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The Articulata hypothesis – or what is a segment?**

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Abstract

The long held view that annelids and arthropods are closely related (Articulata) has been challenged recently by phylogenetic analyses using molecular data. The outcome of these studies is a clade of moulting animals (Ecdysozoa) comprising arthropods and some taxa of the nemathelminth worms. Monophyly of the Ecdysozoa has not yet been shown convincingly on morphological evidence, but is strongly supported by molecular data. The implication of the Ecdysozoa hypothesis is that the type of segmentation found in annelids and arthropods must be either convergent or an ancestral feature of protostomes or even bilaterians. The present review discusses aspects of segmentation in annelids and arthropods at the genetic, cellular, morphogenetic and morphological levels. Based on numerous similarities not shared with other bilaterian taxa it is suggested that segmentation of annelids and arthropods is homologous and apomorphic for a monophyletic Articulata. However, the challenge provided by the molecular analyses should stimulate research programmes gaining more data such as on additional genes, cleavage patterns, molecular developmental biology, and the comparison of nervous systems at the level of single neurons.

Key words: Ecdysozoa, teloblasts, Annelida, Arthropoda, Cycloneuralia

Introduction

The Articulata hypothesis is a very elegant and convincing solution for the phylogenetic relationships of annelids and arthropods within the Bilateria. Accordingly, it has been almost universally accepted for the last decades. As early as 1817, Cuvier unified annelids and arthropods in the taxon ("embranchement") Articulata, based on the evident similarities in the body organisation of the two groups which is characterised by repeated morphological units along the antero-posterior body axis, the so-called segments. In a comparative study on annelid development, Hatschek (1878: 110) stated: "The connections of the phylum arthropods with the annelids are so evident and beyond any doubt that a close relationship of these two groups has to be accepted" (translation by G.S.). Since the monophyly of the Articulata was almost taken for granted by many zoologists, little attention has been paid to actually supporting this taxon with explicit apomorphies. In most textbooks (e.g., Brusca & Brusca 1990, Westheide & Rieger 1996, Ax 1999, Nielsen 2001) and also in phylogenetic studies (e.g., Lauterbach 1972, Weygoldt 1986, Rouse & Fauchauld 1997) one finds only a few characters in favour of the Articulata, and most of those are related to segmentation. The following list of putative synapomorphies for the Articulata is typical of this attitude (a–c after Ax 1999, d–e after Westheide 1996; translations by G.S.):

a: segmentation
b: teloblastic formation of segments
c: longitudinal musculature concentrated in strands
d: homonomous segments with parapodia-like appendages
e: ladder-like central nervous system.

This list is relatively short for two groups this large and diverse, and some of the few characters are even problematic, e.g. the character "ladder-like CNS" is already included in the character "homonomous segments", and teloblasts in arthropods and annelids are most likely convergent (see below).

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The comfortable position concerning the Articulata has been dramatically challenged by two simultaneously published phylogenetic analyses using 18S rDNA (Aguinaldo et al. 1997, Eernisse 1997). In these studies, the arthropods appeared as close relatives of several Nemathelminthes taxa (Fig. 1). Because the members of the resulting clade share the character of moulting a cuticle, the group has been baptised Ecdysozoa (Aguinaldo et al. 1997). The annelids clustered with molluscs and other spiralian groups widely separated from the Ecdysozoa.

The Ecdysozoa hypothesis provoked different reactions within the scientific community. Many scientists enthusiastically adopted the Ecdysozoa, and numerous papers have been published discussing the hypothesis and its implications for the view of bilaterian evolution from the perspectives of morphology, development, Hox genes, and palaeontology (e.g., Schmidt-Rhaesa et al. 1998, Adoutte et al. 1999, De Rosa et al. 1999, Budd & Jensen 2000, Valentine & Collins 2000). On the other hand, many people were reluctant and critical and considered the Ecdysozoa hypothesis to be implausible

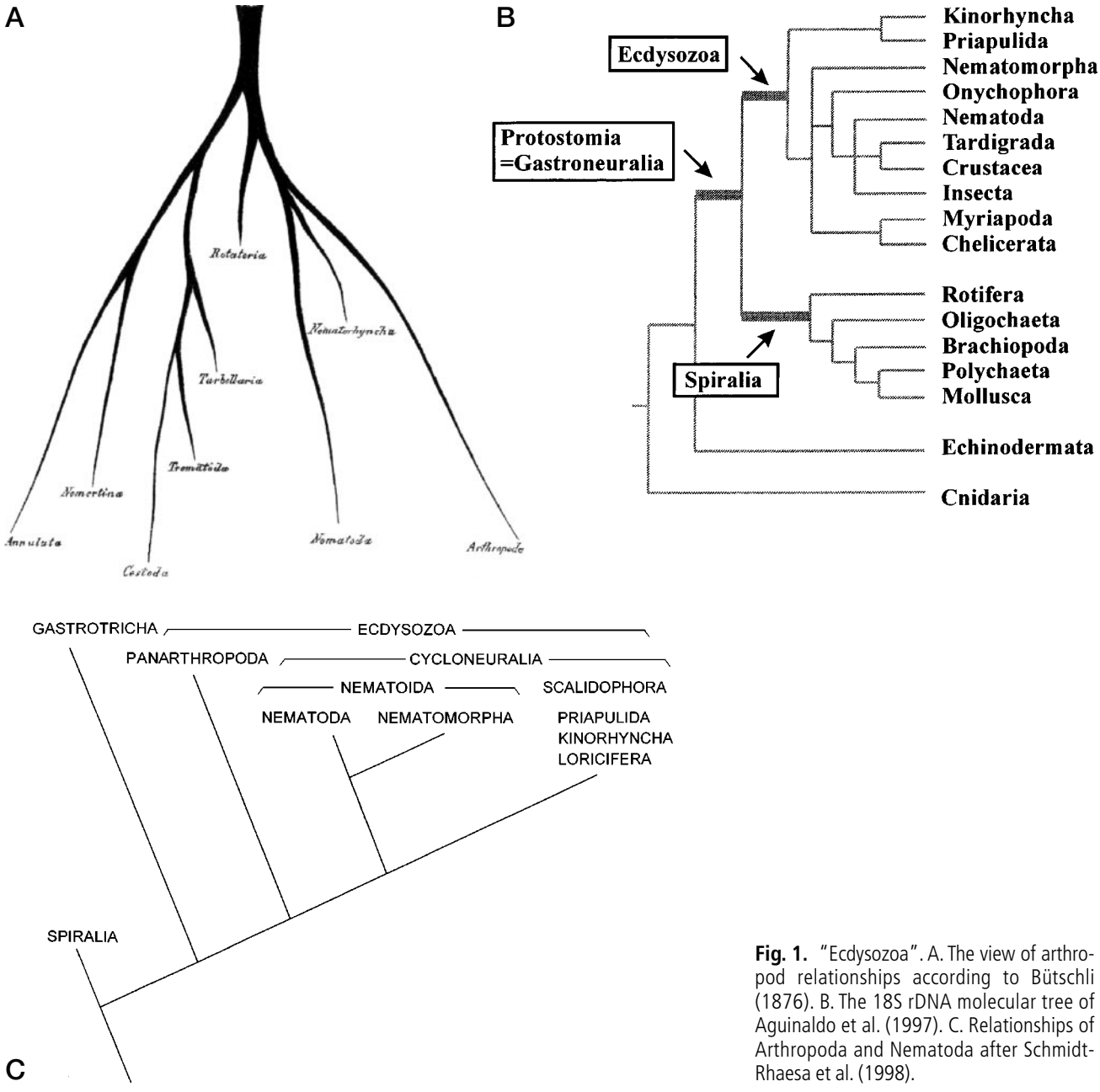


Fig. 1. "Ecdysozoa". A. The view of arthropod relationships according to Bütschli (1876). B. The 18S rDNA molecular tree of Aguinaldo et al. (1997). C. Relationships of Arthropoda and Nematoda after Schmidt-Rhaesa et al. (1998).

(e.g., Ax 1999, Wägele et al. 1999, Hausdorf 2000, Wägele & Misof 2001, Nielsen 2001, Blair et al. 2002, Scholtz in press).

Not too long ago the discussion about the phylogenetic relationships between the various "worms" and about their relationship to arthropods was also highly controversial, and the Articulata hypothesis was just one among many others. As early as 1876, Bütschli developed ideas on arthropod affinities which, translated into a modern cladogram, suggested a sister-group relationship between Arthropoda and a clade comprising Nematoda (including Nematomorpha) and Nematorhyncha (Kinorhyncha and Gastrotricha) (Fig. 1). This idea is not so far away from the phylogeny published by Schmidt-Rhaesa et al. (1998) who considered Cycloneuralia (Nematoda, Nematomorpha, Kinorhyncha, Priapulida, and Loricifera) as sister-group to Arthropoda, together constituting the Ecdysozoa, and Gastrotricha as sister-group to Ecdysozoa (Fig. 1), nor from the view of Garey (2001), based on 18S rRNA data, that the cycloneuralians are paraphyletic with the arthropods being the sister group to Nematoda and Nematomorpha. Among others, Carpenter (1906) claimed an arthropod origin from rotifer-like, unsegmented organisms. In 1909 Rauther published a very detailed study on nematode morphology, in which he suggested that nematodes are in many respects reduced forms which originated from terrestrial arthropods. The characters discussed in favour of the "Ecdysozoa" at the end of the 19th century are the same as today: chitinous cuticle, moulting, absence of cilia, and the shape of the pharynx. The last morphology-based cladistic analysis supporting a nematode/arthropod clade published before 1997 was by Eernisse et al. (1992). Since it placed its focus more on the question of an Annelida-Mollusca sister-group relationship it did not influence the scientific discussion about arthropods very much.

The morphological characters used in support of the Ecdysozoa s.l. – a layered chitinous cuticle, moulting with ecdysone, loss of epidermal locomotory cilia, triradiate pharynx (Schmidt-Rhaesa et al. 1998) – are not very convincing in terms of homology and have been critically evaluated by Wägele et al. (1999), Wägele & Misof (2001), and Nielsen (2001). Also, the data matrices and character scoring of Eernisse et al. (1992) and Zrzavy et al. (1998) have been justifiably criticized by Wägele et al. (1999), Wägele & Misof (2001), and Jenner (2001). I do not want to repeat all the arguments here, but will discuss a further example for the general weakness of the morphological ecdysozoan characters. It has been stressed that one putative apomorphy for Ecdysozoa is the loss of epidermal (motile) cilia (Aguinaldo et al. 1997, Schmidt-Rhaesa et al.1998). In their recent investigation on onychophoran embryonic development, Eriksson et al. (in press) describe the occurrence of cilia in the epidermis of *Euperipatoides kanangrensis*. These cilia occur either spread over the embryonic epidermis or more concentrated in an area which invaginates to form the so-called hypocerebral organ where they persist in a rudimentary form (Eriksson et al. in press). The authors discuss this as putative evidence for a transitional place for onychophorans with respect to ciliary loss in ecdysozoans. However, at least rudimentary epidermal cilia must then have been present in a putative ecdysozoan stem species, and the entire loss of epidermal cilia would be a convergent character of euarthropods and cycloneuralians.

Some new characters supporting Ecdysozoa which deserve further evaluation and investigation have to be mentioned here. Manuel et al. (2000) compared the β-thymosin homologues in Metazoa and found a characteristic pattern of repeats in *Drosophila* and *Caenorhabditis* not shared by other taxa. This is a new and interesting independent character, but the problem of this study is the limited taxon sampling. Another interesting aspect has been brought up by Haase et al. (2001). These authors studied the expression of horseradish peroxidase (HRP) immunoreactivity, an established marker for insect nervous systems, in a variety of animals, and they found expression only in arthropods, nematodes, a priapulid, and a nematomorphan. All representatives of other higher metazoan taxa showed no expression in their nervous systems. Although the HRP antibody is not very specific and binds to a set of various glycoproteins, this is additional independent "morphological" evidence in favour of the Ecdysozoa.

The strongest support for a clade Ecdysozoa, however, still comes from molecular analyses using sequence data and from those combining molecular and morphological data (total evidence) (Zrzavy et al. 1998, Giribet et al. 2000, Peterson & Eernisse 2001; but see Hausdorf 2000, Blair et al. 2002). This is obviously independent of the methods applied for analysing the data, such as distance methods, maximum parsimony, maximum likelihood, etc. A bias is involved by the fact that the molecular as well as the total evidence approaches mainly rely on the 18S rDNA gene sequences. The use of this gene for resolving deep metazoan phylogeny is problematic (for discussions see Abouheif et al. 1998, Wägele et al. 1999, Giribet & Ribera 2000, Wägele & Misof 2001).

Given the metazoan cladograms based on molecular and total evidence analyses, the results have a strong impact on our view concerning the homology and evolution of segmentation. One major implication of the Ecdysozoa hypothesis is that if it is correct, the segmentation we find in annelids and arthropods must be either a convergent or a very ancient character which occurred already either in the stem species of the protostomes or even in that of the Bilateria. In the latter cases segmentation must have been independently lost in many lin-

eages. All these alternatives have been discussed by various authors (e.g., Eernisse 1997, Holland et al. 1997, Schmidt-Rhaesa et al. 1998, Arendt & Nübler-Jung 1999, Davis & Patel 1999, Jenner 2000). At least for chordates it is becoming more and more clear that the body organisation and subsequent segmentation follow an entirely different process compared to "segmented" protostome groups (Meinhardt 2002). However, the whole discussion suffers from the fact that it is often unclear what is meant by segmentation or segments, and as a result segmentation is inaccurately treated in phylogenetic analyses. Here, I do not want to evaluate the conflict between characters supporting the Ecdysozoa versus those in favour of the Articulata. Rather, I want to review what is known about segmentation in annelids and arthropods, and specify what characteristics of the segmentation complex are shared by annelids and arthropods but not by other metazoans.

Review of segmentation

What is a segment?

The most important questions to start with are: What is a segment? What do we mean by segmentation? Is there anything about segmentation that is uniquely shared by arthropods and annelids? Is a segment the region of embryonic gene expression? Is it characterised by genetic regulatory networks? Does it represent a physiological unit? Is it defined by clonal restrictions? Is it a morphological unit? And how are all these levels related to each other?

Depending on one's scientific background, the answers will vary. For example, the molecular geneticist's concept of a segment is different from that of the morphologist. In the model for segmentation developed by Meinhardt (1986), a segment is formed and characterised by three different cell states. Lawrence (1992: 91) defines a segment of the ectoderm as "a pair of compartments, one anterior and one posterior." According to Rauskolb (2001: 4511) "segmentation is a developmental mechanism that subdivides a tissue into repeating functional units." Kroiher et al. (2000: 485) define segmentation as "the formation of a periodic pattern of paralogous blocks of cells." Particularly the latter definition is certainly too general in order to address questions of homology of segmentation between different taxa, because it comprises virtually all cases of repeated structures along the body axis. A useful definition of segmentation has to be more specific by stating what structures are repeated and what the pattern of their arrangement is. Repetitive elements alone are not segmentation. There are all sorts of serially repeated structures along the body axis of several bilaterian groups. These characters comprise elements of the nervous system (e.g. in Platyhelminthes, Solenogastres, Kinorhyncha, Nematoda), muscle patterns (e.g. in Monoplacophora, Kinorhyncha), shell structures (e.g. Polyplacophora), gonads (e.g. Nemertini) or nephridia (e.g. Monoplacophora) (Clark 1980, Neuhaus 1994, Scholtz in press). But labelling all of these as segmentation would mean stretching the term too far. Nevertheless, "true" segmentation might have started from some sort of repeated structures – a scenario discussed by Budd (2001) and Scholtz (in press). The most meaningful definition of a segment in this context is the classical morphological definition:

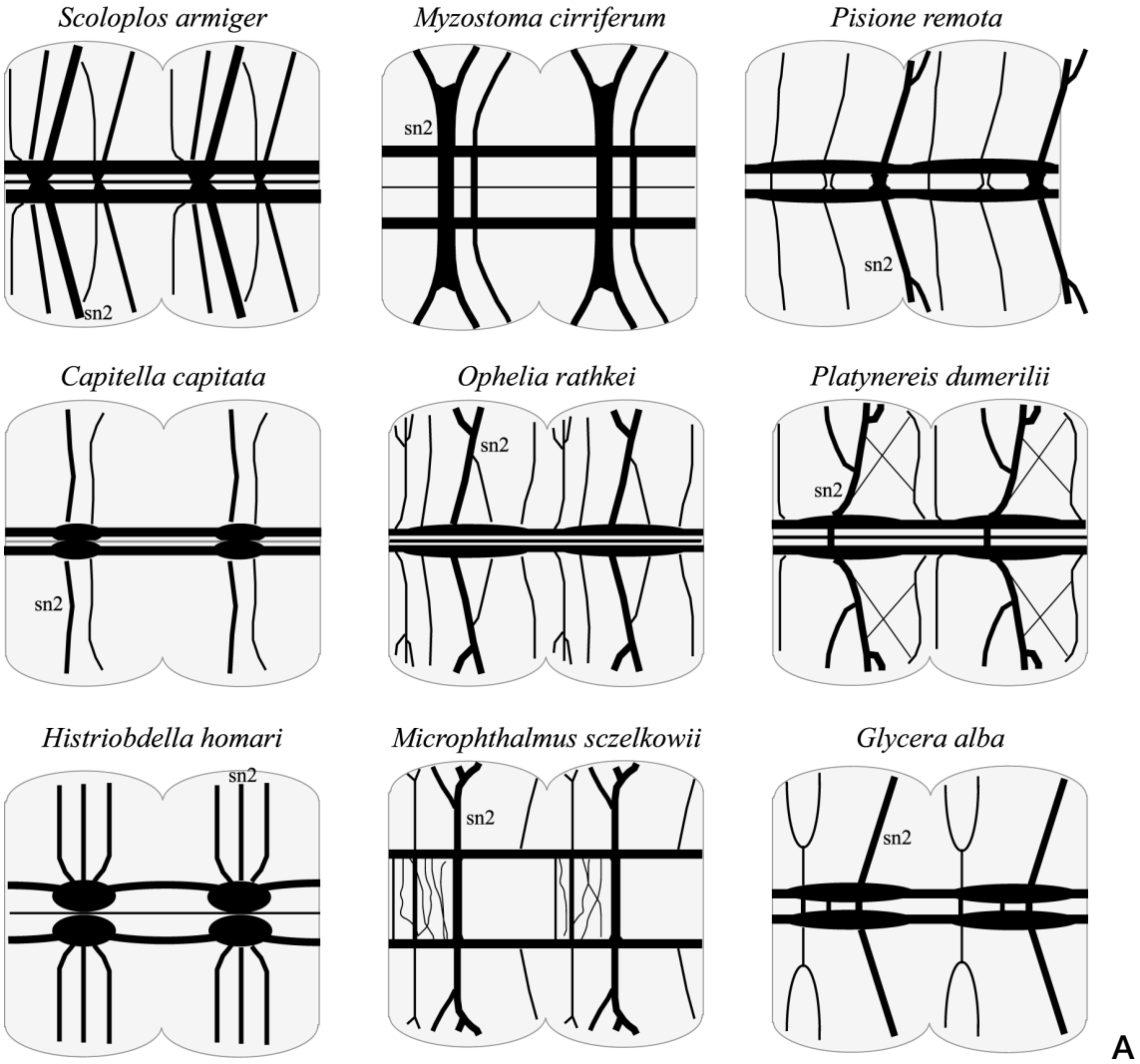
A segment is an antero-posteriorly repeated body unit which can be defined by a set of sub-structures or characters in a specific spatio-temporal correlation. In the case of annelids and arthropods, these are the following features (see Goodrich 1897, Scholtz in press):

– an outer annulus
– one pair of mesodermal hollow spaces
– one pair of ventral ganglia
– one pair of metanephridia
– a set of muscles
– one pair of appendages.

All these structures together characterize segments in arthropods and annelids (but not in chordates) (see Guthrie 1995). This is not merely a list of structures or characters, rather these characters show a distinct spatial pattern (e.g., the nephropores of Annelida and Arthropoda lie in a position ventral to the base of the appendages and lateral of the nerve cord). In addition, one can stress similarities in the ontogeny of segments of annelids and arthropods such as the formation by a posterior mesodermal and ectodermal growth zone and an antero-posterior differentiation process. However, is this enough to claim homology of a specific articulatan segmentation? Perhaps all these characters are necessarily linked to and dependent on each other and, thus, the genetic information underlying segment formation is not very complex. Hence, the homology of annelid and arthropod segments might not be as plausible as often thought.

Testing homology of segments

To make the homology of segments plausible it has to be shown that the similar segmental patterns of annelids and arthropods are complex. Complexity is the most important criterion or test for the plausibility of the homology of characters (Riedl 1975, Dohle 1989, Wägele 2000, Scholtz in press). Homologisation of characters can be done best when the character under question can be subdivided into substructures which together show a distinct pattern (Rieppel & Kearney 2002). The complexity of this pattern can be shown by proving the independence of individual substructures. This is done by comparing the patterns of substructures in different taxa. If individual substructures of the pattern under compari-



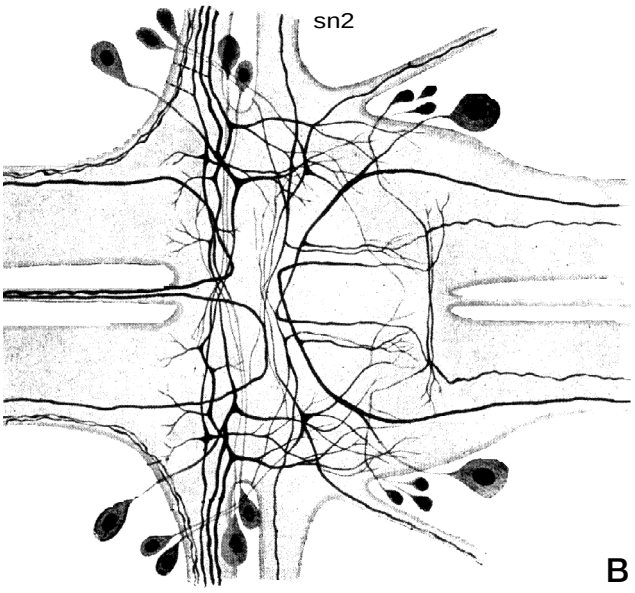


Fig. 2. Central nervous system of Annelida and Arthropoda. A. Schematic representation of various polychaete ganglia with median nerve, connectives, commissures, and segmental nerves (modified after Müller 1999). Two adjacent ganglia of each species are shown, sn2 = second segmental nerve (parapodial nerve). B. A ganglion from *Aeschna* (Insecta, Odonata), (modified after Hanström 1928); as in (A), the connectives, commissures, segmental nerves, and the median nerve are shown.

son have been altered or lost between the taxa without an effect on the general pattern, the independence of these particular substructures is proven. The fact that the substructures occur together despite their independence shows the complexity of the general pattern. Thus, complexity of similarity makes homology likely or plausible. The substructures can also be the subject of a homology analysis applying the same type of complexity test. This hierarchical approach of evaluating the patterns and sub-patterns under comparison makes the assumption of an independent evolution of these patterns very unlikely (see Riedl 1975; Dohle 1976, 1989; Scholtz 1984, in press). The ontogeny of structures can be seen as a sequence of substructures in time. Accordingly, the inclusion of developmental characters can additionally strengthen the confidence in homology of similar characters. An important aspect of homologisation concerns the asymmetry between similarity and difference. The question must be: how many substructures of a pattern must be similar to claim homology of this pattern? The question is not: how many differences must occur to reject the possibility of homology?

Can this complexity test be applied to segments of arthropods and annelids as defined above? I think it can. If we compare the segments of a variety of annelids and arthropods it becomes evident that all the listed parts of a segment can be altered individually all the way to complete loss, and there are numerous examples of segments where one or more of these characters are absent: we find segments without ganglia, without nephridia, without an outer annulus, etc. This proves that the suite of characters that makes up a segment is complex (because in many cases most of the characters appear together although they do not have to), and an independent evolution of the segments of annelids and arthropods is therefore not plausible.

The substructures of substructures

We can go even further with a hierarchical approach and apply the complexity test to the level of the substructures that make up a segment themselves. Here, the same test of complexity can be applied concerning the substructures of the substructures. This principle can be exemplified by a comparison of the ganglia and other parts of the nervous system of annelids and arthropods. The confidence in the homology of the general pattern increases if the substructures again show a high plausibility of homology (Riedl 1975).

***The ganglia and the nervous system*:** The metamerically arranged ganglia of Annelida and Arthropoda are composed of paired neuropils with ventrolaterally lying perikarya. This is not so evident in some groups such as Onychophora or oligochaetes. But even in these cases a neuromere structure is still recognisable (Schürmann 1995) and embryologically evident (Anderson 1973, Eriksson et al. in press). Between the neuropils of the ganglia of each side there are transverse commissures, and the ganglia of adjacent segments are connected by a pair of longitudinal connectives. The number of commissures varies, but in many cases in annelids and arthropods we find two large commissures per segment (Hanström 1928, Whitington 1996, Harzsch et al. 1997, Müller 1999, Müller & Westheide 2000) (Fig. 2). The ganglia are equipped with three main (large) lateral nerves in many arthropods, clitellates and polychaetes (Hanström 1928, Hessling & Westheide 1999, Müller 1999) (Fig. 2). Hanström (1928: 303) considered this to be a good character for unifying annelids and arthropods. However, Müller (1999) showed that in polychaetes the number of segmental nerves varies to a high degree, a situation which is also found in arthropods

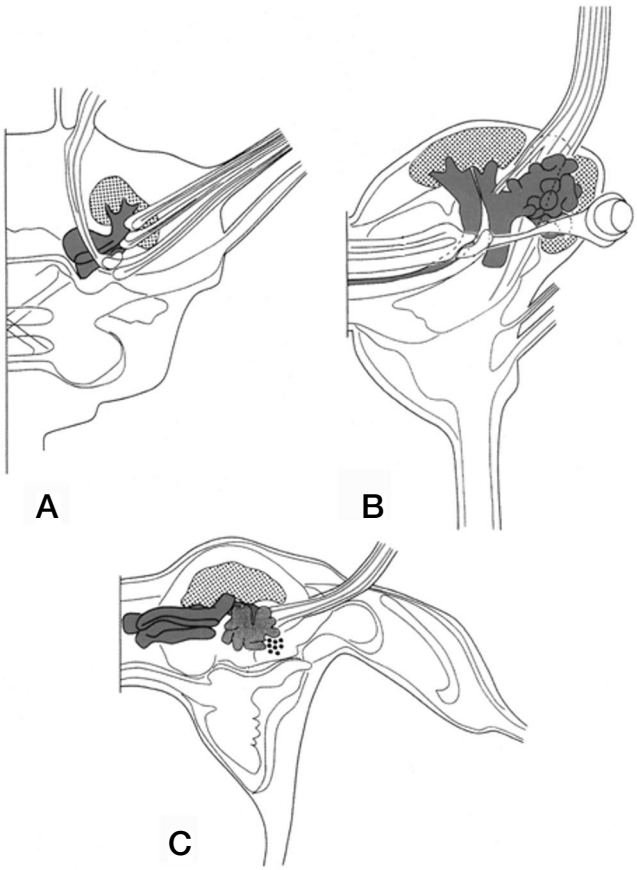


Fig. 3. Mushroom bodies (corpora pedunculata) in the anterior brain of annelids and arthropods (right half of brain, dorsal aspect) (modified from Strausfeld et al. 1995). A. The polychaete *Nereis bicolor*. B. The onychophoran *Euperipatoides leuckartii*. C. The millipede *Orthoporus ornatus*. The characteristic shape and structure of the mushroom bodies with the calyx of globuli cells (stippled) and the lobate peduncles can be seen. In (C) the mushroom body is associated with the olfactory lobe in the deutocerebrum (light grey).

(Heckmann & Kutsch 1995). In this case the problem is the homologisation of the smaller segmental nerves between different taxa. A median nerve running through all ganglia is a character shared by many annelids and arthropods (Hanström 1928, Harzsch et al. 1997, Müller 1999, Gerberding & Scholtz 2001) (Fig. 2).

For many groups of arthropods and annelids the presence of mushroom bodies (corpora pedunculata) in the anterior brain region has been described (Hanström 1928, Åkesson 1963, Bullock & Horridge 1965, Strausfeld et al. 1995, Yoshida-Noro et al. 2000). These neuropil regions are characterised by their mushroom-like shape, their bundles of fibres, and specific arrangements of so-called globuli cells, i.e. neurons with relatively large nuclei which are intensely stained in histological preparations (Fig. 3).

The position and shape of the stomatogastric nervous system is similar between many annelids and arthropods (Hanström 1928, Bullock & Horridge 1965). The paired nerves connecting the central nervous system and the stomatogastric ganglion originate at the posterior region of the brain, the stomatogastric ganglion lies on the dorsal side of the stomach (Hanström 1928, Böhm et al. 2001). According to Bullock & Horridge (1965: 765) "the set of nerves and ganglia (of the stomatogastric nervous system) is a characteristic feature of articulates."

***Limbs*:** It is evident that comparisons of some segmental substructures are problematic, because the complexity of similarity is not very high and thus homology is difficult to test. For instance, the homology between annelid parapodia and arthropod lobopodia and arthropodia has been controversially discussed. However, based on various grounds (anatomy, gene expression, phylogeny) several authors propose a homology between parapodia and arthropod limbs (Lauterbach 1978; Panganiban et al. 1997, Westheide 1997).

Development, substructures in time

As in the case of the static pattern of the substructures, plausibility for homology of segmentation increases with homologies at various developmental levels such as morphogenesis, cell division patterns, and gene expression.

***Cell proliferation and segmentation*:** One can discriminate two developmental processes crucial for segment formation in annelids and arthropods. One is the budding of competent cellular material from a posterior growth zone along the body axis. The other is the subdivision of the body into metamerically repeating units (Dohle 1972, Scholtz 1992).

The material for segmentation is formed by proliferation in a preanal growth zone which comprises the ectoderm and the mesoderm. Elongation of the embryo basically consists of two steps, a posterior cell proliferation and an intercalary cell division or rearrangement spread all over the length of the germ. Whereas this is not so evident in insects (Davis & Patel 2002), it has been clearly demonstrated in clitellates and malacostracan crustaceans where the stereotyped cell division pattern allows tracing of the germ band cells from their origin through several rounds of division all the way to segmental differentiation of their descendants (Dohle & Scholtz 1988, Shankland 1999, Shimizu & Nakamoto 2001).

The segmentation process in annelids and arthropods follows mainly an antero-posterior gradient, with the more anterior segments being the most differentiated whereas the posterior segments develop last (Figs 6, 7). All the data on cell proliferation and segmentation clearly contradict models about a spatial and temporal refinement of segmentation along the length of the embryo (Minelli 2001).

The processes of proliferation and segmentation are often described as teloblastic formation of segments (Anderson 1973, Ax 1999, Nielsen 2001). However, true teloblasts are defined as large stem cells at the posterior end of the germ band giving rise to smaller descendants in an anterior direction by unequal divisions (Siewing 1969) (Fig. 4). Mesodermal teloblasts can be found in annelids, and within the arthropods only in cirripede and malacostracan crustaceans (Anderson 1973, Dohle & Scholtz 1988, Scholtz 2000, Hejnol 2002). The presence of ectodermal teloblasts is even more restricted, they occur only in clitellate annelids (Dohle 1972, 1999) and in malacostracan and probably cirripede crustaceans (Anderson 1973, Dohle & Scholtz 1988, Scholtz 2000). The number and arrangement of teloblasts is very different between annelids/clitellates and malacostracans, and the only similarity is the presence of asymmetrical uni-

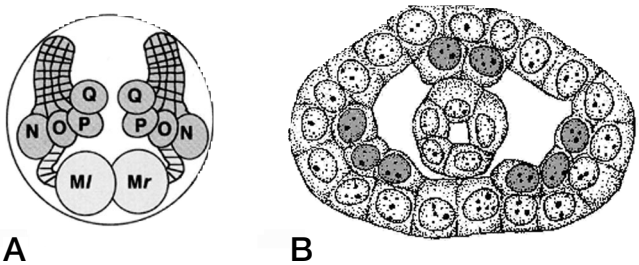


Fig. 4. Pattern of teloblastic growth of the germ band in Clitellata and Malacostraca. A. Arrangement of ecto- and mesoteloblasts in the clitellate *Tubifex* (modified after Shimizu & Nakamoto 2001). There are 4 paired ectoteloblasts N, O, P, Q, and one pair of mesoteloblasts M in a specific pattern. They bud off the primary blast cells of the germ band by asymmetric divisions in anterior direction. B. Ground pattern of teloblasts in malacostracans (modified after Scholtz 2000). A ring of 19 ectoteloblasts (a median ventral cell and 9 paired laterodorsal cells) surrounds an inner ring of 8 mesoteloblasts (grey).

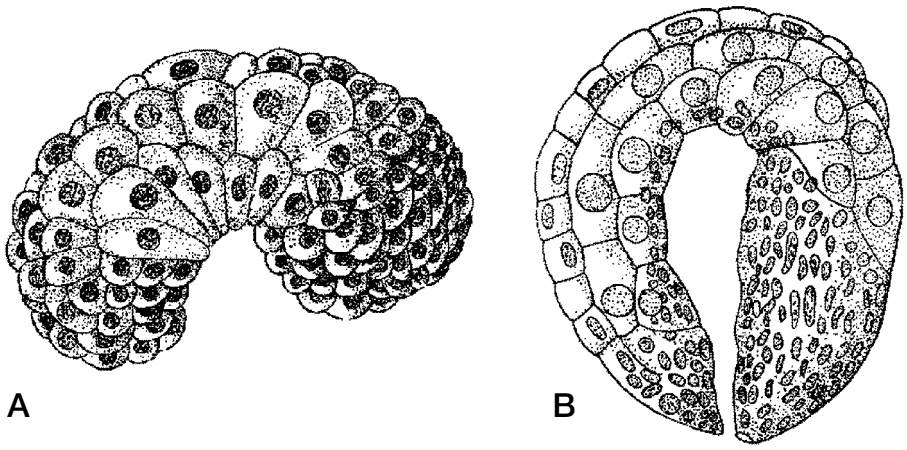


Fig. 5. Embryonic growth in the nematode *Ascaris* (anterior to the right) (modified after Müller 1903). The elongation of the germ (A) early stage is accomplished by change in the cell shape leading to the worm habit (B). A posterior growth zone is not involved.

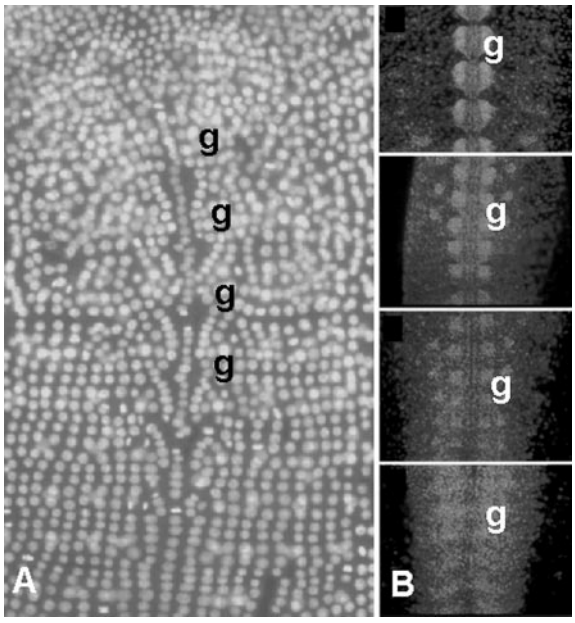


Fig. 6. Neurogenesis. A. Ventral aspect of the post-naupliar germ band of a malacostracan crustacean (Amphipoda), showing the anteroposterior decrease of differentiation. The forming segmental ganglia (g) are recognisable at right and left of midline at an early stage. B. Ganglia forming in the germ band of an annelid (Hirudinea) (modified after Shain et al. 1998). The pattern of segmental ganglion (g) formation is similar to what is seen in the arthropod representative.

directional divisions (Fig. 4). The restriction of mesodermal and ectodermal teloblasts to some crustacean subgroups shows that teloblastic growth is not part of the arthropod ground pattern, and for annelids only mesoteloblasts appear to be plesiomorphic (Dohle 1972, Scholtz 1997). As early as 1895, McMurrich suggested that teloblastic growth in crustaceans and annelids is not homologous but rather an efficient way to generate cells which evolved independently in annelids and arthropods – a view which still holds true.

There is not much information about the growth and extension of gastrotrich and cycloneuralian embryos. Teuchert (1968) reports no posterior growth zone for gastrotrichs. It is known from nematodes that embryonic elongation is achieved by the alteration of cell shape (stretching), not through directional cell division (Müller 1903, Priess & Hirsh 1986) (Fig. 5). Neuhaus (1993, 1995) describes that two zonites (11 and 12) are added by a subcaudal growing zone during the postembryonic development of several species of the Kinorhyncha. However, the figures in Neuhaus's papers and his description of the internal anatomy clearly show that the anlagen of these two additional zonites are already existent at hatching; the "adding" of zonites is merely an intercalary differentiation during postembryonic moults. This reveals that in Kinorhyncha there is no growth zone comparable to that of arthropods and annelids – at least not during postembryonic development.

***Neurogenesis*:** The segmental ganglia of annelids and arthropods originate from paired longitudinal cell strands on each side of the embryonic midline (Hatschek 1878, Bate 1976, Dohle & Scholtz 1988, Scholtz & Dohle 1996, Shain et al. 1998). Together with the other aspects of segmentation, the cells of these strands show iterated specifications and form the segmental ganglion anlagen by internalisation in an antero-posterior sequence (Fig. 6). This means that prior to, or coincident with, internalisation, the ganglion primordia are individualised – a unique character among bilaterians (compare Sulston et al. 1983, Younossi-Hartenstein et al. 2000, Voronezhskaya et al. 2002, Friedrich et al. 2002).

***Coelom formation*:** The mesoderm is also first proliferated and then metamerically subdivided. It forms paired lateral strands to the left and right of the midgut anlage, which develop hollow spaces (Anderson 1966, 1973) (Fig. 7). This so-called schizocoely shows a very similar pattern in annelids and arthropods. The similarity relates

to the sequential arrangement of the coelomic cavities, the antero-posterior sequence of differentiation, the early ventral differentiation of the mesoderm with dorsal migration during development, the facts that all cells become lining cells of the coelomic cavity, and that in annelids as well as in arthropods the lateral outer part of the embryonic coelomic wall is much thicker than that of the inner visceral region (Hatschek 1878; Anderson 1966, 1973; Bartolomaeus & Ruhberg 1999) (Fig. 7). Recent studies on an onychophoran revealed that even at the ultrastructural level the epithelia of these coelomic spaces are very similar between arthropods and annelids, with the exception that the onychophoran coelothel seems to remain in a more undifferentiated

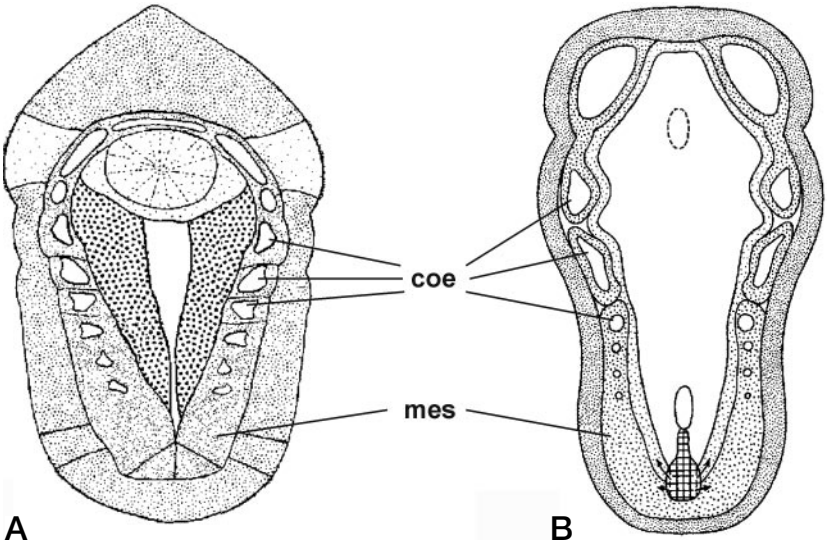


Fig. 7. Coelom formation in embryonic (larval) Annelida and Arthropoda (anterior at top) (modified from Anderson 1966, 1973). A. The annelid *Scoloplos armiger*. B. The onychophoran *Peripatopsis* spec. In both cases lateral mesodermal bands (mes) are formed from the posterior growth zone. Paired hollow metamerical spaces (coelom = coe) are formed in an antero-posterior sequence.

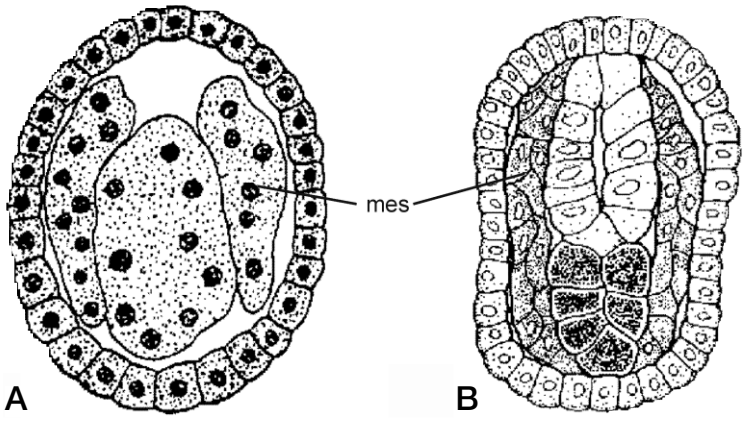


Fig. 8. The embryonic mesoderm of the nematode *Ascaris* (A) and the gastrotrich *Turbanella cornuta* (B) (anterior at top) (modified after Boveri 1899 and Teuchert 1968). Although lateral mesodermal bands (mes) are formed, they are not budded by a posterior growth zone and they never develop paired coelomic spaces.

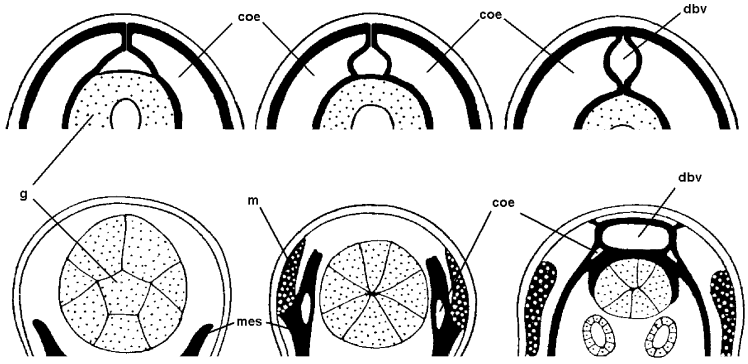


Fig. 9. Schematic cross sections showing the development of the dorsal blood vessel in Annelida (upper line) and Arthropoda (lower line) (modified after Siewing 1969). In either case the dorsally migrating, metamerically arranged coelomic sacs meet in the dorsal midline, forming the dorsal blood vessel by leaving a longitudinal space between the coelothelia.

state (Bartolomaeus & Ruhberg 1999). It must be stressed, however, that during development the coelomic spaces of arthropods are highly transformed, subdivided, and reduced ("mixocoel") (Dohle 1979). Only in onychophorans are parts of them connected with a ciliated funnel bearing metamerically repeated metanephridia (Storch & Ruhberg 1993).

No signs of metamerical schizocoely can be found in any representative of gastrotrichs or cycloneuralians. The lateral mesodermal strands do not form any hollow spaces but become differentiated directly into musculature and other mesodermal derivates (Müller 1903, Teuchert 1968) (Fig. 8). Thus, the resulting body cavity has a different character and is of different origin and neither comparable to a coelom nor to a mixocoel, and this is even true for the Kinorhyncha which show some serially repeated structures (Neuhaus 1994).

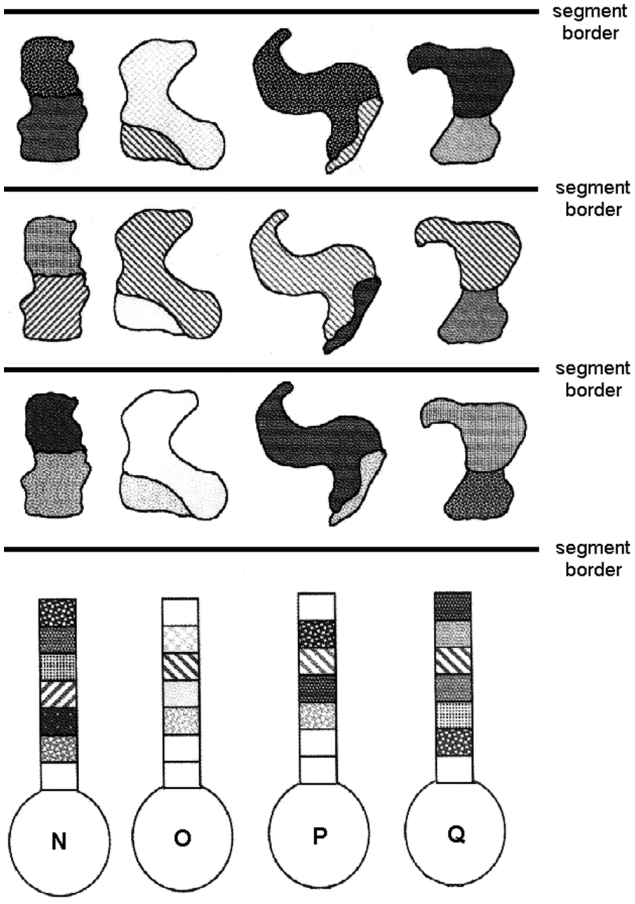


Fig. 10. Cell lineage in the germ band of the clitellate annelid *Tubifex* (modified after Shimizu & Nakamoto 2001). The 4 ectoteloblasts N, O, P, Q produce bandlets of primary blast cells. The progeny of the blast cells form different amounts of segmental ectoderm and ganglion anlagen. The O, P clones straddle the segment borders and contribute to two segments each. Thus, they show a parasegment-like behaviour. In the N, Q lineages, two primary blast cells make up segmental structures. This resembles insect A/P compartment boundaries (Lawrence 1992).

***Blood vessel formation*:** Annelids and arthropods possess a contractile, long, tube-like dorsal blood vessel with a postero-anterior blood flow. This dorsal vessel is formed embryologically between the dorsal parts of the paired coelomic spaces, in a fashion similar between annelids and arthropods (Siewing 1969; Anderson 1966, 1973; Dohle 1979) (Fig. 9). The blood vessel represents a hollow space in the extracellular matrix external to the coelomic epithelia (Westheide 1997). Interestingly annelids and several arthropods – in particular malacostracan crustaceans, myriapods, and scorpions – also share the existence of a supraneural longitudinal ventral blood vessel (Hjelle 1990, Richter & Scholtz 2001, Wirkner & Pass 2002).

Neither Gastrotricha nor Cycloneuralia possess any blood vascular system.

***Cell level*:** Cell lineage studies have shown for clitellates as representatives of annelids and for malacostracan crustaceans among the arthropods that the morphological segments do not match the genealogical units at the cellular levels. The progeny of the primary blast cells of the O and P ectoteloblast lineages of clitellate oligochaetes and leeches straddles the segment border, whereas in the N and Q lineages the derivatives of two adjacent blast cells contribute to one segment (Fig. 10) (Shankland 1999, Shimizu & Nakamoto 2001). Comparably, the descendants of the ectodermal transverse cell rows in malacostracans contribute to parts of two adjacent segments (Fig. 11) (Dohle & Scholtz 1988, Scholtz & Dohle 1996). The clonal situation in malacostracans and clitellates resembles the parasegment of *Drosophila*, which is the primary metameric unit marked by lineage restrictions and gene expression. Furthermore, this parasegment does not match the segment but contributes to parts of two adjacent segments, the posterior compartment of the anterior segment and the anterior compartment of the posterior segment (Lawrence 1992). Recent investigations on the expression patterns of the genes *wingless*, *engrailed*, and *cubitus interruptus* in a spider show that the parasegment is a general arthropod feature (Damen 2002). It must be stated, however, that there is some cellular intermixing across the segment boundary for all five teloblast lineages (M, N, O, P, Q) in hirudineans, which indicates that the genealogical units are not spatially restricted as in insect parasegments (Shankland 1999). Even when we take this difference into account, it seems a common principle for segmentation in annelids and arthropods that the segments are composed of cells from different origins.

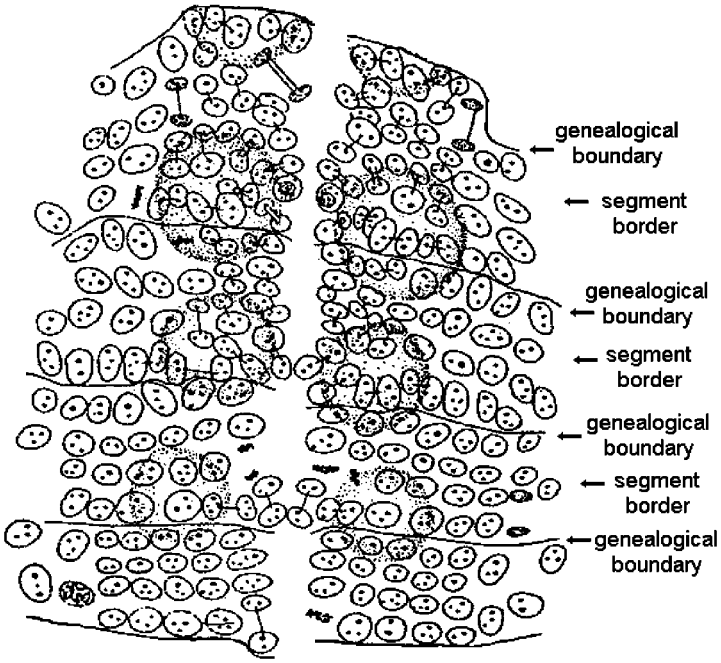


Fig. 11. Cell lineage in the germ band of the malacostracan crustacean *Neomysis integer.* The progeny of ectodermal cell rows behave parasegmentally by forming the posterior parts of a morphological segment and the anterior portion of the next posterior segment. Thus, the genealogical boundaries do not match the segmental borders.

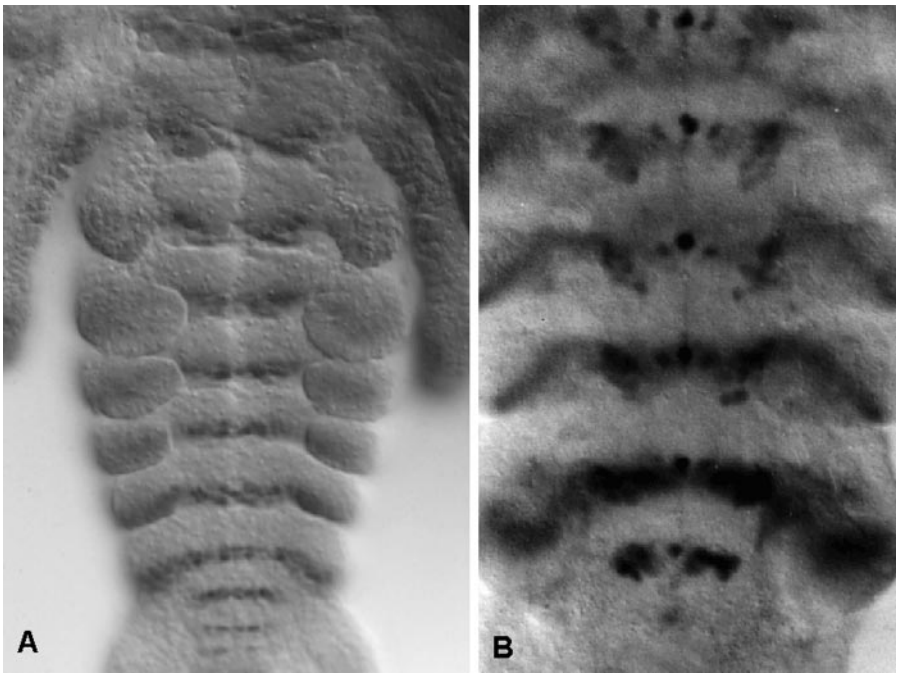


Fig. 12. Expression of *engrailed* in the arthropod *Cherax destructor.* A. Early ectodermal expression in iterated stripes in posterior of each segment in the embryonic pleon (compare Fig. 13A). B. Neuronal expression in forming ganglia in the pleon of a more advanced stage. Note the paired lateral cluster of *engrailed* positive cells in the posterior and the median cluster in the anterior of each ganglion anlage (compare Fig. 13B).

***Segmentation genes*:** It has been shown for numerous representatives of all major euarthropod groups that the segment polarity gene *engrailed* is expressed in transverse stripes in the ectoderm of the posterior portion of forming segments (Patel et al. 1989a, b; Scholtz & Dohle 1996; Damen et al. 1998; Peterson et al. 1998; Telford & Thomas 1998; Queinnec et al. 1999; Arthur 2002; Hughes & Kaufman 2002b). In addition, there is a secondary, neuronal *engrailed* expression in the ganglion anlagen showing a highly similar pattern in crustaceans and insects (Patel et al. 1989a, b; Scholtz 1995; Harzsch et al. 1998; Duman-Scheel & Patel 1999) (Fig. 12). Interestingly, at least in the midline the neuronal *engrailed* expression is not found in clones deriving from cells of the early segmental expression (Gerberding & Scholtz 1999). A metamerically iterated *engrailed* ex-

pression in the posterior region of embryonic segments and putatively in the neurogenetic region has also been reported for an onychophoran species (Wedeen et al. 1997). This pattern appears similar to what is found in euarthropods, although it is not clear whether the early expression is restricted to the mesoderm or the ectoderm (later it seems to be mesodermal or neuronal) (Wedeen et al. 1997). A corresponding sequence of dual *engrailed* expression has been described for leech embryos (Fig. 13). As in arthropods, *engrailed* is first expressed in transverse stripes in the posterior of the segment anlagen, followed by distinct neuronal expression in the ganglia, which resembles the pattern described for insects and crustaceans (compare Figs 12B, 13B) (Wedeen & Weisblat 1991, Lans et al. 1993). Again, there is no general clonal continuity between the early ectodermal and the neuronal expression (Lans et al. 1993). Despite the highly similar expression pattern there seem to be differences in the influence of *engrailed*-expressing cells on the regulation of the fate of neighbouring cells between the leech *Helobdella robusta* and *Drosophila*. In *Helobdella* the normal segmentation is retained even when the *engrailed*-expressing cells are ablated (Seaver & Shankland 2000, 2001). However, a change of function does not necessarily contradict homology of the *engrailed* pattern, but might be due to the highly derived stereotyped cell division pattern found in leeches, and similar processes might be true for malacostracan crustaceans as well. In malacostracans as in clitellates repeated units are marked already at the cell level long before *engrailed* expression and segmental morphogenesis begin. Generally, differences in underlying developmental processes do not refute homology of resulting patterns (Dohle & Scholtz 1988, Scholtz & Dohle 1996). For polychaete representatives the results on *engrailed* expression are contradictory. Prud'homme, de Rosa, Arendt, Julien, Dorresteijn, Adoutte, Wittbrodt & Balavoine (pers. comm.) describe a regular stripe pattern in *Platynereis dumerilii*, which is comparable to what is seen in hirudineans and arthropods. In contrast, Seaver et al. (2001) report for *Chaetopterus variepedatus* a very complex and dynamic pattern of *engrailed* expression in the mesoderm, the ectoderm and in the neurogenetic region, which the authors considered as being too different from the arthropod pattern to claim homology. However, there are some correspondences with other annelids and arthropods. Seaver et al. (2001) describe ventral ectodermal and mesodermal bands of *engrailed* expression correlated to morphological segment formation in at least two tagmata (body regions B and C, although in C morphological segmentation occurs prior to *engrailed* expression), and there is a metamerical expression in the forming ganglia.

Nothing is known about *engrailed* expression from representatives of cycloneuralians or gastrotrichs. Outside annelids and arthropods iterated *engrailed* expression has only been reported from molluscs (Polyplacophora) and chordates (Cephalochordata) (Jacobs et al. 2000, Holland et al. 1997). *Engrailed* expression in chitons is correlated to dorsal shell formation (Jacobs et al. 2000). In chordates a metamerical pattern of *engrailed* expression has been found. However, *AmphiEn* expression in the lancelet *Branchiostoma* (Holland et al. 1997) is restricted to the first eight mesodermal somites and does not show a stripe pattern. The expression in the nervous system of *Branchiostoma* is very different to that observed in annelids and arthropods, showing no comparable metamerical repeats (Holland et al. 1997). The iterated expression of *engrailed* in vertebrates (e.g. in muscle pioneer cells in the zebrafish) occurs only after the establishment of morphological metamerism (Ekker et al. 1992).

***Hox genes*:** The anterior boundary of the expression of the Hox genes *labial, proboscipedia, Deformed, sex combs reduced, Antennapedia,* and the combined domains of *Ultrabithorax* and *abdominal-A* is by and large conserved throughout the euarthropods (Abzhanov & Kaufman 1999, 2000; review by Scholtz 2001; Hughes

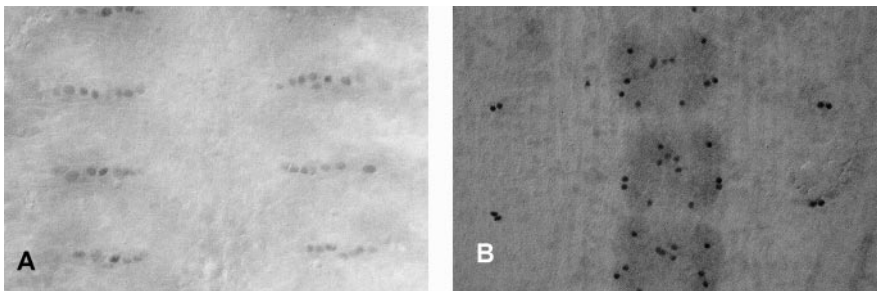


Fig. 13. Expression of *engrailed* in the annelid *Helobdella triserialis* (modified after Weisblat 1994). A. Early embryonic ectodermal expression (N lineage) in iterated stripes in posterior of each segment (compare Fig. 12A). B. Neuronal expression in forming ganglia of a more advanced stage. Note the paired lateral cluster of *engrailed* positive cells in the posterior and the median cluster in the anterior of each ganglion anlage (compare Fig. 12B).

& Kaufman 2002a). The assumption of a conserved anterior boundary led some authors to re-interpret the homology between chelicerate and mandibulate segments (Damen et al. 1998, Telford & Thomas 1998, Damen & Tautz 1999). If we compare this general Hox pattern with the one of annelids, the leech *Helobdella* and the polychaete *Chaetopterus*, we again find a striking similarity of anterior boundaries (Kourakis et al. 1997, Shankland 1999, Irvine & Martindale 2000). On the basis that the prostomial ganglion of annelids is homologous to the protocerebrum of arthropods and that the antennal segment of arthropods is the anteriormost true segment (Scholtz 1997), the annelid pattern is in good agreement with the general euarthropod pattern. For instance, in all cases the anterior boundary of *labial* and *proboscipedia* expression is found in the region of the 1st or 2nd segments. The only exception is seen in *Drosophila* where the anterior border of *proboscipedia* expression is in the 4th segment. *Deformed* expression is found from the middle of the 2nd segment in the leech, at the border between 2nd and 3rd segments in Crustacea, Insecta, Myriapoda, and Chelicerata, and in the posterior of the 3rd segment in *Chaetopterus*. The anterior border of *sex combs reduced* expression spans the region from the middle of the 3rd segment in the leech representative, the anterior border of the 4th segment in crustaceans and chelicerates, and the middle of the 4th segment or the anterior border of the 5th segment in insects and myriapods. The anterior border of *Antennapedia* expression is restricted to the 4th or 5th segments, and the combined expression of *Ultrabithorax* and *abdominal-A* is seen in the 6th and 7th segments in the leech and the arthropods studied. The onychophoran studied concerning the expression of *Ultrabithorax/abdominal-A* does not fit into this pattern, showing expression only in posterior segments (Grenier et al. 1997). This seems to be one of the numerous autapomorphies of Onychophora. Even if the slight differences in segmental register are considered, the resemblance between annelids and arthropods is astonishing. In general there are one to two segments between the anterior borders of two subsequent Hox genes in arthropods and annelids.

A comparison with Hox gene expression in chordates reveals distinct differences with respect to the metamerical register (Holland & Garcia-Fernandez 1996; Prince et al. 1998a, b; Sharman & Brand 1998; Carroll et al. 2001). In vertebrates the gene *Hox1* (*labial*) has its anterior expression border in the 4th rhombomere, the *Hox2* gene (*proboscipedia*) starts in rhombomere 2. The anterior expression border of *Hox4* (*Deformed*) falls together with the boundary between the 6th and 7th rhombomere, whereas that of *Hox5* (*sex comb reduced*) lies posterior to rhombomere 8, etc. This means that the anterior boundaries of two Hox genes span the width of about three metameres.

It is not trivial that the relative size class of segments in relation to Hox gene expression is the same in annelids and arthropods and different from the pattern observed in chordates. Hox gene expression is not strictly related to segmentation and it also occurs in non-segmented bilaterians. For example, *sex combs reduced* is expressed in the anterior mid-body region of an unsegmented gastropod in the area of the forming branchial ganglion (Giusti et al. 2000). Thus, metamerization is an evolutionarily secondary character which became superimposed on the bilaterian body which was patterned by Hox genes along the antero-posterior axis.

Perspectives

It appears that there are numerous and independent correspondences between the segmentation patterns of annelids and arthropods at different levels including development. All the listed characters suggest homology of the specific segmentation of annelids and arthropods. Most of these characters find no correspondence in other animal taxa, particularly not in the representatives of Gastrotricha and Cycloneuralia, but also not in molluscs (Friedrich et al. 2002). Thus, there is good evidence that the complex segmentation pattern is synapomorphic for annelids and arthropods. Accordingly, the bilaterian stem species ("Urbilateria") did not show a corresponding segmentation. In my opinion it is premature to interpret new data, for instance developmental gene expression, exclusively in the light of the Ecdysozoa hypothesis. However, the Ecdysozoa concept is a significant challenge which requires a series of research programmes which should be undertaken open-mindedly.

There is a chance of finding more similarities which possibly can support the Articulata. For instance, we do not have data on individual neurons homologous between annelids and arthropods which are comparable with respect to their position, their axon morphology, and to the expression of transmitters or genes. At least for arthropods there is growing evidence for conservation of such characters (Whitington 1996; Gerberding & Scholtz 1999, 2001; Duman-Scheel & Patel 1999; Harzsch & Waloszek 2000). This is a promising field for further studies to find possible homologues between annelids and arthropods. Furthermore, it is now well established that several genes involved in segmentation and in limb formation show similar expression patterns throughout euarthropods, but we do not yet know much about this in annelids.

On the other hand, there are some characters supporting Ecdysozoa and perhaps there are more to be found. For instance, gene expression data for gastrotrichs, priapulids, kinorhynchs, nematomorphans and loriciferans are entirely lacking. Moreover, we still know too little

about the embryology of most cycloneuralians. Except for nematodes (e.g., Boveri 1899, Müller 1903, Sulston et al. 1983, Schierenberg 2000), there are no data about early cleavage patterns, the formation of germ layers, embryonic growth, or neurogenesis. In particular, the embryonic formation and differentiation of the zonites of Kinorhyncha has never been investigated. I think this is a promising field for our understanding of bilaterian relationships.

The early cleavage pattern has always been used to infer phylogenetic relationships among higher metazoan taxa (Siewing 1969, 1979; Valentine 1997). The most prominent example for this is the taxon Spiralia (Siewing 1979, van den Biggelaar et al. 1997). If the Articulata hypothesis is correct, arthropods are consequently members of the Spiralia. Accordingly, the pattern of holoblastic cleavages occurring in some representatives of arthropods has been interpreted as being spiralian-like by several authors (e.g., Anderson 1969, 1973; Nielsen 2001). However, these spiralian characters of arthropod cleavage have been doubted by Pflugfelder (1962), Siewing (1979), Weygoldt (1979), Dohle (1979, 1989), and Scholtz (1997). Scholtz (1997) instead reconstructed a ground pattern of arthropod cleavage as being a variable modified radial cleavage. This does not necessarily mean, however, that the arthropod cleavage is not

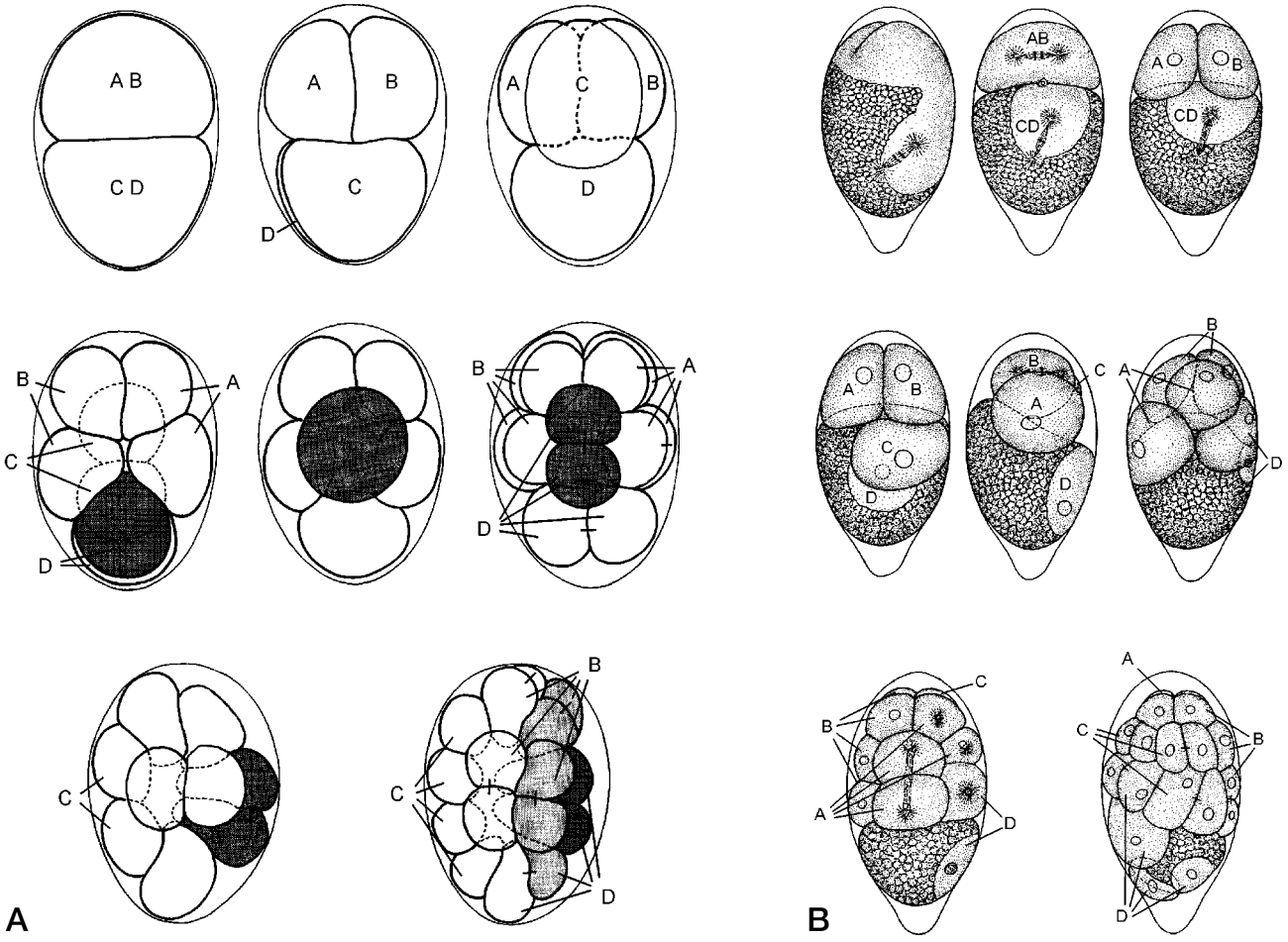


Fig. 14. Cleavage in Gastrotricha and Crustacea. A. Early cleavage up to gastrulation in the gastrotrich *Turbanella cornuta* (modified after Teuchert 1968). Upper row from left to right: 2-cell stage, 4-cell stage, 4-cell stage with blastomere C migrated to anterior (all dorsal views). Middle row from left to right (ventral views): 8-cell stage (the dark grey blastomere is the ventral descendant of D, it forms the entoderm), 8-cell stage with anterior movement of the prospective entoderm cell (dark grey), 16-cell stage. Lower row (lateral views, ventral to the right): 16-cell stage, 30-cell stage (light grey: mesoderm, dark grey: entoderm).
B. Early cleavage in the cirripede crustacean *Tetraclita rosea* (modified after Anderson 1969). Upper row from left to right: first cleavage division, 2-cell stage, beginning 4-cell stage. Middle row from left to right: 4-cell stage (dorsal view), 4-cell stage (lateral view), 8-cell stage (lateral view, dorsal to the right). Lower row from left to right: 15-cell stage (lateral view, dorsal to the right), 28-cell stage (lateral view, dorsal to the left). In all eggs, the entoderm cells (derivates of blastomere D) are shown with yolk granules. In contrast to *Turbanella*, the gastrulation is no immigration, but the large yolk-containing cells of the D quadrant are overgrown by the derivatives of the other quadrants.

derived from ancestral spiral cleavage. In the light of the Ecdysozoa hypothesis the pattern of arthropod holoblastic cleavage could be reconsidered. There are some astonishing resemblances of the early cleavage between some crustaceans and gastrotrichs (Teuchert 1968, Anderson 1969, Hertzler & Clark 1992) (Fig. 14). These similarities concern the directions of the two spindles of the second cleavage, which are at right angles to each other, the resulting cleavage pattern of two crosswise interlocked bands of blastomeres, the blastomeres A, B, and C marking the anterior and dorsal regions of the animal, the D cell giving rise to the posterior/ventral regions, the retardation in division of ur-entoderm cells deriving from the D blastomere of the 4-cell stage, the ur-entoderm cells starting gastrulation, and the origin of the mesoderm from more than one blastomere of the 4-cell stage (Fig. 14). However, these might just be superficial correspondences, and a careful analysis of cell lineage is urgently needed.

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