

Five classes of echinoderm and one school of thought

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ABSTRACT: Whether using molecules or morphology, choice of taxa, density of sampling, faith in homology and character coding are but some of the criteria which affect phylogenetic reconstruction. Early molecular-based phylogenies have been criticised on most of these grounds. To resolve the interrelationships between the echinoderm classes we have determined new nuclear ribosomal gene sequences for crinoids (C), holothuroids (H), asteroids (A) and ophiuroids (O) to complement existing data for the echinoids (E). The molecular data were reduced to 'class consensus sequences', and various combinations of data sets were compared, contrasted and combined with a new revised morphological data set with the aim of reaching a robust and consensus phylogeny for the Phylum Echinodermata. A single most parsimonious, total evidence, solution gave a tree with the topology (outgroup(C(A(O(E,H))))).

1 INTRODUCTION

Controversy over the relationships of echinoderms has been around ever since the term Echinodermata was coined by Bruguière (1791). Although Belon (1553), in providing the first description of a holothurian, recognized its affinities with echinoderms, Bruguière (1791) placed holothurians with the vermes as distinct from echinoderms, whereas Lamarck (1801) grouped asteroids, echinoids and holothurians together with medusoid coelenterates. In spite of the advent of modern methods of phylogenetic reconstruction and the first attempt to resolve the phylogeny of the major echinoderm classes using cladistics (Smith, 1984), there has been disagreement as to the true relationships of the group. There are 5 extant taxa and 105 possible rooted trees and yet we wish to resolve the one single tree that reflects the evolutionary history of the group. Alternative morphologically based phylogenies have been proposed by Smiley (1988) and Pearse & Pearse (1994). At least 7 alternative hypotheses have been proposed by workers using molecular or biochemical data (see review in Littlewood, 1995) and to date there has been little consensus of opinion.

Areas of conflict between the phylogenetic hypotheses of echinoderm class interrelatedness, have arisen due to inconsistency and inaccuracy in the morphological matrices, and insufficient sampling, lack of complementarity and poor alignment amongst the molecular data sets.

We have revised the morphological data set and have added to the existing molecular data sets

utilised by Littlewood (1995) in what was the first "total evidence" approach at resolving class relationships. Here we present a summary of our results which are detailed more extensively elsewhere (Littlewood *et al.*, in press), and concentrate on the phylogenetic signal afforded by each independent data set.

2 MATERIALS AND METHODS

The morphological matrices presented by Smith (1984, 1988), Strathmann (1988) and Smiley (1988) were revised and expanded with reference to new morphological studies (in particular articles in Harrison & Chia, 1994). In total 21 larval and 50 adult morphological characters were available for analysis. Larval and adult data were analysed separately and in combination. For the larval data we scored outgroup and crinoid characters as unknown. Crinoids develop directly and we have no notion as to the homology between larval echinoderms and putative outgroup taxa (i.e. hemichordates).

Although Strathmann (1988) argued for morphological homologies and character polarities between the feeding larvae of echinoderms and those of enteropneust hemichordates, he chose to score these outgroup character states as absent (0; $n=7$), whereas we have been more cautious and have scored them as unknown (?; $n=21$).

New nucleotide sequences for all the classes of echinoderm, except echinoids, were determined for both complete 18S and partial 28S ribosomal RNA genes (rDNA) wherever possible. In total, 37

Table 1. Number of gene sequences available for determining class relationships.

	complete 18S rDNA	partial 28S rDNA
Echinoids	23	19
Holothurians	4	5
Ophiuroids	5	12
Asteroids	3	9
Crinoids	2	1
outgroups	5	2

complete 18S and 46 partial 28S rDNA sequences from various echinoderms were available for analysis (Table 1). Outgroup sequences were taken from GenBank and were *Herdmania momus* (X53538), *Styela plicata* (M97577), *Branchiostoma floridae* (M97571), *Balanoglossus carnosus* (D14359) and *Saccoglossus kowalevskii* (L28054) for the 18S data set; and *Xenopus laevis* (X04025) and *Herdmania momus* (X53538) for the 28S data set.

Although we have analysed our full molecular data sets individually, we present here only the results of our class consensus approach.

Class consensus sequences were determined from full unambiguously aligned regions of our nucleotide alignments following these criteria:

- (i) sequences of all taxa within a single class were aligned as best as possible,
- (ii) any position where the base was invariant amongst sampled members was included,
- (iii) any obvious autapomorphic change was ignored; e.g. if at one position all echinoderms have a 'C', except within asteroids where two taxa have a 'T', while the other asteroids have a 'C' like all other echinoderms, then asteroids were scored as having a 'C' at that position. Autapomorphies can only be removed where site variation is confined to within a single class.
- (iv) At sites where base composition was more varied, the following IUPAC codes were used: - R = A or G; Y = C or T; N = other combinations (transitions and transversions); ? = any base or gap.
- (v) The six consensus sequences (one for each class plus one for the outgroup [*Xenopus* and *Herdmania* for LSU sequence data, and *Balanoglossus*, *Saccoglossus* and *Branchiostoma* for the SSU sequence data]) were then aligned. This was identical to the alignment used for the full analysis. As throughout, only regions which could be aligned without ambiguity were included in the final analysis. In the final analysis 318 unambiguously aligned positions were available from the LSU sequence data set, of which 11 were parsimony-informative, and 1696 unambiguously aligned positions were available from the SSU data, of which 52 were parsimony-informative.

3 RESULTS AND DISCUSSION

The full morphological and molecular data sets are available from either DTJL or ABS. All data were analysed using the exhaustive search option of PAUP (Swofford, 1993) where each tree supported by the data could be found, and a tree length frequency distribution determined.

Total morphology, 18S rDNA, 28S rDNA, total molecular and combined evidence trees consistently supported three tree topologies, namely (out(C(A(O(E,H))))), (out(C(O(A(E,H))))), and (out(C((A,0)(E,H)))). Each data set supported a single most parsimonious tree reflecting one of these topologies except the adult data which supported 2 solutions with equal parsimony. Table 2 illustrates the topologies of the trees best supported by each data set treated singly in combination. Trees derived from the larval data could not be rooted, as stated above, so they are excluded from the table. However, three equally parsimonious unrooted trees were derived from the larval data and alone, these data were inconclusive. This reflects Strathmann's (1988) finding that larval characters are highly homoplasious.

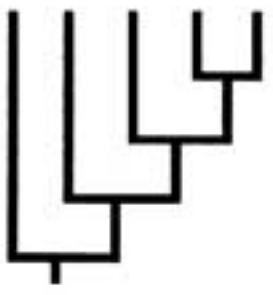
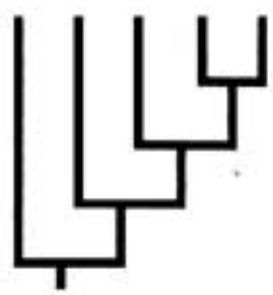
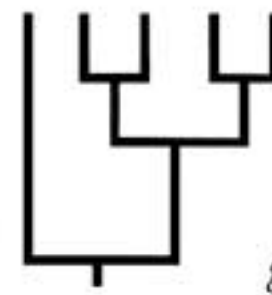
Every data set strongly supported crinoids as the outgroup to all other classes and supported echinoids+holothurians (Eleutherozoa) as the most derived clade. Based on the high negative g_i -statistic values of the exhaustive searches, each data set was highly structured. In other words each data set presented strong hierarchical signal (Hillis & Huelsenbeck, 1992).

Table 2 also indicates the extra number of steps required to achieve the alternative 2 topologies common to these data sets. Most of the data sets supported (out(C(A(O(E,H)))) as the most parsimonious solution although bootstrap support was generally poor for the nodes supporting the asteroid-ophiuroid split. The three competing topologies differ only in the divergence of and relationship between the asteroids and ophiuroids.

When we considered the morphological implications imposed by each of these tree solutions we found little support for the asteroid-echinoid-holothurian clade. Indeed, of the 18 characters which differ in their distribution amongst the three rival topologies, 4 are homoplasious and only 1 supported the grouping [A,H,E]. This character, the presence of internal ampullae, appears to have evolved independently in asteroids after they diverged from echinoids and holothurians (Blake & Guensberg, 1988). Subsequently, we can discount this character and topology 2 (Table 2). This leaves only two trees from the possible 105 as reasonable topologies which reflect both the phylogenetic analyses and the distribution of morphological characters about the trees.

Until such time that further evidence can be invoked to determine which is more likely to reflect the true evolutionary relationships between the extant echinoderm classes, we advocate using both

Table 2. The three most parsimonious solutions supported by the individual and combined data sets. The most parsimonious solution for each data set is labelled BEST, and the number of extra steps (+n) required to change to the two other solutions is indicated. The tree length of each most parsimonious tree is in parentheses after BEST.

Data	topology 1	topology 2	topology 3	g_1 -statistic
	C A O E H	C O A E H	C A O E H	
adult				
adult	BEST(35)	+n	BEST(35)	-0.76
adult+larval	BEST(82)	+2	+1	-0.75
18S rDNA	BEST(294)	+2	+2	-0.85
28S rDNA	BEST(71)	+1	+1	-0.58
total molecular	+3	BEST(360)	+2	-0.75
morph.+molec.	BEST(443)	+1	+2	-0.85

of these phylogenies as the basis for a comparative and phylogenetic approach in evaluating the evolution of extant echinoderms.

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