

The clade Ecdysozoa, perplexities and questions

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Abstract

A relatively new clade, the Ecdysozoa [Aguinaldo et al., 1997. *Nature* 387, 489–493] was raised based on the 18S ribosomal DNA sequences that indicate a close relationship between the moulting phyla (Arthropoda, Tardigrada, Onychophora, Nematoda, Nematomorpha, Kinorhyncha, Lorificera and Priapulida), from which the Annelida, with other phyla, are excluded.

However, the authors here expressed puzzlement about this conclusion. In particular they stressed that: (a) ecdysis might not be an autapomorphy for the Ecdysozoa; (b) some Ecdysozoa phyla are unrelated from one another with regard to morphology and embryogeny; (c) the annelids have a body architecture that is more similar to arthropods than some of the Ecdysozoa; (d) the annelids are moulting animals; (e) some phyla excluded from the new clade (e.g. the gastrotrichs), probably carry out a gradual ecdysis by flaking similar to that of the polychaetes.

The authors concluded that the clade Ecdysozoa appears to be phylogenetically unconvincing.

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Aguinaldo et al. (1997) recently proposed a new clade, Ecdysozoa, which included all moulting protostomes. These authors wrote: "our phylogenetic analysis of 18S ribosomal DNA sequences indicates a close relationship between arthropods, nematodes and all other moulting phyla. The results suggest that ecdysis (moulting) arose once and support the idea of a new clade, Ecdysozoa, containing moulting animals: arthropods, tardigrades, onychophorans, nematodes, nematomorphs, kinorhynchs and priapulids". Annelids, by inference non-

moulting, were excluded from this clade as, to quote: "No support is found for a clade of segmented animals, the Articulata, uniting annelids with arthropods" (Aguinaldo et al. 1997).

The clade of the Ecdysozoa has stimulated a great deal of interest, and is much cited by many zoologists. Our team includes zoologists, comparative anatomists and geneticists, who for many years have been engaged in research projects on animal morphology, systematics and phylogenesis. After much discussion on the subject of the Ecdysozoa clade, which took into account various viewpoints, we felt it would be useful to publish our deliberations.

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The methodology adopted by [Aguinaldo et al. \(1997\)](#) was standard (and in this respect we have no criticism). But we should emphasize that [Wägele et al. \(1999\)](#) and [Wägele and Misof \(2001\)](#) maintained that the monophyly of the Ecdysozoa might be a manifestation caused by chance similarities in 18S rDNA sequences. Regardless of this, the conclusions of [Aguinaldo et al.](#) induced us to raise some questions and to make some comments.

(A) Given that of 15 phyla (out of the 26 investigated)¹ [Aguinaldo et al. \(1997\)](#) tested only one species (or only a few species in each of the other phyla), we wonder how a single species can truly represent the whole phylum. In our opinion it is not possible to exclude the fact that there will be differences between species of the same phylum, and that one species is not representative of the whole.

Within some phyla (e.g. Platyhelminthes, Nematoda) the 18S rDNA sequences evolve at different rates (in some species very rapidly in other species more slowly); in these cases [Aguinaldo et al. \(1997\)](#) selected the slowest evolving species and so compared phyla having sequences with similar evolutionary velocities; however, in our opinion, this does not prove with absolute certainty that such tested species are representative of the whole phylum. Above all, it does not prove that the 18S rDNA sequence of the few tested species is the minimum evolutive velocity of the relative phylum.

With regard to the 18S rDNA our comments seem to be justified due to the fact that, for example, [Le et al. \(2000\)](#) found considerable differences in the mitochondrial gene orders between species of Trematoda from the genus *Schistosoma*, and [Hickerson and Cunningham \(2001\)](#) obtained similar result in crustacean species. Thus [Nielsen \(2003, p. 480\)](#) noted: “the comparisons of species belonging to different phyla can be dubious if only one or few species have been investigated”.

(B) [Aguinaldo et al. \(1997\)](#) wrote: “However, given that all moulting taxa sampled are in this clade, and given the significant anatomical modifications associated with moulting, such as the lack of locomotory cilia, ecdysis appears to be a defining synapomorphy for this group”. But, as [Aguinaldo et al. \(1997\)](#) also noted, the absence of locomotory cilia is not exclusive of the Ecdysozoa (for example acanthocephalans and chaetognaths also lack locomotory cilia). We also believe that the absence of locomotory cilia in the various evolutionary lines is a secondary character, according to the opinion of all the scholars of animal phylogenesis who maintain that the hypothetical common ancestor of the Metazoans was a ciliated organism. Therefore convergences cannot be excluded.

We do wonder why some important differences that divide the various phyla of the Ecdysozoa were neglected. To mention the most obvious we can state that: (i) the priapulids have radial cleavage (or with no elements of spiral cleavage) (see: [Hammarsten 1915](#); [Lang 1939, 1953, 1963](#); [Purasjoki 1944](#); [Zhinkin 1949](#); [Zhinkin and Korsakova 1953](#); [Hyman 1959](#); [Dawydoff 1959](#); [Colosi 1967](#); [Barnes 1980](#); [Van Der Land and Nørrevang 1985](#); [Baccetti et al. 1991](#); [Ruppert and Barnes 1997](#); [Brusca and Brusca 2003](#)), while all other Ecdysozoa have spiral cleavage; [Aguinaldo et al. \(1997\)](#), and other advocates of the Ecdysozoa, do not specify what the triploblastic ancestor common to priapulids and the other Ecdysozoa should be. Did it have radial or spiral cleavage? Perhaps they meant to imply that the priapulids, with radial cleavage, could be derived from an ancestor with spiral cleavage; if so, this hypothesis should be discussed. (ii) Nematodes, nematomorphs and kinorhynchs have a blastocoelom while all the others have a mesodermic coelom. (iii) Some phyla are metameric and others have no trace of metamerism, not even in the most primitive species. (iv) Some phyla have an unsclerotised cuticle, while others have a sclerotised cuticle. (v) Some phyla have appendices while others do not (not even in the most primitive species). (vi) [Aguinaldo et al. \(1997\)](#), and other advocates of Ecdysozoa, did not take into consideration other characters, for example the organisation of the intracellular joints of the midgut in nematodes and arthropods although the data about this are contradictory (see [Marinari Palmisano et al. 1989](#); [Dallai 2002](#)). [Marinari Palmisano et al. \(1989\)](#) wrote: “The case of superficial resemblance of the midgut junction in nematodes and arthropods indicates not a relationship between these two groups, but merely a parallel evolution of the organization of this junctional model”.

It seems superfluous to continue this list as according to [Aguinaldo et al. \(1997\)](#) all these differences, and others that we have not mentioned, are less important than the similarities of the 18S rDNA sequences.

For these authors ([Aguinaldo et al. 1997](#)) the differences based on these sequences are, together with the absence of ecdysis, the main character to support the more distant phylogenetic position of the annelids from the arthropods than that of the nematodes or priapulids. Therefore they excluded the annelids from the Ecdysozoa despite the morphological similarities between annelids and onychophorans, tardigrades and arthropods. Similarities are also emphasized by others (See: [La Greca 1981](#); [Westheide and Rieger 1996](#); [Schram 1991](#); [Backeljau et al. 1993](#); [Schram and Ellis 1994](#); [Nielsen et al. 1996](#); [Wägele et al. 1999](#); [Ax 2000](#); [Sørensen et al. 2000](#); [Nielsen 2001](#); [Scholtz 2002](#); [Brusca and Brusca 2003](#) and many others).

(C) [Aguinaldo et al. \(1997\)](#) consider ecdysis as a synapomorphy within Ecdysozoa. Before drawing any

¹Actually the number of phyla investigated was 24 because Pogonophora and Vestimentifera are today considered polychaete annelids ([Rouse and Pleijel 2001](#)).

conclusions we would first like to present some considerations. Obviously the acquisition of ecdysis is not to be underestimated, but it is also apparent that the evolutive thrust towards ecdysis depends on the characteristics of the tegument. No animal with a sclerotised cuticle would have survived if it had not at the same time acquired the capacity to carry out ecdysis. Animals with very flexible cuticles might or might not acquire ecdysis. It seems clear that only those strains that possess the pre-requisites that could lead to ecdysis (e.g. the production of ecdysteroids) were able to acquire ecdysis and harden their cuticle. There are two possible scenarios: (a) the pre-requisites could have been present in more than one ancient ancestor. In this case we can imagine that similar pre-requisites were selected for in quite dissimilar groups; or (b) the pre-requisites could have been present in a unique ancestor. In this case it is possible that not all the descendant strains modified the cuticle and/or acquired the capacity to carry out ecdysis.

In fact, taking into account that ecdysteroids have been found both in moulting animals and in non-moulting animals: e.g. cnidarians (Giribet 2003), nemertean (Okazaki et al. 1998), Platyhelminthes (Sauber et al. 1983) and are also present in plants (Lafont 1997), we wonder if two events could have taken place. Is it possible that: (a) phylogenetically distant groups independently acquired the capacity to moult? The ecdysis of snakes and other reptiles is not homologous to that of the Ecdysozoa, but its existence shows that the capacity to substitute the superficial portion of the tegument has appeared more than once in different groups, and that this takes place with different mechanisms according to the structure of the tegument. (We will speak of another type of moulting later). In other words, how can we exclude that the capacity to carry out ecdysis is, at least sometimes, the product of convergence? And, (b) in two phylogenetically close taxa one modified its cuticle and acquired the capacity to carry out ecdysis while the other did not undergo these modifications? This is at least theoretically possible.

Given that, as we believe, the answer to these two questions is affirmative, it seems that we cannot rule out that ecdysis has appeared more than once in different animal groups, in both phylogenetically distant and close evolutionary lines. From this we can conclude that the ecdysis might not be an autapomorphy for the Ecdysozoa clade. This is also the opinion of Wägele et al. (1999, p. 218) who wrote: “There any acceptable evidence for the homology of moulting is lacking”.

(D) As regards the clade of the Ecdysozoa, Aguinaldo et al. (1997, p. 491) reported: “The name reflects the property that all members of this group, and only members of this group, undergo ecdysis during at least part of their life cycles”. However, the authors only studied the 18S rDNA from 24 phyla (and only from a single species in 15 of these) while sequences of several

other phyla have not been investigated (i.e. Placozoa, Rhombozoa, Orthonectida, Porifera, Gnathostomulida, Entoprocta, Cycliophora, Pentastomida, Hemichordata). We therefore wonder if we can exclude without previous investigation these other groups, or whether they might contradict the conclusions of Aguinaldo et al. (1997)? In other words, can we exclude that one of the not investigated and not moulting groups, has 18S rDNA sequences similar to those of the Ecdysozoa? Obviously this is only a hypothetical possibility, but it raises some doubts about the correctness of drawing conclusions, with less than full data. These dilemmas could be easily be eliminated by specific investigations.

(E) To the series of concerns expressed above, we must add a rather significant fact (not a hypothesis), which in our opinion questions the validity of the Ecdysozoa clade. The annelids, conversely to the opinion expressed by Aguinaldo et al. (1997), and the other advocates of the Ecdysozoa clade, carry out ecdysis. This property had been indicated by Leydig in 1849 for leeches (see: Sauber et al. 1983) and it was also noted in various zoological textbooks (e.g. Harant and Grassé 1959; Colosi 1967; Baccetti et al. 1991 and others). Leeches in moult are relatively easy to find. Sauber et al. (1983) emphasized the presence of ecdysone and 20-hydroxyecdysone in leeches and in two oligochaetes, *Eisenia foetida* (Savigny, 1826) and *Dendrobaena subrubicunda* (today *Dendrodrilus subrubicundus*) (Eisen, 1874), and hypothesized that “chez les Annélides, les ecdystéroïds contrôlent les cycles tégumentaires comme ils le font chez les Arthropodes”. Giribet (2003, p. 314), (an exponent of the pro Ecdysozoa fraction), noted: “Furthermore, ecdysteroids are also found in leeches, where they are thought to be involved in cuticular shedding (Sauber et al. 1983). However, these characteristics, irrespective of whether Articulata or Ecdysozoa (or neither) are accepted, can only be explained by convergence, since leeches are generally considered derived annelids.” We do not understand how Giribet overlooked that Sauber et al. (1983) also found ecdysone and 20-hydroxyecdysone in oligochaetes. With regard to the polychaetes (certainly not the most derived annelids), Pilato et al. (1989,1990), Pilato and La Rosa (1990, 1992) and La Rosa and Pilato (1993) studied the cuticle ultrastructure of five species from four different families and showed that in the polychaetes the cuticle is also a dynamic structure. They noted that, in all the species studied, the superficial portion of the cuticle undergoes modifications in preparation of substitution, and that the superficial layers detached by a form of flaking (Figs. 1–3). Thus polychaetes perform a particular form of gradual ecdysis. Whilst we have not personally studied the oligochaetes, the structure of their cuticle, and the observations of Postwald (1971) and Sauber et al. (1983) lead us to believe that the oligochaete cuticle behaves in

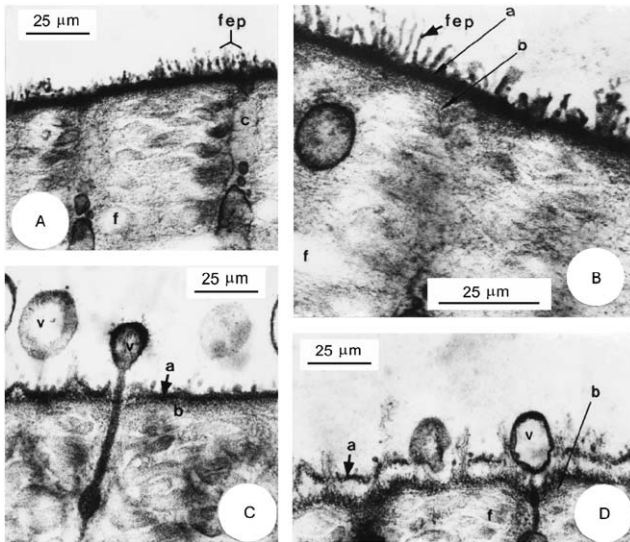


Fig. 1. Cuticle of *Lumbrineris crosslandi* Perkins, 1979 (Polychaeta, Lumbrineridae). (A, B) Cuticle at rest. The epicuticle is visible (with the electrondense layers a and b and the epicuticular filaments) above the fibrous portion. Two microvillar canals are also visible in (A). (C) The distal portion of a microvillus became the electrondense peduncle of a vesicle that will come off. (D) The layer a is splitted and the more superficial layer is coming off by flaking. Epicuticular filaments are already pulled off. a, b = layers of the epicuticle; c = microvillar canal; f = cuticular fiber; fep = epicuticular filaments; v = distal portion of a microvillus formed a terminal vesicle (from Pilato et al. 1990).

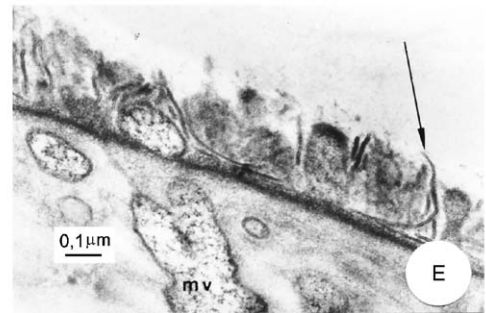
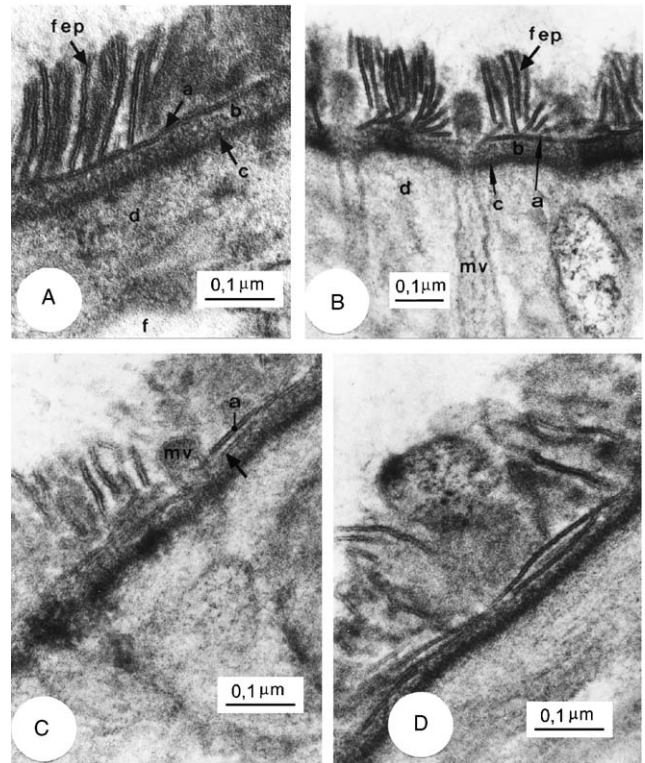


Fig. 2. Cuticle of *Marphysa sanguinea* Montagu, 1815 (Polychaeta, Eunicidae). (A, B) Cuticle at rest. The epicuticle with the layers a, b, c, d and the filamentous epicuticular formations is visible; two microvillus are also visible in (B). (C) A new layer a is forming (arrow) beneath the more superficial layer a. (D) Two layers a are formed the more superficial of which is partially coming out. (E) The more superficial layer a is partially coming out (arrow); some filamentous epicuticular formations are already pulled off. a, b, c, d = layers of the epicuticle; f = fibers of the fibrous layer of the cuticle; fep = epicuticular filaments; mv = microvillus (from Pilato and La Rosa 1990).

the same way as that of the polychaetes. Therefore, we believe moulting in annelids is a primitive character of the whole phylum and not an exclusive secondary character confined to leeches as hypothesized by Giribet (2003). This confirms our opinion that moulting could have appeared more than once using different mechanisms, according to the characteristics of the tegument, in descendants of ancestors that already possessed ecdysteroids. Therefore we believe that, as far as moulting is concerned, convergence cannot be completely ruled out.

It would also be appropriate to verify whether other groups that were excluded from the Ecdysozoa clade also moult in a similar way to the polychaetes. Some figures from Rieger and Rieger (1977) on the ultrastructure of the gastrotrich cuticle (particularly Figs. 1, 2, 6A–D, 7B and 10A) suggest that these animals probably moult by flaking. Also some observations by Rieger and Rieger (1977), such as the number of epicuticular layers, appear to confirm our opinion. Rieger and Rieger (1977, p. 84) reported: “the cuticle consisted always of an outer part of membranous sheaths of varying numbers”; also (p. 85): “in the latter the numbers vary considerably within the specimen studied”; and, most importantly, (p. 87): “Whereas in one specimen most of the layer was gone completely

(except for small areas...) the second specimen clearly showed the lamellar layer continuous along the animal surface but... bi- or monolayers could not be clearly distinguished”. How else could these images and statements be interpreted if they do not provide evidence of moulting (by flaking) in gastrotrichs?

Among the phyla included in the clade of the Ecdysozoa there exist differences relative to the structure of the cuticle; furthermore, it would be necessary to

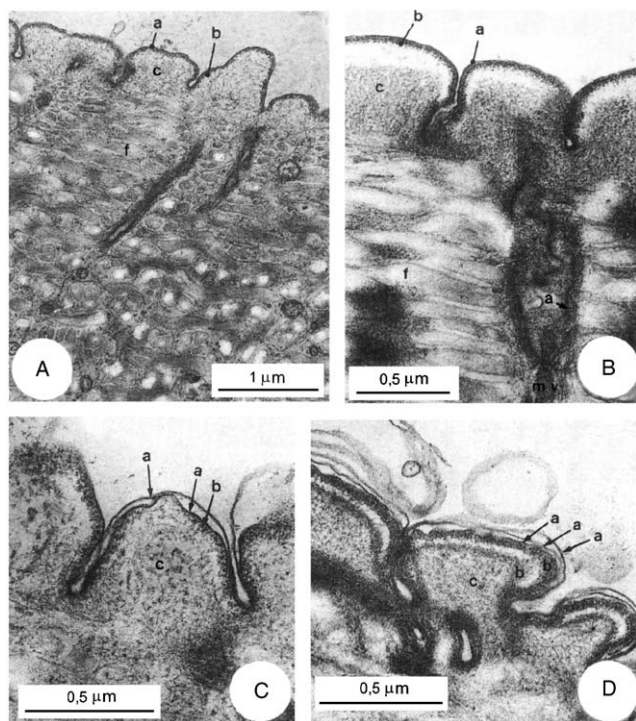


Fig. 3. Cuticle of *Nereis diversicolor* O. F. Muller, 1776 (Polychaeta, Nereidae). (A) Cuticle at rest. The epicuticle with its layers a, b and c is visible above the fibrous portion. Two microvillous are also visible. (B) The superficial layers a and b tend to come off from the layer c. (C) The layer a partially came off and a new layer a is formed. (D) The superficial layers a and b tend to come off from the layer c which is forming a new layer b. Three layer a partially coming off are evident. a, b, c, = layers of the epicuticle; f = fiber of the fibrous portion of the cuticle (from Pilato and La Rosa 1992).

verify whether there are also differences in type of ecdysis, and to evaluate whether such differences are derived characters.

In particular, it seems important for us to emphasize that the points discussed above for annelids (and potentially for gastrotrichs) would appear to be sufficient to invalidate the statement referring to the Ecdysozoa that: "... only member of this group undergo ecdysis during at least part of their life cycle" (Aguinaldo et al. 1997).

If ecdysis is not exclusive to the phyla that make up the clade of Ecdysozoa, then these phyla are grouped together based solely on the similarity of the 18S rDNA sequences. A similarity that is at odds with the morphological differences (some of which we have already mentioned), and with those of a molecular nature that were reported by Aguinaldo et al. (1997) (e.g.: Winnepeninckx et al. 1995; Boore et al. 1995). This single point of similarity does not appear to be sufficient to cluster nematodes or priapulids more closely to arthropods than annelids. Felsenstein (1978)

and McHugh (1998) reported on the probability that a high rate of substitution can lead to similar nucleotide sequences in taxa that are phylogenetically distant; Wägele et al. (1999) and Wägele and Misof (2001) also expressed puzzlement over grouping within the Ecdysozoa clade. Taking these statements into consideration, we believe that the phyla included in the Ecdysozoa clade (Aguinaldo et al. 1997) do not form a homogeneous phyletic line, and that it is unjustified to consider the annelids phylogenetically more distant from the arthropods than nematodes, nematomorphs, kinorhynchs and priapulids. We therefore submit that this clade, at least paraphyletic, is not phylogenetically justified.

We do agree that there are some characters that are common to the phyla included in the Ecdysozoa clade (Schmidt-Rhaesa et al. 1998; Manuel et al. 2000; Haase et al. 2001; Kristensen, 2003). However, we will not discuss these similarities, as we think that some of them could be caused by convergence, while others may be the trace of an extremely ancient common ancestor from which the annelids also are derived (Pilato 1992).

In conclusion we believe that the comparison of the 18S rDNA sequences, and other molecular sequences that are in fashion today, can undoubtedly give useful information and help resolve various taxonomical and phylogenetic problems. We consider all molecular data very important and we are deeply convinced that it is necessary to recognize their contribution. Nevertheless, we do not believe it is justifiable to neglect the indications from morphology, and embryology, and establish phylogenesis giving preference to molecular data. Particularly if this data is in contrast to the evaluation suggested by morphology and embryogeny, and at odds with other molecular data. Some of these contradictions include: (a) previous work on 18S rRNA gene sequences which indicated that tardigrades represent a sister group of an assemblage that included nemertean, molluscs, sipunculids, annelids and arthropods, and that they represent a discrete lineage apart from nematodes (Moon and Kim 1996); (b) nematodes differ from the other blastocoelomates due to the type of collagen, haemoglobin and rRNA sequences (Willmer 1990); (c) the onychophorans are grouped with the arthropods when 12S mitochondrial rRNA sequences are considered (Ballard et al. 1992), but emerge as a sister group when the 18S rDNA is applied (Wheeler et al. 1993); (d) the 18S rDNA sequences support, according to Wheeler et al. (1993) a close annelid-arthropod link, but Van de Peer et al. (1993) expressed a different opinion, which was supported by Abele et al. (1989) and Turbeville et al. (1991) who studied the 18S rRNA sequences, and Ballard et al. (1992) who studied the 12S mitochondrial rRNA sequences; (e) the results from 18S rDNA analysis indicate that priapulids belong to Ecdysozoa, but the amino acid sequences of

hemerythrin indicate an affinity between priapulids and brachiopods (Runnegar and Curry 1992).

We also cannot accept the evident disregard for established facts regarding the presence of moulting in annelids, and are bemused by the continued disregard of this aspect despite the level of debate excited by the Ecdysozoa clade. Indeed, some authors are adamant when they express their opinion, e.g. Schmidt-Rhaesa et al. (1998, p. 271): “The cuticle is moulted in panarthropods and cycloneuralians, but not in annelids”. We think it appropriate to remove this misunderstanding, and we also think that the conclusions maintained by a misunderstanding are at least debatable. For example, Schmidt-Rhaesa et al. (1998, p. 274) noted: “In gastrotrichs, several trilaminate layers are found (Ruppert 1991) and this multiplication is probably an autapomorphic character of the taxon”; but it has been known for a long time that polychaete annelids can have an epicuticle with many trilaminate layers (see Figs. 2C, D, E and 3C, D and Westheide and Rieger (1978): Figs. 5–7). As a consequence we think that the opinion of Schmidt-Rhaesa et al. (1998) about the position of gastrotrichs is at least debatable. In addition, the advocates of the Ecdysozoa clade have, without discussion, included priapulids (to which many authors attribute a radial cleavage), in a group that is considered to derive from an ancestor with spiral cleavage. In doing so they have overlooked the problem of how radial cleavage would be derived from spiral cleavage.

We have no intention of discussing here all the pros and cons of the Ecdysozoa and Articulata hypotheses (such discussions can be found in the papers by Schmidt-Rhaesa et al. (1998), Giribet (1999, 2003), Giribet and Wheeler (1999), Wägele et al. (1999), Wägele and Misof (2001), Zrzavý (2001, 2003), Scholtz (2002), Nielsen (2003) and many others). Also we do not think that it is appropriate to insist on considering those two phyletic groups as two well-defined and radically divergent evolutionary lines. It seems to us more probable that many protostome phyla were derived, in an “explosive” manner, from a unique very remote ancestor, with some phyla running more parallel to one another (Pilato 1992). Although we have different ideas to the solution proposed by Nielsen (2003) for the Articulata–Ecdysozoa controversy, we do agree with him about the opportunity and the possibility of overcoming that controversy. Here we should like to encourage protagonists to overcome the radical disagreements between morphological and embryological data on the one hand and molecular data on the other. All data are useful but all data must be taken into consideration. This is also the opinion of other scientists; Wheeler (2004, p. 573) reported: “Molecular phylogenies have retraced ground covered long ago by morphologists, ecologists, and behaviourists. Without this historical background knowledge of interesting

anatomical structures or behaviours, these molecular phylogenies would have little or no interest to science”. And (p. 574): “We need to re-establish parity between morphological research and molecular”.

Today people speak of “molecular phylogenesis”, or “new phylogenesis” but we believe that no adjectives should be added to the word phylogenesis. Phylogenesis should be traced using all the data that we can obtain without attempting to give precedence to one type over another. Nor should data be ignored as it could contribute to avoiding errors and resolving problems.

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