzsch, S. (2003) Ontogeny of the ventral nerve cord in malacostracan crustaceans: a common plan for neuronal development in Crustacea, Hexapoda and other Arthropoda? *Arthropod Struct. Dev.*, 32, 17–37.
- Harzsch, S. & Glötzner, J. (2002) An immunohistochemical study of structure and development of the nervous system in the brine shrimp *Artemia salina* Linnaeus, 1758 (Branchiopoda, Anostraca) with remarks on the evolution of the arthropod brain. *Arthropod Struct. Dev.*, 30, 251–270.
- Harzsch, S., Sandeman, D. & Chaigneau, J. (2012) Morphology and development of the central nervous system. In: J. Forest & J.C. von Vaupel Klein (Eds) *Treatise on Zoology – The Crustacea Vol. 3*, pp. 7–221. Brill Academic Publishers, Leiden, Boston.
- Høeg, J.T. (1987) The relation between cypris ultrastructure and metamorphosis in male and female *Sacculina carcini* (Crustacea, Cirripedia). *Zoomorphology*, 107, 299–311.
- Høeg, J.T., Deutsch, J., Chan, B.K.K. & Semmler Le, H. (2015) “Crustacea”: Cirripedia. In: A. Wanninger (Ed) *Evolutionary Developmental Biology of Invertebrates 4*, pp. 153–181. Springer, Vienna.
- Høeg, J.T. & Lützen, J. (1995) Life cycle and reproduction in the Cirripedia Rhizocephala. *Oceanogr. Mar. Biol. Annu. Rev.*, 33, 427–485.
- Høeg, J.T., Maruzzo, D., Okano, K., Glenner, H. & Chan B.K.K. (2012) Metamorphosis in balanomorph, pedunculated, and parasitic barnacles: A video-based analysis. *Integr. Comp. Biol.*, 52, 337–347.
- Høeg, J.T. & Møller, O.S. (2006) When similar beginnings lead to different ends: Constraints and diversity in cirripede larval development. *Invertebr. Reprod. Dev.*, 49, 125–142.
- Høeg, J.T., Perez-Losada, M., Glenner, H., Kolbasov, G. A. & Crandall, K. A. (2009) Evolution of morphology, ontogeny and life cycles within the Crustacea Thecostraca. *Arthropod Syst. Phylo.*, 67, 199–217.

- Jägersten, G. (1972) *Evolution of the Metazoan Life Cycle: A Comprehensive Theory*. Academic Press, London, New York.
- Jiríkowski, G., Wolff, C. & Richter, S. (2015) Evolution of eumalacostracan development – new insights into loss and reacquisition of larval stages revealed by heterochrony analysis. *EvoDevo*, 6, 1–30.
- Jensen P.G., Moyses, J., Høeg, J.T. & Al Yahya, H. 1994. Comparative SEM studies of lattice organs: putative sensory structures on the carapace of larvae from Ascothoracida and Cirripedia (Crustacea Maxillopoda Thecostraca). *Acta Zool.*, 75, 124–142.
- Jones, L.W.G. & Crisp, D.J. (1954) The larval stages of the barnacle *Balanus improvisus* Darwin. *Proc. Zool. Soc. London*, 123, 765–780.
- Kauri, T. (1962) On the frontal filaments and nauplius eye in *Balanus*. *Crustaceana*, 4, 131–142.
- Kauri, T. (1966) On the sensory papilla x organ in cirriped larvae. *Crustaceana*, 11, 115–122.
- Lagersson, N.C. & Høeg, J.T. (2002) Settlement behavior and antennular biomechanics and in cypris larvae of *Balanus amphitrite* (Crustacea: Thecostraca: Cirripedia). *Mar. Biol.*, 141, 513–526.
- Lagersson, N.C., Garm, A.L. & Høeg, J.T. (2003) Notes on the ultrastructure of the setae on the fourth antennular segment of the *Balanus amphitrite* cyprid (Crustacea: Cirripedia: Thoracica). *J. Mar. Biol. Assoc. U.K.*, 83, 361–365.
- Lang, W.H. (1979) Larval development of shallow water barnacles of the Carolinas (Cirripedia: Thoracica) with keys to the naupliar stages. *NOAA Tech. Rep. NMFS Circ.*, 421, 1–39.
- Luther, G. (1987) Seepocken der deutschen Küstengewässer. *Helgol. Meeresunters.*, 41, 1–43.
- Maruzzo, D., Aldred, N., Clare, A.S. & Høeg, J.T. (2012) Metamorphosis in the cirripede crustacean *Balanus amphitrite*. *PLoS ONE*, 7, 1–8.
- Noever, C., Keiler, J. & Glenner, H. (2016) First 3D reconstruction of the rhizocephalan root system using MicroCT. *J. Sea Res.*, 113, 58–64.
- Obukhova, A.L., Voronezhskaya, E.E. & Malakhov, V.V. (2015) The morphology of the frontal filaments in the nauplii of *Verruca stroemia* (Müller, 1776) and *Hesperibalanus hesperius* (Pilsbry, 1916) (Cirripedia: Thoracica). *Russ. J. Mar. Biol.*, 41, 363–371.
- Obukhova, A.L., Voronezhskaya, E.E. & Malakhov, V.V. (2016) Fine morphology of frontal filaments in nauplii of cirriped crustaceans. *Dokl. Biol. Sci.*, 468, 351–353.
- Passano, L.M. (1961) The regulation of crustacean metamorphosis. *Integr. Comp. Biol.*, 1, 89–95.
- Pechenik, J.A., Rittschof, D. & Schmidt, A.R. (1993) Influence of delayed metamorphosis on survival of juvenile barnacles *Balanus amphitrite*. *Mar. Biol.*, 294, 287–294.
- Ponomarenko, E.A. (2014) *The Embryonic Development of Elminius modestus Darwin, 1854 (Thecostraca: Cirripedia)*. Diploma-Thesis, Humboldt-Universität zu Berlin, Mathematisch-Naturwissenschaftliche Fakultät I.
- Richter, S., Loesel, R., Purschke, G., Schmidt-Rhaesa, A., Scholtz, G., Stach, T., Vogt, L., Wanninger, A., Brenneis, G., Döring, C., Faller, S., Fritsch, M., Grobe, P., Heuer, C.M., Kaul, S., Møller, O.S., Müller, C.H.G., Rieger, V., Rothe, B.H., Stegner, M.E.J. & Harzsch, S. (2010) Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. *Front. Zool.*, 7, 29.
- Richter, S., Stach, T. & Wanninger, A. (2016) Perspective – Nervous system development in bilaterian larvae: testing the concept of “primary larvae”. In: A. Schmidt-Rhaesa, S. Harzsch & G. Purschke (Eds) *Structure and Evolution of the Invertebrate Nervous Systems*, pp. 313–324. Oxford University Press.
- Rybakov, A.V., Høeg, J.T., Jensen, P.G. & Kolbasov, G.A. (2003) The chemoreceptive lattice organs in cypris larvae develop from naupliar setae (Thecostraca: Cirripedia, Ascothoracida and Facetotecta). *Zool. Anz.*, 242, 1–20.
- Schachtner, J., Schmidt, M. & Homberg, U. (2005) Organization and evolutionary trends of primary olfactory brain centers in Tetraconata (Crustacea+Hexapoda). *Arthropod Struct. Dev.*, 34, 257–299.

- Semmler, H., Høeg, J.T., Scholtz, G. & Wanninger, A. (2009) Three-dimensional reconstruction of the naupliar musculature and a scanning electron microscopy atlas of nauplius development of *Balanus improvisus* (Crustacea: Cirripedia: Thoracica). *Arthropod Struct. Dev.*, 38, 135–145.
- Semmler, H., Wanninger, A., Høeg, J.T. & Scholtz, G. (2008) Immunocytochemical studies on the naupliar nervous system of *Balanus improvisus* (Crustacea, Cirripedia, Thecostraca). *Arthropod Struct. Dev.*, 37, 383–395.
- Stegner, M.E.J. & Richter, S. (2011) Morphology of the brain in *Hutchinsoniella macracantha* (Cephalocarida, Crustacea). *Arthropod Struct. Dev.*, 40, 221–243.
- Stegner, M.E.J. & Richter, S. (2015) Development of the nervous system in Cephalocarida (Crustacea): early neuronal differentiation and successive patterning. *Zoomorphology*, 134, 183–209.
- Stegner, M.E.J., Fritsch, M. & Richter, S. (2014) The central complex in Crustacea. In: J.W. Wägele & T. Bartolomaeus (Eds) *Deep Metazoan Phylogeny: The Backbone of the Tree of Life*, pp. 361–384. De Gruyter, Berlin.
- Stemme, T., Iliffe, T.M., von Reumont, B.M., Koenemann, S., Harzsch, S. & Bicker, G. (2013) Serotonin-immunoreactive neurons in the ventral nerve cord of Remipedia (Crustacea): support for a sister group relationship of Remipedia and Hexapoda? *BMC Evol. Biol.*, 13, 119.
- Takenaka, M., Suzuki, A., Yamamoto, T., Yamamoto, M. & Yoshida, M. (1993) Remodeling of the nauplius eye into the adult ocelli during metamorphosis of the barnacle, *Balanus amphitrite hawaiiensis*. *Dev. Growth Differ.*, 35, 245–255.
- Ungerer, P., Geppert, M. & Wolff, C. (2011) Axogenesis in the central and peripheral nervous system of the amphipod crustacean *Orchestia cavimana*. *Integr. Zool.*, 6, 28–44.
- Vilpoux, K., Sandeman, R. & Harzsch, S. (2006) Early embryonic development of the central nervous system in the Australian crayfish and the marbled crayfish (Marmorkrebs). *Dev. Genes Evol.*, 216, 209–223.
- von Reumont, B.M., Jenner, R.A., Wills, M.A., Dell'Ampio, E., Pass, G., Ebersberger, I., Meyer, B., Koenemann, S., Iliffe, T.M., Stamatakis, A., Niehuis, O., Meusemann, K. & Misof, B. (2012) Pancrustacean phylogeny in the light of new phylogenomic data: support for Remipedia as the possible sister group of Hexapoda. *Mol. Biol. Evol.*, 29, 1031–1045.
- Walker, G. (1974) The fine structure of the frontal filament complex of barnacle larvae (Crustacea: Cirripedia). *Cell Tissue Res.*, 152, 449–465.
- Walker, G. (1985) The cypris larvae of *Sacculina carcini* Thompson (Crustacea: Cirripedia: Rhizocephala). *J. Exp. Mar. Biol. Ecol.*, 93, 131–145.
- Walker, G. & Lee, V.E. (1976) Surface structures and sense organs of the cypris larva of *Balanus balanoides* as seen by scanning and transmission electron microscopy. *J. Zool.*, 178, 161–172.
- Walley, L.J. (1969) Studies on the larval structure and metamorphosis of *Balanus balanoides* (L.). *Philos. Trans. R. Soc. B Biol. Sci.*, 256, 237–280.
- Webster, S.G. (1998) Peptidergic neurons in barnacles: An immunohistochemical study using antisera raised against crustacean neuropeptides. *Biol. Bull.*, 195, 282–289.
- Werner, E.E. (1988) Size, scaling, and the evolution of complex life cycles. In: B. Ebenman & L. Persson (Eds) *Size-Structured Populations: Ecology and Evolution*, pp. 60–81, Springer, Berlin, Heidelberg.
- Wolfe, J.M. (2017) Metamorphosis is ancestral for crown euarthropods, and evolved in the Cambrian or earlier. *Integr. Comp. Biol.*, 57, 499–509.

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