FORUM

Cladistics (1994) 10:331–337

METAZOAN RELATIONSHIPS: A REBUTTAL

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Received for publication 9 May 1994; accepted 30 September 1994

Backeljau et al. (1993) offer a reappraisal of the published cladistic analysis of metazoan phylogenetic relationships of Schram (1991). They state that the original study appeared erroneous in a few points as to both method and interpretation of characters. They based their comments on this short article rather than the larger work (Meglitsch and Schram, 1991) from which that paper was derived. This latter book contains a survey of the entire array of metazoan phyla, and it deals extensively with many of the exact critical character issues that Backeljau et al. address in their paper.

The analysis in Meglitsch and Schram (1991) was the first attempt to cladistically treat all the phyla with an explicit matrix handled by a computer program. It was developed mainly as a pedagogical device to show students how this kind of analysis can be done and what the results could be in terms of clarifying, in a testable way, the possible relationships between animal groups. The paper by Schram (1991) was directed to the issue of the use of fossils in phylogenetic analysis and was designed for the specific purpose of demonstrating how even the small amount of information available from fossils can be treated in a more analytical way than has often been the case in the past. Schram emphasized in both these instances that the issue of method used had greater importance than the question of actual result. All previous morphologic analyses of the phylogenetic relationships of all the animal phyla had received treatment only in an evolutionary systematic context. For example, one of the most recent attempts in this vein, that of Wilmer (1990), while exhaustive in its survey of the pertinent literature, concluded that one can make few actual connections between animal phyla and only a phylogenetic guess can characterize phylum level relationships (e.g., Fig. 14.2, p. 361). Schram believed that it was important to break with this tradition and move the debate about metazoan relationships into a phylogenetic systematic context. That this effort succeeded so quickly (see not only Backeljau et al., 1993; but also Eernisse et al., 1992) is a tribute to the method more than to any of our individual efforts *per se*.

Nevertheless, the paper of Backeljau et al. (1993) does express concern over some vexing issues about the analyses of specific characters as well as the treatment of large data sets, concerns which we share. However, we think the issues here must be more clearly and accurately drawn than they have done.

Backeljau et al. rightly point out that not all of the original 77 features are phylogenetically informative. In the pedagogic context of Meglitsch and Schram, in which the original presentation of the results occurred, Schram believed that it was more informative to students to retain all the characters in the cladograms in order

that they could see the optimization of the *entire* data set. Indeed, to rely entirely on consensus trees in this regard is not effective.

Backeljau et al. also expressed separate concerns about 7 of the original characters as possibly improperly interpreted. Consultation of Meglitsch and Schram (1991) would have addressed these issues, since the features that they questioned involve the shifting understanding of metazoan comparative anatomy and ontogeny. A real effort was made in Meglitsch and Schram to incorporate the latest information and interpretations of controversial points of the anatomy of invertebrates that occur in the current literature. Many of these 7 challenged characters in fact deal with such new information and understanding.

The question of retrocerebral organs in rotifers and chaetognaths is vexing (Meglitsch and Schram's character 27). Hyman (1959) herself first made the comparison between these. Ultrastructural studies of both (e.g. see Clément and Wurdak, 1991, for rotifers; Bone and Goto, 1991, for chaetognaths) point to a gland with secretory organelles and many microtubules. Are these similarities explainable by convergence due to function, or are these true homologs with similar locations and derivations? We still do not know for sure, although the analysis of Meglitsch and Schram would suggest these act as homoplasies in closely related phyla. Bone and Goto interestingly draw comparisons of the chaetognath retrocerebral organ not to rotifers, but to the crustacean, *Leptodora*.

Concerning the eutely or non-eutely of priapulids (Meglitsch and Schram's character 28) the work of Shapeero (1961) is a questionable source, its main conclusion concerning true mesenteries in Priapulida was disproven by MacLean (1984). Fixed cell number in priapulids can remain a legitimate point of debate, but it would appear that the entire issue of eutely in aschelminths no longer figures as one of great importance (e.g. see various papers in Harrison and Ruppert, 1991).

Molting in pentastomids (Meglitsch and Schram's character 30) does in fact occur, but the "phylum" really should be deleted from the matrix. It is now agreed that these peculiar animals are not only crustaceans, but also closely related to the branchiurans or fish lice (e.g. see Wingstrand, 1972; Abele et al., 1989).

The question of a lorica in rotifers (Meglitsch and Schram's character 40) in juxtaposition to that of priapulids and loriciferans is actually rather clear. Not all rotifers are loricate, and for those that possess a "lorica" the structure is quite unique. The lorica in those rotifers that have one is an intracytoplasmic laminated skeleton in the epidermal tissues. Kristensen (1991: 353) states that similarities between rotifer and other "loricas" remains an "example of convergence". The epidermally secreted, decorated plates of priapulid and loriciferan loricas remains an important apomorphy between those two groups.

The entire issue of lophophores in invertebrates (Meglitsch and Schram's character 45) stands as a fine example of what rigid adherence to "definition" can do to obscure potential relationships and close-off open discussion of the issues at hand. Few would deny that the "tentaculate structures" that arise from the heads of several phyla do bear a striking similarity to each other. Their location, derivation, and function certainly must involve our attentions when trying to sort out metazoan phylogenetic relationships. A valid point is raised by Backeljau et al. when they inject certain aspects of the old classic definitions of a lophophore, viz., mouth and anal locations. The lophophore, however, is really too complex an organ to be

reduced to a single presence or absence character. It is not one character, but several. The tentaculate arms represent one feature, location of the anus another, location of the mouth yet another, relationship to the coelom, blood system, and nervous system constitute additional distinct features, and the ciliary arrangements and functions still others (Halanych, 1993). Future data bases should score these features as independent. However, in the context of the Meglitsch and Schram more limited data base we think it valid to utilize lophophores as an apomorphic feature of several phyla; and certainly other features act to unite the so-called lophophorate and deuterostome phyla into a coherent clade.

Embryonic origins of coeloms (Meglitsch and Schram's character 52) is another issue where "tradition" often obscures fact. If one goes back and reads the original description of early development of chaetognaths (Burfield, 1927) it is clear that the cavity in the "mesodermal outpocketings" disappears entirely even before the mesodermal tissues completely separate from the gut; in other words, there is no enterocoel in chaetognaths. Again, Hyman (1959) was among the first to draw attention to the fact that the early ontogenetic delineation of germ layers in chaetognaths was more akin to that seen in pseudocoelomates. When this and other dissimilarities to deuterostomes are acknowledged in the data base (e.g. see Bone et al., 1991), the similarities of chaetognaths to pseudocoelomates emerge as quite more important in a cladistic analysis. Furthermore, the status of the chaetognaths separate from the deuterostomes is a position that now finds some support from recent molecular studies (e.g. see Telford and Holland, 1993; and Wada and Satok, 1994).

Finally, Backeljau et al. point out that Mollusca *do* have a true hemocoel (Meglitsch and Schram's character 67). The scoring in Meglitsch and Schram was due to a typographical error in the original data base and indeed should be corrected.

In conclusion, the result of the character inspection of Backeljau et al. uncovered only one real lapsus and a number of features for which either tradition has obscured discussion, or which exist as genuine points of debate and for which more work must be done to resolve the issues. Their criticisms of the data set of Meglitsch and Schram (1991) is more reflective of old "orthodoxies" and incorporates little new information.

There remains some concern about methods used by Meglitsch and Schram (1991). Backeljau et al. utilized Hennig86 and report that they discovered little similarity with the trees they got from the data base and ensuing trees in either Meglitsch and Schram (1991) or Schram (1991). Their strict consensus revealed a series of "combs" connoting grand uncertainty about the data matrix's efficacy. They then performed a series of operations, deleting parts of the data set in an effort to get more resolution, and only partially succeeded.

At the time of the original analysis, Schram had easy access to a main frame loaded with the program PHYSIS and utilized several options available with that package. Before going to press with the book, he was able to run the data set with an early version of PAUP for MS-DOS. The results were comparable. A small number of variant, but equally parsimonious trees were obtained with both programs. Schram picked one tree to illustrate the results. Nonetheless, the resulting trees were all more or less equivalent. So when Backeljau et al. published their reappraisal with its consensus of grand polychotomies from 2444 equally parsimonious trees, it was quite surprising.

We ran 1600+ repetitions of a heuristic search on the corrected data set using PAUP 3.1.1 (Swofford, 1993), with further examination of the results using MacClade 3.01 (Maddison and Maddison, 1992). We corrected the mistake on Mollusca, deleted 16 uninformative characters (viz., 1, 3, 9, 10, 15, 17, 29, 35, 36, 38, 59, 60, 65, 68, 70, and 74), and removed the taxa "ancestor" and Pentastomida. Strictly speaking an "ancestor" is not necessary in this case, it forces character states into plesiomorphic/apomorphic dichotomies not always easily justified. The analysis was run with characters unweighted and unordered. At each repetition a tree was built by random addition and subsequently improved by branch swapping. Branch swapping used tree bisection-reconnection. Other options were tried as well for shorter numbers of repetitions, but produced the same results. All minimal length trees were kept; their maximal number was set at 3000, a limit that was never reached.

We obtained 30 trees of minimal length 123. (Not collapsing zero-length branches yielded 54 trees of the same length.) Figure 1 shows the resulting strict consensus tree which has, in the notation of MacClade, a length of 119+ steps ($ci = 0.51$, $rc = 0.39$, and $ri = 0.76$). This tree is in fact quite close to that published in Meglitsch and Schram (1991: fig. 38.2), which had a tree length = 140 using all 77 characters and a $ci = 0.55$, and substantially like that of Eernisse et al. (1992). We chose here to root the tree between the Mesozoa, Placozoa, and Porifera. We did not attempt a branch and bound analysis. However, we gave the data matrix, including the "ancestor", to a colleague to run on Hennig86 and he obtained the same consensus tree.

Correcting the mistake in the original data with regard to molluscan hemocoels allows complete resolution of the relationship of the "trochophorates". This resulted in an arrangement of trochophorate phyla that is in agreement with the findings of Eernisse et al. (1992), which used a different character data set. Furthermore, the most parsimonious trees in all these analyses present Cnidaria and Ctenophora arranged paraphyletically between Porifera and the "higher" metazoans. Manipulations within MacClade to produce a single "radiate" clade resulted in a tree with 121+ steps, only two steps longer than the strict consensus.

From our analysis, it appears that the basic data set of Meglitsch and Schram, contrary to the contentions of Backeljau et al., is indeed quite effective in sorting relationships among the phyla of invertebrates. Each new generation of computer programs for cladistic analysis brings advancements in technique and improvements in the results. It is the patterns of relationships that really interests us all, but the data put into the matrixes of course determines the output of pattern. That is why we think that it is not particularly helpful to get into debates about programs used, permutations of data and procedures tried, and arguments about particular data sets or scoring aside from correcting obvious mistakes. The effort of Meglitsch and Schram (1991) was the first attempt to try a cladistic analysis of morphologic and ontogenetic features for all metazoan phyla. That many aspects of the classic Hyman view of metazoan phylogeny were confirmed is comforting. That other aspects of the traditional view were not should cause us not to argue about specific data bases but to look for more and better data to test such hypotheses. A quantum step in this latter direction was taken by Eernisse et al. (1992) who made a concerted effort to expand the total list of morphological characters appropriate to sorting invertebrate phylogenetic relationships. That Eernisse et al. did not apply

Fig. 1. Strict consensus tree derived from 30 trees produced by PAUP 3.1.1 and the amended data matrix of Meglitsch and Schram (1991)—see text for details.

these features to all the animal phyla is unfortunate, but at the time they were only seeking to test a part of the Meglitsch and Schram tree.

That morphology and ontogeny alone can give us so much information about metazoan phylogeny is reassuring. However, this is not to denigrate the efforts of various molecular laboratories from offering their own source of data. That interpretation of these data at times have been contradictory (e.g. compare Raff et al., 1989; Lake, 1989), probably reflects the fact that molecular data cannot and should not be used exclusively. Wheeler et al. (1993) offer an interesting object lesson in regard to the comparative use of morphological and molecular data when they

tried to further elucidate the relationships among arthropods. They found that only when molecular methods were combined with the morphologic data did completely resolved trees emerge. Interestingly, it appears that in the Wheeler et al. analyses, the tree resulting from the total evidence approach largely confirmed the overall pattern of relationships between arthropod groups that resulted from the morphologic data utilized alone. We suspect the same may prove true when molecules are combined with morphology to sort out metazoan phylogeny.

We have come a long way in recent years in the field of invertebrate phylogenetics. However, we should not be misled into thinking that we will arrive at ultimate truth as regards the relationships of the phyla. We can nevertheless better organize the information that we have, and this in turn can guide our efforts to find new information. Hyman (1959: 697) remarked concerning the arrangement of the animal kingdom that "a lack of decisive facts permits every zoologist to have his own opinion". We think that we are approaching a time when that may no longer prove true.

Acknowledgments

We are indebted to the stimulation and encouragement of Dr Richard Brusca, University of Charleston, and Dr Edward C. Wilson, Natural History Museum of Los Angeles County to take up these issues again. Dr Douglas J. Eernisse and another anonymous reviewer offered valuable comments on the draft manuscript. Dr Harry ten Hove checked our results with that of Hennig86.

REFERENCES

- ABELE, L. G., W. KIM AND B. E. FELGENHAUER. 1989. Molecular evidence for the inclusion of the phylum Pentastomida in the Crustacea. *Mol. Biol. Evol.* 6: 685–691.
- BACKELJAU, T., B. WINNEN AND L. DE BRUYN. 1993. Cladistic analysis of metazoan relationships: a reappraisal. *Cladistics* 9: 167–181.
- BONE, Q., H. KAPP AND A. C. PIERROT-BULTS. 1991. The Biology of Chaetognaths. Oxford Univ. Press, Oxford.
- BONE, Q., H. KAPP AND A. C. PIERROT-BULTS. 1991. Introduction and relationships of the group. In Q. Bone, H. Kapp and A. C. Pierrot-Bults (eds) *The Biology of Chaetognaths*. Oxford Univ. Press, Oxford, pp. 1–4.
- BONE, Q. AND T. GOTO. 1991. The nervous system. In Q. Bone, H. Kapp and A. C. Pierrot-Bults (eds) *The Biology of Chaetognaths*. Oxford Univ. Press, Oxford, pp. 18–31.
- BURFIELD, A. 1927. *Sagitta*. *Proc. and Trans. Liverpool Biol. Soc.* 41 (appendix 2): 1–104, pls. 1–12.
- CLÉMENT, P. AND E. WURDAK. 1991. Rotifera. In F. W. Harrison and E. E. Ruppert (eds) *Microscopic Anatomy of the Invertebrates*, vol. 4, Aschelminthes. Wiley-Liss, New York, pp. 219–297.
- EERNISSE, D. J., J. S. ALBERT AND F. E. ANDERSON. 1992. Annelida and Arthropoda are not sister taxa: a phylogenetic analysis of spiralian metazoan morphology. *Syst. Biol.* 41: 305–330.
- HALANYCH, K. M. 1993. Suspension feeding by the lophophore-like apparatus of the pterobranch hemichordate *Rhabdopleura normani*. *Biol. Bull.* 185: 417–427.
- HARRISON, F. W. AND E. E. RUPPERT. 1991. *Microscopic Anatomy of the Invertebrates*, vol. 4, Aschelminthes. Wiley-Liss, New York.
- HYMAN, L. H. 1959. *The Invertebrates: Smaller Coelomate Groups*. McGraw-Hill Book Co., New York.
- KRISTENSEN, R. M. 1991. Loricifera. In F. W. Harrison and E. E. Ruppert (eds) *Microscopic Anatomy of the Invertebrates*, vol. 4, Aschelminthes. Wiley-Liss, New York, pp. 351–375.

- LAKE, J. A. 1990. Origin of the Metazoa. *Proc. Nat. Acad. Sci., USA* 87: 763–766.
- MADDISON, W. P. AND D. R. MADDISON. 1992. *MacClade: Analysis of Phylogeny and Character Evolution*, Version 3.01. Sinauer, Sunderland, Mass.
- MEGLITSCH, P. AND F. R. SCHRAM. 1991. *Invertebrate Zoology*, third edition. Oxford Univ. Press, New York.
- MACLEAN, N. 1984. Amoebocytes in the lining of the body cavity and mesenteries of *Priapulus caudatus*. *Acta Zool.* 65: 75–78.
- RAFF, R. A., et al. 1989. Metazoan phylogeny based on analysis of 18S ribosomal RNA. In B. Fernholm, K. Bremer and H. Jönvall (eds). *The Hierarchy of Life*. Excerpta Medica (Elsevier), Amsterdam, pp. 247–260.
- SCHRAM, F. R. 1991. Cladistic analysis of the metazoan phyla and the placement of the fossil problematica. In A. M. Simonetta and S. Conway Morris (eds) *The Early Evolution of the Metazoa and the Significance of Problematic Taxa*. Cambridge Univ. Press, Cambridge, pp. 35–46.
- SHAPEERO, W. L. 1961. Phylogeny of the Priapulid Science 133: 879–880.
- SWOFFORD, D. L. 1993. *PAUP: Phylogenetic Analysis Using Parsimony*, Version 3.1.1. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.
- TELFORD, M. J. AND P. W. H. HOLLAND. 1993. The phylogenetic affinities of the chaetognaths: a molecular analysis. *Mol. Biol. Evol.* 10: 660–676.
- WADA, H. AND N. SATOK. 1994. Details of the evolutionary history of invertebrates to vertebrates, as deduced from the sequencing of 18S rDNA. *Proc. Nat. Acad. Sci., USA* 91: 1801–1804.
- WHEELER, W. C., P. CARTWRIGHT AND C. Y. HAYASHI. 1993. Arthropod phylogeny: a combined approach. *Cladistics* 9: 1–39.
- WILMER, P. 1990. *Invertebrate Relationships: Patterns in Animal Evolution*. Cambridge Univ. Press, Cambridge.
- WINGSTRAND, K. G. 1972. Comparative spermatology of a pentastomid, *Raillietiella hemidactyli*, and a branchiuran crustacean, *Argulus foliaceus*, with a discussion of pentastomid relationships. *Kong. Danske Vidensk. Selsk. Biol. Skrift.* 19(4): 1–72, 23 pls.