The nervous and visual systems of onychophorans and tardigrades: learning about arthropod evolution from their closest relatives

Christine Martin[1], Vladimir Gross[1], Lars Hering[1], Benjamin Tepper[1], Henry Jahn[1], Ivo de Sena Oliveira[1,2], Paul Anthony Stevenson[3], Georg Mayer[1]

✉   Georg Mayer

georg.mayer@uni-kassel.de

[1]Department of Zoology, University of Kassel, Heinrich-Plett-Str. 40, D-34132 Kassel, Germany

[2]Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, 31270-901 Belo Horizonte, Brazil.

[3]Physiology of Animals and Behaviour, Institute of Biology, University of Leipzig, Talstraße 33, D-04103 Leipzig, Germany

Abstract

Understanding the origin and evolution of arthropods requires examining their closest outgroups, the tardigrades (water bears) and onychophorans (velvet worms). Despite the rise of molecular techniques, the phylogenetic positions of tardigrades and onychophorans in the panarthropod tree (onychophorans + tardigrades + arthropods) remain unresolved. Hence, these methods alone are currently insufficient for clarifying the panarthropod topology. Therefore, the evolution of different morphological traits, such as one of the most intriguing features of panarthropods — their nervous system — becomes essential for shedding light on the origin and evolution of arthropods and their relatives within the Panarthropoda. In this review, we summarise current knowledge of the evolution of panarthropod nervous and visual systems. In particular, we focus on the evolution of segmental ganglia, the segmental identity of brain regions, and the visual system from morphological and developmental perspectives. In so doing, we address some of the many controversies surrounding these topics, such as the homology of the onychophoran eyes to those of arthropods as well as the segmentation of the tardigrade brain. Finally, we attempt to reconstruct the most likely state of these systems in the last common ancestors of arthropods and panarthropods based on what is currently known about tardigrades and onychophorans.

Background

Arthropods (chelicerates, myriapods, crustaceans and hexapods) comprise the most abundant and diverse animal group worldwide (Zhang 2011, 2013). Accordingly, they attract attention from countless researchers seeking to unlock the secrets behind their evolutionary success. The complex organisation of their bodies, with different levels of segmentation and tagmatisation, is considered to have greatly contributed to their successful speciation. However, despite concerted efforts from several researchers (e.g., Fortey and Thomas 1998; Minelli et al. 2013), many gaps remain in our understanding of their evolution. For example, it remains unclear how the arthropod nervous system, which reflects to a great extent the organisation of the body, arose and how such a complex system might have evolved over time. However, learning about evolution, irrespective of whether molecular or morphological, always requires comparative analyses (Harzsch 2006; Richter et al. 2010). It is, therefore, crucial to first have a closer look at the arthropods' closest relatives: the tardigrades (water bears) and onychophorans (velvet worms).

Arthropods, tardigrades and onychophorans have commonly been classified within a major group named Panarthropoda, which together with the Cycloneuralia comprises the Ecdysozoa (moulting animals; Fig. 1a–d). Nevertheless, relationships within each of these two major ecdysozoan clades remain unresolved. Several competing hypotheses have been suggested attempting to describe firstly the interrelationships among the three panarthropod groups (Fig. 1d) and second the phylogenetic placement of the remaining ecdysozoan lineages (Rota-Stabelli et al. 2013; Borner et al. 2014). The main hypotheses surrounding the panarthropod tree differ mainly in that they consider either the tardigrades as the sister group of a clade formed by onychophorans + arthropods (Rota-Stabelli et al. 2013) or the onychophorans as the sister group of tardigrades + arthropods (Fig. 1d; Campbell et al. 2011; Mayer et al. 2013a). Other, less-supported hypotheses (Fig. 1d) place tardigrades + onychophorans as the sister group to arthropods (Mallatt et al. 2004; Mallatt and Giribet 2006;

Rota-Stabelli et al. 2010) or even recover Panarthropoda as a paraphyletic group, with tardigrades falling within the Cycloneuralia (Dunn et al. 2008; Hejnol et al. 2009; Rehm et al. 2011). Since the paraphyly of Panarthropoda is presumably a phylogenetic artefact caused by long-branch attraction, this hypothesis has commonly been ruled out (Edgecombe 2010; Rota-Stabelli et al. 2010). Despite the controversies surrounding the topology of ecdysozoans and panarthropods, tardigrades and onychophorans still remain the best candidates for shedding light on the evolution of arthropods and their organ systems.

Tardigrades are microscopic invertebrates rarely, exceeding 1 mm in length that inhabit diverse aquatic and semi-terrestrial localities (Dewel et al. 1993; Kinchin 1994; Nielsen 2012; Nelson et al. 2015). To date, more than 1300 tardigrade species have been described and subdivided into two major subgroups: Eutardigrada and Heterotardigrada (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Degma et al. 2014). These animals have garnered interest due to their ability to tolerate extreme conditions, such as freezing (Hering et al. 2016; Tsuijimoto et al. 2016), osmotic stress (Heidemann et al. 2016), high radiation levels (Fernandez et al. 2016) and exposure to outer space (Jönsson 2001; Møbjerg et al. 2011; Rebecchi et al. 2011), yet the mechanisms behind this resistance are only beginning to be understood (Ramløv and Westh 2001; Hengherr et al. 2008; Schokraie et al. 2011; Halberg et al. 2013; Hashimoto et al. 2016). The body of tardigrades consists of only five segments: an anterior head with a subterminal or ventral mouth and four subsequent leg-bearing segments (Fig. 2a; Dewel et al. 1993; Nielsen 2012; Smith et al. 2016). As opposed to arthropods, tardigrades possess non-articulated, lobopodous appendages. Furthermore, due to their miniaturised body size, tardigrades might have lost several characters secondarily, namely those associated with respiratory and circulatory systems such as tracheae/gills or a heart, respectively (Dewel and Dewel 1997; Schmidt-Rhaesa 2001).

Onychophorans are soft-bodied, exclusively terrestrial invertebrates that inhabit tropical and temperate forests of the southern hemisphere (Brinck 1957; Allwood et al. 2010;

4

Ruhberg and Mayer 2013). Currently, the >200 onychophoran species described are subdivided into two major subgroups, which most likely diverged from each other more than 374 million years ago: the Peripatidae and the Peripatosidae (Mayer and Oliveira 2011, 2013; Oliveira et al. 2012, 2016; Murienne et al. 2014). The elongated onychophoran body consists of an anterior head with a ventral mouth, a multilegged trunk and a posterior anal cone (Fig. 3a; Ruhberg and Mayer 2013). The head comprises the three anterior-most body segments, each of which is equipped with a pair of specialised appendages: the antennae, the jaws, and the slime papillae, respectively (Mayer and Koch 2005; Mayer et al. 2010, 2015a; Ou et al. 2012). The latter are a unique feature of onychophorans and are used for ejecting a sticky slime to catch their prey and as a defence mechanism (Baer and Mayer 2012; Mayer et al. 2015a). The number of leg-bearing segments in onychophorans varies in different species between 13–43, and even intraspecific variation is known in many taxa (Oliveira et al. 2011; Oliveira and Mayer 2013). As in tardigrades, each trunk segment is equipped with a pair of non-articulated, lobopodous limbs used for walking. The posterior-most anal cone is the sole segment that lacks any appendages or their derivatives, although it is still considered a true segment based on the occurrence of embryonic coelomic cavities, nephridial derivatives and the expression of segmentation genes (Mayer and Koch 2005; Mayer et al. 2005; Franke and Mayer 2015).

The open question of the phylogenetic relationships within Panarthropoda underlines the importance of understanding the evolution of both tardigrades and onychophorans. Previous attempts to resolve this were based on a wide range of experimental approaches ranging from molecular phylogenetics to comparative morphology of living and fossil specimens (e.g., Poinar 2000; Hou et al. 2004; Strausfeld et al. 2006a, 2016; Mayer and Harzsch 2007, 2008; Zantke et al. 2008; Campbell et al. 2011; Legg et al. 2012; Ou et al. 2012; Persson et al. 2012, 2014; Mayer et al. 2013a, 2013b, 2015b; Rota-Stabelli et al. 2013; Cong et al. 2014; Smith and Ortega-Hernández 2014; Boothby et al. 2015; Arakawa 2016;

Bemm et al. 2016; Hashimoto et al. 2016; Hering et al. 2016; Koutsovoulos et al. 2016; Oliveira et al. 2016; Martin et al. 2017; Ortega-Hernández et al. in press). Nevertheless, even despite the recent surge in molecular phylogenetic analyses on many animal groups, thus far these methods have retrieved inconclusive results for clarifying the affinities among panarthropods. Hence understanding transformation of morphological characters becomes essential for elucidating the evolutionary history of these lineages (Harzsch et al. 2006; Richter et al. 2010; Hejnol and Lowe 2015).

In this respect, the central nervous system together with the sensory systems associated with it, such as the visual system, has been one of the most important morphological features for clarifying the evolution of many animal groups (Strausfeld et al. 1993; Harzsch et al. 2006; Homberg 2008; Loesel et al. 2013; Hejnol and Lowe 2015; Schmidt-Rhaesa et al. 2016). In arthropods, the central nervous system consists of an anterior brain composed of fused segmental neuromeres and segmental trunk ganglia, each of which can, in turn, show further fusions into functional units (Osorio et al. 1995; Loesel et al. 2013). While much is known about these ganglia regarding their structure and function (e.g., Pflüger and Stevenson 2005; Schmidt-Rhaesa 2007; Richter et al. 2010; Loesel et al. 2013), our understanding of their origin and evolution is rather vague. Again, this is partly due to the unclear panarthropod phylogeny and depends largely on which outgroup is most closely related to arthropods (Hejnol and Lowe 2015). On the other hand, recent approaches that integrate morphological and molecular techniques, such as evolutionary developmental biology (EvoDevo), have already offered new insights into previously highly controversial topics, such as clarifying the presence of a deutocerebrum in chelicerates (Damen et al. 1998; Telford and Thomas 1998), and promise to continue doing so. Therefore, combining comparative morphological and developmental analyses of anatomical features, like those of the nervous system, are likely to be pivotal for understanding the evolution of panarthropods and reconstructing their last common ancestor.

In this review, we summarise current knowledge of morphological and developmental characters of the tardigrade and onychophoran nervous systems. In doing so, we highlight outstanding issues surrounding panarthropod nervous systems and their evolution, including age-old controversies such as the segmental composition of the onychophoran and tardigrade brains. Additionally, we give a short overview of the visual system and its evolution in these animal groups, as it also plays an important role in our interpretation of the last common ancestor of panarthropods.

Ventral and peripheral nervous system in tardigrades

Despite their diminutive size (Fig. 2a), tardigrades have been studied since the 18th century (reviewed in Nelson et al. 2015), yet one of the earliest detailed descriptions of their nervous system was first published in 1840 (Doyère 1840). Since that time, further studies on the nervous system of tardigrades have been published (Greeff 1865; Basse 1905; Marcus 1929; Greven and Kuhlmann 1972; Dewel et al. 1993, 1999; Castano et al. 1996; Dewel and Dewel 1996; Wiederhöft and Greven 1999; Zantke et al. 2008; Persson et al. 2012, 2014; Mayer et al. 2013a, 2013b; Schulze et al. 2014; Smith and Jockusch 2014; Gross and Mayer 2015; Gross et al. 2015), but large gaps in knowledge still remain, partly due to methodological difficulties.

The tardigrade nervous system consists of an anterior dorsal brain and a subsequent chain of four segmentally arranged ventral trunk ganglia with minor variation in morphology (Fig. 2b–d), a situation also seen in arthropods (Harrison et al. 1995; Osorio et al. 1995; Harzsch 2004a). Furthermore, as in arthropods, the ganglia are shifted to an anterior position with respect to the legs of the corresponding segment (Deutsch 2004; Mayer et al. 2013a). This shift is less evident in the first trunk ganglion but becomes more pronounced in the subsequent three ganglia (Fig. 2d). The shifted condition, referred to as parasegmental in arthropods, represents the initial type of segmentation that is present during embryonic

7

development and differs from the segmental arrangement of metameric units in adult arthropods (Deutsch 2004; Damen 2007). The parasegmental arrangement in tardigrades is, so far, only indicated by position of the ganglia relative to the legs (Mayer et al. 2013a) and thus requires additional molecular investigations, such as expression patterns of the segment polarity genes *engrailed*, *Wnt/wingless*, *hedgehog* and *cubitus interruptus*, which are known to be expressed at the parasegmental boundaries in arthropods (Ingham and Martinez Arias 1992; Damen 2002; Simonnet et al. 2004; Carroll et al. 2005; Damen 2007), but show no evidence for parasegments in onychophorans (Franke and Mayer 2014).

Comparable to arthropods, the ganglia of tardigrades are connected to each other via a pair of somata-free connectives (Fig. 2c, d; Harrison et al. 1995; Osorio et al. 1995; Harzsch 2004a; Richter et al. 2010; Mayer et al. 2013a), whereas two pairs of connectives are present between the first trunk ganglion and the brain (inner and outer connectives in Fig. 2b, d; Mayer et al. 2013b; Gross and Mayer 2015). Each ventral ganglion consists of two hemiganglia, which are connected to each other via a commissural fibre mass (Fig. 2c), unlike arthropods, which exhibit two or more clearly distinguishable commissures (Whitington and Bacon 1997; Harzsch 2003; Mittmann and Scholtz 2003; Harzsch 2004a). In the first description of the tardigrade nervous system, Doyère (1840) also described the presence of two distinguishable commissures connecting the hemiganglia of one segment, but this was not evident in subsequent analyses using confocal and transmission electron microscopy in adults and embryos, which rather revealed individual fibres and bundles of neurites projecting contralaterally within the commissural fibre mass (Mayer et al. 2013a; Gross and Mayer 2015).

Interestingly, the tardigrade nervous system shows additional commissures in front of the second, third, and fourth ganglia, which were mentioned already in 1865 (referred to as "Quercommissuren" by Greeff 1865) but subsequently neglected until an immunohistochemical study by Mayer and colleagues (2013a) drew attention to them again.

8

Additional nerves arise laterally from each ganglion (i.e., peripheral nerves) and project dorsally (Mayer et al. 2013a). They are interconnected by a laterally paired longitudinal nerve spanning through the whole animal from anterior to posterior (Fig. 2c, d). Within the first trunk segment this peripheral nerve seems to arise from the outer connectives linking the brain with the first ganglion. However, developmental studies show that this nerve actually originates from the first trunk ganglion (Gross and Mayer 2015). Other prominent nerves arising laterally from the ganglia are the two nerve pairs (i.e., an anterior and a posterior pair), which innervate the legs of the corresponding segment (Zantke et al. 2008; Mayer et al. 2013a). Additionally, each anterior pair of leg nerves projects to a peripheral ganglion, which is situated within the leg itself (Mayer et al. 2013a; Gross and Mayer 2015).

Ventral and peripheral nervous system in onychophorans

Although first mentioned in the 19th century (Guilding 1826), onychophorans aroused the interest of evolutionary biologists partly due to their status as one of the closest relatives of arthropods. Their far larger body size compared to tardigrades facilitates their examination, thus leading to many more studies, including detailed investigations of their nervous system (Balfour 1883; Sedgwick 1885; von Kennel 1888; Evans 1901; Holmgren 1916; Hanström 1928; Fedorow 1929; Hanström 1935; Henry 1948; Pflugfelder 1948; Schürmann and Sandeman 1976; Schürmann 1987, 1995; Eriksson and Budd 2000; Eriksson et al. 2003; Strausfeld et al. 2006a, 2006b; Mayer and Harzsch 2007, 2008; Whitington 2007; Mayer and Whitington 2008; Mayer et al. 2010, 2013a, 2014, 2015b; Whitington and Mayer 2011; Martin and Mayer 2014, 2015; Mayer et al. 2015b; Mayer 2016; Schumann et al. 2016; Martin et al. 2017). The onychophoran nervous system consists of an anterior dorsal brain that proceeds into a paired ventral nerve cord (Fig. 3b–d; Holmgren 1916; Hanström 1928; Mayer and Harzsch 2007). The two widely separated nerve cords span fairly uniformly throughout the length of the body. Thus a division into ganglia and somata-free connectives is completely

lacking, as both neuropil and a perikaryal layer are present throughout the entire nerve cords (Mayer and Harzsch 2007; Mayer 2016; Martin et al. 2017).

Nonetheless, the nerve cords show slight swellings that are segmentally arranged and occur at the same level as the leg pairs (Balfour 1883; Sedgwick 1895; Schürmann 1995; Mayer and Harzsch 2007; Martin et al. 2017), in contrast to the anterior shift exhibited by the ganglia of tardigrades and arthropods. These swellings bear the most prominent, segmentally arranged nerves — the paired leg nerves. Together with the nephridial nerves, these are the sole segmentally arranged nerves of the onychophoran ventral nervous system (Mayer and Harzsch 2008). In addition, the nerve cords are connected via numerous median commissures on the ventral side and by ring commissures that arise laterally but run dorsally along the body wall (Fig. 3c, d). Both median commissures and ring commissures are serially repeated but in a non-segmental fashion, i.e., the occurrence of these commissures differs slightly in number and position per leg-bearing segment (Balfour 1883; Fedorow 1929; Mayer and Harzsch 2008; Mayer and Whitington 2009a). The only nerve occurring in the trunk that does not show a bilaterally paired arrangement is the heart nerve (Fig. 3c), which instead lies dorso-medially and runs from anterior to posterior through the onychophoran trunk (Mayer and Harzsch 2008; Mayer and Whitington 2009a; Whitington and Mayer 2011; Mayer 2016). However, its origin and the question of whether or not it is associated with the brain remain to be clarified. Lateral to the heart nerve, a pair of dorsolateral nerves also proceeds through the length of the animal, thereby interconnecting the ring commissures (Fig. 3d).

Evolution of the peripheral nervous system and nerve cords in panarthropods

Despite all their differences, both the onychophoran and tardigrade nervous systems show an orthogonal arrangement — i.e., longitudinal nerve cords interconnected regularly by transverse commissures at right angles (*sensu* Reisinger 1925, 1972) — although this pattern is partially incomplete in tardigrades. Therefore, the question arises of whether or not an

orthogonal pattern of the nervous system is an ancestral feature of all panarthropods, albeit reduced in several recent arthropod lineages. Interestingly, "intersegmental nerves" similar to the commissures observed in onychophorans and tardigrades have also been reported from many crustacean species such as the mystacocarid *Derocheilocaris remanei* (Brenneis and Richter 2010), the phyllocarid *Nebalia bipes* (Harzsch and Wasloszek 2000), the cephalocarid *Hutchinsoniella macracantha* (Stegner et al. 2014) and the anostracan *Branchinella* sp. (Frase and Richter 2013). Each of these intersegmental nerves splits off from the connective between two consecutive ganglia and projects dorsally, where it branches further. At least in *D. remanei*, the posterior-most branch of these nerves shows a dorsal closure, thus resembling an orthogonal-like nervous system in these animals (Brenneis and Richter 2010). Additionally, the hypothesis suggesting an orthogonal-like nervous system as a ground pattern of panarthropods (Mayer and Harzsch 2008), or even ecdysozoans (Yang et al. 2016), receives further support from comparisons with the closest panarthropod relatives, the Cycloneuralia, which also exhibit an orthogonal-like pattern of the nervous system (Schmidt-Rhaesa and Henne 2016).

More recently, Yang and colleagues (2016) described an exceptionally well preserved early Cambrian fossil, the fuxianhuiid †*Chengjiangocaris kunmingensis* belonging to the stem-group arthropods with a fossilised nervous system. In their work, they discovered, in contrast to extant arthropods, that the ventral nervous system of †*C. kunmingensis* exhibited incomplete ring commissures as observed in tardigrades, only more numerous (Mayer et al. 2013a; Yang et al. 2016). Considering that all cycloneuralians, onychophorans, tardigrades and several crustaceans (to some extent), and at least one known example of a stem-group arthropod exhibit an orthogonal-like nervous system, it is reasonable to assume that this feature was already present in the last common ancestor of ecdysozoans. The presence of an orthogonal nervous system in several other distantly related protostomes, including Platyhelminthes, Rotifera, Gastrotricha and Annelida suggests that this arrangement may even

represent the ancestral state at least of protostomes (Reisinger 1972; Kotikova 1995; Reuter et al. 1998; Mayer and Harzsch 2008; Whitington and Mayer 2011, Müller and Westheide 2002; Hochberg and Litvaitis 2003; Purschke 2016). If this hypothesis holds true, then it is likely that the median and ring commissures, observed in onychophorans, were reduced within the tardigrade/arthropod lineage (Fig. 4a). Alternatively, if tardigrades represent the basal-most panarthropod group, one would have to assume that ring and median commissures have independently been reduced to one incomplete ring-like commissure per segment, as seen in extant tardigrades and many extant crustaceans and most likely completely lost in the majority of other arthropods, while they persisted in onychophorans (Fig. 4b). At present, it is reasonably clear that two pairs of nerves innervating each leg most likely represent the panarthropod ground pattern, but whether the leg nerves underwent a fusion or a loss within the lineage leading to arthropods remains to be resolved.

One of the most controversial topics concerning the panarthropod nervous system surrounds the evolution of segmental ganglia. Ganglia — i.e. discrete concentrations of neuronal cell bodies — are present in various animal groups, including both subclades of protostomes: the Spiralia and the Ecdysozoa (Hejnol and Lowe 2015; Schmidt-Rhaesa et al. 2016). A previous study even suggested the presence of ganglionated ventral nerve cords in the lobopodian fossil †*Paucipodia inermis* (Hou et al. 2004), but this interpretation has since been viewed critically (Edgecombe et al. 2015; Yang et al. 2016). Even so, this finding further fuelled the view that segmental ganglia were lost within the onychophoran lineage and that the swellings in the leg-bearing regions of the nerve cords represent remnants of those ganglia (Eriksson et al. 2005a; Campbell et al. 2011). However, recent morphological evidence speaks against this previous assumption. First, the position of these swellings corresponds to the position of the legs and their nerve pairs, which arise laterally from these swellings and are supplied by a number of motor neurons, which is, in fact, over 10 times greater than the number of neurons supplying an adjacent ring commissure (Martin et al. 2017). Second, the

vesicle-binding protein synapsin, a marker of presynaptic endings of nerve fibres, is distributed ubiquitously along the entire onychophoran nerve cord neuropil and, in addition shows slight peaks in signal intensity at the basis of each leg nerve (Fig. 5a). In contrast, synapsin in tardigrades and arthropods is localized almost exclusively within the neuropil of segmental ganglia (Fig. 5b, c). These findings, combined with the higher number of neurons supplying the legs, therefore favour the hypothesis that the swellings within the onychophoran nerve cord are actually a secondary product of their function rather than remnants of ganglia.

Based on current understanding of the nervous systems of living representatives, the last common ancestor of panarthropods most likely had a pair of medullary rather than ganglionated nerve cords (Yang et al. 2016). However, whether the paired condition is apomorphic for Panarthropoda or Ecdysozoa as a whole is difficult to answer as long as the monophyly and relationships of Cycloneuralia remain unresolved (Hejnol and Lowe 2015). Irrespective of that, this hypothesis contradicts the assumption that segmental ganglia evolved only once within the protostomes (Scholtz 2002; Northcutt 2012) but were lost in some lineages, including onychophorans. Instead, it favours multiple ganglionisation events of the ventral nerve cords occurring independently during evolution of bilaterians, such as in annelids and arthropods (Mayer and Whitington 2009a; Hejnol and Lowe 2015). Within Panarthropoda, for example, two possible hypotheses emerge regarding the evolution of ganglia, depending on the assumed position of tardigrades in the tree (Fig. 4a, b). If tardigrades are regarded as the basal-most group within panarthropods, a ganglionated nervous system would have evolved twice within Panarthropoda (Fig. 4b). On the other hand, if tardigrades represent the closest relatives to arthropods, segmental ganglia most likely evolved only once in the tardigrade/arthropod lineage (Fig. 4a) and is, therefore, the most parsimonious hypothesis.

Structure of the tardigrade brain

13

Water bears possess a dorsal ganglionic brain with a shape resembling a set of three lobes: inner, outer, and median (Fig. 6a; Smith and Goldstein, in press, reviewed by Schulze and Persson 2016). The outer and inner lobes are paired while the median lobe, lying ventral to the former two, has been alternatively described as either paired (Persson et al. 2012, 2014) or unpaired (Mayer et al. 2013b; Smith and Jockusch 2014). Although the brain consists of a relatively small number of cells (~200; Mayer et al. 2013b), the distribution of various neuronal markers is complex and specialised (Fig. 6b–e; Mayer et al. 2013b). Strong serotonin-like immunoreactivity is concentrated in the structures surrounding the mouth opening and the region surrounding the central brain neuropil (Fig. 6b), the latter also shows vivid labelling with markers against RFamide, perisulfakinin and allatostatin (Fig. 6c–d). Markers against serotonin, RFamide and allatostatin also weakly label the outer connectives that connect the lateral brain to the first trunk ganglion and represent an autapomorphy of the group (Fig. 6b, c; Mayer et al. 2013a).

Although a lobate organisation is widely accepted as a feature of all tardigrade brains investigated to date, the lobes themselves remain a topic of discussion in terms of brain segmentation. In fact, the segmentation of the tardigrade brain and head in general has been perhaps the most widely discussed issue in tardigrade evolutionary biology (reviewed by Smith and Goldstein, in press). The inner, outer and median brain lobes have been homologised with the arthropod proto-, deuto-, and tritocerebrum, respectively (Kristensen 1983), following the argument that each lobe is a modified ganglion itself as evidenced by the presence of commissures within each lobe (Persson et al. 2012, 2014). However, this observation is insufficient for addressing the question of segmentation (Zantke et al. 2008), as the number of brain commissures does not correspond to the number of brain segments, for example in onychophorans either (Mayer et al. 2010). Instead, most recent studies report the tardigrade head to be derived from a single segment with the strongest evidence based on the position of the stomatogastric ganglion (Mayer et al. 2013b) and Hox gene expression

14

patterns (Smith et al. 2016). Thus, the entire tardigrade brain likewise consists of one segmental region that has been homologised with the arthropod protocerebrum (Mayer et al. 2013b; Gross and Mayer 2015).

In addition to the brain, early studies of the tardigrade nervous system (e.g., Marcus 1929) also depicted a subpharyngeal ganglion located ventrally in the head. However, despite a few recent reports of a subpharyngeal ganglion (Persson et al. 2012, 2014), most studies find no evidence for this structure neither in adults (Mayer et al. 2013b; Schulze et al. 2014; Smith and Jockusch 2014) nor in embryos (Hejnol and Schnabel 2005; Gross and Mayer 2015). Instead, the structure identified as a subpharyngeal ganglion by Persson et al. (2012, 2014) is probably an extension of the brain itself (Hejnol and Schnabel 2005), possibly representing the ventral-most region of the horseshoe-shaped median lobe (Smith and Jockusch 2014). Studies detailing the interconnections of the neuronal processes in the head as well as markers against proneural and neurogenic genes during embryonic development would go a long way towards resolving these issues.

The small body size of tardigrades should be taken into account when considering the evolution of the nervous system. In fact, the miniaturisation of tardigrades cannot be achieved without consequences for the construction and function of their nervous system (see Niven and Farris 2012 for a general appraisal). To put in perspective, an entire tardigrade brain would easily fit in the soma of an insect (e.g., locust) neuron of 50 μm diameter. However, compared to arthropods, their brains contain far fewer neurons, totalling approximately 200 as judged from counting stained nuclei (Mayer et al. 2013). Clearly, reduction in cell number must go in hand with increased specialisation if functional integrity is to be maintained, and indeed there is some evidence for this in tardigrades. As is typical for many arthropods (Hoyle 1983; Kutsch and Breidbach 1994; Bullock, 2000; Brenneis and Richter 2010; Brenneis and Scholtz 2015), immunohistochemical staining for neurotransmitters indicates that tardigrade neurons are uniquely identifiable with respect to their position, shape, and function (Mayer et

al. 2013; Gross and Mayer 2015). The size of their somata, however, does not appear to be exceptionally small as seen, e.g., in confocal microscopy images. They reveal single neuronal cell bodies of around 3–5 μm in diameter (Fig. 6a; Mayer et al. 2013; Gross & Mayer 2015), which almost approaches the size of motor neurons in the onychophoran nerve cords (~8 μm in diameter, Martin et al. 2017) as well as some of the smallest neurons in insects (e.g., Kenyon cells associated with the mushroom bodies, which are 6–20 μm in diameter; Mizunami et al. 1998). The main concession of single neurons to size limitations appears to be a reduction in the expanse of their dendritic trees. Immunohistochemical staining for an RFamide-like neuropeptide, indicates that individual neurons in tardigrades seem to have exceptionally few terminals within any given ganglion, possibly even as few as only two in some cases (Mayer et al. 2013). These putative synaptic boutons, with diameters of ~2.5 μm are slightly smaller than their parent soma, but in fact in the same size range as glutamatergic synaptic terminals in the mammalian brain, for example (~2 μm in diameter; Petrof and Sherman, 2013). Thus, miniaturisation of the tardigrade nervous system is achieved largely by reducing cell numbers, neuropil branches and the number of synaptic connections, while, at the same time, increasing functional specificity. Nonetheless, given the proximity of synaptic neighbours, these adaptations are unlikely to compromise functionality to any great degree.

Structure of the onychophoran brain

Like the tardigrade brain, the onychophoran brain has been an important focus for research (e.g., Balfour 1883; von Kennel 1888; Holmgren 1916; Hanström 1928, 1935; Fedorow 1929; Henry 1948; Pflugfelder 1948; Schürmann 1987, 1995; Eriksson and Budd 2000; Eriksson et al. 2003; Strausfeld et al. 2006a, 2006b; Mayer et al. 2010, 2014, 2015b; Whitington and Mayer 2011; Martin and Mayer 2014, 2015; Mayer 2016). The anterior ganglionic brain of onychophorans is connected to the nerve cords via the "connecting cords", a part of the nervous system greatly resembling the ventral nerve cords except for the lack of ring and

median commissures (Fig. 3b, d; Martin and Mayer 2015). The brain itself consists of two bilateral hemispheres comprising the major neuropils: the anterior, central and lateral neuropils, the dorsal midline central body ("arcuate body" *sensu* Strausfeld et al. 2006a), as well as the paired ventro-lateral mushroom bodies (Fig. 7b; Holmgren 1916; Hanström 1928; Fedorow 1929; Schürmann 1987; Strausfeld et al. 2006a; Mayer 2016). The mushroom bodies consist primarily of three lobes that project anteriorly to calyces. They are connected to each other via an additional lobe and to the central body via a pair of pedunculi (Fig. 7b; Strausfeld et al. 2006a). The central body consists of an anterior and a posterior lamina and its lateral portions receive input from the optic tract (Fig. 7b; Mayer 2006; Strausfeld et al. 2006a, 2006b). Surrounding the central body are the anterior brain neuropil, paired lateral neuropils, and a central neuropil (Mayer 2016). Additionally, the onychophoran brain exhibits a prominent cortex (perikaryal rind): an accumulation of more or less uniform neuronal cell bodies encompassing the brain neuropils, with a thin dorsal layer and a thicker ventral layer (Mayer 2016). Finally, the brain is associated ventrally with a pair of hypocerebral organs — structures with a putative neurosecretory function (Fig. 3d; Sanchez 1958; Eriksson et al. 2005b).

Obvious boundaries delineating specific segments of the onychophoran nervous system are absent, thus leading to difficulties assigning each region to corresponding body segments (Holmgren 1916; Hanström 1928; Schürmann 1987; Mayer et al. 2010; Martin and Mayer 2015). Based on various methods (e.g., homologising appendages with those of arthropods or using the presence of hypocerebral and "ventral organs" as landmarks), the initial assumption was a tripartite division of the onychophoran brain — a hypothesis that was, for a long time, well accepted by researchers (see discussion by Pflugfelder 1948). Eventually, this idea was revisited using more recent methods, such as immunohistochemistry in conjunction with confocal microscopy (Mayer et al. 2010). Due to the lack of well-defined segments, appendages with determined segmental identity have been used as unambiguous

landmarks for understanding the segmental organisation of the onychophoran brain. For example, the tracts supplying the onychophoran antennae, which represent the appendages of the first body segment, are associated with the segment homologous to the protocerebral segment in arthropods; the nerves supplying the onychophoran jaws, which belong to the second body segment, are supplied by the segment homologous to the deutocerebral segment; and the nerve pair supplying the appendages of third onychophoran body segment, the slime papillae, innervate the segment homologous to the tritocerebral segment in arthropods. Moreover, neuronal tracing experiments exposing the position of somata associated with each head appendage revealed that only the somata of the two anterior-most head appendages (i.e. the antennae and jaws) are located within the brain (Fig. 7a; Mayer et al. 2010). On the other hand, the neuronal cell bodies of the third segmental appendages (slime papillae) are located outside the brain within the connecting and nerve cords (Fig. 7a; Mayer et al. 2010), thus rendering the onychophoran brain as a bipartite structure. Interestingly, this assumption had already been suggested by Holmgren (1916) and Hanström (1935) in the early 20th century.

Evolution of the panarthropod brain and segmental identity of the frontal appendages

The segmental composition of brains varies among panarthropods, with two segments being present in the onychophoran brain (Mayer et al. 2010), one in tardigrades (Mayer et al. 2013b; Smith et al. 2016) and at least three in most arthropods (Harzsch 2004b; but see Kirsch and Richter 2007 for an exception in branchiopod crustaceans). The tripartite compound brain of arthropods most likely evolved by a fusion of initially separate ganglia (Richter et al. 2010). In onychophorans, however, an analysis of post-oral commissures (Fig. 8c) suggests that their bipartite brain rather evolved by a successive anterior movement of non-ganglionised neuronal cell bodies of the second segment, indicating independent evolution of their composite brain (Martin and Mayer 2015). This hypothesis contradicts the assumption that a bipartite brain was already present in the last common ancestor of onychophorans and

18

arthropods (Mayer et al. 2010; see discussion by Scholtz 2016). Depending on the position of tardigrades and onychophorans in the panarthropod tree, this would suggest either a loss of one brain segment in tardigrades (if onychophorans are sister to tardigrades plus arthropods) or a convergent evolution of segmental ganglia in tardigrades and arthropods (if tardigrades branch off from the bottom of the panarthropod tree). Nonetheless, taking into account that the onychophoran and arthropod brains probably evolved by different mechanisms, the presence of a bipartite brain in the last common ancestor of onychophorans and arthropods appears to be questionable.

Unfortunately, immunohistochemical and neuronal tracing analyses of the onychophoran brain are scarce compared to arthropods (Eriksson and Budd 2000; Eriksson et al. 2003; Strausfeld et al. 2006a; Mayer et al. 2010, 2014, 2015b, 2015c; Martin and Mayer 2015; Schumann et al. 2016) and therefore still leave many questions unanswered. For example, there is so far no evidence for individually identifiable neurons within the onychophoran brain, in contrast to arthropods and tardigrades. However, Gaffron (1884) mentioned the presence of approximately eight giant cell bodies ("Riesenganglienzellen" *sensu* Gaffron, 1884), which are 6–10 times larger than the remaining neuronal cell bodies. Although Holmgren (1916) and Schürmann (1987) also observed giant neurons associated with giant nerve fibres projecting into the ventral nerve cords, their exact number, pathways, and functions remain unknown.

We believe that future immunohistochemical investigations will foster a better understanding of the nature of the major brain neuropils, including their origin, exact pathways, and functions. For instance, a recent study on the non-visual opsin arthropsin in onychophorans revealed a strong signal only within the median lobes of the protocerebral mushroom bodies (Schumann et al. 2016), suggesting a putative functional partition of the separate lobes. Additionally, little is known, for example about the so-called "olfactory lobes" of onychophorans, which are situated antero-laterally within each brain hemisphere and

19

consist of ~30 glomeruli each (Fig. 7a, b; Hanström 1928; Schürmann 1987). Although their appearance greatly resembles the arthropod glomeruli, they differ from the latter in that those of onychophorans occur within the protocerebrum, whereas in arthropods they appear in different segments of the nervous system. For example, myriapods, crustaceans and hexapods possess glomeruli in the deutocerebrum (Richter et al. 2010), while in chelicerates these structures are located either within the segment bearing the olfactory appendages (Strausfeld et al. 2006) or within each trunk ganglion as in pycnogonids (Brenneis 2016). Therefore, the homology between the onychophoran and arthropod olfactory glomeruli has not yet been demonstrated, but the fact that these structures occur in non-homologous segments of the central nervous system and are mainly associated with non-homologous structures, i.e., the antennae in onychophorans and most arthropod groups (Mayer et al. 2010), may suggest convergent evolution of these structures. The morphological similarities between olfactory glomeruli in these groups, therefore, most likely resulted from functional constraints.

The onychophoran brain is equipped with several nerves and nerve tracts, almost all of which are paired; a single medial dorsal nerve is the only exception (Hanström 1928; Fedorow 1929; Henry 1948; Eriksson and Budd 2000; Mayer 2016). The most prominent tracts originating from the brain supply the appendages of the first body segment (i.e., the antenna) and differ from all other cephalic nerves/tracts due to their medullary structure (Eriksson and Budd 2000). Additionally, the brain is associated with several other protocerebral nerves and tracts, including the tracts supplying the eyes and the tongue nerves, the latter originating lateral to the hypocerebral organs. As stated above, a pair of nerves originating from the deutocerebral region innervates the appendages of the second body segment, the jaws (Fig. 7a; Hanström 1928; Fedorow 1929; Henry 1948; Mayer et al. 2010). Moreover, two of the three nerve pairs supplying the lip papillae originate from the brain — one from the protocerebral and other from the deutocerebral segment. In contrast, the third pair arises from the connecting cords, anterior to the slime papilla nerves (Fig. 7a; Eriksson

and Budd 2000; Martin and Mayer 2014). Indeed, the fact that the onychophoran lip papillae are supplied by three different parts of the central nervous system led to the previous assumption of a circumoral brain similar to that of cycloneuralians as being present in onychophorans (Eriksson and Budd 2000).

More recently, phylogenetic analyses recovered cycloneuralians as paraphyletic, with either Nematoda or Nematoida being closer related to panarthropods than to other cycloneuralians (Campbell et al. 2011; Rota-Stabelli et al. 2013; Borner et al. 2014), suggesting that a ring-shaped brain is an ancestral feature of all ecdysozoans (Hejnol and Lowe 2015). However, neuroanatomical tracing experiments of all three lip papillae nerves in the onychophoran *Euperipatoides rowelli* did not reveal a circumoral arrangement of these nerves or their supplying somata (Fig. 8a). This firmly opposes the hypothesis of a cycloneuralian-type brain being an ancestral condition for panarthropods (Martin and Mayer 2014). Evidence for such a structure is not present in arthropods or tardigrades either. Although the lip nerves do show an association with the three anterior-most segments of the onychophoran nervous system, their great majority is supplied by the deutocerebrum (Fig. 8a). Interestingly, neuronal tracing experiments further revealed that the two pairs of nerves innervating the pharynx in onychophorans are also supplied by the deutocerebrum (Martin and Mayer 2015). The fact that the jaw nerves are also associated with the deutocerebrum leads to an indication of this region of the brain being the main integration centre controlling feeding functions in Onychophora, while the tritocerebrum plays this role in arthropods.

Despite previous studies, many aspects of the onychophoran nervous system are still poorly understood. For example, a putative prefrontal ganglion identified in a recently discovered Cambrian stem-arthropod fossil, the anomalocaridid †*Lyrarapax unguispinus*, was homologised with a prefrontal part of the nervous system in onychophorans (Cong et al. 2014). With that, the authors suggested a pre-protocerebral affinity of the so-called great appendages — a view that is not supported by any current hypotheses, which rather indicate

21

either a protocerebral (Budd 2002; Legg and Vannier 2013; Legg et al. 2013; Ortega-Hernández et al. in press) or deutocerebral (Chen et al. 2004; Waloszek et al. 2005; Scholtz and Edgecombe 2005, 2006; Stein 2010; Haug et al. 2012) identity of these structures in the stem-group arthropods. Additionally, the authors claimed the presence of a hitherto unknown structure in onychophorans — a frontal, pre-protocerebral ganglion. However, further investigations based on several immunohistochemical markers as well as histology in both embryos and adult specimens of different species (including that studied by Cong and colleagues) do not show any evidence of an additional ganglion associated with the onychophoran antenna (Fig. 7c–i; Mayer et al. 2014 contra Cong et al. 2014).

Due to their phylogenetic position, onychophorans are often used as an outgroup for clarifying the ancestral arthropod body plan. The evolution of the arthropod head — its composition, nervous system, and homology of appendages — has been part of a major debate for many years (e.g., Budd 2002; Waloszek et al. 2005; Scholtz and Edgecombe 2006; Bitsch and Bitsch 2010; Ou et al. 2012; Richter et al. 2013; Janssen 2017). Although many homologies have been established, several structures remain with an unresolved origin, the arthropod labrum being one of most enigmatic and controversially discussed structures. At the centre of the debate is whether or not the labrum is appendicular, i.e. derived from the pair of limbs of the first segment and consequently, serially homologous to the remaining limbs. Its appendicular origin is mainly supported by developmental investigations, showing that the unpaired labrum develops in most arthropods from a paired bud-like anlage (see, e.g., Ungerer and Wolff 2005; Mittmann and Wolff 2012 for labrum development), a situation also seen during the development of appendages like antennae or walking legs, and by expression data of genes associated with limb development, such as *Distal-less* (*dll*) or *dachshund* (Rogers and Kaufman 1997; Prpic et al. 2001; Kimm and Prpic 2006), which show signal in the labrum and in subsequent appendages in arthropods. Boyan and colleagues (Boyan et al.

2003) further found neuroanatomical similarities between the labrum and the hind leg in the locust *Shistocerca gregaria*.

Nonetheless, many researchers questioned the appendicular origin of the arthropod labrum (reviewed by Scholtz and Edgecombe 2006) as *dll*, for example, is not expressed solely in the segmental appendage anlagen, thus rendering this marker as rather non-informative for this purpose. A recent gene expression study shows that, although a single gene (*six3*) is expressed in both the arthropod labrum and the onychophoran antennae (Eriksson et al. 2013a), another gene (*FoxQ2*) is expressed exclusively within the anlage of the labrum but not in the antennal anlagen of onychophorans (Janssen 2017). Therefore, it has been suggested that conclusions concerning labrum homology based on the expression of single genes should be considered carefully (Janssen 2017). An alternative hypothesis suggests that the labrum does not have an appendicular origin, but is instead homologous to the anterior-most pair of lip papillae of onychophorans, while the onychophoran antennae, in turn, might be homologous to the frontal filaments of some crustaceans (Frase and Richter 2013, 2016; Richter et al. 2013). The latter assumption is based on the fact that the frontal filaments in the anostracan crustaceans *Branchinella* sp. and *Artemia franciscana* are believed to have a similar innervation pattern as the onychophoran antennae (Frase and Richter 2013, 2016; Scholtz 2016). Nevertheless, more detailed investigations are still required in order to clarify which of these hypotheses holds true, whereas our current knowledge suggests that a homology of onychophoran antennae to the crustacean frontal filaments seems to be unlikely, as the crustacean frontal filaments are said to be rather simple evaginations of the body wall and most likely not homologous to the limbs (Ortega-Hernández and Budd 2016). Furthermore, a homology of the onychophoran frontal lip papillae to the arthropod labrum also seems unlikely as (i) the onychophoran mouth and its surrounding lip papillae are most likely a unique feature of Onychophora (Ou et al. 2012) and (ii) the labrum of arthropods is mainly innervated by the tritocerebrum (Boyan et al. 2002), whereas the anterior-most pair of

lip papillae in onychophorans is supplied by a different part of the brain, namely the protocerebrum (Martin and Mayer 2014). Meanwhile, it is still unclear whether the labrum represents a derived feature of arthropods or whether homologous structures occur in other animal groups. This underlines the importance of further studying the anatomy of outgroups, such as onychophorans and tardigrades.

Neural development in tardigrades

Although the development of the nervous system in tardigrades was first detailed during the early 20th century by Marcus (1929), our knowledge of this process on the whole remains in its infancy. According to Marcus's (1929) observations, the nervous system is formed from a single region represented by a thickening of the ectoderm along the entire ventral side of the embryo, from the tissue surrounding the stomodaeum to the anterior border of the proctodaeum. This thickening, i.e., the anlage of the entire central nervous system, gives rise to the brain and all four trunk ganglia simultaneously. Although Eibye-Jacobsen (1996/97) largely maintained Marcus's (1929) model — except for the precise timing of developmental events — later studies have not found support for this hypothesis. Instead, Hejnol and Schnabel (2005) show that each ganglion arises from a neural progenitor cell in the eutardigrade *Thulinius stephaniae* (Bertolani, 2003), and that these cells are never part of a unitary structure. Furthermore, the progenitor cells of the brain were shown to immigrate before those of the trunk ganglia, contradicting Marcus's (1929) hypothesis that all main parts of the central nervous system are formed simultaneously. Gabriel et al. (2007) make no mention of a unitary anlage in *Hypsibius dujardini* either, instead describing the appearance of the ganglia as aggregations of cells.

A more recent immunohistochemical study using a marker against acetylated α-tubulin in *H. dujardini* revealed further details regarding the development of the central nervous system (Gross and Mayer 2015). In accordance with a previous study (Hejnol and Schnabel

24

2005), the brain and each trunk ganglion were found to develop individually as separate, bilaterally symmetric structures (Fig. 9e–h). The stomodaeal nervous system, associated with the sensory structures around the mouth, develops separately and, although it appears to consist of several rings, is also bilaterally symmetric (Fig. 9e; Gross and Mayer 2015). Like the buccopharyngeal apparatus it innervates, this structure probably represents an autapomorphy of tardigrades and is homologous neither to the circumpharyngeal brain of the cycloneuralians, which has a perikarya-neuropil-perikarya pattern arranged in rings (Rothe and Schmidt-Rhaesa 2010; Brenneis and Scholtz 2014), nor to the circumoral nerve ring of arthropods, which is formed by the three paired neuromeres of the brain (Harzsch 2006; Scholtz and Edgecombe 2006; Brenneis et al. 2008). The observation of a single developing central brain neuropil (Fig. 9e–g), together with the lack of any additional cerebral or subpharyngeal ganglia, suggests that the tardigrade brain consists of a single segment, as opposed to at least three in arthropods. Unfortunately, neural or proneural gene expression data are missing entirely from tardigrades but will be crucial for fully understanding neural development in these animals.

Neural development in onychophorans

In contrast to the large invaginations of cell clusters found in chelicerates/myriapods and the neuroblasts of hexapods/crustaceans, onychophorans form their nerve cords via individual neural precursor cells, which originate from the ventral neuroectoderm (Mayer and Whitington 2009b; Whitington and Mayer 2011). The ingression of a large number of these cells forms an additional basal layer of the ectoderm that then generates the neuropil dorsally. The axons of the neuropil arise in an anterior-to-posterior cascade but extend only in the anterior direction towards the axons that have already formed (Mayer and Whitington 2009a). The leg nerves as well as the median and ring commissures are generated after the longitudinal nerve cords have been established (Fig. 9a–d), with the ring commissures further

contributing to the heart and dorsolateral nerves (Mayer and Whitington 2009a). Three uncharacterised neuropils of the onychophoran brain have previously been homologised with the proto-, deuto-, and tritocerebrum of arthropods (Strausfeld et al. 2006a). Subsequent studies have since shown that these structures arise sequentially from a single central brain neuropil during development, thus excluding their putative origin from three separate segments and consequently their homology to the arthropod proto-, deuto-, and tritocerebrum (Fig. 9b–d; Mayer et al. 2010).

In contrast to tardigrades, expression data from onychophorans are available for several genes involved in neural development. As in *Drosophila melanogaster*, the cells that will give rise to the neuropil express an *achaete-scute* homolog in onychophorans (Skeath et al. 1992; Eriksson and Stollewerk 2010a, 2010b). The genes *Delta* and *Notch* are likewise involved in the formation of the nervous system in arthropods (Chipman and Stollewerk 2006; Whitington and Mayer 2011) and were therefore proposed to have the same role in onychophorans (Eriksson and Stollewerk 2010a, 2010b; Eriksson et al. 2010). However, while this may hold true for the brain (Eriksson and Stollewerk 2010b), these two genes are probably involved in the development of segmental structures named ventral and preventral organs in the trunk instead (Oliveira et al. 2013). As opposed to several arthropods (e.g., chelicerates and myriapods), where homonymous albeit not homologous ventral organs play a role in neurogenesis as segmental anlagen of the nervous system (Heymons 1901; Tiegs 1940a, 1940b; Brenneis and Scholtz 2014), the ventral and preventral organs in onychophorans arise after the formation of the nervous system and rather represent attachment sites for the ventral limb musculature (Mayer and Whitington 2009a; Oliveira et al. 2013). Despite the data available from onychophorans, additional comparative studies are still vital for functional inference of various genes during embryonic development of these animals.

Evolution of visual organs in panarthropods

Vision — the ability to perceive light — is an essential sensory property of the vast majority of metazoan animals, which show a remarkable diversification of visual systems (Arendt and Wittbrodt 2001; Arendt et al. 2009; Land and Nilsson 2012; Nilsson 2013). Within arthropods, there are two main types of visual organs: the compound eyes and the median ocelli (Fig. 10c; Paulus 1979, 2000; Bitsch and Bitsch 2005), with the latter considered ancestral for panarthropods (Mayer 2006). The compound eyes are considered autapomorphic for the arthropods, as the radiodontans (stem-group arthropods) are the earliest fossil forms in which such eyes have been found (Paterson et al. 2011; Ortega-Hernández et al. in press). Tardigrades and onychophorans each possess a single type of eyes: the lateral eyes, which are comparatively simple in appearance (Figs 10a, b; 11a; Mayer 2006; Greven 2007). Several onychophoran species, such as the peripatopsids *Tasmanipatus anophthalmus* Ruhberg et al., 1991 (Fig. 11b) and *Peripatopsis alba* Lawrence, 1931, and the peripatids *Speleoperipatus spelaeus* Peck, 1975 and *Typhloperipatus williamsoni* Kemp, 1913 completely lack eyes and are therefore thought to be blind. The absence of eyes in these species is presumably a result of independent losses, as simple structured eyes were already present in Cambrian lobopodians (~520 million years old), such as †*Miraluolishania haikouensis* and †*Onychodictyon ferox* (Liu et al. 2004; Schoenemann et al. 2009; Ou et al. 2012), as well as in the oldest fossil onychophoran preserved in amber — the ~100 million years old †*Cretoperipatus burmiticus* (Oliveira et al. 2016). Remarkably, cryosections labelled with a DNA marker as well as synchrotron radiation-based X-ray microtomography (SRμCT) data from the Australian species *T. anophthalmus* do not reveal a total lack of eyes, but instead confirm the presence of rudimentary structures corresponding in position to the eyes of other onychophoran species (Fig. 11c, d; see also Ruhberg et al. 2001). This raises the question of whether eyeless onychophorans are truly without visual capabilities or whether these remnants still allow light perception.

Nonetheless, the origin of the onychophoran and the tardigrade eyes and the evolution of their respective visual systems is a controversial topic that attracts the attention of researchers from many fields such as molecular, ultrastructural, and neuroanatomical research (Mayer 2006; Strausfeld et al. 2006a; Greven 2007; Hering et al. 2012; Hering and Mayer 2014). For many years, researchers attempted to determine the origin of the onychophoran eye, hoping to reconstruct the anatomy of the ancestral eye within panarthropods. Due to an even array of straight cylindrical microvilli — a feature they share with the arthropod compound eye — the onychophoran eyes were thought to be homologous to the compound eyes rather than to the median ocelli of arthropods (Eakin and Westfall 1965; Hermans and Eakin 1974). However, further investigations into their ontogenetic origin and innervation patterns instead support a homology of onychophoran eyes with the median ocelli of arthropods (Mayer 2006). Both the onychophoran eyes and the arthropod median ocelli develop from an ectodermal groove (or pit), whereas the compound eyes of arthropods originate from a proliferation zone during ontogeny (Sedgwick 1887; von Kennel 1888; Paulus 2000; Eriksson et al. 2003; Walker and Tait 2004; Mayer et al. 2015c). Furthermore, although the onychophoran eyes are located laterally on the head, it is the median neuropil of the brain, i.e., the central body ("arcuate body" *sensu* Strausfeld et al. 2006a; Richter et al. 2010), that receives input from the eyes (Mayer 2006; Strausfeld et al. 2006a), similar to the condition observed for the median ocelli at least in chelicerates (Calman et al. 1991; Harzsch et al. 2005; Lehmann et al. 2012).

Another yet unresolved issue concerns the number of visual neuropils supplied by the onychophoran eyes. While it is accepted that a lateral neuropil is associated directly with the eye (Mayer 2006; Strausfeld et al. 2006a), the nature of the bifurcated optic tract that extends medially from the eye remains unresolved. Strausfeld et al. (2006a) describe both branches of the optic tract as two parts of an additional paired neuropil: the anterior ventral branch connects to the mushroom bodies while the posterior dorsal branch flanks the central body.

On the contrary, Mayer (2006) describes the posterior dorsal branch not as flanking the central body but rather entering it directly, thereby arguing against the optic tract being a separate neuropil. Therefore, the precise pathway and connections of the optic tract in onychophorans remain unresolved, with the visual neuropils numbering either one or two. Clarifying this issue may greatly contribute to our understanding of the evolution of the ancestral condition of the panarthropod eye.

Even less is known about the optical system in tardigrades. Many eutardigrades possess lateral visual organs resembling an inverse pigment-cup ocellus (Marcus 1929; Kristensen 1978; Dewel et al. 1993; Greven 2007), but these do not show many similarities to the compound eyes or median ocelli of arthropods, thus leaving the question open of whether the tardigrade eyes are homologous to any type of arthropod eyes, or alternatively represent a a derived characteristic occurring exclusively within their own lineage. Tardigrade eyes are sometimes even referred to as cerebral eyes, suggesting that they are part of the brain (Greven 2007), although Kristensen (1983) shows that they retain a connection to the epidermal layer. To clarify the origin of the tardigrade eye, therefore, the innervation pattern of the eyes needs to be resolved, for example by ultrastructural and immunohistochemical investigations. Due to their putative phylogenetic position as one of the closest relatives of arthropods, more information about the visual system in tardigrades would be crucial for elucidating the evolution of vision in panarthropods.

Several recent phylogenetic analyses have focused on the evolution of opsins, the main metazoan photoreceptor proteins (e.g., Plachetzki et al. 2007; Shichida and Matsuyama 2009; Feuda et al. 2012; Hering et al. 2012; Porter et al. 2012; Hering and Mayer 2014; Ramirez et al. 2016). The last common ancestor of Bilateria most likely possessed nine different opsins belonging to four major subclades (I-canonical visual opsins, II-chaopsins, III-xenopsins and IV-tetraopsins) two of which (i.e. II-chaopsins and III-xenopsins) were apparently lost in the ecdysozoan lineage (Ramirez et al. 2016). Transcriptomic data suggest

that the last common ancestor of Panarthropoda most likely possessed only five opsins in total belonging to the major opsin subclades I and IV (Fig. 10d; Hering and Mayer 2014; Ramirez et al. 2016). In arthropods, vision is mainly provided by the canonical rhabdomeric opsins (=r-opsins), in contrast to the canonical ciliary opsins (=c-opsins) in vertebrates (Arendt and Wittbrodt 2001; Arendt 2003; Plachetzki et al. 2007; Vopalensky and Kozmik 2009). However, further analyses revealed that only one of the two ancestral canonical r-opsins in panarthropods has a visual role, represented by onychopsin, in Onychophora (Hering et al. 2012). The second canonical r-opsin in onychophorans, arthropsin, is expressed within the inner lobes of the mushroom bodies, thus suggesting its putative role in olfactory pathways (Schumann et al. 2016). *In situ* hybridisation experiments confirm the presence of *onychopsin* mRNA within the photoreceptor cells of the onychophoran eye (Fig. 10e; Beckmann et al. 2015), thus contradicting earlier hypotheses of a potential influence of canonical c-opsins on vision in onychophorans (Eriksson et al. 2013b). The presence of one visual r-opsin in onychophorans and tardigrades (Hering et al. 2012; Hering and Mayer 2014) leads to the assumption that monochromatic vision is an ancestral feature of all panarthropods and that colour vision most likely evolved within the arthropod lineage, where the r-opsins are highly diverse (Fig. 10d; e.g., Briscoe 2000; Porter et al. 2007; Koyanagi et al. 2008; Kashiyama et al. 2009; Katti et al. 2010; Battelle et al. 2015; Henze and Oakley 2015). Indeed, electrophysiological and behavioural experiments on onychophorans confirm a single peak in the visible light spectrum around 480 nm (blue-green light) and therefore support the hypothesis of monochromatic vision in these animals (Beckmann et al. 2015). Interestingly, the two onychophoran subgroups show a similar spectral sensitivity, suggesting that this feature most likely evolved before the split of Peripatidae and Peripatopsidae over 374 million years ago (Murienne et al. 2014). Behavioural and gene expression studies in tardigrades might provide a more detailed picture of the evolution of vision in Panarthropoda.

References

Allwood J, Gleeson D, Mayer G, Daniels S, Beggs JR, Buckley TR (2010) Support for vicariant origins of the New Zealand Onychophora. J Biogeogr 37:669–681

Arakawa K (2016) No evidence for extensive horizontal gene transfer from the draft genome of a tardigrade. Proc Natl Acad Sci USA 113:E3057–E3057

Arendt D (2003) Evolution of eyes and photoreceptor cell types. Int J Dev Biol 47:563–571

Arendt D, Wittbrodt J (2001) Reconstructing the eyes of Urbilateria. Philos Trans R Soc B Biol Sci 356:1545–1563

Arendt D, Hausen H, Purschke G (2009) The 'division of labour' model of eye evolution. Philos Trans R Soc B Biol Sci 364:2809–2817

Baer A, Mayer G (2012) Comparative anatomy of slime glands in Onychophora (velvet worms). J Morphol 273:1079–1088

Balfour FM (1883) The anatomy and development of *Peripatus capensis*. Q J Microsc Sci 23:213–259

Basse A (1905) Beiträge zur Kenntnis des Baues der Tardigraden. Z Wiss Zool:259–281

Battelle B-A, Kempler KE, Saraf SR, Marten CE, Dugger DR, Speiser DI, Oakley TH (2015) Opsins in *Limulus* eyes: characterization of three visible light-sensitive opsins unique to and co-expressed in median eye photoreceptors and a peropsin/RGR that is expressed in all eyes. J Exp Biol 218:466–479

Beckmann H, Hering L, Henze MJ, Kelber A, Stevenson PA, Mayer G (2015) Spectral sensitivity in Onychophora (velvet worms) revealed by electroretinograms, phototactic behaviour and opsin gene expression. J Exp Biol 218:915–922

Bemm F, Weiß CL, Schultz J, Förster F (2016) Genome of a tardigrade: horizontal gene transfer or bacterial contamination? Proc Natl Acad Sci USA 113:E3054–E3056

Bitsch C, Bitsch J (2005) Evolution of eye structure and arthropod phylogeny. In: Koenemann S (ed) Crustacea and Arthropod Relationships. CRC Press, Taylor & Francis Book Inc., New York, pp 81–111

Bitsch J, Bitsch C (2010) The tritocerebrum and the clypeolabrum in mandibulate arthropods: segmental interpretations. Acta Zool 91:249–266

Boothby TC, Tenlen JR, Smith FW, Wang JR, Patanella KA, Nishimura EO, Tintori SC, Li Q, Jones CD, Yandell M (2015) Evidence for extensive horizontal gene transfer from the draft genome of a tardigrade. Proc Natl Acad Sci USA:201510461

Borner J, Rehm P, Schill RO, Ebersberger I, Burmester T (2014) A transcriptome approach to ecdysozoan phylogeny. Mol Phylogenet Evol 80:79–87

Boyan GS, Williams JLD, Posser S, Bräunig P (2002) Morphological and molecular data argue for the labrum being non-apical, articulated, and the appendage of the intercalary segment in the locust. Arthropod Struct Dev 31:65–76

Boyan GS, Bräunig P, Posser S, Williams JLD (2003) Embryonic development of the sensory innervation of the clypeo-labral complex: further support for serially homologous appendages in the locust. Arthropod Struct Dev 32:289–302

Brenneis G (2016) Pycnogonida. In: Schmidt-Rhaesa A, Harzsch S, Purschke G (eds) Structure and Evolution of Invertebrate Nervous Systems. Oxford University Press, Oxford, pp 419–427

Brenneis G, Richter S (2010) Architecture of the nervous system in Mystacocarida (Arthropoda, Crustacea) — an immunohistochemical study and 3D reconstruction. J Morphol 271:169–189

Brenneis G, Scholtz G (2014) The 'ventral organs' of Pycnogonida (Arthropoda) are neurogenic niches of late embryonic and post-embryonic nervous system development. PLoS ONE 9:e95435

Brenneis G, Scholtz G (2015) Serotonin-immunoreactivity in the ventral nerve cord of Pycnogonida – Support for individually identifiable neurons as ancestral feature of the arthropod nervous system. BMC Evol Biol 15:136

Brenneis G, Ungerer P, Scholtz G (2008) The chelifores of sea spiders (Arthropoda, Pycnogonida) are the appendages of the deutocerebral segment. Evol Dev 10:717–724

Brinck P (1957) Onychophora, a review of South African species, with a discussion on the significance of the geographical distribution of the group. In: Hanström B, Brinck P, Rudebeck G (eds) South African Animal Life. Almqvist & Wiksell, Stockholm, Sweden, pp 7–32

Briscoe AD (2000) Six opsins from the butterfly *Papilio glaucus*: molecular phylogenetic evidence for paralogous origins of red-sensitive visual pigments in insects. J Mol Evol 51:110–121

Budd GE (2002) A palaeontological solution to the arthropod head problem. Nature 417:271–275

Bullock TH (2000) Revisiting the concept of identifiable neurons. Brain Behav Evol 55:236–240

Calman BG, Lauerman MA, Andrews AW, Schmidt M, Battelle BA (1991) Central projections of *Limulus* photoreceptor cells revealed by a photoreceptor-specific monoclonal antibody. J Comp Neurol 313:553–562

Campbell LI, Rota-Stabelli O, Edgecombe GD, Marchioro T, Longhorn SJ, Telford MJ, Philippe H, Rebecchi L, Peterson KJ, Pisani D (2011) MicroRNAs and phylogenomics resolve the relationships of Tardigrada and suggest that velvet worms are the sister group of Arthropoda. Proc Natl Acad Sci USA 108:15920–15924

Carroll SB, Grenier JK, Weatherbee SD (2005) From DNA to Diversity. Molecular Genetics and the Evolution of Animal Design. Blackwell Publishing, Malden, USA

Castano RA, Castano D, Dewel WC (1996) Arthropod head segmentation: an integrated approach. Am Zool 36:132A

Chen J, Waloszek D, Maas A (2004) A new "great appendage" arthropod from the Lower Cambrian of China and homology of chelicerate chelicerae and raptorial antero-ventral appendages. Lethaia 37:3–20

Chipman AD, Stollewerk A (2006) Specification of neural precursor identity in the geophilomorph centipede *Strigamia maritima*. Dev Biol 290:337–350

Cong P, Ma X, Hou X, Edgecombe GD, Strausfeld NJ (2014) Brain structure resolves the segmental affinity of anomalocaridid appendages. Nature 513:538–542

Damen WGM (2002) Parasegmental organization of the spider embryo implies that the parasegment is an evolutionary conserved entity in arthropod embryogenesis. Development 129:1239–1250

Damen WGM (2007) Evolutionary conservation and divergence of the segmentation process in arthropods. Dev Dyn 236:1379–1391

Damen WGM, Hausdorf M, Seyfarth EA, Tautz D (1998) A conserved mode of head segmentation in arthropods revealed by the expression pattern of Hox genes in a spider. Proc Natl Acad Sci USA 95:10665–10670

Degma P, Guidetti R (2007) Notes to the current checklist of Tardigrada. Zootaxa 1579:41–53

Degma P, Bertolani R, Guidetti R (2014) Actual checklist of Tardigrada species. http://www.tardigrada.modena.unimo.it/miscellanea/Actual%20checklist%20of%20Tardigrada.pdf

Deutsch JS (2004) Segments and parasegments in arthropods: a functional perspective. BioEssays 26:1117–1125

Dewel RA, Dewel WC (1996) The brain of *Echiniscus viridissimus* Peterfi, 1956 (Heterotardigrada): a key to understanding the phylogenetic position of tardigrades and the evolution of the arthropod head. Zool J Linn Soc 116:35–49

Dewel RA, Dewel WC (1997) The place of tardigrades in arthropod evolution. In: Fortey RA, Thomas RH (eds) Arthropod Relationships. Chapman & Hall, London, pp 109–123

Dewel RA, Nelson DR, Dewel WC (1993) Tardigrada. In: Harrison FW, Rice ME (eds) Microscopic Anatomy of Invertebrates. Wiley-Liss, New York, pp 143–183

Dewel RA, Budd GE, Castano DF, Dewel WC (1999) The organization of the suboesophageal nervous system in tardigrades: insights into the evolution of the arthropod hypostome and tritocerebrum. Zool Anz 238:191–203

Doyère M (1840) Mémoire sur les Tardigrades. Ann Sci Nat (Paris), Zool, Ser 2 14:269–361

Dunn CW, Hejnol A, Matus DQ, Pang K, Browne WE, Smith SA, Seaver E, Rouse GW, Obst M, Edgecombe GD, Sørensen MV, Haddock SHD, Schmidt-Rhaesa A, Okusu A, Kristensen RM, Wheeler WC, Martindale MQ, Giribet G (2008) Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452:745–749

Eakin RM, Westfall JA (1965) Fine structure of the eye of peripatus (Onychophora). Z Zellforsch Mikrosk Anat 68:278–300

Edgecombe GD (2010) Arthropod phylogeny: an overview from the perspectives of morphology, molecular data and the fossil record. Arthropod Struct Dev 39:74–87

Edgecombe GD, Ma X, Strausfeld NJ (2015) Unlocking the early fossil record of the arthropod central nervous system. Philos Trans R Soc B Biol Sci 370:20150038

Eibye-Jacobsen J (1996/97) New observations on the embryology of the Tardigrada. Zool Anz 235:201–216

Eriksson BJ, Budd GE (2000) Onychophoran cephalic nerves and their bearing on our understanding of head segmentation and stem-group evolution of Arthropoda. Arthropod Struct Dev 29:197–209

Eriksson BJ, Stollewerk A (2010a) The morphological and molecular processes of onychophoran brain development show unique features that are neither comparable to insects nor to chelicerates. Arthropod Struct Dev 39:478–490

Eriksson BJ, Stollewerk A (2010b) Expression patterns of neural genes in *Euperipatoides kanangrensis* suggest divergent evolution of onychophoran and euarthropod neurogenesis. Proc Natl Acad Sci USA 107:22576–22581

Eriksson BJ, Tait NN, Budd GE (2003) Head development in the onychophoran *Euperipatoides kanangrensis* with particular reference to the central nervous system. J Morphol 255:1–23

Eriksson BJ, Larson ET, Thörnqvist P-O, Tait NN, Budd GE (2005a) Expression of *engrailed* in the developing brain and appendages of the onychophoran *Euperipatoides kanangrensis* (Reid). J Exp Zool Part B Mol Dev Evol 304B:1–9

Eriksson BJ, Tait NN, Norman JM, Budd GE (2005b) An ultrastructural investigation of the hypocerebral organ of the adult *Euperipatoides kanangrensis* (Onychophora, Peripatopsidae). Arthropod Struct Dev 34:407–418

Eriksson BJ, Tait NN, Budd GE, Janssen R, Akam M (2010) Head patterning and Hox gene expression in an onychophoran and its implications for the arthropod head problem. Dev Genes Evol 220:117–122

Eriksson BJ, Samadi L, Schmid A (2013a) The expression pattern of the genes *engrailed*, *pax6*, *otd* and *six3* with special respect to head and eye development in *Euperipatoides kanangrensis* Reid 1996 (Onychophora: Peripatopsidae). Dev Genes Evol 223:237–246

Eriksson BJ, Fredman D, Steiner G, Schmid A (2013b) Characterisation and localisation of the opsin protein repertoire in the brain and retinas of a spider and an onychophoran. BMC Evol Biol 13:186

Evans R (1901) On two new species of Onychophora from the Siamese Malay States. Q J Microsc Sci 44:473–538

Fedorow B (1929) Zur Anatomie des Nervensystems von Peripatus. II. Das Nervensystem des vorderen Körperendes und seine Metamerie. Zool Jahrb Abt Anat Ontog Tiere 50:279–332

Fernandez C, Vasanthan T, Kissoon N, Karam G, Duquette N, Seymour C, Stone J (2016) Radiation tolerance and bystander effects in the eutardigrade species *Hypsibius dujardini* (Parachaela: Hypsibiidae). Zool J Linn Soc 178:919–923

Feuda R, Hamilton SC, McInerney JO, Pisani D (2012) Metazoan opsin evolution reveals a simple route to animal vision. Proc Natl Acad Sci USA 109:18868–18872

Fortey RA, Thomas RH (1998) Arthropod Relationships. Chapman & Hall, London

Franke FA, Mayer G (2014) Controversies surrounding segments and parasegments in Onychophora: insights from the expression patterns of four "segment polarity genes" in the peripatopsid *Euperipatoides rowelli*. PLoS ONE 9:e114383

Frase T, Richter S (2013) The fate of the onychophoran antenna. Dev Genes Evol 223:247–251

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

Gabriel WN, McNuff R, Patel SK, Gregory TR, Jeck WR, Jones CD, Goldstein B (2007) The tardigrade *Hypsibius dujardini*, a new model for studying the evolution of development. Dev Biol 312:545–559

Gaffron E (1884) Kurzer Bericht über fortgesetze Peripatus-Studien. Zool Anz 7:336–339

Geidies H (1954) Abgeänderte Azan− Methoden. Mikrokosmos 42:239–240

Greeff R (1865) Ueber das Nervensystem der Bärthierchen, Arctiscoida C.A.S. Schultze (Tardigraden Doyère) mit besonderer Berücksichtigung der Muskelnerven und deren Endigungen. Arch mikrosk Anat 1:101–123

Greven H (2007) Comments on the eyes of tardigrades. Arthropod Struct Dev 36:401–407

Greven H, Kuhlmann D (1972) Die Struktur des Nervengewebes von *Macrobiotus hufelandi* C.A.S. Schultze (Tardigrada). Z Zellforsch 132:131–146

Gross V, Mayer G (2015) Neural development in the tardigrade *Hypsibius dujardini* based on anti-acetylated α-tubulin immunolabeling. EvoDevo 6:12

Gross V, Treffkorn S, Mayer G (2015) Tardigrada. In: Wanninger A (ed) Evolutionary Developmental Biology of Invertebrates. Springer, Berlin, pp 35–52

Guidetti R, Bertolani R (2005) Tardigrade taxonomy: an updated check list of the taxa and a list of characters for their identification. Zootaxa 845:1–46

Guilding L (1826) Mollusca Caribbaeana. Zoological Journal 2:437–449

Halberg KA, Jørgensen A, Møbjerg N (2013) Desiccation tolerance in the tardigrade *Richtersius coronifer* relies on muscle mediated structural reorganization. PLoS ONE 8:e85091

Hanström B (1928) Onychophora. Vergleichende Anatomie des Nervensystems der Wirbellosen Tiere unter Berücksichtigung seiner Funktion. Springer, Berlin, pp 341–351

Hanström B (1935) Bemerkungen über das Gehirn und die Sinnesorgane der Onychophoren. Lunds Univ Årsskr 31:1–37

Harrison PJ, Macmillan DL, Young HM (1995) Serotonin immunoreactivity in the ventral nerve cord of the primitive crustacean *Anaspides tasmaniae* closely resembles that of crayfish. J Exp Biol 198:531–535

Harzsch S (2003) Evolution of identified arthropod neurons: the serotonergic system in relation to *engrailed*-expressing cells in the embryonic ventral nerve cord of the American lobster *Homarus americanus* Milne Edwards, 1873 (Malacostraca, Pleocyemata, Homarida). Dev Biol 258:44–56

Harzsch S (2004a) Phylogenetic comparison of serotonin-immunoreactive neurons in representatives of the Chilopoda, Diplopoda, and Chelicerata: implications for arthropod relationships. J Morphol 259:198–213

Harzsch S (2004b) The tritocerebrum of Euarthropoda: a "non-drosophilocentric" perspective. Evol Dev 6:303–309

Harzsch S (2006) Neurophylogeny: architecture of the nervous system and a fresh view on arthropod phyology. Integr Comp Biol 46:162–194

Harzsch S, Wildt M, Battelle B, Waloszek D (2005) Immunohistochemical localization of neurotransmitters in the nervous system of larval *Limulus polyphemus* (Chelicerata, Xiphosura): evidence for a conserved protocerebral architecture in Euarthropoda. Arthropod Struct Dev 34:327–342

Harzsch S, Sandeman D, Chaigneau J (2006) Morphology and development of the central nervous system. Koninklijke Brill Academic Publishers, Leiden, Netherlands, pp 1–84

Hashimoto T, Horikawa DD, Saito Y, Kuwahara H, Kozuka-Hata H, Shin T, Minakuchi Y, Ohishi K, Motoyama A, Aizu T (2016) Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein. Nat Commun 7

Haug JT, Waloszek D, Maas A, Liu Y, Haug C (2012) Functional morphology, ontogeny and evolution of mantis shrimp-like predators in the Cambrian. Palaeontology 55:369–399

Heidemann NW, Smith DK, Hygum TL, Stapane L, Clausen LK, Jørgensen A, Hélix-Nielsen C, Møbjerg N (2016) Osmotic stress tolerance in semi-terrestrial tardigrades. Zool J Linn Soc 178:912–918

Heidenhain M (1915) Über die mallorysche Bindegewebsfärbung mit Karmin und Azokarmin als Vorfarben. Z Wiss Mikrosk 33:361–372

Hejnol A, Schnabel R (2005) The eutardigrade *Thulinia stephaniae* has an indeterminate development and the potential to regulate early blastomere ablations. Development 132:1349–1361

Hejnol A, Lowe CJ (2015) Embracing the comparative approach: how robust phylogenies and broader developmental sampling impacts the understanding of nervous system evolution. Philos Trans R Soc B Biol Sci 370:20150045

Hejnol A, Obst M, Stamatakis A, Ott M, Rouse GW, Edgecombe GD, Martinez P, Baguñà J, Bailly X, Jondelius U, Wiens M, Müller WEG, Seaver E, Wheeler WC, Martindale MQ, Giribet G, Dunn CW (2009) Assessing the root of bilaterian animals with scalable phylogenomic methods. Proc R Soc B Biol Sci 276:4261–4270

Hengherr S, Heyer AG, Köhler HR, Schill RO (2008) Trehalose and anhydrobiosis in tardigrades — evidence for divergence in responses to dehydration. FEBS J 275:281–288

Henry LM (1948) The nervous system and the segmentation of the head in the Annulata. Microentomology 13:27–48

Henze MJ, Oakley TH (2015) The dynamic evolutionary history of pancrustacean eyes and opsins. Integr Comp Biol 55:830–842

Hering L, Mayer G (2014) Analysis of the opsin repertoire in the tardigrade *Hypsibius dujardini* provides insights into the evolution of opsin genes in Panarthropoda. Genome Biol Evol 6:2380–2391

Hering L, Henze MJ, Kohler M, Kelber A, Bleidorn C, Leschke M, Nickel B, Meyer M, Kircher M, Sunnucks P, Mayer G (2012) Opsins in Onychophora (velvet worms) suggest a single origin and subsequent diversification of visual pigments in arthropods. Mol Biol Evol 29:3451–3458

Hering L, Bouameur J-E, Reichelt J, Magin TM, Mayer G (2016) Novel origin of lamin-derived cytoplasmic intermediate filaments in tardigrades. eLife 5:e11117

Hermans CO, Eakin RM (1974) Fine structure of the eyes of an alciopid polychaete, *Vanadis tagensis* (Annelida). Z Morphol Tiere 79:245–267

Heymons R (1901) Die Entwicklungsgeschichte der Scolopender. Zoologica 33:1–244

Hochberg R, Litvaitis MK (2003) Ultrastructural and immunocytochemical observations of the nervous systems of three macrodasyidan gastrotrichs. Acta Zool 84:171–178

Holmgren NF (1916) Zur vergleichenden Anatomie des Gehirns von Polychaeten, Onychophoren, Xiphosuren, Arachniden, Crustaceen, Myriapoden, und Insekten. Vorstudien zu einer Phylogenie der Arthropoden. Kungl Svenska Vetenskapsakad Handlingar [Ser 2] 56:1–303

Homberg U (2008) Evolution of the central complex in the arthropod brain with respect to the visual system. Arthropod Struct Dev 37:347–362

Hou XG, Ma XY, Zhao J, Bergström J (2004) The lobopodian *Paucipodia inermis* from the Lower Cambrian Chengjiang fauna, Yunnan, China. Lethaia 37:235–244

Hoyle G (1983) On the way to neuroethology: the identified neuron approach. Neuroethology and behavioral physiology. Springer, pp 9–25

Ingham P, Martinez Arias A (1992) Boundaries and fields in early embryos. Cell 68:221–235

Janssen R (2017) Comparative analysis of gene expression patterns in the arthropod labrum and the onychophoran frontal appendages, and its implications for the arthropod head problem. EvoDevo 8:1

Jönsson KI (2001) The nature of selection on anhydrobiotic capacity in tardigrades. Zool Anz 240:409–417

Kashiyama K, Seki T, Numata H, Goto SG (2009) Molecular characterization of visual pigments in Branchiopoda and the evolution of opsins in Arthropoda. Mol Biol Evol 26:299–311

Katti C, Kempler K, Porter ML, Legg A, Gonzalez R, Garcia-Rivera E, Dugger D, Battelle B-A (2010) Opsin co-expression in *Limulus* photoreceptors: differential regulation by light and a circadian clock. J Exp Biol 213:2589–2601

Kimm MA, Prpic NM (2006) Formation of the arthropod labrum by fusion of paired and rotated limb-bud-like primordia. Zoomorphology 125:147–155

Kinchin IM (1994) The Biology of Tardigrades. Portland Press Inc., London

Kirsch R, Richter S (2007) The nervous system of *Leptodora kindtii* (Branchiopoda, Cladocera) surveyed with Confocal Scanning Microscopy (CLSM), including general remarks on the branchiopod neuromorphological ground pattern. Arthropod Struct Dev 36:143–156

Kotikova EA (1995) Localization and neuroanatomy of catecholaminergic neurons in some rotifer species. Hydrobiologia 313/314:123–127

Koutsovoulos G, Kumar S, Laetsch DR, Stevens L, Daub J, Conlon C, Maroon H, Thomas F, Aboobaker AA, Blaxter M (2016) No evidence for extensive horizontal gene transfer in the genome of the tardigrade *Hypsibius dujardini*. Proc Natl Acad Sci USA 113:5053–5058

Koyanagi M, Nagata T, Katoh K, Yamashita S, Tokunaga F (2008) Molecular evolution of arthropod color vision deduced from multiple opsin genes of jumping spiders. J Mol Evol 66:130–137

Kristensen RM (1978) Notes on marine Heterotardigrades l. Description of two new *Batillipes* species, using the electron microscope. Zool Anz 200:1–17

Kristensen RM (1983) The first record of cyclomorphosis in Tardigrada based on a new genus and species from Arctic meiobenthos. Z Zool Syst Evolutionsforsch 20:249–270

Kutsch W, Breidbach O (1994) Homologues structures in the nervous system of Arthropoda. Adv Insect Physiol 24:1–113

Land MF, Nilsson D-E (2012) Animal eyes. Oxford University Press

Legg DA, Vannier J (2013) The affinities of the cosmopolitan arthropod *Isoxys* and its implications for the origin of arthropods. Lethaia 46:540–550

Legg DA, Sutton MD, Edgecombe GD, Caron J-B (2012) Cambrian bivalved arthropod reveals origin of arthrodization. Proc R Soc B Biol Sci 279:4699–4704

Legg DA, Sutton MD, Edgecombe GD (2013) Arthropod fossil data increase congruence of morphological and molecular phylogenies. Nat Commun 4

Lehmann T, Heß M, Melzer RR (2012) Wiring a periscope — ocelli, retinula axons, visual neuropils and the ancestrality of sea spiders. PLoS ONE 7:e30474

Liu J, Shu D, Han J, Zhang Z (2004) A rare lobopod with well-preserved eyes from Chengjiang Lagerstätte and its implications for origin of arthropods. Chin Sci Bull 49:1063–1071

Loesel R, Wolf H, Kenning M, Harzsch S, Sombke A (2013) Architectural principles and evolution of the arthropod central nervous system. In: Minelli A, Boxshall G, Fusco G (eds) Arthropod Biology and Evolution – Molecules, Development, Morphology. Springer, Heidelberg, pp 299–342

Mallatt J, Giribet G (2006) Further use of nearly complete 28S and 18S rRNA genes to classify Ecdysozoa: 37 more arthropods and a kinorhynch. Mol Biol Evol 40:772–794

Mallatt JM, Garey JR, Shultz JW (2004) Ecdysozoan phylogeny and Bayesian inference: first use of nearly complete 28S and 18S rRNA gene sequences to classify the arthropods and their kin. Mol Phylogenet Evol 31:178–191

Marcus E (1929) Tardigrada. In: Bronn HG (ed) Klassen und Ordnungen des Tierreichs. Akademische Verlagsgesellschaft, Leipzig, pp 1–609

Martin C, Mayer G (2014) Neuronal tracing of oral nerves in a velvet worm — implications for the evolution of the ecdysozoan brain. Front Neuroanat 8:7:1–13

Martin C, Mayer G (2015) Insights into the segmental identity of post-oral commissures and pharyngeal nerves in Onychophora based on retrograde fills. BMC Neurosci 16:53

Martin C, Gross V, Pflüger H-J, Stevenson PA, Mayer G (2017) Assessing segmental versus non-segmental features in the ventral nervous system of onychophorans (velvet worms). BMC Evol Biol 17:3

Mayer G (2006) Structure and development of onychophoran eyes — what is the ancestral visual organ in arthropods? Arthropod Struct Dev 35:231–245

Mayer G (2016) Onychophora. In: Schmidt-Rhaesa A, Harzsch S, Purschke G (eds) Structure and Evolution of Invertebrate Nervous Systems. Oxford University Press, Oxford, pp 390–401

Mayer G, Koch M (2005) Ultrastructure and fate of the nephridial anlagen in the antennal segment of *Epiperipatus biolleyi* (Onychophora, Peripatidae) — evidence for the onychophoran antennae being modified legs. Arthropod Struct Dev 34:471–480

Mayer G, Harzsch S (2007) Immunolocalization of serotonin in Onychophora argues against segmental ganglia being an ancestral feature of arthropods. BMC Evol Biol 7:118

Mayer G, Harzsch S (2008) Distribution of serotonin in the trunk of *Metaperipatus blainvillei* (Onychophora, Peripatopsidae): implications for the evolution of the nervous system in Arthropoda. J Comp Neurol 507:1196–1208

Mayer G, Whitington PM (2008) Insights into neural anatomy and development in Onychophora. J Morphol 269:1463–1464

Mayer G, Whitington PM (2009a) Neural development in Onychophora (velvet worms) suggests a step-wise evolution of segmentation in the nervous system of Panarthropoda. Dev Biol 335:263–275

Mayer G, Whitington PM (2009b) Velvet worm development links myriapods with chelicerates. Proc R Soc B Biol Sci 276:3571–3579

Mayer G, Oliveira IS (2011) Phylum Onychophora Grube, 1853. In: Zhang, Z.-Q. (Ed.) Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Rrichness. Zootaxa 3148:98

Mayer G, Oliveira IS (2013) Phylum Onychophora Grube, 1853. In: Zhang, Z.-Q. (Ed.) Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Rrichness (Addenda 2013). Zootaxa 3703:15–16

Mayer G, Bartolomaeus T, Ruhberg H (2005) Ultrastructure of mesoderm in embryos of *Opisthopatus roseus* (Onychophora, Peripatopsidae): Revision of the "long germ band" hypothesis for *Opisthopatus*. J Morphol 263:60–70

Mayer G, Whitington PM, Sunnucks P, Pflüger H-J (2010) A revision of brain composition in Onychophora (velvet worms) suggests that the tritocerebrum evolved in arthropods. BMC Evol Biol 10:255

Mayer G, Martin C, Rüdiger J, Kauschke S, Stevenson PA, Poprawa I, Hohberg K, Schill RO, Pflüger H-J, Schlegel M (2013a) Selective neuronal staining in tardigrades and onychophorans provides insights into the evolution of segmental ganglia in panarthropods. BMC Evol Biol 13:230

Mayer G, Kauschke S, Rüdiger J, Stevenson PA (2013b) Neural markers reveal a one-segmented head in tardigrades (water bears). PLoS ONE 8:e59090

Mayer G, Martin C, de Sena Oliveira I, Franke FA, Gross V (2014) Latest anomalocaridid affinities challenged. Nature 516:E1–E2

Mayer G, Oliveira IS, Baer A, Hammel JU, Gallant J, Hochberg R (2015a) Capture of prey, feeding, and functional anatomy of the jaws in velvet worms (Onychophora). Integr Comp Biol 55:217–227

Mayer G, Hering L, Stosch JM, Stevenson PA, Dircksen H (2015b) Evolution of pigment-dispersing factor neuropeptides in Panarthropoda: insights from Onychophora (velvet worms) and Tardigrada (water bears). J Comp Neurol 523:1865–1885

Mayer G, Franke FA, Treffkorn S, Oliveira IdS (2015c) Onychophora. In: Wanninger A (ed) Evolutionary Developmental Biology of Invertebrates. Springer, Berlin, pp 53–98

Minelli A, Boxshall G, Fusco G (2013) Arthropod biology and evolution: molecules, development, morphology. Springer Science & Business Media

Mittmann B, Scholtz G (2003) Development of the nervous system in the "head" of *Limulus polyphemus* (Chelicerata: Xiphosura): morphological evidence for a correspondence between the segments of the chelicerae and of the (first) antennae of Mandibulata. Dev Genes Evol 213:9–17

Mittmann B, Wolff C (2012) Embryonic development and staging of the cobweb spider *Parasteatoda tepidariorum* C. L. Koch, 1841 (syn.: *Achaearanea tepidariorum*; Araneomorphae; Theridiidae). Dev Genes Evol 222:189–216

Mizunami M, Iwasaki M, Okada R, Nishikawa M (1998) Topography of four classes of Kenyon cells in the mushroom bodies of the cockroach. J Comp Neurol 399:162–175

Møbjerg N, Halberg KA, Jørgensen A, Persson D, Bjørn M, Ramløv H, Kristensen RM (2011) Survival in extreme environments – on the current knowledge of adaptations in tardigrades. Acta Physiol 202:409–420

Müller MCM, Westheide W (2002) Comparative analysis of the nervous systems in presumptive progenetic dinophilid and dorvilleid polychaetes (Annelida) by immunohistochemistry and cLSM. Acta Zool 83:33–48

Murienne J, Daniels SR, Buckley TR, Mayer G, Giribet G (2014) A living fossil tale of Pangean biogeography. Proc R Soc B Biol Sci 281:1471–2954

Nelson DR, Guidetti R, Rebecchi L (2015) Phylum Tardigrada. In: Thorp J, Rogers DC (eds) Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates. Academic Press, pp 347–380

Nielsen C (2012) Animal Evolution: Interrelationships of the Living Phyla. Oxford University Press, Oxford, UK

Nilsson D-E (2013) Eye evolution and its functional basis. Vis Neurosci 30:5–20

Niven JE, Farris SM (2012) Miniaturization of nervous systems and neurons. Curr Biol 22:R323–R329

Northcutt RG (2012) Evolution of centralized nervous systems: two schools of evolutionary thought. Proc Natl Acad Sci USA 109:10626–10633

Oliveira IS, Mayer G (2013) Apodemes associated with limbs support serial homology of claws and jaws in Onychophora (velvet worms). J Morphol 274:1180–1190

Oliveira IS, Lacorte GA, Fonseca CG, Wieloch AH, Mayer G (2011) Cryptic speciation in Brazilian *Epiperipatus* (Onychophora: Peripatidae) reveals an underestimated diversity among the peripatid velvet worms. PLOS ONE 6:e19973

Oliveira IS, Franke FA, Hering L, Schaffer S, Rowell DM, Weck-Heimann A, Monge-Nájera J, Morera-Brenes B, Mayer G (2012) Unexplored character diversity in Onychophora (velvet worms): a comparative study of three peripatid species. PLOS ONE 7:e51220

Oliveira IS, Tait NN, Strübing I, Mayer G (2013) The role of ventral and preventral organs as attachment sites for segmental limb muscles in Onychophora. Front Zool 10:73

Oliveira IS, Bai M, Jahn H, Gross V, Martin C, Hammel JU, Zhang W, Mayer G (2016) Earliest onychophoran in amber reveals Gondwanan migration patterns. Curr Biol 26:2594–2601

Ortega-Hernández J, Budd GE (2016) The nature of non-appendicular anterior paired projections in Palaeozoic total-group Euarthropoda. Arthropod Struct Dev 45:185–199

Ortega-Hernández J, Janssen R, Budd GE (in press) Origin and evolution of the panarthropod head–A palaeobiological and developmental perspective. Arthropod Struct Dev

Osorio D, Averof M, Bacon JP (1995) Arthropod evolution: great brains, beautiful bodies. Trends Ecol Evol 10:449–454

Ou Q, Shu D, Mayer G (2012) Cambrian lobopodians and extant onychophorans provide new insights into early cephalization in Panarthropoda. Nat Commun 3:1261

Paterson JR, García-Bellido DC, Lee MSY, Brock GA, Jago JB, Edgecombe GD (2011) Acute vision in the giant Cambrian predator *Anomalocaris* and the origin of compound eyes. Nature 480:237–240

Paulus HF (1979) Eye structure and the monophyly of the Arthropoda. In: Gupta AP (ed) Arthropod Phylogeny. Van Nostrand Reinhold Company, New York, pp 299–383

Paulus HF (2000) Phylogeny of the Myriapoda — Crustacea — Insecta: a new attempt using photoreceptor structure. J Zool Syst Evol Res 38:189–208

Persson DK, Halberg KA, Jørgensen A, Møbjerg N, Kristensen RM (2012) Neuroanatomy of *Halobiotus crispae* (Eutardigrada: Hypsibiidae): tardigrade brain structure supports the clade Panarthropoda. J Morphol 273:1227–1245

Persson DK, Halberg KA, Jørgensen A, Møbjerg N, Kristensen RM (2014) Brain anatomy of the marine tardigrade *Actinarctus doryphorus* (Arthrotardigrada). J Morphol 275:173–190

Petrof I, Sherman SM (2013) Functional significance of synaptic terminal size in glutamatergic sensory pathways in thalamus and cortex. J Physiol 591:3125–3131

Pflüger HJ, Stevenson PA (2005) Evolutionary aspects of octopaminergic systems with emphasis on arthropods. Arthropod Struct Dev 34:379–396

Pflugfelder O (1948) Entwicklung von *Paraperipatus amboinensis* n. sp. Zool Jahrb Abt Anat Ontog Tiere 69:443–492

Plachetzki DC, Degnan BM, Oakley TH (2007) The origins of novel protein interactions during animal opsin evolution. PLoS ONE 2(10):e1054

Poinar G (2000) Fossil onychophorans from Dominican and Baltic amber: *Tertiapatus dominicanus* n.g., n.sp. (Tertiapatidae n.fam.) and *Succinipatopsis balticus* n.g., n.sp. (Succinipatopsidae n.fam.) with a proposed classification of the subphylum Onychophora. Invertebr Biol 119:104–109

Porter ML, Cronin TW, McClellan DA, Crandall KA (2007) Molecular characterization of crustacean visual pigments and the evolution of pancrustacean opsins. Mol Biol Evol 24:253–268

Porter ML, Blasic JR, Bok MJ, Cameron EG, Pringle T, Cronin TW, Robinson PR (2012) Shedding new light on opsin evolution. Proc R Soc B 279:3–14

Prpic N-M, Wigand B, Damen WG, Klingler M (2001) Expression of *dachshund* in wild-type and *Distal-less* mutant *Tribolium* corroborates serial homologies in insect appendages. Dev Genes Evol 211:467–477

Purschke G (2016) Annelida: basal groups and Pleistoannelida. In: Schmidt-Rhaesa A, Harzsch S, Purschke G (eds) Structure and evolution of invertebrate nervous systems. Oxford Univesity Press, Oxford, UK, pp 254–312

Ramirez M, Pairett A, Pankey M, Serb J, Speiser D, Swafford A, Oakley T (2016) The last common ancestor of most bilaterian animals possessed at least nine opsins. Genome Biol Evol:3640–3652

Ramløv H, Westh P (2001) Cryptobiosis in the eutardigrade *Adorybiotus* (*Richtersius*) *coronifer*: tolerance to alcohols, temperature and *de novo* protein synthesis. Zool Anz 240:517–523

Rebecchi L, Altiero T, Cesari M, Bertolani R, Rizzo AM, Corsetto PA, Guidetti R (2011) Resistance of the anhydrobiotic eutardigrade *Paramacrobiotus richtersi* to space flight (LIFE–TARSE mission on FOTON-M3). J Zool Syst Evol Res 49 (Suppl. 1):98–103

Rehm P, Borner J, Meusemann K, von Reumont BM, Simon S, Hadrys H, Misof B, Burmester T (2011) Dating the arthropod tree based on large-scale transcriptome data. Mol Phylogenet Evol 61:880–887

Reisinger E (1925) Untersuchungen am Nervensystem der *Bothrioplana semperi* Braun. (Zugleich ein Beitrag zur Technik der vitalen Nervenfärbung und zur vergleichenden Anatomie des Plathelminthennervensystems.). Z Morph Ökol Tiere5:119–149

Reisinger E (1972) Die Evolution des Orthogons der Spiralier und das Archicölomatenproblem. Z zool Syst Evolutionsforsch 10:1–43

Reuter M, Mäntylä K, Gustafsson MKS (1998) Organization of the orthogon — main and minor nerve cords. Hydrobiologia 383:175–182

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 CM, Kaul S, Møller OS, Müller CHG, Rieger V, Rothe BH, Stegner MEJ, Harzsch S (2010) Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. Front Zool 7:29

Richter S, Stein M, Frase T, Szucsich NU (2013) The arthropod head. In: Minelli A, Boxshall G, Fusco G (eds) Arthropod Biology and Evolution. Springer, Heidelberg, pp 223–240

Rogers BT, Kaufman TC (1997) Structure of the insect head in ontogeny and phylogeny: a view from *Drosophila*. Int Rev Cytol 174:1–84

Rota-Stabelli O, Kayal E, Gleeson D, Daub J, Boore J, Telford M, Pisani D, Blaxter M, Lavrov D (2010) Ecdysozoan mitogenomics: evidence for a common origin of the legged invertebrates, the Panarthropoda. Genome Biol Evol 2:425–440

Rota-Stabelli O, Daley AC, Pisani D (2013) Molecular timetrees reveal a Cambrian colonization of land and a new scenario for ecdysozoan evolution. Curr Biol 23:1–7

Rothe BH, Schmidt-Rhaesa A (2010) Structure of the nervous system in *Tubiluchus troglodytes* (Priapulida). Invertebr Biol 129:39–58

Ruhberg H, Mayer G (2013) Onychophora, Stummelfüßer. In: Westheide W, Rieger G (eds) Spezielle Zoologie Teil 1: Einzeller und Wirbellose Tiere. Springer-Verlag, Berlin, pp 457–464

Ruhberg H, Tiemann H, Mette A, Rhode B (2001) Evolutionäre Aspekte der Augen-Rückbildung beim hemiedaphischen Onychophoren *Tasmanipatus anophthalmus* Ruhberg et al., 1991 (Peripatopsidae). Mitt Hambg Zool Mus Inst 98:31–50

Sanchez S (1958) Cellules neurosécrétrices et organes infracérébraux de *Peripatopsis moseleyi* Wood (Onychophores) et neurosécrétion chez *Nymphon gracile* Leach (Pycnogonides). Arch Zool Exp Gen [Notes Rev] 96:57–62

Schoenemann B, Liu JN, Shu DG, Han J, Zhang ZF (2009) A miniscule optimized visual system in the Lower Cambrian. Lethaia 42:265–273

Schmidt-Rhaesa A (2001) Tardigrades — Are they really miniaturized dwarfs? Zool Anz 240:549–555

Schmidt-Rhaesa A (2007) The Evolution of Organ Systems. Oxford University Press, Oxford

Schmidt-Rhaesa A, Henne S (2016) Cycloneuralia (Nematoda, Nematomorpha, Priapulida, Kinorhyncha, Loricifera). In: Schmidt-Rhaesa A, Harzsch S, Purschke G (eds) Structure and evolution of invertebrate nervous systems. Oxford University Press, Oxford, UK, pp 368–381

Schmidt-Rhaesa A, Harzsch S, Purschke G (2016) Structure and Evolution of Invertebrate Nervous Systems. Oxford University Press, Oxford

Schokraie E, Hotz-Wagenblatt A, Warnken U, Frohme M, Dandekar T, Schill RO, Schnölzer M (2011) Investigating heat shock proteins of tardigrades in active versus anhydrobiotic state using shotgun proteomics J Zool Syst Evol Res 49 (Suppl. 1):111–119

Scholtz G (2002) The Articulata hypothesis — or what is a segment? Org Divers Evol 2:197–215

Scholtz G (2016) Perspective—heads and brains in arthropods: 40 years after the'endless dispute'. In: Schmidt-Rhaesa A, Harzsch S, Purschke G (eds) Structure and Evolution of Invertebrate Nervous Systems. Oxford University Press, Oxford, pp 402–410

Scholtz G, Edgecombe GD (2006) The evolution of arthropod heads: reconciling morphological, developmental and palaeontological evidence. Dev Genes Evol 216:395–415

Schulze C, Persson D (2016) Tardigrada. In: Schmidt-Rhaesa A, Harzsch S, Purschke G (eds) Structure and Evolution of Invertebrate Nervous Systems. Oxford University Press, Oxford, pp 383–389

Schulze C, Neves RC, Schmidt-Rhaesa A (2014) Comparative immunohistochemical investigation on the nervous system of two species of Arthrotardigrada (Heterotardigrada, Tardigrada). Zool Anz 253:225–235

Schumann I, Hering L, Mayer G (2016) Immunolocalization of Arthropsin in the onychophoran *Euperipatoides rowelli* (Peripatopsidae). Front Neuroanat 10

Schürmann FW (1987) Histology and ultrastructure of the onychophoran brain. In: Gupta AP (ed) Arthropod Brain, its Evolution, Development, Structure, and Functions. John Wiley & Sons, New York, pp 159–180

Schürmann FW (1995) Common and special features of the nervous system of Onychophora: a comparison with Arthropoda, Annelida and some other invertebrates. In: Breidbach O, Kutsch W (eds) The Nervous System of Invertebrates: An Evolutionary and Comparative Approach. Birkhäuser, Basel, pp 139–158

Schürmann FW, Sandeman DC (1976) Giant fibres in the ventral nerve cord of *Peripatoides leuckarti* (Onychophora). Naturwissenschaften 63:580–581

Sedgwick A (1885) The development of *Peripatus capensis* Proc R Soc Lond B Biol Sci 38:354–361

Sedgwick A (1887) The development of the Cape species of peripatus. Part III. On the changes from stage A to stage F. Q J Microsc Sci 27:467–550

Sedgwick A (1895) Peripatus. In: Harmer SF, Shipley AE (eds) Peripatus , Myriapods and Insects, part I. MacMillan & Co., London, pp 3–26

Shichida Y, Matsuyama T (2009) Evolution of opsins and phototransduction. Philos Trans R Soc B Biol Sci 364:2881–2895

Simonnet F, Deutsch J, Quéinnec E (2004) *hedgehog* is a segment polarity gene in a crustacean and a chelicerate. Dev Genes Evol 214:537–545

Skeath JB, Panganiban G, Selegue J, Carroll SB (1992) Gene regulation in two dimensions: the proneural *achaete* and *scute* genes are controlled by combinations of axis-patterning genes through a common intergenic control region. Genes Dev 6:2606–2619

Smith FW, Goldstein B (in press) Segmentation in Tardigrada and diversification of segmental patterns in Panarthropoda. Arthropod Struct Dev

Smith FW, Jockusch EL (2014) The metameric pattern of *Hypsibius dujardini* (Eutardigrada) and its relationship to that of other panarthropods. Front Zool 11:66

Smith FW, Boothby TC, Giovannini I, Rebecchi L, Jockusch EL, Goldstein B (2016) The compact body plan of tardigrades evolved by the loss of a large body region. Curr Biol 26:224–229

Smith MR, Ortega-Hernández J (2014) *Hallucigenia*'s onychophoran-like claws and the case for Tactopoda. Nature 514:363–366

Stein M (2010) A new arthropod from the Early Cambrian of North Greenland, with a 'great appendage'-like antennula. Zool J Linn Soc 158:477–500

Strausfeld NJ, Weltzien P, Barth FG (1993) Two visual systems in one brain: neuropils serving the principal eyes of the spider *Cupiennius salei*. J Comp Neurol 328:63–75

Strausfeld NJ, Strausfeld C, Stowe S, Rowell D, Loesel R (2006a) The organization and evolutionary implications of neuropils and their neurons in the brain of the onychophoran *Euperipatoides rowelli*. Arthropod Struct Dev 35:169–196

Strausfeld NJ, Strausfeld CM, Loesel R, Rowell D, Stowe S (2006b) Arthropod phylogeny: onychophoran brain organization suggests an archaic relationship with a chelicerate stem lineage. Proc R Soc Biol Sci Ser B 273:1857–1866

Strausfeld NJ, Ma X, Edgecombe GD (2016) Fossils and the evolution of the arthropod brain. Curr Biol 26:R989–R1000

Telford MJ, Thomas RH (1998) Expression of homeobox genes shows chelicerate arthropods retain their deutocerebral segment. Proc Natl Acad Sci USA 95:10671–10675

Tiegs OW (1940) The embryology and affinities of the Symphyla, based on a study of *Hanseniella agilis*. Q J Microsc Sci 82:1–225

Tiegs OW (1947) The development and affinities of the Pauropoda, based on a study of *Pauropus silvaticus*. Part I. Q J Microsc Sci 88:165–267

Tsujimoto M, Imura S, Kanda H (2016) Recovery and reproduction of an Antarctic tardigrade retrieved from a moss sample frozen for over 30 years. Cryobiology 72:78-81

Ungerer P, Wolff C (2005) External morphology of limb development in the amphipod *Orchestia cavimana* (Crustacea, Malacostraca, Peracarida). Zoomorphology 124:89–99

von Kennel J (1888) Entwicklungsgeschichte von *Peripatus edwardsii* Blanch. und *Peripatus torquatus* n. sp. II. Theil. Arb Zool-Zootom Inst Würzburg 8:1–93

Vopalensky P, Kozmik Z (2009) Eye evolution: common use and independent recruitment of genetic components. Philos Trans R Soc B Biol Sci 364:2819–2832

Walker MH, Tait NN (2004) Studies on embryonic development and the reproductive cycle in ovoviviparous Australian Onychophora (Peripatopsidae). J Zool 264:333–354

Waloszek D, Chen J, Maas A, Wang X (2005) Early Cambrian arthropods — new insights into arthropod head and structural evolution. Arthropod Struct Dev 34:189–205

Whitington PM (2007) The evolution of arthropod nervous systems: insights from neural development in the Onychophora and Myriapoda. In: Striedter GF, Rubenstein JLR (eds) Theories, Development, Invertebrates. Academic Press, Oxford, pp 317–336

Whitington PM, Bacon JP (1997) The organization and development of the arthropod ventral nerve cord: insight into arthropod relationships. In: RA F, RH T (eds) Arthropod Relationships. Chapman & Hall, London, pp 349–367

Whitington P, Mayer G (2011) The origins of the arthropod nervous system: insights from the Onychophora. Arthropod Struct Dev 40:193–209

Wiederhöft H, Greven H (1999) Notes on head sensory organs of *Milnesium tardigradum* Doyère, 1840 (Apochela, Eutardigrada). Zool Anz 238:338–346

Yang J, Ortega-Hernández J, Butterfield NJ, Liu Y, Boyan GS, Hou J-b, Lan T, Zhang X-g (2016) Fuxianhuiid ventral nerve cord and early nervous system evolution in Panarthropoda. Proc Natl Acad Sci USA 113:2988–2993

Zantke J, Wolff C, Scholtz G (2008) Three-dimensional reconstruction of the central nervous system of *Macrobiotus hufelandi* (Eutardigrada, Parachela): implications for the phylogenetic position of Tardigrada. Zoomorphology 127:21–36

Zhang Z-Q (2011) Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Richness. Zootaxa 3148:1–237

Zhang Z-Q (2013) Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Richness (Addenda 2013). Zootaxa 3703:1–82

Figure Legends

Fig. 1 Representatives of the three major panarthropod groups and their current phylogenetic positions within the Ecdysozoa. **a** Macrograph of the onychophoran *Principapillatus hitoyensis*. **b** False-coloured scanning electron micrograph of the tardigrade *Hypsibius dujardini*. **c** Macrograph of the hexapod *Acheta domesticus*. **d** Phylogenetic tree of all ecdysozoan subclades with alternative hypotheses of the position of tardigrades (*green*) (modified from Mayer et al. 2013a)

Fig. 2 Organisation of the tardigrade nervous system. Anterior is left in all images. **a** Light micrograph of the tardigrade *Hypsibius dujardini*. **b, c** Anti-α-tubulin immunolabelling showing the brain (**b**) and trunk ganglia 2 and 3 (**c**) of *Macrobiotus* cf. *harmsworthi*. **d** Simplified diagrams illustrating the organisation of the tardigrade nervous system (in ventral and lateral views) based on anti-α-tubulin immunolabelling (original data from Mayer et al. 2013a, creative common license of BMC Evol Biol). *al*, anterior leg nerve; *as*, anterolateral sensory field; *br*, brain; *cn*, connectives; dn, dorsal nerve; *ey*, eye; *ga1–4*, ganglia 1–4; gu, gut; *ic*, inner connective; *ip*, interpedal commissure; *lg*, leg ganglion; *le1–4*, legs 1–4; *ln*, lateral nerve; *mo*, mouth; *ne*, neurites supplying the peribuccal lamellae; *np*, central brain neuropil; *nr*, nerve ring; *oc*, outer connective; *pg*, peripheral ganglion; *pl*, posterior leg nerve; *pn*, peripheral nerve; *ps*, posterolateral sensory field. *Scale bars* **a** 25 μm, **b, c** 10 μm

Fig. 3 Organisation of the onychophoran nervous system. Anterior is left in all images. **a** Macrograph of a walking specimen of the onychophoran *Euperipatoides rowelli*. **b, c** Anti-α-tubulin immunolabelling in the brain (**b**) as well as the ventral and peripheral nervous system (**c**) of *Principapillatus hitoyensis*. Asterisks indicate regions of the leg nerves. **d** Simplified diagrams showing the organization of the onychophoran nervous system (in dorsal and lateral views; for the sake of clarity, only the brain and the ventral nervous system are illustrated in dorsal view; modified from Martin et al. 2017). *an*, antenna; *at*, antennal tract; *br*, brain; *cc*, connecting cords; *dl*, dorsolateral nerve; *ey*, eye; *hn*, heart nerve; *ho*, hypocerebral organ; *jn*, jaw nerve; *le*, leg; *ln*, leg nerves; *mc*, median commissure; *mo*, mouth; *nc*, nerve cords; *np*, central brain neuropil; *ph*, pharyngeal loop nerve; *rc*, ring commissure; *sn*, slime papilla nerves; *sp*, slime papilla. *Scale bars* **b, c** 100 μm

Fig. 4 Two alternative hypotheses of the evolution of ganglia within Panarthropoda depending on the position of onychophorans as sister to tardigrades + arthropods (**a**) or sister to arthropods (**b**). Note that both the evolution of ganglia and the reduction of commissures

might have occurred convergently in arthropods and tardigrades in scenario **a**. *grey dots*, distribution patterns of generic neuronal cell bodies; *orange dots*, distribution of motor neurons supplying the legs. Note that two pairs of segmental leg nerves are present in onychophorans and tardigrades while only one pair occurs in arthropods. Ground patterns based on Yang et al. (2016) and Martin et al. (2017). Diagrams of the onychophoran and arthropod nervous systems based on Mayer and Harzsch (2007, 2008) and Richter et al. (2010), respectively

Fig. 5 Anti-synapsin immunoreactivity in the panarthropod ventral nervous system. **a** Dissected ventral nerve cord of the onychophoran *Euperipatoides rowelli*. **b** Two consecutive trunk ganglia of the tardigrade *Hypsibius dujardini*; whole mount. C Two dissected, consecutive abdominal ganglia and connectives of the arthropod *Schistocerca gregaria*. Maximum projections of confocal micrographs. Anterior is up in all images. Note the ubiquitous labelling of synapsin immunoreactivity in the neuropil of the onychophoran nerve cord, whereas both tardigrades and arthropods show a strong immunoreactivity only within their ganglia. The weak signal in the connectives of *S. gregaria* is background due to synapsin transport between ganglia. *ag* abdominal ganglion; *cn*, connective; *ga2, ga3*, second and third trunk ganglia; *ln*, leg nerve; *mc*, median commissure; *rc*, ring commissure. *Scale bars* **a, c** 100 μm, **b** 5 μm

Fig. 6 Organisation of the tardigrade brain as revealed by different neuronal markers. Anterior is right in all images. Maximum projection confocal micrographs labelled with markers against **a** DNA (*dashed line* demarcates the brain), **b** serotonin and **c** RFamide in *Macrobiotus* cf. *harmsworthi* **d** perisulfakinin in *Hypsibius dujardini* and **e** allatostatin in *Paramacrobiotus richtersi* (original data from Mayer et al. 2013a, creative common license of BMC Evol Biol). *ic*, inner connectives; *il*, inner lobe of the brain; *ml* median lobe of the brain; *oc*, outer connectives; *ol*, outer lobe of the brain. *Scale bars* **a, b, d, e** 10 μm, **c** 5μm

Fig. 7 Organisation of the onychophoran brain. Anterior is up in all images. **a** Diagram illustrating the segmental composition of the brain based on the position of somata of nerves supplying the three anterior-most pairs of segmental appendages, antennae (*blue*), jaws (*purple*), and slime papillae (*yellow*), respectively (indicated only on the left side). **b** Diagram of the onychophoran brain in dorsal view showing the major neuropils (*blue*) (modified from Martin and Mayer 2014; Mayer 2016 based on Schürmann 1987; Strausfeld et al. 2006a; Mayer et al. 2010). **c–i** Light micrographs of histological sections through the onychophoran brain labelled with the AZAN technique (Heidenhain 1915, modified by Geidies 1954).

Arrows point to the position of a putative frontal ganglion as suggested by Cong et al. (2014), which remains controversial (see critical discussion by Mayer et al. 2014). *ag*, antennal glomeruli; *al*, accessory lobe of the mushroom body; *an*, antenna; *at*, antennal tract; *cb*, central body; *dc*, deutocerebrum; *ey*, eye; *jn*, jaw nerve; *L1–L3*, lip papillae nerves 1 to 3; *mb*, mushroom body; *mc*, median commissure; *np*, major neuropil; *pc*, protocerebrum; *ot*, optic tract; *pd*, pedunculus; *ph1, ph2*, pharyngeal nerves 1 and 2; *pl*, perikaryal layer; *sn*; slime papilla nerves; *sp*, slime papilla; *vn*, visual neuropil. *Scale bar* **c** 100 μm

Fig. 8 Simplified diagrams of neuronal fibres and cell bodies supplying the lip papillae nerves (*green*), pharyngeal nerves (*blue*) and the six anterior-most post-oral commissures (*glow*) as revealed by neuronal tracing experiments. Diagram of lip papillae nerves is modified from Martin and Mayer (2014), diagrams illustrating pharyngeal nerves as well as post-oral commissures are modified after Martin and Mayer (2015). *cc*, connecting cord; *dc*, deutocerebrum; *ey*, eye; *jn*, jaw nerve; *L1–L3*, lip papillae nerves 1–3; *mc*, median commissure; *nc*, nerve cord; *pc*, protocerebrum; *ph1, ph2*, pharyngeal nerves 1 and 2; *sn*, slime papilla nerves; *#1–6*, post-oral commissures 1 to 6

Fig. 9 Development of the nervous system in onychophorans (**a–d**) and tardigrades (**e–f**). For the onychophoran, only the anterior 4 (in **a, b**) or 5 (in **c, d**) segments are shown. For the tardigrade, the entire body (five segments) is shown. Arrowheads (in **c, d**) represent developing brain neuropils. *Black*, central nervous system; *blue*, median/transverse commissures; *green*, leg nerves; *orange*, stomodaeal nervous system; *red*, ring commissures/peripheral nerves. Diagram of tardigrade development modified from Gross and Mayer (2015); onychophoran development based on Mayer and Whitington (2009a) and Mayer et al. (2010). *an*, antenna; *av*, anteroventral cells; *c1*, developing commissure of the first ganglion; *cb*, developing central brain neuropil; *g1–g4*, anlagen of ganglia 1 to 4; *jw*, jaw; *l1, l2*, legs 1 and 2; *lg*, developing leg ganglion; *nc*, developing neuropil of the nerve cord; *oc*, outer connective; *pl*, posterior leg nerve; *sp*, developing slime papilla

Fig. 10 Visual organs and evolution of opsins in Panarthropoda. **a** Macrograph showing the position of the eye (*orange arrow*) in the onychophoran *Euperipatoides rowelli*. **b** Light micrograph showing the eyes (*orange arrows*) of the tardigrade *Hypsibius dujardini*. **c** Macrograph of the head of the arthropod *Gryllus bimaculatus. Orange arrows* point to ocelli, *orange arrowheads* indicate compound eyes. **d** Hypothesis of the evolution of opsins suggesting the presence of five opsin genes in the last common ancestor of Panarthropoda: two IV-tetraopsins (*green*) and three I-canonical visual opsins [two canonical r-opsins (*blue*)

and one canonical c-opsin (*red*)]. Three copies of c-opsins might have evolved within the tardigrade lineage, while one r-opsin copy may have been lost. Modified from Hering and Mayer (2014) and Ramirez et al. (2016) **e** Cryosection showing the expression of the visual r-opsin *Er-onychopsin* exclusively in the photoreceptor layer (*black arrowhead*) in the eye of *E. rowelli*. *ey*, eye. *Scale bar* **e** 250 μm

Fig. 11 Position of the eyes in two onychophoran species: *Euperipatoides rowelli* (**a, c**) and the eyeless, putatively blind species *Tasmanipatus anophthalmus* (**b, d**). Anterior is left in **a, b** and up in **c, d**. (**a, b**) Scanning electron micrographs of the cuticle covering the eyes in *E. rowelli* and *T. anophthalmus* (*arrows*). Note the absence of external eye structure in *T. anophthalmus*. (**c, d**) Artificially highlighted eyes (*glow*) of horizontal cryosections labelled with a DNA marker and μCT image (*insets*) showing the eye in *E. rowelli* and the remnants of the eye in *T. anophthalmus* (*arrows*). *an*, antenna; *br*, brain; *co*, cornea; *pc*, photoreceptor cell layer; *vb*, vitreous body. *Scale bars* 50 μm

Onychophora (a)

Tardigrada (b)

Arthropoda (c)

(d)

Cycloneuralia

Nematomorpha

Kinorhyncha

Priapulida

Loricifera

Nematoda

Tardigrada

Tardigrada

Onychophora

Tardigrada

Tardigrada

Arthropoda

Panarthropoda
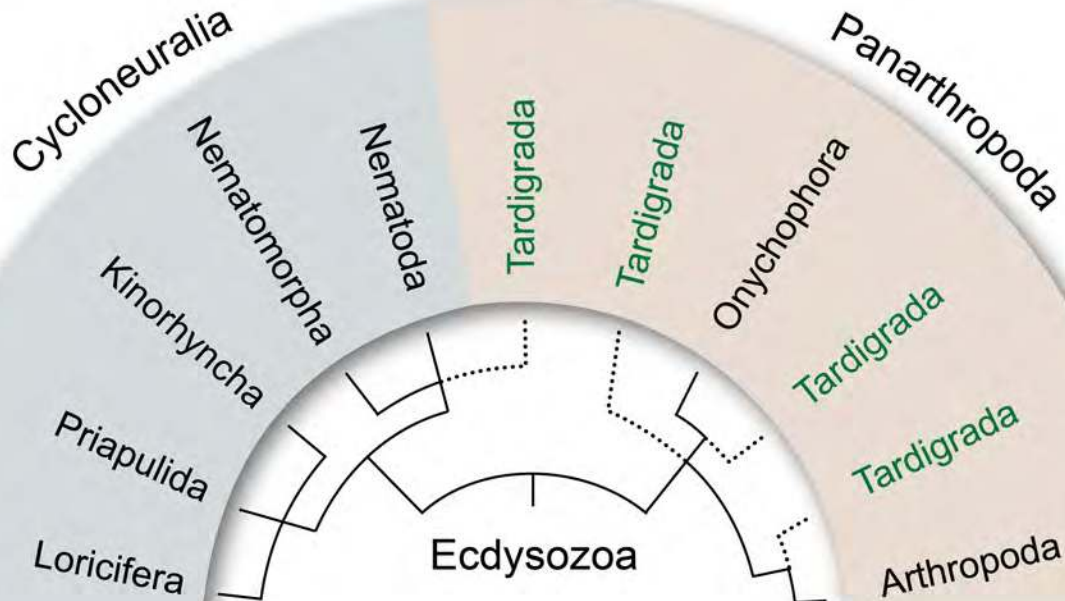
Ecdysozoa
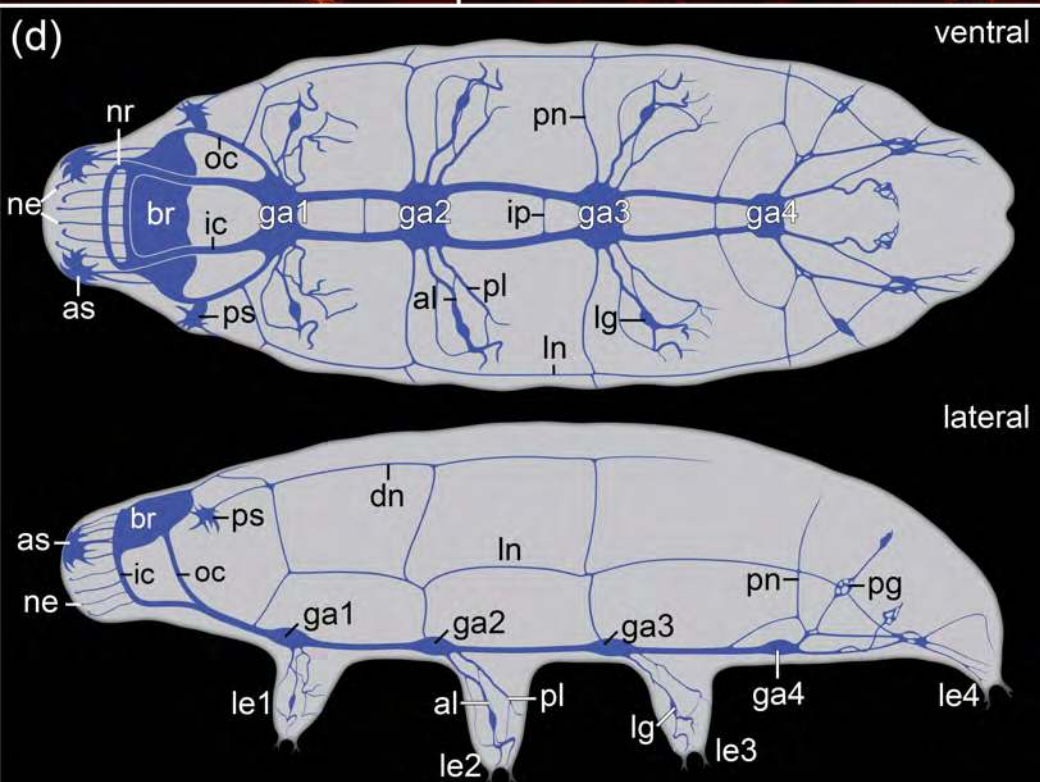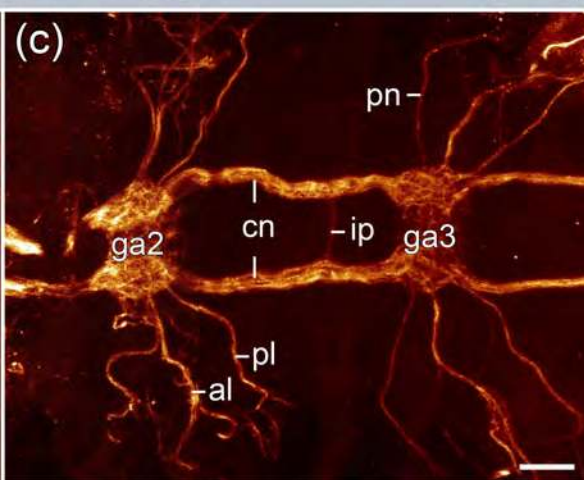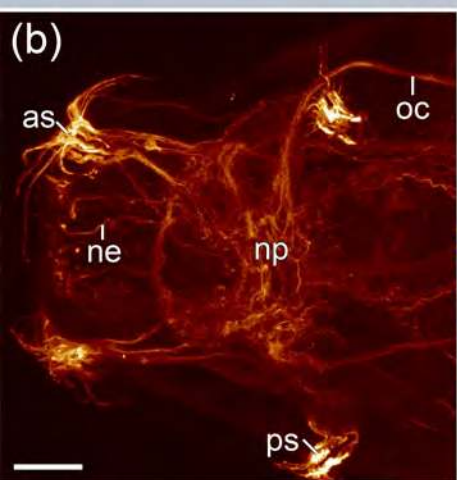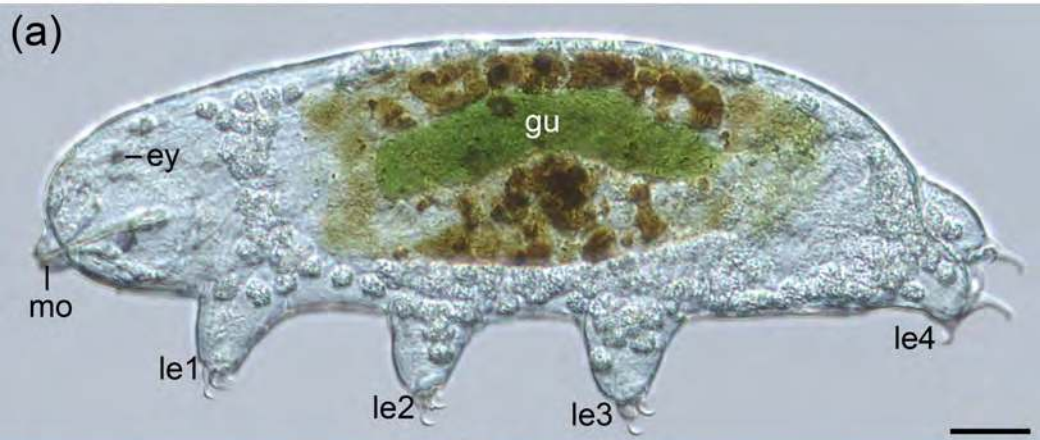
Tardigrada
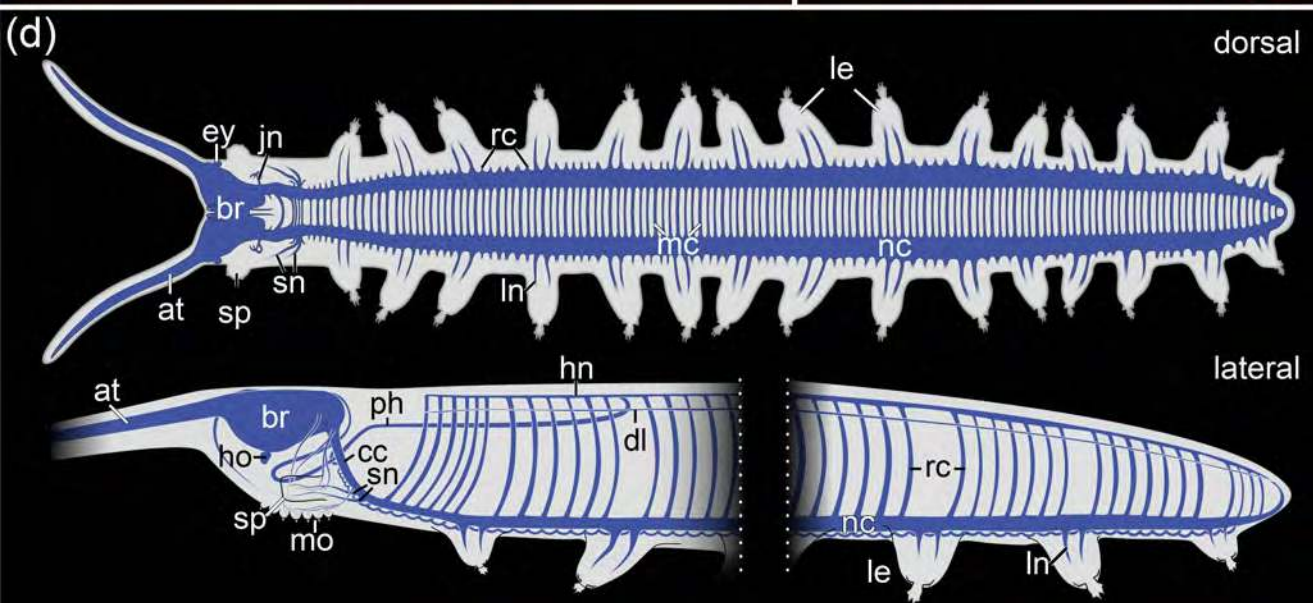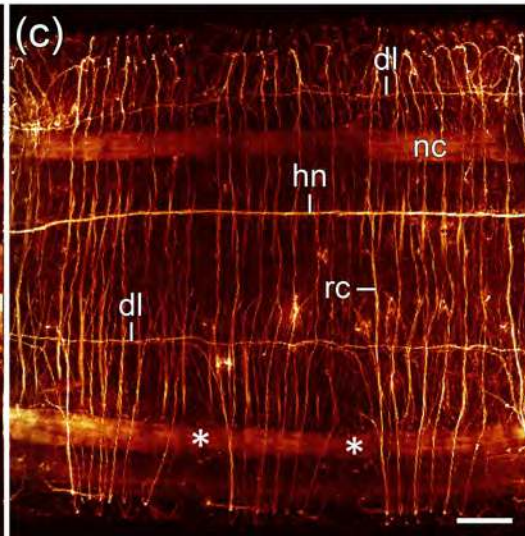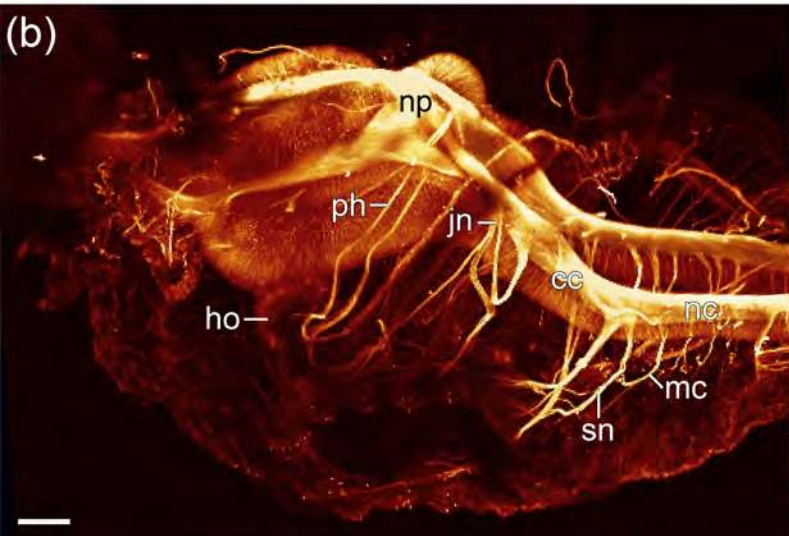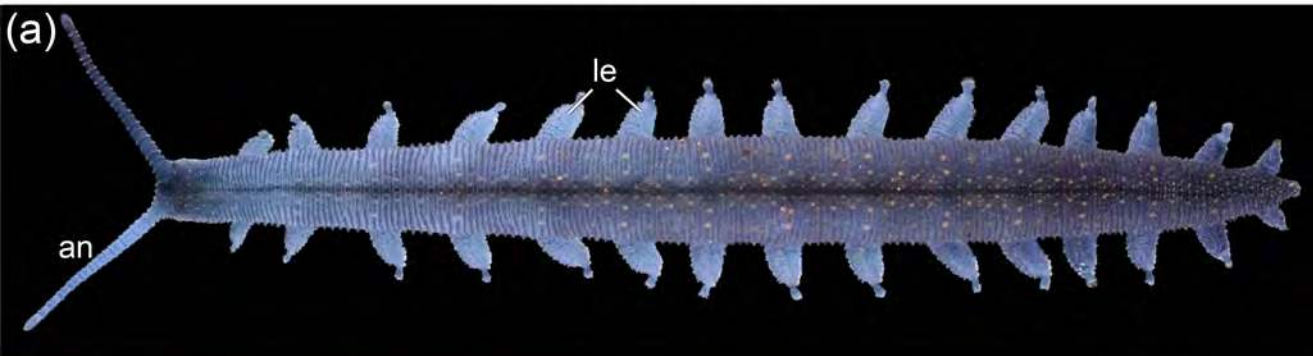
Onychophora

Cycloneuralia   Onychophora   Arthropoda   Tardigrada
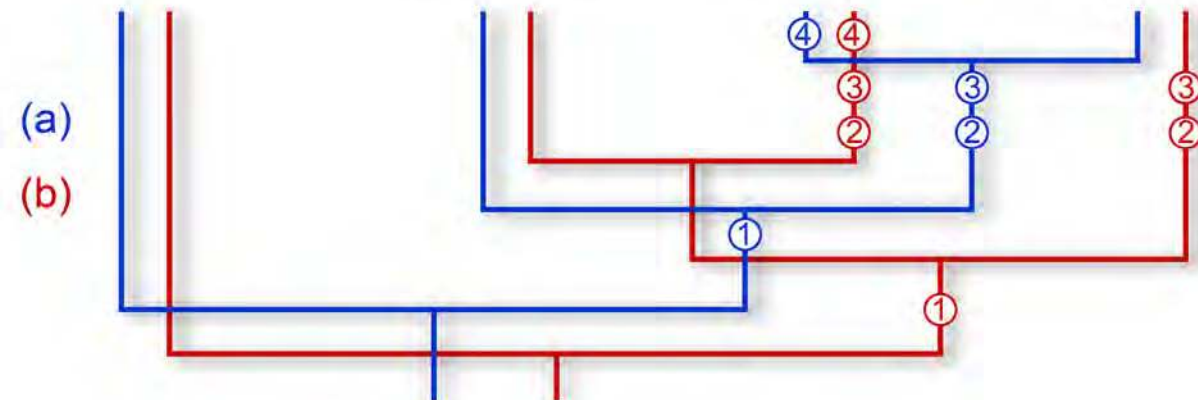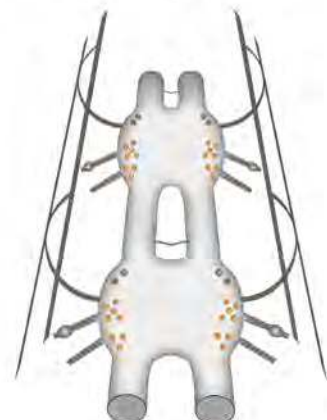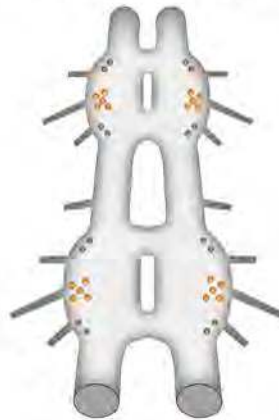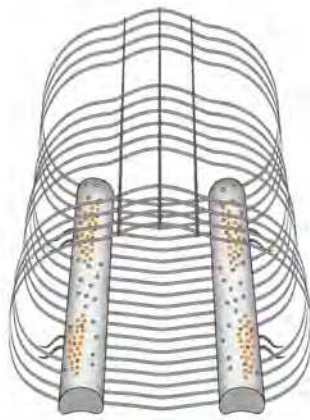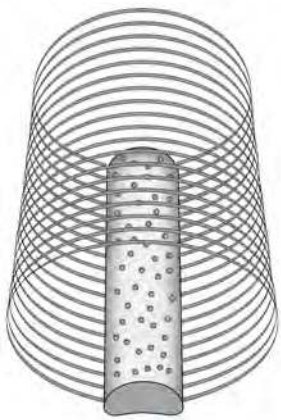
(a)

(b)
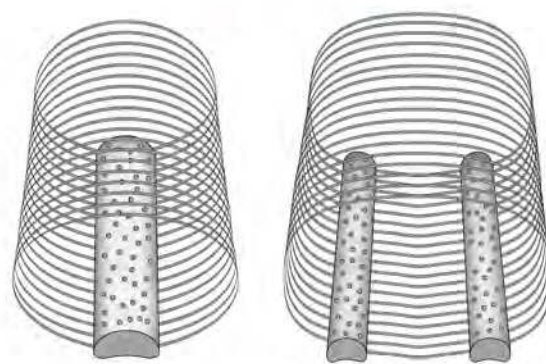
Last common ancestor of Ecdysozoa

① Paired, segmentally repeated leg nerves

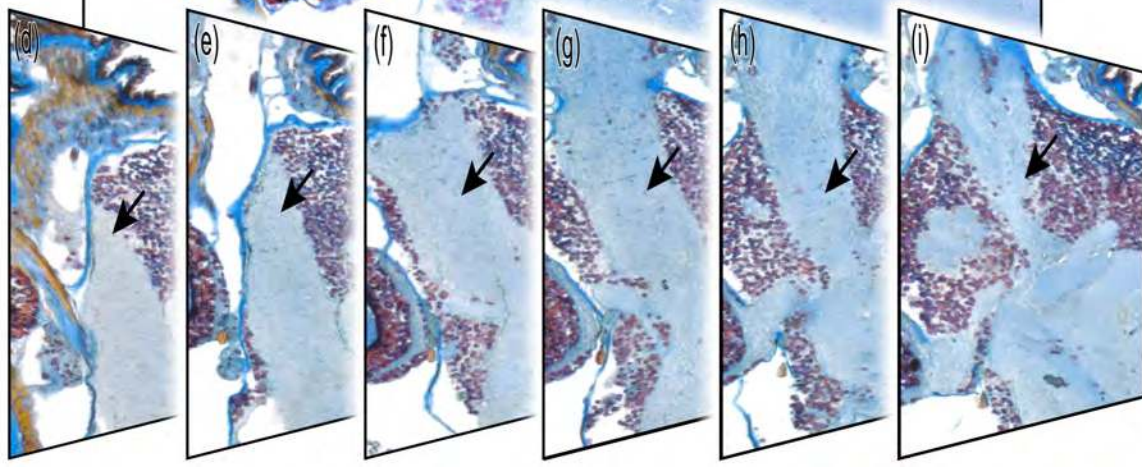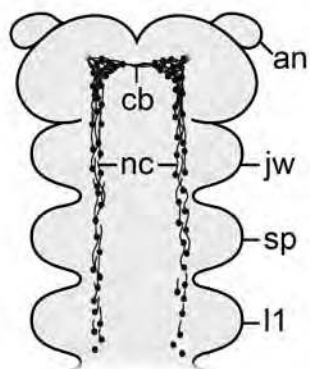② Segmental ganglia

③ Reduction of ring and median commissures

④ Reduction/fusion of leg nerves

Onychophora	Tardigrada	Arthropoda

Tardigrada

(a) nuclear marker — ol, il, ml

(b) anti-serotonin-like — oc, oc

(c) anti-RFamide-like — oc, ic, oc

(d) anti-perisulfakinin-like — ic

(e) anti-allatostatin-like
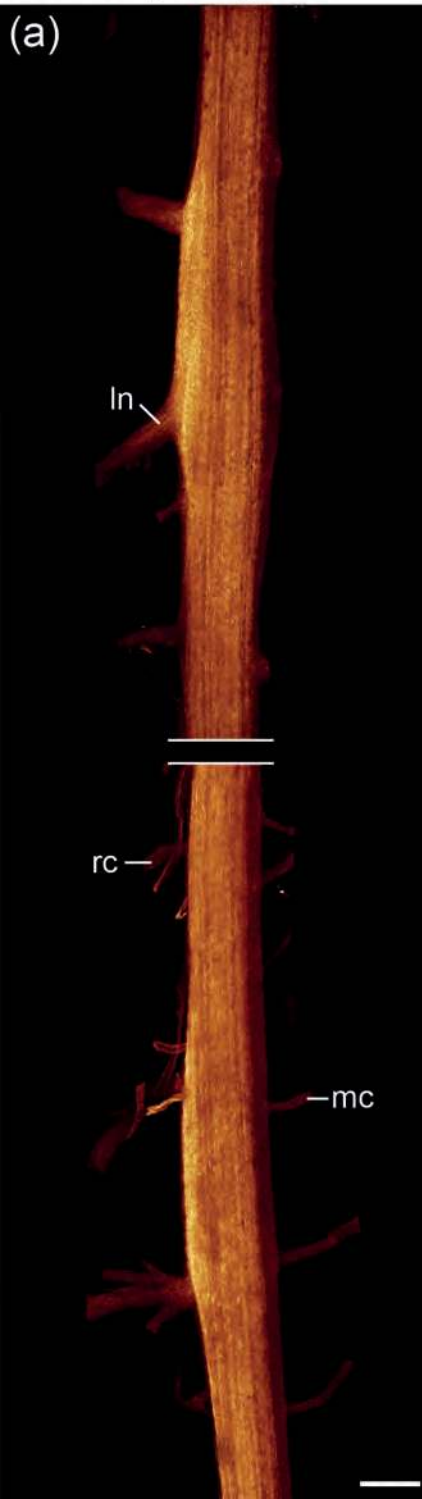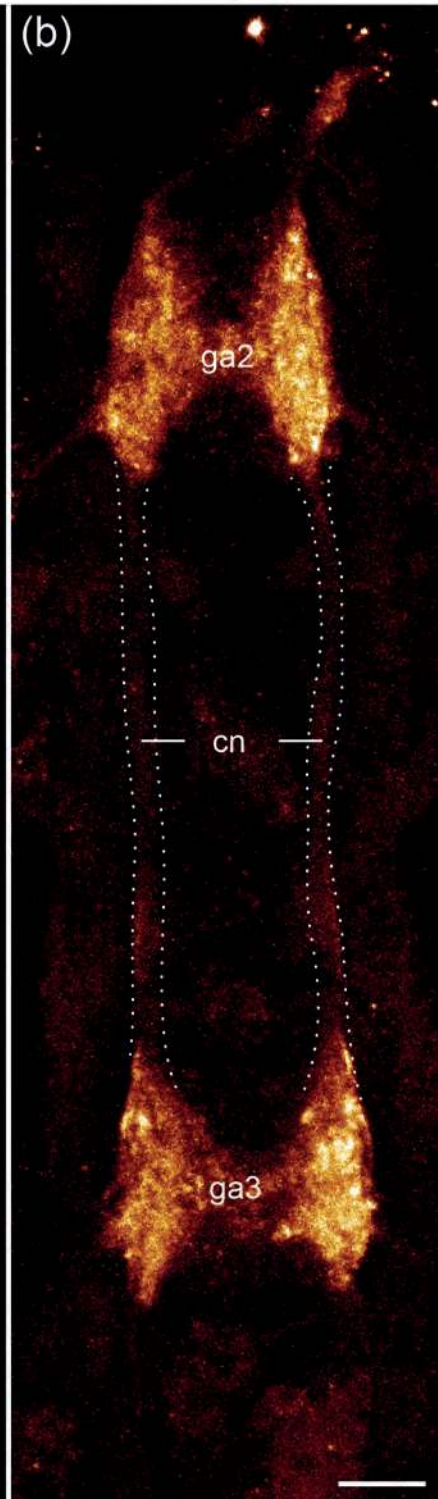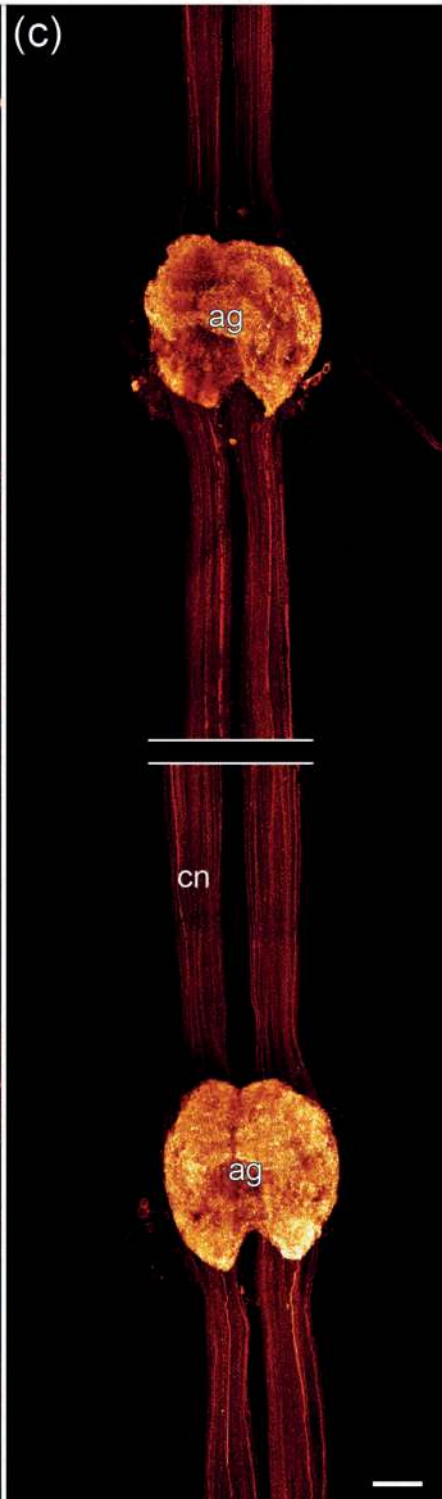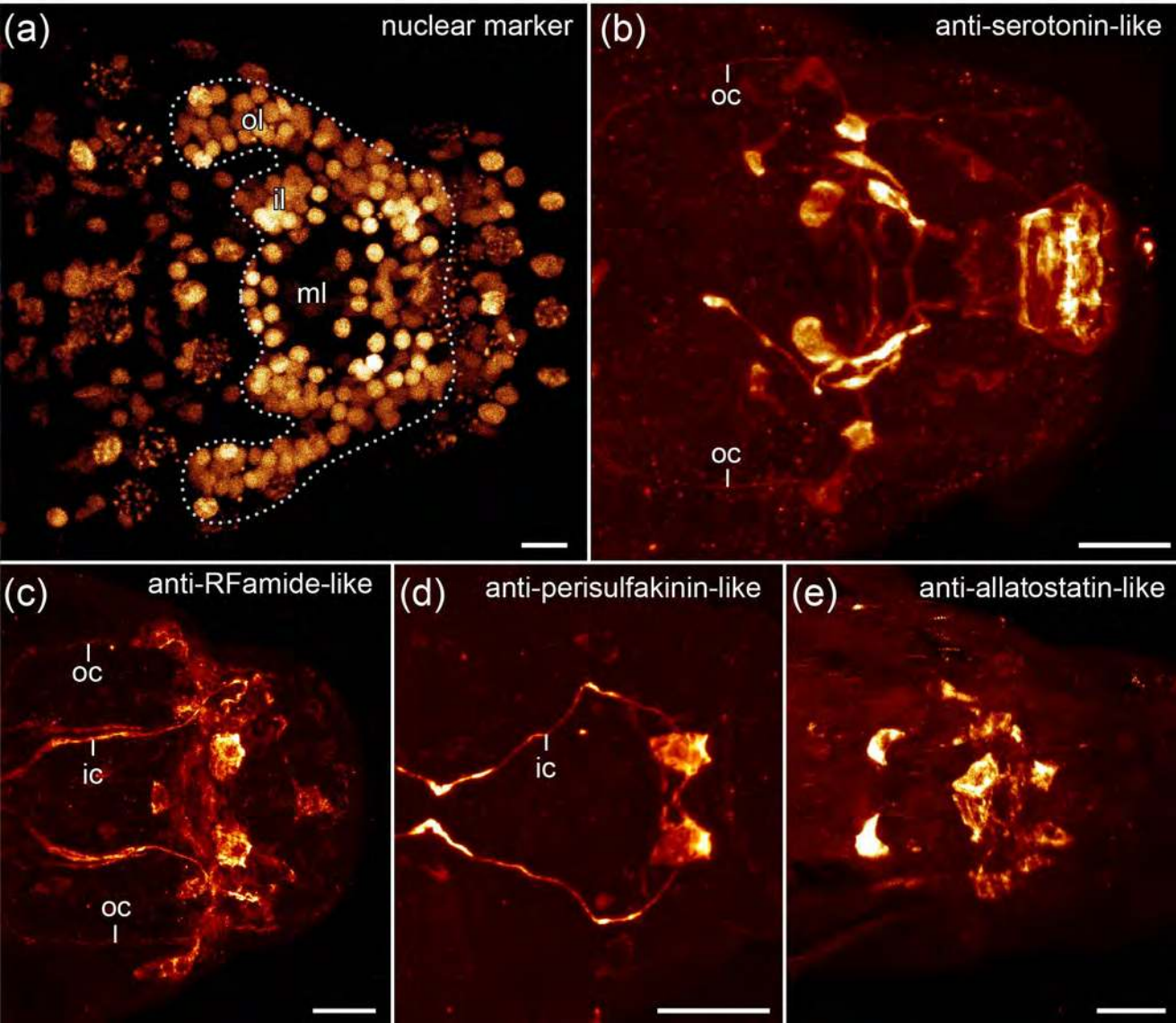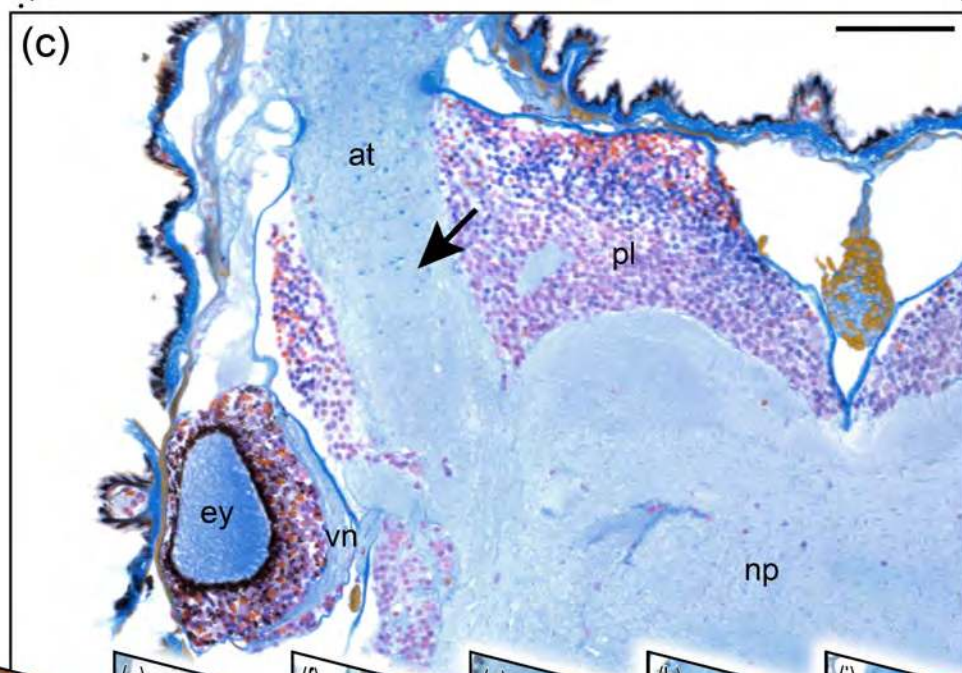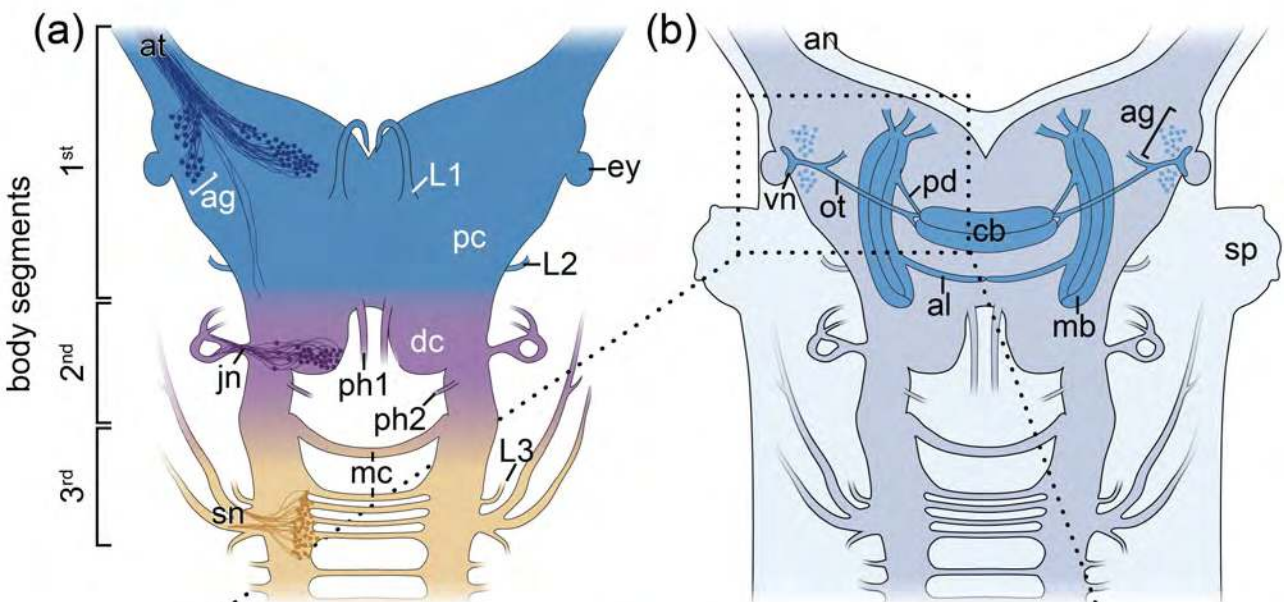
Onychophora

Onychophora
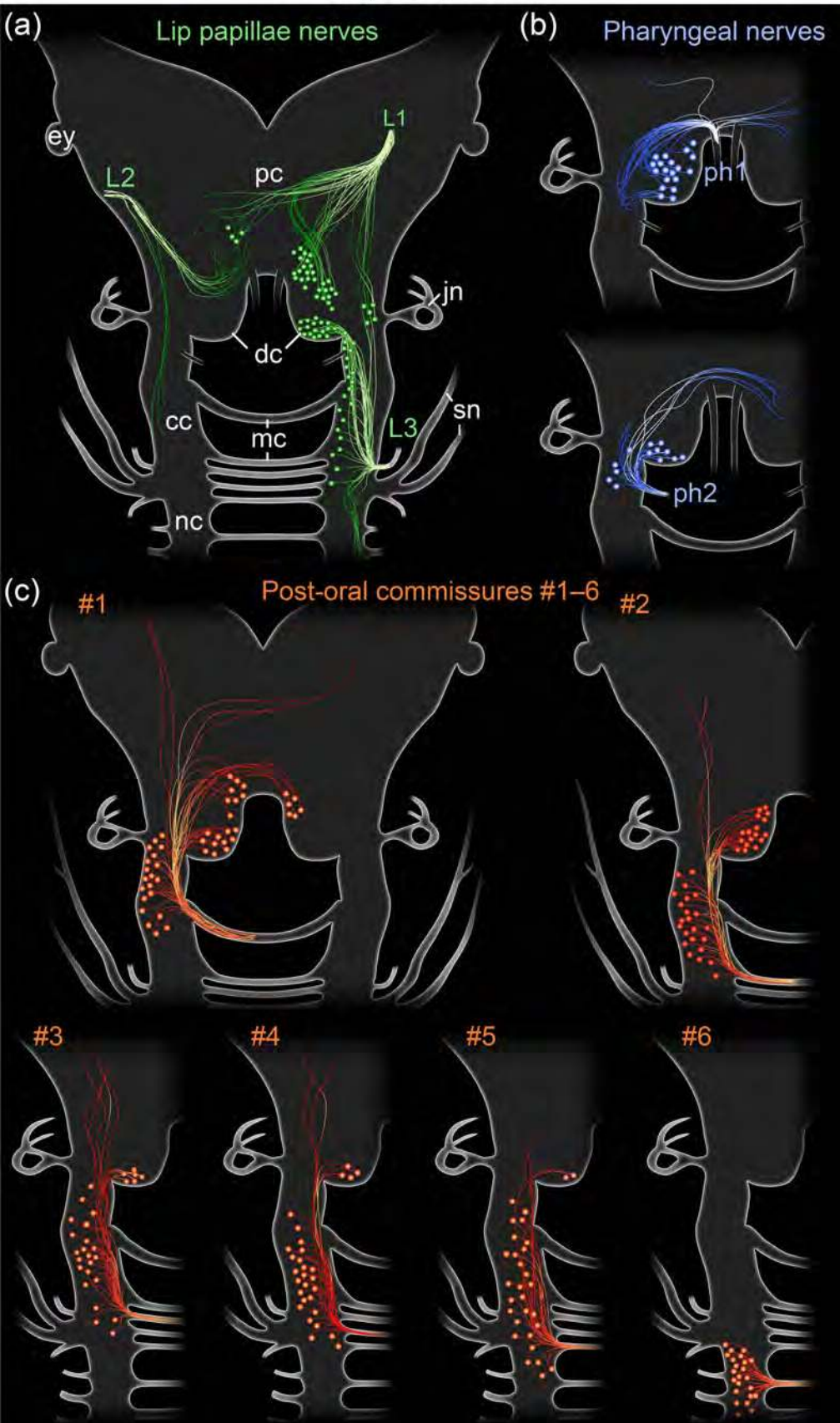
(a) Lip papillae nerves

(b) Pharyngeal nerves

(c) Post-oral commissures #1–6

Onychophora

Tardigrada

(a)
- an
- cb
- nc
- jw
- sp
- l1

(e)
- cb
- av
- g1
- g2
- g3

(b)
- an
- cb
- jw
- sp
- l1

(f)
- cb
- g1
- g2
- g3
- g4

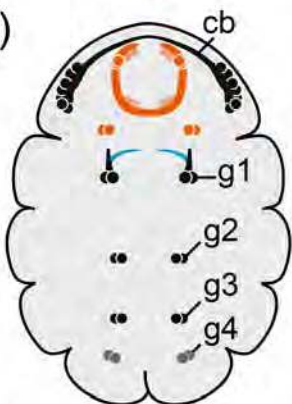(c)
- an
- sp
- l1
- l2

(g)
- cb
- c1
- lg

(d)
- an
- sp
- l1
- l2

(h)
- oc
- pl

(a)

(b)

(c)

(d) Onychophora   Tardigrada   Arthropoda

(e) *Er-onychopsin*

ey

duplication ●
gene loss ✕
terminal diversification ◢
canonical-opsins ▬
neuropsins ▬
peropsins ▬
arthropsins ▬
visual r-opsin ▬
IV-tetraopsins ▭
I-canonical visual opsins ▭

canonical r-opsins
canonical c-opsins

IV   I
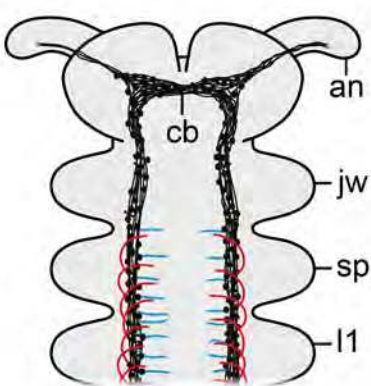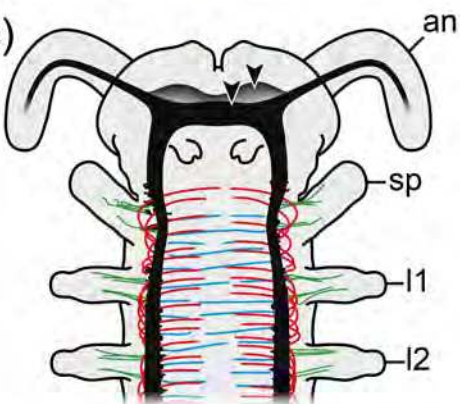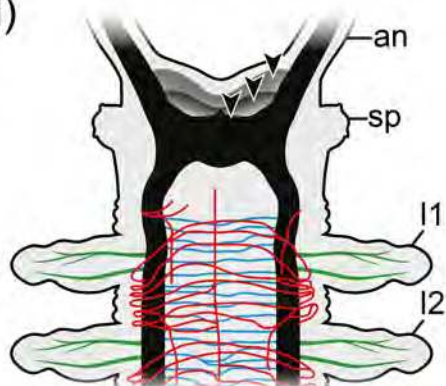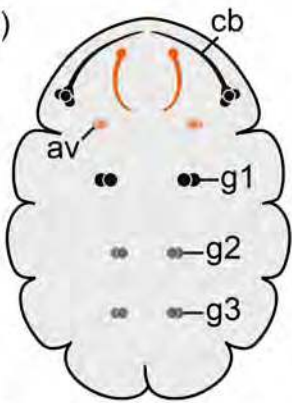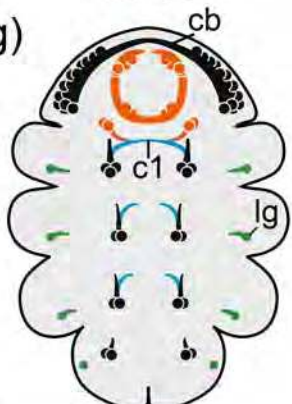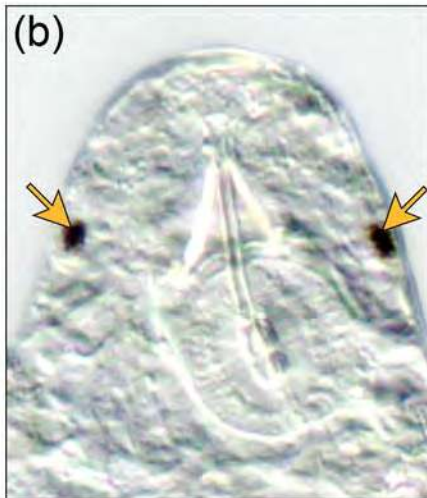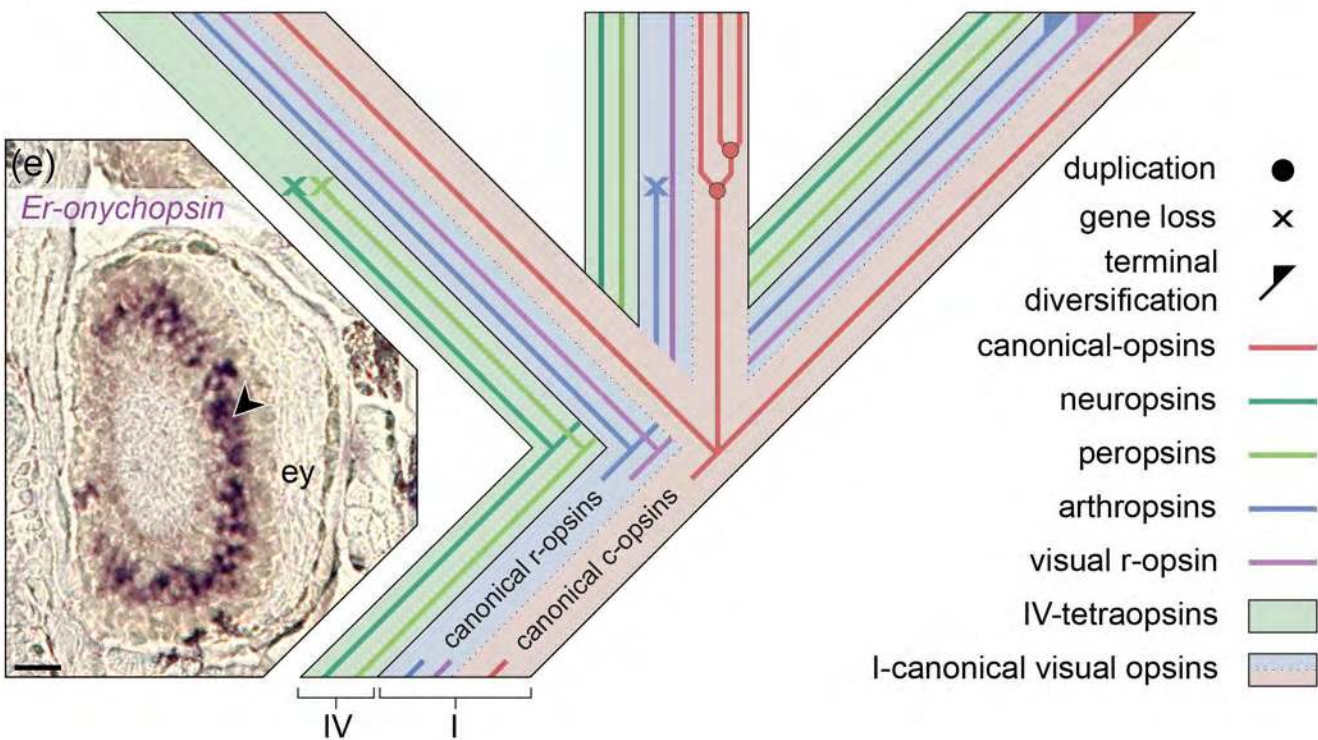
Onychophora

Euperipatoides rowelli　　　*Tasmanipatus anophthalmus*