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The dawn of bilaterian animals: the case of acoelomorph flatworms

Jaume Baguñà* and Marta Riutort

Summary

The origin of the bilaterian metazoans from radial ancestors is one of the biggest puzzles in animal evolution. A way to solve it is to identify the nature and main features of the last common ancestor of the bilaterians (LCB). Recent progress in molecular phylogeny has shown that many platyhelminth flatworms, regarded for a long time as basal bilaterians, now belong to the lophotrochozoan protostomates. In contrast, the LCB is now considered a complex organism bearing several features of modern bilaterians. Here we discuss an alternative view, in which acoelomorph (Acoela + Nemertodermatida) flatworms, which do not belong to the Platyhelminthes, represent the earliest extant bilaterian clade. Sequences from ribosomal and other nuclear genes, Hox cluster genes, and reinterpretation of some morphological features strongly support the basal position of acoelomorphs arguing against a complex LCB. This reconstruction backs the old planuloid–acoeloid hypothesis and may help our understanding of the evolution of body axes, Hox genes and the Cambrian explosion. *BioEssays* 26: 1046–1057, 2004. © 2004 Wiley Periodicals, Inc.

Introduction

A deep mystery: what the Last Common Bilaterian (LCB) Ancestor looked like

Among the unsolved puzzles in animal evolution, the origin of the bilaterian metazoans from radial ancestors and the ensuing Cambrian explosion, is outstandingly important. What makes it so special is the large structural and functional gap that it represents and the apparent swiftness with which it occurred. Moreover, the legacy that it left is the most eloquent proof of how important it was: 34 out of the 38 living phyla and over 99 per cent of described living species are bilaterians, far more complex in structure and far more diverse in morphology and ecology than their radial forebears. Therefore, the nature

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of the LCB or, in other words, what the LCB looked like, is of enormous interest not only for understanding where most of the animal life came from but to infer the genetic, molecular and cellular changes that implemented the radial–bilaterian transition.

Most bilaterians share several defining characteristics. (1) A basic set of body plan features about which there is overall consensus comprises bilateral symmetry, two orthogonal body axes (anteroposterior and dorsoventral), triploblasty (appearance of mesoderm as a third layer), and a nervous system with an anterior concentration of nerve cells from which nerve tracts extends posteriorly. (2) Further characters have also been considered potential bilaterian synapomorphies: a through-gut (mouth + anus), an excretory system, primitive eyes and, more recently, the presence of an extended Hox cluster comprising at least 7 or 8 colinear genes. (3) More problematic features include the presence of a secondary body cavity or coelom, segments, a heart, and, even, the type of appendages. Whenever an hypothetical LCB with the first or with the second and third sets of apomorphies is compared to a radial organism bearing none of them and from which the former originated, one is left to wonder how this actually took place.

The main conflicting hypothesis on the radial–bilaterian transition

The two main scenarios are represented in a simplified form in Fig. 1. The first (Fig. 1A), which epitomises the work of Libbie Hyman[1,2] and many others, conceives such a transition in small steps.[3] This is best illustrated using the origin of the coelom. The lack of coelom (acoelomate, as seen in plathelminthes and thought to be in nemerteans) is considered as the primitive bilaterian condition. Bilaterians are then arranged in an acoelomate–pseudocoelomate–coelomate series, which reflects successive branches of these three grades of increasing structural organization and complexity. Similar reasoning can be applied to other characters such as the gut, with bilaterians such as plathelminthes, which have sack-like guts (no anus) like cnidarians, considered primitive, and the rest derived. The view of acoelomate with sack-like guts as the earliest-emerging bilaterian clades is linked to a specific hypothesis as to how bilaterians
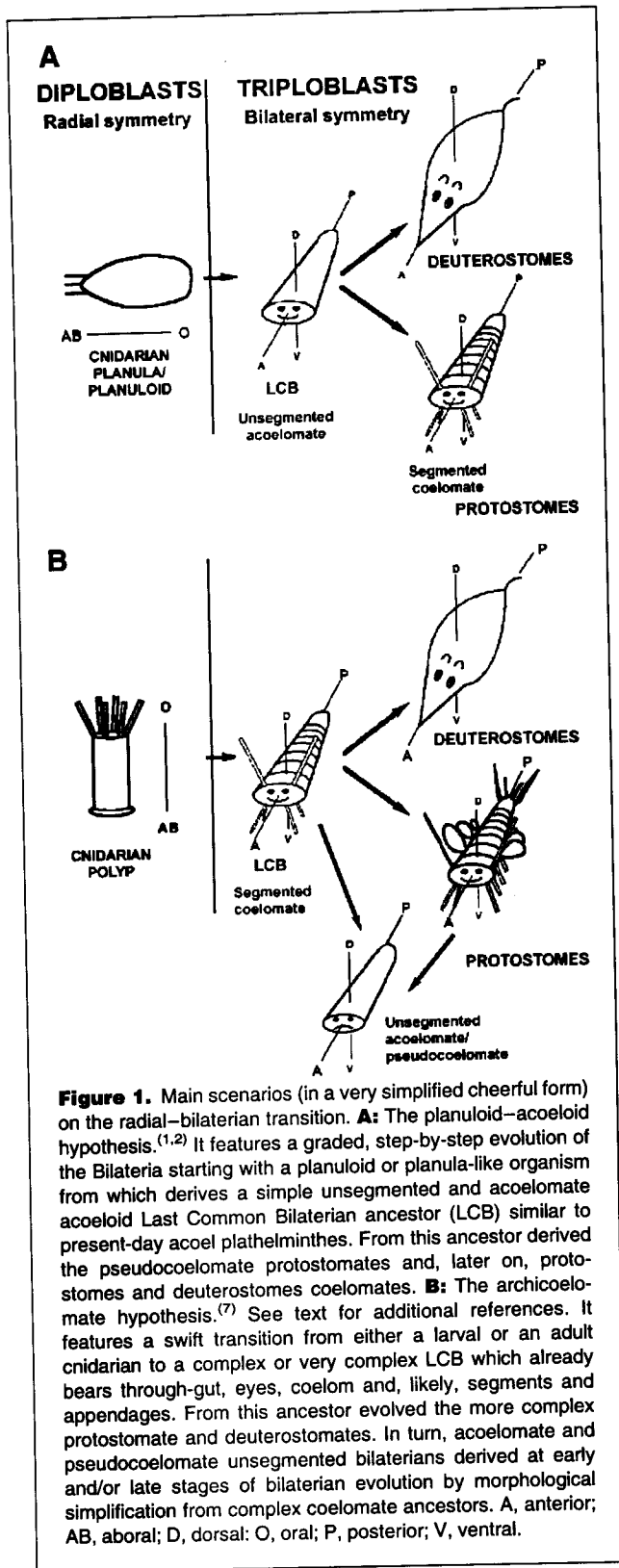
Figure 1. Main scenarios (in a very simplified cheerful form) on the radial–bilaterian transition. **A:** The planuloid–acoeloid hypothesis.[1,2] It features a graded, step-by-step evolution of the Bilateria starting with a planuloid or planula-like organism from which derives a simple unsegmented and acoelomate acoeloid Last Common Bilaterian ancestor (LCB) similar to present-day acoel plathelminthes. From this ancestor derived the pseudocoelomate protostomates and, later on, proto-stomes and deuterostomes coelomates. **B:** The archicoelo-mate hypothesis.[7] See text for additional references. It features a swift transition from either a larval or an adult cnidarian to a complex or very complex LCB which already bears through-gut, eyes, coelom and, likely, segments and appendages. From this ancestor evolved the more complex protostomate and deuterostomates. In turn, acoelomate and pseudocoelomate unsegmented bilaterians derived at early and/or late stages of bilaterian evolution by morphological simplification from complex coelomate ancestors. A, anterior; AB, aboral; D, dorsal: O, oral; P, posterior; V, ventral.
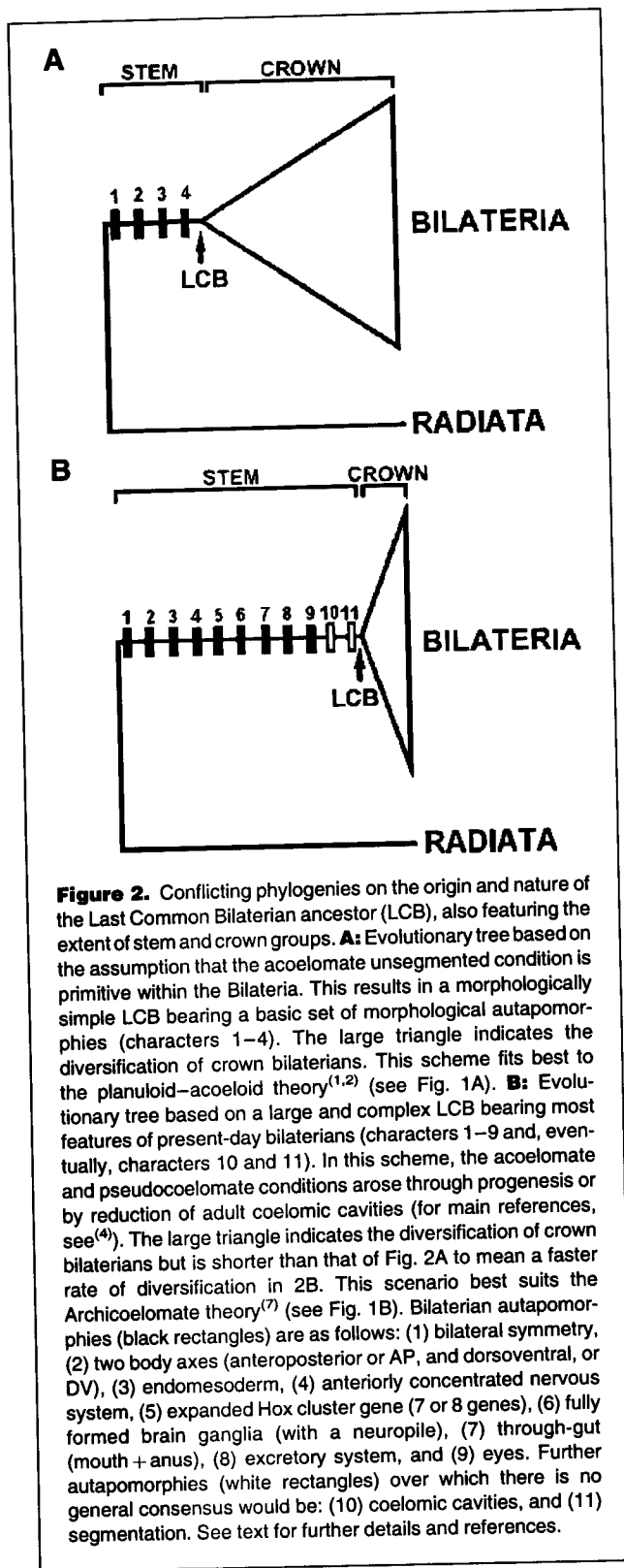
originated from radially constructed organisms (e.g. cnidar-ians and ctenophores): the planuloid–acoeloid hypothesis (for main references[4]). This scenario begins with a planuloid resembling the planula larva of a modern cnidarian but able to reproduce (initially, the planuloid was considered the common precursor of cnidarians and bilaterians). The planuloid evolved into the first bilaterian, which was similar to present-day acoel platyhelminths, probably by shifting the posteriorly placed blastopore (mouth/anus) to a central (ventral) position.

The second scenario of the radial–bilaterian transition considers the gastral pouches of cnidarians homologous to the gastral pouches (enterocoels) that form the coelomic cavities of deuterostomes (for general references[4]). Closing of the gastral pouches in the polyp or the late larva gave rise to the coelomic cavities of the bilaterian ancestor. In parallel, the polyp's oral opening (actually oval) elongated and sealed centrally except at the two ends (which gave the mouth and anus). Such feature is claimed to be 'recapitulated' in the closing of the blastopore lips by amphistomy in today's pro-tostome embryos.[5,6] Such views are encapsulated in the Archicoelomate Theory, also called the enterocoel theory, first proposed by Sedgwick[7] and later expanded in the bilaterogastraea and cyclomerism theories.[8,9] Variations of this "amphistomic" scenario have been suggested[10–12] and recently updated[13] and will not be dealt with here. All result in a common ancestor of protostomes and deuterostomes that was relatively complex anatomically (Fig. 1B). The main casualties of these views are the acoelomate Platyhelminthes and the pseudocoelomates, which had to originate from coelomate ancestors by reduction of coelomic cavities in the adult[14] or by progenesis from larval forms.[15]

Stem bilaterian groups, the LCB and crown bilaterians

The large structural (and functional) gap separating extant radial and bilaterian organisms suggests that several, now extinct, lineages intervened between the two forms. Such lineages comprise by definition fossil forms making the so-called stem bilaterian groups, which bear transitional mor-phologies leading to the crown bilaterians (the last common ancestor of extant bilaterians (LCB) and all its descendants; Fig. 2).[16] During stem group evolution, the ancestor of the crown group likely acquired its features in a stepwise fashion together with losing some of its plesiomorphic characters. However, our views on what the LCB looked like rest largely upon hypothesis about the characteristic bodyplan features that it bears. Under the gradist view (Fig. 1A), the LCB displays a limited number of features of extant bilaterians (Fig. 2A; characters 1–4), the rest being likely acquired during the evolution of the stem protostome and deuterostome lineages. Instead, the archicoelomate view (Fig. 1B) starts with a morphologically complex LCB bearing the main features of crown bilaterians (Fig. 2B; characters 1–9 or 1–11, see figure
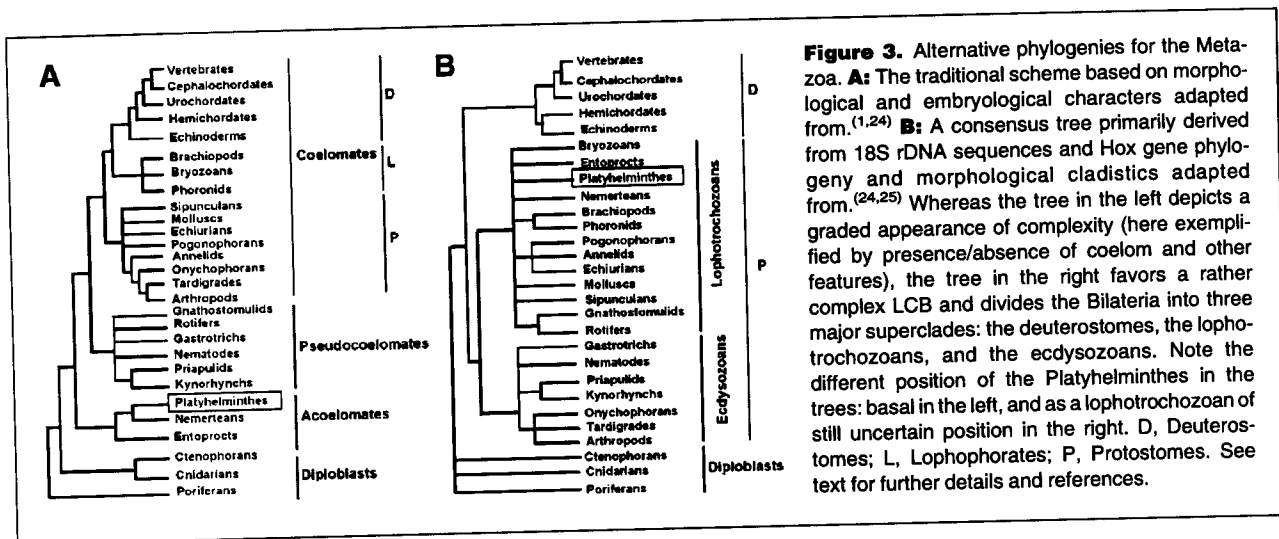
Figure 2. Conflicting phylogenies on the origin and nature of the Last Common Bilaterian ancestor (LCB), also featuring the extent of stem and crown groups. **A:** Evolutionary tree based on the assumption that the acoelomate unsegmented condition is primitive within the Bilateria. This results in a morphologically simple LCB bearing a basic set of morphological autapomorphies (characters 1–4). The large triangle indicates the diversification of crown bilaterians. This scheme fits best to the planuloid–acoeloid theory[1,2] (see Fig. 1A). **B:** Evolutionary tree based on a large and complex LCB bearing most features of present-day bilaterians (characters 1–9 and, eventually, characters 10 and 11). In this scheme, the acoelomate and pseudocoelomate conditions arose through progenesis or by reduction of adult coelomic cavities (for main references, see[4]). The large triangle indicates the diversification of crown bilaterians but is shorter than that of Fig. 2A to mean a faster rate of diversification in 2B. This scenario best suits the Archicoelomate theory[7] (see Fig. 1B). Bilaterian autapomorphies (black rectangles) are as follows: (1) bilateral symmetry, (2) two body axes (anteroposterior or AP, and dorsoventral, or DV), (3) endomesoderm, (4) anteriorly concentrated nervous system, (5) expanded Hox cluster gene (7 or 8 genes), (6) fully formed brain ganglia (with a neuropile), (7) through-gut (mouth + anus), (8) excretory system, and (9) eyes. Further autapomorphies (white rectangles) over which there is no general consensus would be: (10) coelomic cavities, and (11) segmentation. See text for further details and references.

legend). Although the last scenario is compatible with a deep but cryptic evolution of stem bilaterians as well as with a swift appearance and diversification of these lineages, most authors supporting a complex LCB have adopted the last view.[17–19] Moreover, in hypothesizing a complex LCB, it is also necessary to postulate the simplification of many metazoan body plans such as those of extant acoelomate and pseudocoelomate unsegmented bilaterians (Fig. 1B).

A good fossil record is essential to distinguish between the hypotheses depicted in Fig. 2A and B and especially to uncover and characterize stem bilaterian groups. However, the fossil record for stem bilaterian groups is presently very poor.[20] Moreover, it is often not taken into account that with elapsed time both the disparity among clades and the opportunity for extinction of intermediate forms increase.[21] Both features enlarge the phylogenetic distance between extant sister clades (witness the long branch separating Radiata from Bilateria in Fig. 2B), which reinforces the mirage that extant crown groups (phyla and superphyla) appeared at once in their present modern form, and affects how much one can infer about the morphology of the LCB from any contemporary bilaterian. The poor fossil record for stem bilaterian groups and the difficulties in choosing a consensus morphology-based hypothesis on bilaterian origins, required novel methods of analysis to set the relationships between extant metazoan groups. So far, the most succesful method has proved to be gene sequencing and the ensuing molecular phylogenies.

The new molecular phylogeny

Molecular phylogenies, namely based on sequencing of the ribosomal small subunit RNA gene (18S rDNA), soon contested some tenets of morphologically based trees[17,22–24] (Fig. 3). Among several major changes and shifts (e.g. the demise of the Articulata, the grouping of the lophophorates with the protostomes, and the split of the latter into Lophotrochozoa and Ecdysozoa), two items are worth stressing. First, acoelomates (basically the Platyhelminthes and the Nemertea) were brought, through 18S rDNA, within the new superclade Lophotrochozoa. Second, pseudocoelomate clades, or 'Aschelminthes',[1] exploded with some groups (Nematoda among them) together with Arthropods and related groups forming the new superclade Ecdysozoa,[23] whereas other phyla (e.g. Rotifera, Gastrotricha) took uncertain positions either with lophotrochozoans or with ecdysozoans.

This new paradigm was reinforced when genes from the Hox cluster of key metazoan groups were sequenced. All bilaterians studied (with some exceptions, the nematode *Caenorhabditis elegans* among them) had a large Hox cluster comprising at least 7 or 8 genes. More importantly, specific amino acid signatures and flanking regions were found for each of the three superclades.[26,27] Such features unequivocally included the Platyhelminthes (actually, only three

Figure 3. Alternative phylogenies for the Metazoa. **A:** The traditional scheme based on morphological and embryological characters adapted from.[1,24] **B:** A consensus tree primarily derived from 18S rDNA sequences and Hox gene phylogeny and morphological cladistics adapted from.[24,25] Whereas the tree in the left depicts a graded appearance of complexity (here exemplified by presence/absence of coelom and other features), the tree in the right favors a rather complex LCB and divides the Bilateria into three major superclades: the deuterostomes, the lophotrochozoans, and the ecdysozoans. Note the different position of the Platyhelminthes in the trees: basal in the left, and as a lophotrochozoan of still uncertain position in the right. D, Deuterostomes; L, Lophophorates; P, Protostomes. See text for further details and references.

species from the highly derived Order Tricladida were analyzed) within the Lophotrochozoa.[26,28,29] In summary, the new molecular phylogeny and data from Hox cluster genes supported the existence of three large bilaterian superclades: Deuterostomia, Ecdysozoa and Lophotrochozoa, with acoelomate and pseudocoelomate groups now displaced to much higher positions inside the tree.[17,24,27] Hopes of finding extant 'intermediates' in the bilaterian lineage were considered doomed and the gradist interpretation of early bilaterian evolution eclipsed.[5,17,24]

The view that the LCB was a large organism similar in complexity to modern bilaterians was also supported by the amazing conservation of the genetic toolkit across the Bilateria, together with the apparently homologous expression of key developmental genes, namely in insects and vertebrates.[18,30] Both were taken as evidence of a deep conservation across the Bilateria of morphogenetic developmental programs and their ensuing morphological characters.[5,17,19,24] Moreover, it favored the archicoelomate (now renamed 'complex Urbilateria',[31,32] hypothesis at the expense of the planuloid–acoeloid hypothesis.

Such unbounded optimism proved premature. (1) As Jenner[3] cogently pointed out, most of the new phylogenies backing a complex LCB were heavily prunned, leaving out several 'minor' phyla, namely basal ecdysozoa and lophotrochozoans, to which most pseudocoelomates and acoelomates belong. (2) Hox cluster sequences came from a restricted set of phyla represented by single species, usually from the most derived groups. (3) The proposed gene expression pattern homologies were variable[33] and drawn from a rather limited set of taxa. (4) It was unclear whether the presumed conservation was at the level of cell-type specification and cell differentiation or related to specific morphogenetic processes.[34] (5) A large LCB might have been expected to have left traces of its presence in the Neoproterozoic fossil

record, but none has so far been found.[34,35] Altogether, the emerging molecular view of animal evolution, however new and interesting, was grounded on poor sampling, unresolved phylogenies and uncertain claims of homology.

The uncertain monophyletic status of the Platyhelminthes and the position of the acoels

The lack of morphological synapomorphies for the Platyhelminthes

Platyhelminthes have traditionally been considered a single phylum[1] and, within them, three clearly monophyletic groups have recently been recognized[36–38]: the Acoelomorpha (Acoela + Nemertodermatida), the Catenulida, and the Rhabditophora, which comprises all other plathelminthes including the parasitic classes. Each of them is defined by well-accepted synapomorphies.[37] However, lack of robust morphological synapomorphies that would unite these three major clades questions the monophyly of the Platyhelminthes.[38] Nonetheless, the phylum was generally believed to be monophyletic because Acoelomorpha, Catenulida and Rhabditophora share a long list of characters, including soft bodies, solid body form, hermaphroditism, internal fertilization, filiform and biflagellate sperm, and multiciliated epidermal cells. Such features, however, are symplesiomorphies or homoplasies and, as such, have no place in cladistic pylogenetics.[39] Other characters considered, such as spiral cleavage and a ladder-like ventral nervous system (see[40] for general references) are shared with phyla other than Platyhelminths (e.g. most protostomates) and likely are symplesiomorphies or homoplasies.

Two final features have been considered potential characters that would unite Acoelomorpha, Catenulida and Rhabditophora: lack of mitosis in differentiated somatic cells[37] and presence of a sack-like gut (which means lack
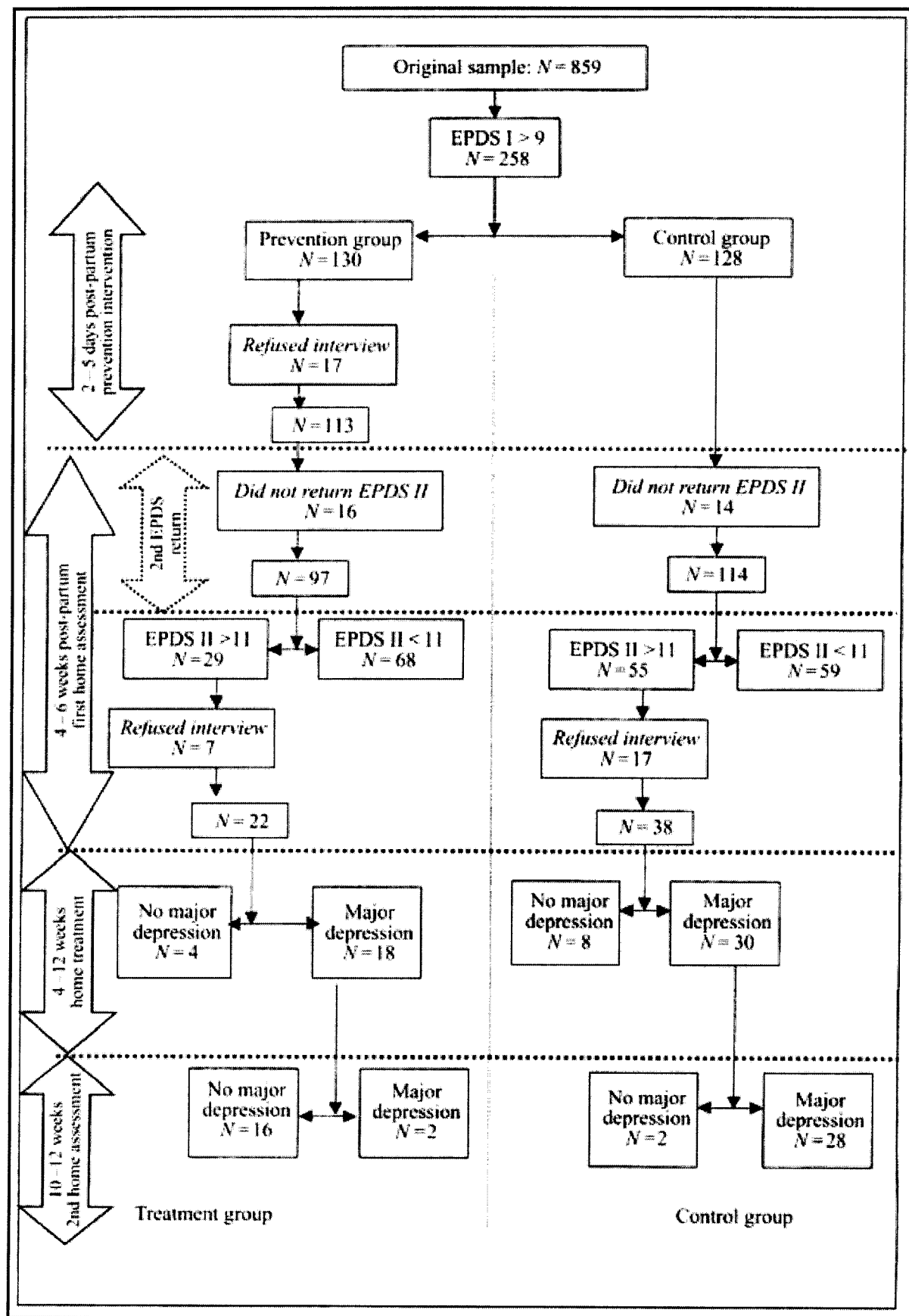
Fig. 1. Flow diagram for subjects through all study phases.

Evaluation of the efficacy of the prevention intervention

Among the 859 subjects, 258 (30%) had EPDS scores of >= 9 in the first days post-partum. Among the 130

subjects eligible for the prevention group, 17 (13%) declined to participate. All subjects eligible for the control group agreed to participate. The prevention group included 113 subjects (mean age = 30.3, S.D. = 4) and the control group, 128 subjects (mean age = 29.6, S.D. = 5). Thirty subjects, 16 (14%) in the prevention group and 14 (11%) in the control group, did not return the EPDS. Thus, the study compared the outcome of 97 treated subjects to 114 control subjects. Table 1 shows the demographic and clinical characteristics of these subjects. None of the differences was statistically significant. Altogether the drop-out rates (refusals plus no return of the second EPDS) in the first part of the study were 25.4% (33/130) in the intervention group and 10.9% (14/128) in the control group. There was no significant difference between drop-outs and completers on any demographic or clinical variables.

	Prevention		Home-treatment	
	Prevention group ($N = 97$)	Control group ($N = 114$)	Treated group ($N = 18$)	Control group ($N = 30$)
Mean age (S.D.)	30·4 (4)	29·6 (5)	30·5 (4·3)	30 (5)
Parity, % primiparous	46·6	48·4	66·7	61·8
Mean number of children, N	1·4	1·5	1·4	1·6
Type of delivery				
Spontaneous vertex, %	76·3	79·3	85·5	75
Caesarean section, %	23·7	20	14·5	25
Marital status				
Single, %	5	0	0	0
Married/Cohabiting, %	95	100	100	100
Employed, %	96	84·5	75	83·8
None of these differences was statistically significant.				

Table 1. Demographic and clinical characteristics of participants

At 4 to 6 weeks post-partum, subjects in the prevention group had significant reduction in the frequency of probable depression, as defined by a score of $>= 11$ on the EPDS. EPDS scores suggested a probable depression in 29 subjects of the prevention group (30.2%) *versus* 55 subjects of the control group (48.2%) ($[chi]^2 = 7.36$, df = 1, $P = 0.0067$). The repeated-measures ANOVA revealed a significant group × assessment occasion interaction ($F_{1,212} = 30.9$, $P < 0.0000001$). The intensity of depressive symptoms measured by the mean score on the EPDS was significantly lower in the prevention group than in the control group (8.5, S.D. = 4 *v.* 10.3, S.D. = 4.4, $t = 3.06$, df = 209, $P = 0.0024$). However, these analyses indicated a medium effect size only (ES = 0.42).

An 'intention to treat' analysis was also performed, in which all subjects with an EPDS score $>= 9$ in the first days post-partum were included. It comprised both subjects who refused interview and subjects who did not return the second EPDS. For these subjects, the first EPDS scores were carried forward. Despite this, a repeated-measures analysis of variance revealed a significant group × assessment interaction in favour of prevention ($F_{1,255} = 6.8$, $P < 0.01$).

Evaluation of the efficacy of the treatment for post-partum depression

Twenty-two (76%) of the subjects with probable depression, who were in the prevention group, accepted participation in the home visit programme of evaluation and treatment. Among the subjects with probable depression in the control group, 38 (69%) agreed to be evaluated at home. Among the 22 subjects with probable depression in the prevention group, 18 (85.7%) met the DSM-IV criteria for a major depressive episode *versus* 30 (79%) of the subjects in the control group ($[chi]^2 = 0.08$, $P = 0.77$). Table 1 shows the demographic and clinical characteristics of these subjects. None of the differences was statistically significant. There were no significant differences between drop-outs and completers on any demographic or clinical

variables.

Table 2 presents the baseline HDRS, BDI and EPDS scores that were consistent with mild to moderate depression. No significant difference was observed between the two groups on all the rating scales. The correlation between HDRS and BDI scores was high ($r = 0.91$, $P < 0.05$).

	Treated group (N = 18)	Control group (N = 30)	t	df	P
Baseline*					
HDRS	16·4 ± 4·5	17·2 ± 4·4	0·56	49	0·57
BDI	16·8 ± 3·9	16·7 ± 3·9	0·09	49	0·92
EPDS	12·8 ± 3·7	13·7 ± 3	0·93	49	0·35
Outcome†					
HDRS	5·7 ± 3·3	16·2 ± 4·5	8·4	49	< 0·0001
BDI	4·7 ± 3	15·7 ± 4·4	9	49	< 0·0001
EPDS	5·9 ± 2·7	13·7 ± 3·6	7·7	49	< 0·0001

* 4–12 weeks post-partum.
† 10–12 weeks post-partum.

Table 2. Evaluation of the home-intervention: baseline and outcome HDRS, BDI, EPDS scores (mean ± *S.D.*) in the treated and control subjects with major depressive episode

The mean number of home visits in the treated group was 6.1 (S.D. = 1.6). All subjects in both groups completed the protocol. The MANOVA detected a significant group × assessment occasion interaction in favour of treatment (Wilks [lambda] = 0.43, Rao $R_{3,54} = 24.5$, $P < 0.0000001$). Repeated-measures ANOVAs showed a significant group × assessment interaction for each treatment outcome: HDRS ($F_{1,57} = 62.1$, $P < 0.0000001$); BDI ($F_{1,57} = 48.4$, $P < 0.0000001$); EPDS ($F_{1,57} = 42.7$, $P < 0.0000001$). Table 2 presents the depression outcomes. There were significant differences between the two groups on all rating scales. The correlation between HDRS and BDI scores was high ($r = 0.89$, $P < 0.05$).

Recovery and response to treatment rates are shown in Table 3. Recovery was defined as an HDRS score of <= 6 or a BDI score of <= 3. Recovery rates were significantly greater in the treated group than in the control group. According to the HDRS scores, recovery rates were 66.6% for the treated group *versus* 6.6% for the control group. Response to treatment was defined as >= 50% reduction in symptoms. Based on HDRS, BDI and EPDS scores, a significantly greater proportion of subjects in the intervention group responded to treatment than subjects in the control group. A clearly therapeutic response to treatment was observed in the treated group with a mean reduction in HDRS score of 10.2 (S.D. = 6.7) from baseline compared with the control group (mean = 1.3, S.D. = 3.3) ($t = 6.2$, df = 49, $P < 0.00001$).

	Treated group (N = 18) N (%)	Control group (N = 30) N (%)	χ^2	P
Recovery (HDRS < 7)	12 (66·6)	2 (6·6)	16·8	< 0·0001
Recovery (BDI < 4)	11 (61·1)	1 (3·3)	17·7	< 0·0001
Reduction of HDRS scores ≥ 50%	15 (83·3)	4 (13·3)	20·2	< 0·0001
Reduction of BDI scores ≥ 50%	16 (88·8)	4 (13·3)	23·4	< 0·0001
Reduction of EPDS scores ≥ 50%	13 (72·2)	1 (3·3)	22·6	< 0·0001

Table 3. Evaluation of the home-intervention for post-partum depression: recovery and response to treatment rates

An 'intention to treat' analysis was also performed, in which all subjects with an EPDS score >= 11 during the period 4 to 6 weeks post-partum were included. It comprised both subjects who refused interview and

subjects with no major depression on the MINI. For these subjects, the second EPDS scores were carried forward. Despite this, a repeated-measures analysis of variance revealed a significant group × assessment interaction in favour of treatment ($F_{1,84} = 32.1$, $P < 0.0000001$).

DISCUSSION

To our knowledge, the present study is one of the first to provide a systematic examination of a well-defined early intervention in a relatively large community sample and is the first to evaluate a structured programme for prevention and treatment of post-partum depression. The prevention intervention resulted in a significant reduction in depressive symptoms and in the frequency of probable depression at 4 to 6 weeks post-partum. However, the effect size was only medium. This result suggests that the prevention intervention did not have a clinically significant effect on the whole prevention group. This confirmed the necessity of adding a treatment intervention for the subjects who developed a post-partum depression despite prevention. The home-based treatment significantly reduced depressive symptoms relative to the control group as attested by the proportion of women who no longer met DSM-IV criteria for major depressive disorder and who met HRSD and BDI criteria for recovery and for response to treatment. Women in the control showed little improvement during the 5 to 8 weeks between the two assessments. These findings suggest that this programme is an efficacious mean of prevention and treatment for post-partum depression. Altogether the drop-out rates (refusals plus no return of the second EPDS) in the first part of the study was 25.4% in the prevention group. Refusals rate was 24% in the home-based treatment and no drop-outs from therapy occurred. These findings suggest that this programme had a reasonable acceptability. They cannot be directly compared with the two largest recent studies where decline in participation rates were respectively 53% and 49% and drop-out rates were 29.8% and 20%, because the times chosen for measuring these differed (Appleby et al. 1997; O'Hara et al. 2000). The brief prevention intervention had the advantage of developing a collaborative relationship with the therapist facilitating the organization of a treatment if depression appeared. Home-visits are a convenient way of treatment for post-partum depressed women who are often tired and overwhelmed. This prevention and treatment programme might also have high applicability as all mothers could be routinely contacted while still in the maternity hospital or clinic. Moreover, it does not require experienced therapists, having shown efficacy being implemented by inexperienced but trained therapists.

One potential limitation of this study is that the outcome clinical evaluation and the treatment were implemented by the same clinical therapist that the subject became acquainted with during the first meeting at the clinic. We chose not to use independent evaluators for two reasons. First, we thought that it would have been almost impossible to keep the interviewers blind to the treatment condition when one group was receiving treatment and other group was not, given that subjects could reveal whether or not they were receiving treatment. Secondly, we felt that this disposition where evaluators and subjects became acquainted could enhance the acceptability of the programme and reduce attrition rates. For the same reasons, a non-blind assessment was also chosen in a recent study of interpersonal therapy for post-partum depression (O'Hara et al. 2000). Moreover, O'Hara et al. had verified that the bias due to this procedure was negligible (by blind assessments of the tapes of the therapists' assessments). As in their study, the low overall decline in participation and dropout rate in our study suggests the usefulness of therapists' assessments. The fact that BDI and HDRS scores were highly correlated and gave similar results suggests that the lack of an independent evaluator did not compromise the reliability of HDRS scores.

Another limitation of the study is that the procedure did not allow us to know the time of onset of these depressions (e.g. it did not allow for identification of depression evolving continuously after delivery or exacerbation of prepartum depression after delivery). A consequence is that the preventive intervention was actually curative for some women.

The sample size in the second part of the study is quite small. This may reduce the generalizability of the results. It should be noted that major depressions were of mild to moderate severity. It is likely that our results cannot be generalized to more severe forms of post-partum depression.

Another limitation is the lack of a follow-up study. This limitation is common to other treatment studies in post-partum depression. Further studies are needed to assess the efficacy of actual treatments in prevention of relapse and recurrence of depression and in the prevention of emotional disorders in children.

The findings of this study indicate that a programme based on an intervention at obstetric clinics and on home visits is reasonably efficacious and well-accepted for the prevention, detection and treatment of post-partum depression. The combination of early prevention and treatment might be a solution to the low detection and compliance to treatment rates in post-partum depression.

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of a proper anus). The first refers to the inability of most differentiated somatic cells to divide, with new cells being generated from undifferentiated cells which, in Platyhelminthes, are known as neoblasts.[41] However, this character is imprecisely defined, since differentiated cells in animals usually do not divide. Moreover, it has not been properly scored for many phyla. As for the second character, it has been suggested that the sack-like gut of Platyhelminthes (including Acoelomorpha) has been derived by paedomorphosis from the through-gut of a more complex ancestor.[15,42] The new placement of Platyhelminthes within the Lophotrochozoa (Fig. 3), most of which bear through-guts, supported the change from a through-gut to a sack-like gut as an apomorphy for the Platyhelminthes. However, this hinged upon the uncertain monophyletic or polyphyletic nature of the Platyhelminthes and the actual position of the Acoelomorpha.

Molecular trees support the polyphyly of the Platyhelminthes and the basal position of the Acoela

The first comprehensive molecular tree of the Platyhelminthes[43] featured full-length 18S sequences of 16 platyhelminthes species, covering most of its orders, and a wide range of metazoans. Platyhelminthes were found to be polyphyletic or paraphyletic, with Acoela and Catenulida branching out in sequence as the first bilaterians whereas the Rhabditophora branched within the Protostomia. However, the acoel species used had extremely long branches indicative of unusually high substitution rates (fast-clock organisms). Such species artificially group with other sequences with long branches regardless of the actual phylogentic relationship of the taxa, causing the well-known long-branch attraction artifacts.[44] To avoid long-branch artifacts, complete 18S rDNA of 18 species of acoels identified one species, *Paratomella rubra*, that evolved at a sufficiently slow rate to avoid such effects. Used together with gene sequences of representative species of a wide set of metazoan taxa that evolved at a relatively uniform rate, the resulting tree[45] reproduced the three large clades: Deuterostomia, Ecdysozoa and Lophotrochozoa, with acoels as the first offshot after the diploblasts. Moreover, and in agreement with the metazoan molecular tree in Fig. 3, the Rhabditophora and the Catenulida clustered within, but at the base of, the Lophotrochozoa.

The proposal that acoels are a basal bilaterian clade and do not belong to the Platyhelminthes was soon contested.[46–48] Such criticisms however proved unfounded because of improper alignment and poor sampling[49] or due to the pervasive use of fast-clock acoels.[47,48] The major contention against acoels as basal bilaterians resulted from the odd position of the representative of the other acoelomorph group (*Nemertinoides elongatus*, Order Nemertodermatida), which grouped unambiguously with the rhabditophoran Platyhelminthes.[45] Because acoels and nemertodermatids share robust morphological characters (ciliary rootlet system, cilia with shelf-like

transition and, likely, duet-spiral type of cleavage), most zoologists believed that placement of acoels was probably erroneous.[19,24,50] However, complete 18S rDNA sequences from three additional species of nemertodermatids unambiguously showed them to cluster with acoels at the base of the bilaterians and not with the rhabditophoran Platyhelminthes.[51] It was suggested that the previous contradictory data for nemertodermatids resulted from the incorrect assignment to *Nemertinoides elongatus* of the single specimen from which sequences were obtained.[51]

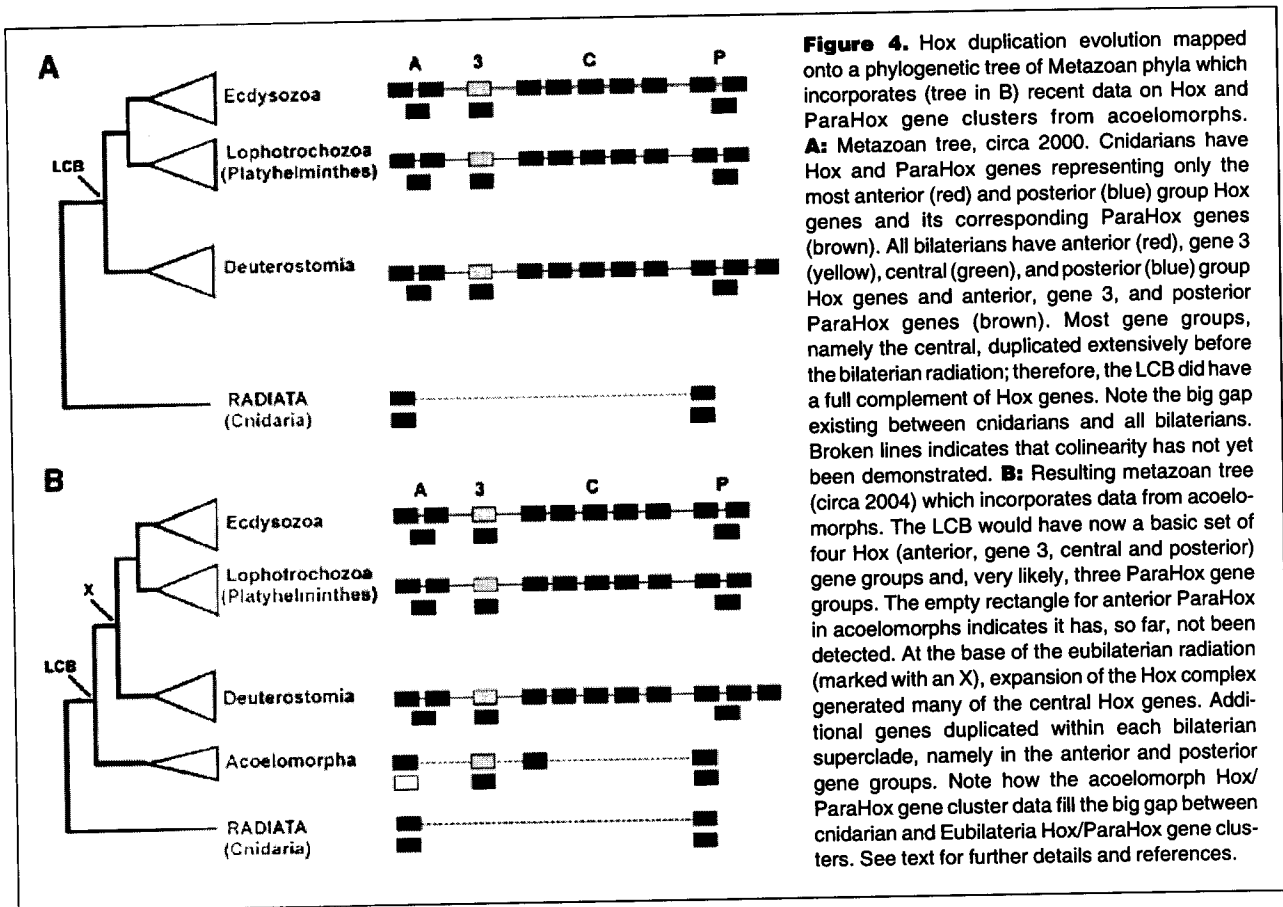## Further molecular arguments for acoelomorphs as basal bilaterians

Nuclear genes and mitochondrial genes

The basal position of the Acoelomorpha and the polyphyly of the Platyhelminthes have recently been tested and corroborated using (1) sequences from the myosin heavy chain type II (myosin II) gene from a large set of metazoans, including acoels and nemertodermatids[52] (2) a combined 18S rDNA + myosin II data set,[52] (3) a combined dataset of 18S + 28S rDNA gene sequences[53] and (4) the expression of the heterochronic gene *let-7*, first described in the nematode *Caenorhabditis elegans*. This gene, which is an essential regulator of developmental timing, has been found consistently in samples from all bilaterians (including triclad and polyclad rhabditophoran plathelminthes) but is absent from all diploblasts tested and from acoels.[54] Finally, phylogenetic analysis of the amino acid sequences inferred from the sequenced mitochondrial genes of a large set of metazoans also confirms the basal position of acoelomorphs.[55]

Hox/ParaHox cluster genes

Finding a full set of Hox cluster genes in acoelomorphs and, especially, the discovery of lophotrochozoan or protostomian molecular signatures in acoelomorph Hox genes, would help confirm that they are not basal bilaterians separate from the lophotrochozoan plathelminthes. Recent work from the acoels *Symsagitifera roscoffensis* and *Paratomella rubra*[56] and from the acoel *Convoluta pulchra* (Martinez, Saló and Baguñà, unpublished data) have, after very extensive searches, identified Hox genes that only fall into four paralogy groups: anterior, group 3, central and posterior (Fig. 4). Whereas acoel Hox genes are more similar to their bilaterian counterparts than to cnidarian genes, none of these genes bear lophotrochozoan or ecdysozoan molecular signatures. In addition, two representatives (Xlox-like and Cad-like) from the ParaHox cluster genes have also been identified.

The finding of similar Hox gene sets in distantly related acoels and, more specifically, in both acoels and nemertodermatids, suggests that this reduced gene complement may be ancestral in acoelomorphs, and that acoelomorphs may have diverged from other bilaterians before the full set of

Figure 4. Hox duplication evolution mapped onto a phylogenetic tree of Metazoan phyla which incorporates (tree in B) recent data on Hox and ParaHox gene clusters from acoelomorphs. **A:** Metazoan tree, circa 2000. Cnidarians have Hox and ParaHox genes representing only the most anterior (red) and posterior (blue) group Hox genes and its corresponding ParaHox genes (brown). All bilaterians have anterior (red), gene 3 (yellow), central (green), and posterior (blue) group Hox genes and anterior, gene 3, and posterior ParaHox genes (brown). Most gene groups, namely the central, duplicated extensively before the bilaterian radiation; therefore, the LCB did have a full complement of Hox genes. Note the big gap existing between cnidarians and all bilaterians. Broken lines indicates that colinearity has not yet been demonstrated. **B:** Resulting metazoan tree (circa 2004) which incorporates data from acoelomorphs. The LCB would have now a basic set of four Hox (anterior, gene 3, central and posterior) gene groups and, very likely, three ParaHox gene groups. The empty rectangle for anterior ParaHox in acoelomorphs indicates it has, so far, not been detected. At the base of the eubilaterian radiation (marked with an X), expansion of the Hox complex generated many of the central Hox genes. Additional genes duplicated within each bilaterian superclade, namely in the anterior and posterior gene groups. Note how the acoelomorph Hox/ParaHox gene cluster data fill the big gap between cnidarian and Eubilateria Hox/ParaHox gene clusters. See text for further details and references.

8–10 gene Hox clusters that characterizes most bilaterians was elaborated. The "intermediate" position for acoelomorphs as regards its Hox/ParaHox gene complement further supports a simple LCB (Fig. 2A).

Morphology versus molecules: towards a compromise?

Is the basal position of the Acoelomorpha supported by morphological characters? First, acoels generate only endomesoderm,[57] in contrast to the rest of the Platyhelminthes (and protostomes in general) that have both ectomesoderm and endomesoderm. Endomesoderm is considered the ancestral form. Second, acoelomorphs have an anterior concentration of nerve cells without forming a "true brain" with neuropile[58,59] though some workers[60] have disputed this. Moreover, while other bilaterians, including catenulids and rhabditophorans, have longitudinal nerve cords that are distinctly dorsal or ventral, acoelomorphs have a radial arrangement, interpreted here as being primitive or separately derived.

In turn, this new phylogeny compels a reinterpretation of the evolution of some features often used in support of a derived

condition for acoels. First, the lack of protonephridia for Acoelomorpha is regarded in traditional schemes as having been derived by loss from their platyhelminth ancestor. However, under the new scenario, this lack of protonephridia may be the retention of a primitive condition, a state shared with diploblasts. Second, the duet-spiral type of embryonic cell cleavage in acoels has been usually considered to be derived from the quartet spiral cleavage of other plathelminthes. Duet cleavage, however, has very distinct features compared to the typical quartet cleavage.[57] Hence, we interpret duet-spiral cleavage as having originated either from a form of radial or biradial cleavage characteristic of the more primitive programs in the Metazoa, whereas quartet spiral cleavage would have originated independently within the Lophotrochozoa. Finally, the sack-like gut of the Acoelomorpha may now be a symplesiomorphy shared with the similar state of diploblasts, followed by the appearance of a through-gut in the rest of the Bilateria, within which the Platyhelminthes (without the Acoelomorpha) separately adopted a similar sack-like gut condition. Moreover, given the uncertain position of the Platyhelminthes (without the Acoelomorpha) within the Lophotrochozoa, the presumed derived condition of its sack-like gut[9,15] should

be studied with molecular markers to track adult vestiges or embryonic rudiments, if any, of a trough-gut (namely the hind-gut).

A new systematic proposal for the Bilateria

In summary, molecular and morphological evidence suggest that the Acoelomorpha are the most-basal extant triploblastic Bilateria and the Platyhelminthes are a polyphyletic group. Awaiting new corroborative evidence, we propose to divide the Bilateria into two inclusive groups: a broad Bilateria including acoelomorphs, and a more derived Bilateria, or Eubilateria, excluding this clade (Fig. 5). If this scheme holds, Acoelomorpha should be established as a new phylum.[45,61] Further, this suggests that the LCB was small, simple, acoelomate, unsegmented and, very likely, a direct developer, reinforcing again the planuloid-acoeloid theory on the origin of the Bilateria. The new position of the Acoelomorpha puts back in time the origin of the LCB, reduces the extent of bilaterian stem lineages, and increases the cryptic precambrian history elapsed between the appearance of stem bilaterian lineages and the LCB and the rapid diversification of the crown bilaterians with its unequivocal appearance in the fossil record.[20]

Do acoelomorphs as basal bilaterians help us to understand the radial–bilaterian transition and the Cambrian explosion?

A previous question: are the Radiata radial and diploblast?

Biradial and bilateral symmetry features in some cnidarian anthozoans (e.g. slit-shaped mouth, internal mesenteries, asymmetryc syphonoglyphs) have recently been claimed as evidence for primary bilaterality in cnidarians.[62,63] However, their external radial symmetry is complete: a non-specialized ectoderm with a ring of tentacles that collect food from any direction and a diffuse nerve net with radially distributed sense organs. Hence, arguments for primary bilateral symmetry seem overstated, as such extant bilateral features, mostly from endodermal origin, could have evolved secondarily. Besides, because most, if not all, genes involved in mesoderm development in bilaterians are also expressed in cnidarians, it has also been claimed that some cnidarians do have mesodermal structures.[64] However, these genes are mostly expressed at the blastopore and in the endoderm. Because mesoderm evolved from endoderm, such genes in cnidarians might instead be involved in germ-layer specification.[63] In contrast to polyps and medusae, planula larvae are fully radial and diploblast and have distinct anterior and posterior ends. In addition, they swim with the anterior part (which corresponds to the aboral end, or foot, of the polyp, and bears a relatively concentrated nerve net) in front, while at the posterior end the mouth opening (a remnant of the blastopore, usually closed) occurs.
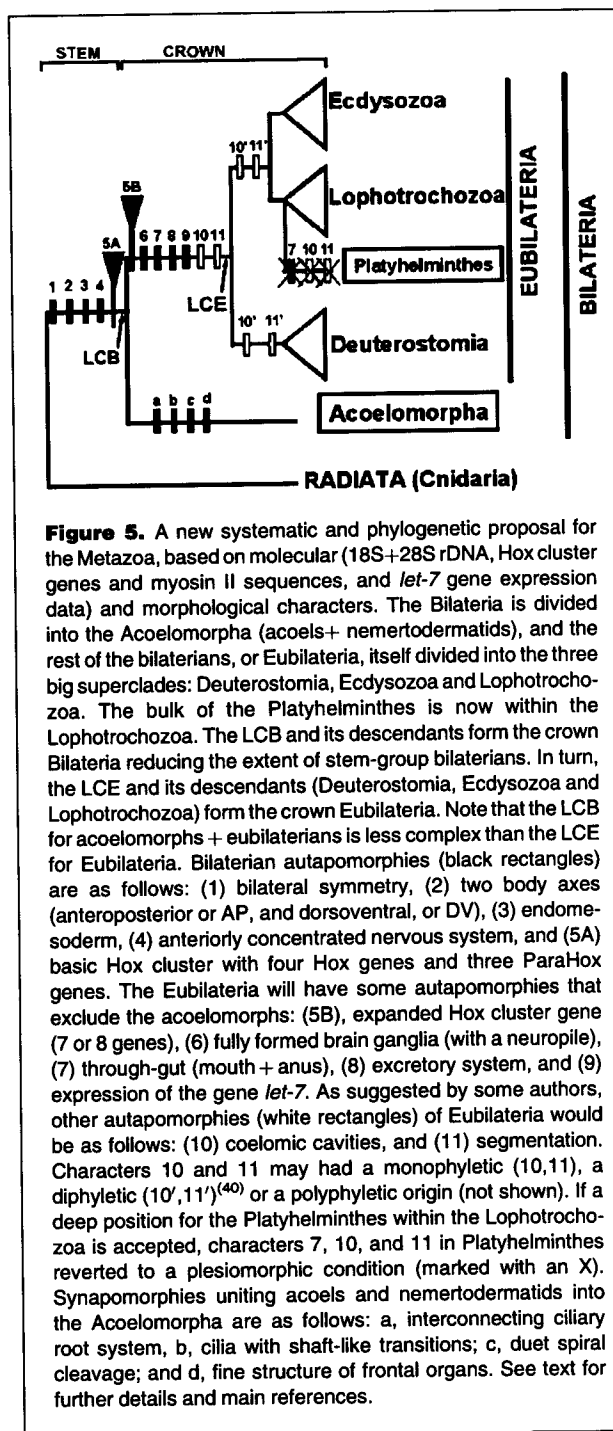

Figure 5. A new systematic and phylogenetic proposal for the Metazoa, based on molecular (18S+28S rDNA, Hox cluster genes and myosin II sequences, and let-7 gene expression data) and morphological characters. The Bilateria is divided into the Acoelomorpha (acoels+ nemertodermatids), and the rest of the bilaterians, or Eubilateria, itself divided into the three big superclades: Deuterostomia, Ecdysozoa and Lophotrochozoa. The bulk of the Platyhelminthes is now within the Lophotrochozoa. The LCB and its descendants form the crown Bilateria reducing the extent of stem-group bilaterians. In turn, the LCE and its descendants (Deuterostomia, Ecdysozoa and Lophotrochozoa) form the crown Eubilateria. Note that the LCB for acoelomorphs + eubilaterians is less complex than the LCE for Eubilateria. Bilaterian autapomorphies (black rectangles) are as follows: (1) bilateral symmetry, (2) two body axes (anteroposterior or AP, and dorsoventral, or DV), (3) endomesoderm, (4) anteriorly concentrated nervous system, and (5A) basic Hox cluster with four Hox genes and three ParaHox genes. The Eubilateria will have some autapomorphies that exclude the acoelomorphs: (5B), expanded Hox cluster gene (7 or 8 genes), (6) fully formed brain ganglia (with a neuropile), (7) through-gut (mouth+anus), (8) excretory system, and (9) expression of the gene let-7. As suggested by some authors, other autapomorphies (white rectangles) of Eubilateria would be as follows: (10) coelomic cavities, and (11) segmentation. Characters 10 and 11 may had a monophyletic (10,11), a diphyletic (10′,11′)[40] or a polyphyletic origin (not shown). If a deep position for the Platyhelminthes within the Lophotrochozoa is accepted, characters 7, 10, and 11 in Platyhelminthes reverted to a plesiomorphic condition (marked with an X). Synapomorphies uniting acoels and nemertodermatids into the Acoelomorpha are as follows: a, interconnecting ciliary root system, b, cilia with shaft-like transitions; c, duet spiral cleavage; and d, fine structure of frontal organs. See text for further details and main references.

The planuloid–acoeloid theory revisited: a glimpse at mechanisms

The overall similarities (not so apparent when looked in detail[42]) between the planula larva of extant cnidarians and the acoelomorphs is the basis upon which rests the
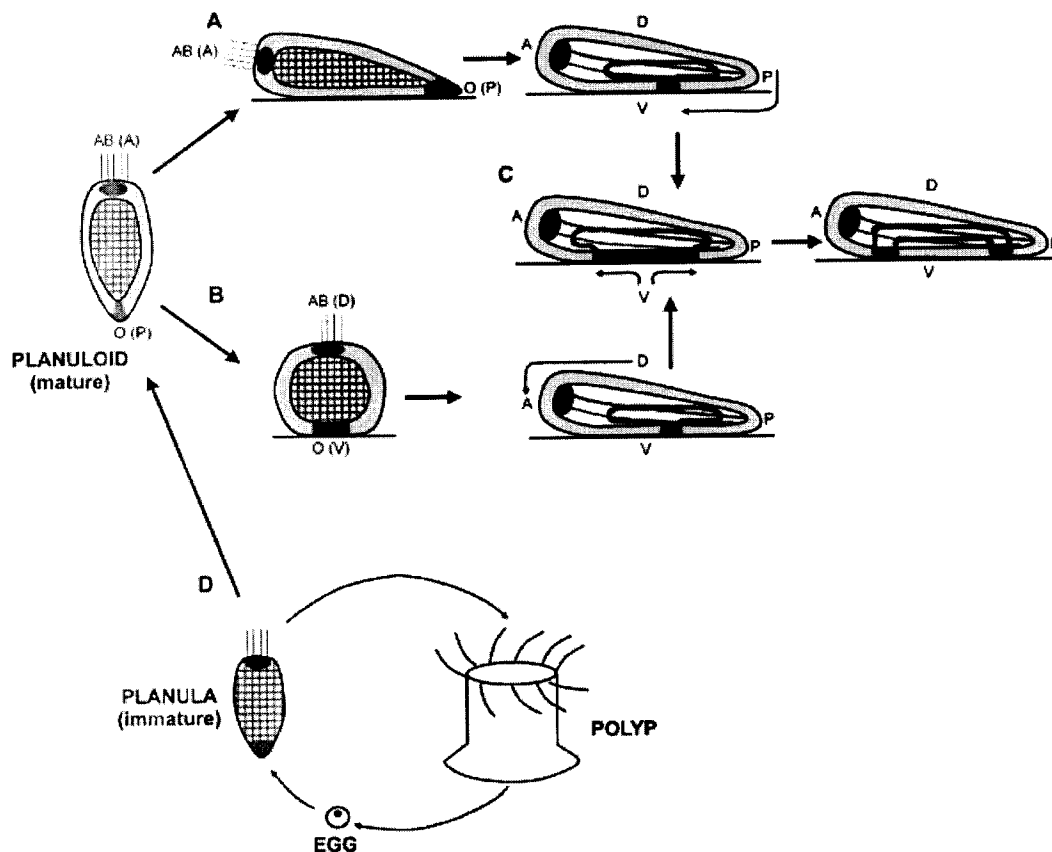
Figure 6. Planuloid–acoeloid scenarios at the radial–bilaterian transition. The ciliated planuloid (left) represents a mature organism bounded by a monolayered ectoderm (yellow) and bearing a condensed nerve net (blue), a tuft of cilia at the apical end (aboral, AB), and a blastoporal mouth/anus (red) at the basal end (oral, O). Chequered red/white indicates the solid endoderm/mesoendoderm. **A:** The evolutionary intermediate settles at the substratum along its O-AB axis, and evolves into a DV asymmetric acoeloid due to the forward movement (curved thin arrow) of the blastoporal mouth/anus to the ventral region. The brain is now at the anterior end from which several longitudinal nerve cords (thin blue lines) run ventrally (in protostomates) or dorsally (in deuterostomates) along the AP axis. Meanwhile, the mesodendoderm has sorted out into mesoderm (white spaces) and endoderm (red). **B:** The evolutionary intermediate settles on the blastopore down to the substratum and elongates by compressing the O–AB axis (homologous to the DV axis). A new AP axis is established by movement of the apical brain area forward (curved thin arrow). **C:** From 6A or 6B, the blastoporal mouth/anus elongates along the AP axis (middle, thin arrows) and seals centrally by amphistomy giving an organism with a through-gut (mouth and anus). **D:** A mature planuloid (left) derives by a paedomorphic mechanism (e.g. progenesis[15]) from an immature planula larva of a cnidarian with a similar life-cycle to extant cnidarians. From the planuloid, an acoeloid derives following either the scenario 6A or 6B. For other symbols, see legend of Fig. 1.

planuloid–acoeloid hypothesis. In its original form,[2] the planuloid was considered a simple, radially symmetrical, solid mature organism from which derived the cnidarian and ctenophores lines and, after differentiating biaxially and acquiring the third layer, the bilaterally symmetrical acoeloid ancestors. As for the later transition, three plausible scenarios could be contemplated (Fig. 6). The first (Fig. 6A) considers the planuloid oral–aboral axis homologous to the anteroposterior axis of the bilaterians, with aboral corresponding to anterior and oral to posterior. It features a swimming planuloid crawling upon the substrate with the blastoporal end at the rear. Interestingly, a basal family of acoels, the Diopisthopor-

idae, bears the mouth at the rear end of the body. Under this situation, the dorsoventral axis is formed anew. A second scenario (Fig. 6B) proposes a swimming planuloid settling on the substratum with its blastopore-end down. Under this scenario, the oral–aboral axis is homologous to the dorsoventral axis of bilaterians, whereas the anteroposterior axis is a new appearance. A third, and less parsimonious, scenario (Fig. 6D) generates an acoeloid ancestor by progenesis from an inmature planula of a cnidarian ancestor with the main life-cycle features of modern cnidarians.

How did the main features defining the Bilateria (see Figs. 2 and 5) appear along the planuloid–acoeloid transition? During

the embryonic development of extant organisms, the transition from a one-axis radial-symmetric embryo to a bilateral embryo with two axes requires a symmetry-breaking event. Common breaking events are the asymmetric invagination of the endomesoderm through and perpendicular to the blastopore along a midline that elongates by convergent extension movements parallel to the AP axis,[65] and the asymmetric migration of a subset of mesodermal cells (the 4d blastomere) to one side (which will become ventral) in most spiralian embryos. In acoel embryos,[57,66] the vegetal macromere duet sinks into the mass of surrounding micromeres and give rise to the entire mesoderm and endoderm. This gives support to the presence of a population of bipotential cells for mesoderm and endoderm early in evolution.[67,68] The dorsoventral asymmetry in acoels only becomes apparent when the mouth opens at the ventral side of the embryo, though the mechanism specifying it is unknown. A latent dorsoventral asymmetry might well occur in form of asymmetric expression of genes at the blastoporal area. An interesting precedent, without any morphological consequences, is the asymmetric expression of a BMP2/4-like gene in the planula of the coral Acropora.[69]

Assuming Fig. 6A as the most-likely scenario, such symmetry breaking would represent the key event in the evolution of the Bilateria as it produced at once a third layer and a new orthogonal body axis (likely the DV) with its concurrent bilateral symmetry. Moreover, an anterior concentration of nerve cells was already in place. To diversify the expanded AP pattern a novel set of central Hox genes (see below) needed to be added between the anterior and posterior Hox genes already present in cnidarians. Such ideas could be tested by studying the expression of Hox/ParaHox and other anterior (e.g. orthodenticle, empty spiracles, distaless, Pax6), posterior (caudal, Brachyury, forkhead, wingless), dorsal (decapentaplegic/Bone Morphogenetic Protein), ventral (short gastrultion/chordin), and mesoendodermal (snail, twist, GATA factors) genes in embryos and adults of selected acoelomorph species. Results could then be compared to the expression data that are accumulating from planula larvae and polyps of several species of cnidarians.[69–71]. Interestingly, an impressive number of these genes, namely expressed at posterior regions in bilaterians, is expressed in a radial manner around the blastoporal region in planulae.[62–64,72–74] This supports the homology between the aboral–oral axis of cnidarians and the AP axis of bilaterians, oral in planula corresponding to posterior in bilaterians.[65]

How did the new features defining the Eubilateria (through-gut, a true brain with asymmetrically placed longitudinal nerve cords, an excretory system, and an expanded set of Hox genes) evolve from an acoeloid-like ancestor? So far, this is anyone's guess. However, during or after the forward movement of the blastoporal mouth/anus region along the ventral region, an elongated blastopore might have fused centrally

leaving the extremes open.[12] In this way, a one-way gut would easily form (Fig. 6C), though other mechanisms could also be contemplated.[13,65] In addition, the radially distributed longitudinal nerve cords in acoelomorphs (and, likely, in the acoeloid) could facilitate, through dorsal or ventral suppresion, the evolution of protostome (ventral) and deuterostome (dorsal) nerve cords[75] without recurse to dorsoventral inversion.[12,30] Further features, such as the excretory system, coelom, segmentation and formation of appendages might have occurred independently several times, despite the surprising conservation of its underlying genetic machinery, in lineages of acoelomate/pseudocoelomate and unsegmented basal lophotrochozoans, ecdysozoans and deuterostomates.

The bearing of acoelomorphs on the Cambrian explosion

The new phylogenetic scheme depicted in Fig. 5, if proved correct, may also have a bearing on our understanding of the so-called Cambrian explosion.[35] The strong support for acoelomorphs as a sister group to the rest of the Bilateria backs the gradist view and may help to bridge our understanding of the radial–bilaterian gap. However derived (which actually they are; see autapomorphies in Fig. 5), the acoelomorphs may represent one among a plethora of stem bilaterian lineages that radiated in the precambrian, well ahead Cambrian times.[20,21,76] One of these lineages, different from that giving rise to acoelomorphs, became the crown ancestor of the Eubilateria and continued to diversify to produce the stem ancestors of the Lophotrochozoa, Ecdysozoa and Deuterostomia.[20,76]

Recent estimates of metazoan divergence times[77,78] using data sets from concatenated amino acid sequences conclude that the cnidarians and bilaterians split between 600 and 630 million years ago (Ma), that the LCE (the last common ancestor of Eubilateria; see Fig. 5) evolved around 570 Ma and that the last common ancestor of protostomes evolved around 550 Ma.[78] Moreover, the origin of total-group Bilateria (comprising stem groups and the crown group bilaterians) has been extrapolated to be around 600–615 Ma, almost 45 Myr before the appearance and diversification of the LCE and the eubilaterian crown group and around 60 Myr before their appearance in the fossil record (555 Ma).[78] Because the new position of acoelomorphs puts the LCB further back in time in relation to the LCE (Fig. 5), the origin of the LCB should occur sometime between 600–615 and 570 Ma. Altogether, this reinforces the existence of a cryptic precambrian history of small, benthic, stem-group bilaterians, which left no trace fossils.[20,21] The explosion of life forms during the Cambrian and thereafter[35] would result from increasing body size and complexity linked to the emergence of indirectly developing larval forms[79,80] in some lineages of stem deuterostomates, ecdysozoans and lophotrochozoans.[81]

Do the simple intermediate Hox cluster of Acoelomorpha have a bearing on the evolution of the Hox/ParaHox clusters?

If a simple Hox gene cluster (Fig. 4B) is substantiated in other acoelomorphs and shown to be colinear, some of the 8–10 genes of the cannonical bilaterian cluster had to be generated (likely by gene duplication) after the split between acoels and the rest of the Bilateria but before the splitting of the three big superclades. Moreover, the bilaterian ancestor had to be structurally simpler (requiring a small number of Hox genes) than the later-evolving and more-complex eubilaterian ancestor.

The existence of a simple, intermediate, Hox gene cluster of acoelomorphs is difficult to reconcile with the presence of an even simpler Hox gene cluster of cnidarians within the evolution of Hox/ParaHox gene clusters. Despite extensive samplings, cnidarians have anterior and posterior Hox and ParaHox genes, but lack intermediate (group 3 and central) Hox and ParaHox genes. This result is puzzling because, after the first description of the ParaHox cluster,[82] models of Hox/ParaHox evolution[83,84] suggest that a protoHox cluster with four genes (anterior, group 3, central, and posterior) duplicated before the Cnidaria/Bilateria split into an ancestral Hox cluster with four genes and an ancestral ParaHox cluster with three genes, which included a PG3 gene (Xlox) but not the central counterpart. If so, the central ParaHox had to be lost in all animals while cnidarians as a whole secondarily lost the central Hox and ParaHox genes and the group 3 ParaHox.

These losses in cnidarians have never been satisfactorily explained, though they fit current phylogenetic trees drawn from Hox/ParaHox gene sequences.[83,84] However, if the planuloid–acoeloid hypothesis is considered in its original formulation,[2] we could envisage a planuloid bearing a protoHox cluster lacking intermediate members (central and group 3 genes). This simple gene set was kept in the lineage leading to cnidarians. Instead, in the lineage leading to stem bilaterian groups and the LCB, central and group 3 Hox genes could evolve through unequal crossing-over between the anterior and posterior genes.[85]

Conclusions

Acoelomorph flatworms are very simply organized bilaterian organisms traditionally considered to belong to the phylum Platyhelminthes. In the past decade, analysis of diverse molecular datasets, namely 18S and 28S ribosomal gene sequences as well as other nuclear and mitochondrial genes, together with reassessment of morphological features strongly suggest that acoelomorph flatworms do not belong to the Platyhelminthes, but are the most basal of known triploblastic Bilateria. This is also supported by recent Hox cluster gene data, which define a basic set of Hox and ParaHox genes for acoels in contrast to the fully extended Hox set for the rest of bilaterians. These results give acoelomorphs a strategic place in the evolutionary tree and have deep implications for our understanding of the radial-bilaterian transition. Firstly, their basal position suggest that bilaterians descend from an acoelomate, unsegmented ancestor with a simple nervous system and a sack-like gut, but no excretory system. This supports the old planuloid–acoeloid hypothesis. Secondly, the 'intermediate' set of Hox genes in acoelomorphs fills the existing gap between cnidarians and higher bilaterians and could be instrumental in a better understanding of the Hox/ParaHox gene cluster evolution. Thirdly, because acoelomorphs are direct developers, their basal position may give some support to the view that ancestral bilaterians were also direct developers, rather than indirect developers with planktonic larvae. Fourthly, it is likely that there were several precambrian lineages of stem bilaterians, of which acoelomorphs are, so far, the only known remnants. Such small lineages might easily have escaped detection in the fossil record. Later, the invention of planktonic larvae and metamorphosis would foster the introduction and diversification of complex life histories, which may explain the amplitude and swiftness of the so-called Cambrian explosion. Finally, because cnidarians already possess, apart from central Hox/ParaHox genes, the genetic toolkit used by all Precambrian and Cambrian bilaterians to develop their adult body plans, bilaterian innovations should be the consequence of novel gene and gene networks redeployments. This could be tested analyzing and comparing the expression of the main AP, DV, mesoendodermal and nervous system genes and its interactions between extant embryos of acoelomorphs and planula larvae of cnidarians.

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