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The interrelationships of the echinoderm classes: morphological and molecular evidence

D. T. J. LITTLEWOOD[1,2] F.L.S., A. B. SMITH[3] F.L.S., K. A. CLOUGH[1] AND R. H. EMSON[2] F.L.S.

Departments of Zoology[1] and Palaeontology[3], The Natural History Museum, Cromwell Road, London SW7 5BD
[2]Division of Life Sciences, King's College, University of London, Campden Hill Road, London W8 7AH

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The relationship between the five echinoderm classes has perplexed phylogeneticists for some time. Although each of the crinoids (C), asteroids (A), ophiuroids (O), echinoids (E) and holothuroids (H) are morphologically distinct, evidence from larval and adult morphology, molecular biology, and stratigraphy have failed to provide a single consensus solution. We have reviewed all available morphological and molecular data, added new data and reanalysed independent data sets individually and in combination, in order to resolve echinoderm class relationships. In total, we present 21 larval and 50 adult morphological characters, partial 28S-like large subunit rRNA gene data for 39 taxa and complete 18S-like small subunit rRNA gene data for 37 taxa. For a 5 taxon problem there are 105 possible rooted tree topologies, and yet we were consistently presented with three competing hypotheses when data sets were analysed both individually and in combination. The total evidence solution favoured (outgroup(C(A(O(E,H))))) although the alternative tree topology, (outgroup(C(O(A(E, H))))) was only one step longer and (outgroup(C((A,O),(E,H)))) was two steps longer. Only these three trees are serious contenders and the distribution of morphological characters suggests we should discount the solution placing ophiuroids as sister group to an asteroid + echinoid + holothurian clade. Thus we are left with (outgroup(C(A(O(E,H))))) and (outgroup(C((A,O),(E,H)))) as the two most plausible phylogenetic hypotheses. Our data showed high levels of phylogenetic signal and these trees best fit the available data.

ADDITIONAL KEY WORDS:—ribosomal RNA – cladistics – phylogeny – systematics – fossil record – total evidence.

CONTENTS

Correspondence to D. T. J. Littlewood. e mail. dtl@nhm.ac.uk

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INTRODUCTION

Only five classes of echinoderm are extant, although there have been a further 18 distinct classes of echinoderm recognized from the fossil record (Paul, 1979), and one other proposed for an aberrant modern asteroid (Baker, Rowe & Clark, 1986). Fossil evidence suggests that the crinoids, asteroids, ophiuroids, echinoids and holothuroids diverged relatively rapidly some 500 million years ago in the Cambrian and Ordovician (Paul & Smith, 1984; Smith, 1988). As with the phylum to which they belong, each of the classes is well defined in terms of morphology and yet the interrelationships between the classes, and the phylogeny of the group, have been a matter of some debate. In part this reflects the rapid radiation suggested by the fossil record and the difficulties in establishing a sufficient number of potentially homologous characters that are phylogenetically informative. However, in spite of a number of attempts at resolving the phylogeny of extant echinoderm classes by various morphological and molecular means there have been at least eight competing scenarios published (see seven reviewed in Littlewood, 1995 and a further topology in Matsumura & Shigei, 1988). Here, we present a reassessment of available evidence, both old and new, and take into account the concerns of combining morphological and molecular data in phylogenetic reconstruction particularly as it pertains to resolving echinoderm class relationships.

Background and aims

There are 105 possible rooted phylogenetic tree topologies with any five taxa (Felsenstein, 1978) and our aim was to find which of these topologies best reflects all the available evidence describing the interrelationships of the echinoderm classes. We have taken independent data sets and analysed them individually and in combination. A similar combined morphological and molecular approach has been successfully applied to resolving relationships within individual echinoderm classes involving a greater number of individual taxa (asteroids: Lafay, Smith & Christen, 1995; echinoids: Littlewood & Smith, 1995; ophiuroids: Smith, Patterson & Lafay, 1995) and we extend this now to address class relationships.

Morphological characters

To date, a number of phylogenies have been presented on the basis of morphological evidence. Smith, (1984) was the first to compute a phylogeny based on cladistic methodology. Smith's (1984) analysis used both larval and adult characters and recognized holothurians and echinoids as sister groups (the Echinozoa) placing ophiuroids as sister group to those two taxa combined. However, in that analysis he wrongly mixed larval character states derived from direct and indirect developing taxa, thus incorporating a number of erroneous character scorings. Strathmann (1988) reanalysed just the larval characters of echinoderms, based on indirect larval development alone, and came to the conclusion that no topology was strongly supported. Furthermore, no matter which was correct, he concluded that there must be striking homoplasy in larval form. Smiley (1988) also presented a cladistic analysis of class relationships based largely on larval characters and a new interpretation of the development of holothurians. He came to a rather different conclusion from Strathmann, and found that holothurians were sister group to all other echinoderm classes. Pearse & Pearse (1994) generated a new data set based primarily on adult features adding some further characters. Unfortunately, the character states that they employed in this analysis are not specified and are largely unrecoverable from the paper. Their analysis found support for the echinoid-holothurian pairing, but also found support for an ophiuroid-asteroid pairing with crinoids as sister group to both pairs of taxa. Thus the four principal cladistic analyses of class relationships have come to surprisingly different conclusions.

During this period there have also been a number of important reviews published, notably on reproduction and development (Giese, Pearse & Pearse, 1991), neurobiology (Cobb, 1987) and microanatomy (Harrison & Chia, 1994) that have brought together information on the comparative structure of echinoderms.

We present a critical re-analysis of these data sets and identify conflict and congruity between them. Subsequently, on this basis, we present a new, internally consistent morphological data matrix and add new data from recently published literature.

Molecular characters

As with many unresolved phylogenetic problems, the interrelationships of the echinoderm classes have received the attention of molecular systematists. In itself the history of molecular systematics is widely reflected in its approach to the echinoderm class relationship question, highlighting the problems associated with choice of taxa, choice of molecule (gene, gene order etc.), density of taxa and the incorporation, or non-incorporation, of morphological evidence. Although attempts have been made to generate multiple independent molecular sequence data sets, there exist only two data sets where each echinoderm class is represented. These are both from the ribosomal RNA gene (rDNA), or gene product (rRNA); namely the 18S-like small subunit (SSU) and the 28S-like large subunit (LSU) components.

There have been no less than five competing phylogenies based on nucleotide sequence alignments alone (Field *et al.*, 1988; Raff *et al.*, 1988; Ratto & Christen, 1990; Wada & Satoh, 1994a,b; Marshall, 1994; Philippe, Chenuil & Adoutte, 1994; see also Littlewood, 1995 in which these data sets are further reviewed). SSU data has been used to support all five of these topologies and LSU data two. We can highlight a number of possible reasons as to why these solutions are in conflict, including method of phylogenetic reconstruction, alignment of sequence data and subsequent identification of unambiguously aligned sites, choice of taxa, number of representative taxa and choice of outgroup (see Littlewood, 1995).

Here we present new data to add to each of the SSU and LSU data sets from selected taxa, thereby increasing sampling density and taxonomic diversity within the data sets. Although these new molecular data sets may not satisfy all the criticisms levelled at previous molecular analyses they do go some way in achieving taxonomic diversity and high species density. Thus, we are in a position to reassess echinoderm class relationships with a high degree of confidence.

MATERIAL AND METHODS

Morphological data

Here we reanalyse all the available morphological data. We have taken into account all previously suggested characters as far as possible, and separated the data into pre- and post-metamorphosis (larval and adult) traits. This is done in order to separate phylogenetic signal coming from larval and adult morphology. The profound changes that take place at metamorphosis mean that few of the larval characters are correlated or connected with adult characters used. Nevertheless, care has been taken not to duplicate any characters and to avoid possible correlated features. Both data sets can be considered to be estimating the 'true' phylogeny but with error (in the form of homoplasy).

For the larval data set, no outgroup was included because of the difficulty in recognizing homologous characters in pterobranchs or enteropneusts, i.e. hemichordates. Furthermore, character states were drawn from the most extended indirect development in each group. Unfortunately, few of the larval traits for crinoids could be scored because this group shows only direct development. For morphological adult characters, the extinct carpoid group Soluta was selected as outgroup. Solutes have a mesodermal skeleton of stereom and a single asymmetric ambulacrum and are therefore closely related to crown group echinoderms but represent pre-radiate forms (Ubaghs, 1975; Paul & Smith, 1984). As the closest fossil stem group to extant echinoderms they provide the most appropriate outgroup.

The full list of morphological characters, with sources, coding and explanations appears in Appendix 1. In total, 21 larval and 50 adult characters have been identified.

Molecular data

Published sequences were taken from GenBank/EMBL or directly from their original publications; see Table 1 for full list of taxa and gene sequences used. New sequences were obtained using established DNA extraction, purification, PCR amplification and sequencing protocols. Genomic DNA was extracted from gonad tissues (0.01–0.1g) from *Astropecten irregularis*, *Porania pulvillus*, *Ophiomyxa brevirima*, *Astrobrachion constrictum*, *Ophiocomina nigra*, *Cucumaria sykion*, *Trochodota dunedinensis*, and *Endoxocrinus parrae*, and body wall from *Lipotrapeza vestiens*, and *Psychropotes longicauda* by grinding in 150 µl TE, pH 8.0, 0.5% SDS followed by a 3–4 hour digest in 6 µl proteinase K (10 mg/ml). DNA was phenol-chloroform extracted and precipitated with sodium acetate and ethanol according to Sambrook, Fritsch & Maniatis (1989) and redissolved in 50 µl dH_2O or TE. A genomic DNA pellet of *Ophiocanops fugiens* was supplied by John Pearse (University of California at Santa Cruz).

Complete 18S rDNA and partial 28S rDNA fragments were amplified using primers and protocols detailed in Littlewood & Smith (1995). Amplification products were either cloned into pGEM-T vector (Promega), grown in JM109 *E. coli* and purified with QIAGEN midi-prep columns, or purified with Wizard Mini-preps (Promega). Gene fragments were sequenced on an ABI 373 automated sequencer using the manufacturers protocols (Applied Biosystems Inc., Perkin-Elmer) and sequencing primers detailed in Littlewood & Smith (1995).

Consensus sequences were assembled and proof-read from individual sequence reactions using Sequencher v.3.0 for the Macintosh (Gene Codes Corporation, Michigan). All sequences were verified using at least three individual contigs and both strands were sequenced. Only approximately 450 bases of the 5′ end of the 28S rDNA fragment were sequenced fully and included in this study.

It is worth noting that not all taxa studied yielded DNA of sufficient quantity or quality for successful PCR amplification. In particular, seven species of stemmed crinoid yielded no detectable amounts of DNA, probably because only small pieces of stems and arms, with little associated tissue, were available. Also, success with 18S rDNA amplification did not guarantee success with partial 28S rDNA amplification from the same genomic DNA extracts.

Published sequences were selected for use as outgroups when rooting trees. For the partial 28S rDNA data set the chordate *Xenopus laevis* (X04025) and the tunicate *Herdmania momus* (X53538) were chosen. For the 18S rDNA set the tunicates *Herdmania momus* (X53538), *Styela plicata* (M97577), the cephalochordate *Branchiostoma floridae* (M97571) and the enteropneust hemichordates *Balanoglossus carnosus* (D14359) and *Saccoglossus kowalevskii* (L28054) were chosen.

Sequence alignment

Multiple sequence alignments were generated with Clustal W (Thompson, Higgins & Gibson, 1994) using default gap opening and gap extension penalties (10 and 0.05, respectively) with transitions unweighted. Initially, sequences were aligned for each echinoderm class. Completed class alignments were then aligned with one another using the ‘profile alignment’ option. The full echinoderm alignments were then aligned to the outgroup taxa, and finally the full alignment was edited by eye and with reference to published secondary structure models (Gutell, 1994) of selected outgroup taxa with GDE (Genetic Data Environment) suite of programs. The pre-aligned sequences of *Herdmania momus*, *Styela plicata*, *Branchiostoma floridae* and *Xenopus laevis* were taken from the ‘subalignment extraction’ option of the Ribosomal

TABLE 1. Echinoderms used in this analysis. [SSU] = SSU rDNA or rRNA sequence available; [LSU] = LSU rDNA or rRNA sequence available. 1 = this study; 2 = published sequence from Smith et al. (1992); 3 = published sequence from Wada & Satoh (1994a); 4 = published sequence from Smith *et al.* (1995); 5 = published sequence from Lafay *et al.* (1995); 6 = published sequence from Littlewood & Smith (1995); 7 = published sequence from Turbeville *et al.* (1994); 8 = unpublished sequence from Richard Christen (Villefranche-sur-Mer)

- Phylum Echinodermata
- Class Echinoidea
  - Order Cidaroidea
    - Family Cidaridae
      - *Cidaris cidaris* (Lamarck) [LSU2]
      - *Eucidaris tribuloides* (Lamarck) [SSU6]
  - Order Echinothurioidea
    - Family Echinothuriidae
      - *Araeosoma ijimai* Yoshiwara [LSU6]
      - *Asthenosoma owstoni* Mortensen [SSU6, LSU6]
  - Order Diadematoida
    - Family Diadematidae
      - *Diadema setosum* (Leske) [SSU6]
      - *Centrostephanus coronatus* (Verrill) [SSU6]
  - Order Arbacioida
    - Family Arbaciidae
      - *Arbacia lixula* (Linnaeus) [SSU6, LSU2]
  - Order Phymosomatoida
    - Family Stomopneustidae
      - *Stomopneustes variolaris* (Lamarck) [SSU6]
  - Order Temnopleuroida
    - Family Temnopleuridae
      - *Temnopleurus hardwickii* (Gray) [SSU6]
      - *Mespilia globulus* (Linnaeus) [SSU6]
      - *Salmacis sphaeroides* (Linnaeus) [SSU6]
  - Order Echinoida
    - Family Echninidae
      - *Echinus esculentus* Linnaeus [SSU6, LSU2]
      - *Paracentrotus lividus* (Lamarck) [LSU2]
      - *Psammechinus miliaris* (Müller) [SSU6, LSU2]
    - Family Echinometridae
      - Subfamily Strongylocentrotidae
        - *Strongylocentrotus intermedius* Agassiz [SSU3]
        - *Strongylocentrotus purpuratus* Stimpson [SSU7]
      - Subfamily Echinometridae
        - *Colobocentrotus atratus* (Linnaeus) [SSU6]
      - Subfamily Toxopneustidae
        - *Sphaerechinus granularis* (Lamarck) [SSU6, LSU2]
        - *Tripneustes gratilla* (Linnaeus) [SSU6]
        - *Lytechinus variegatus* (Lamarck) [LSU2]
  - Order Cassiduloida
    - Family Cassidulidae
      - *Cassidulus mitis* Krau [SSU6]
  - Order Clypeasteroida
    - Suborder Clypeasterina
      - Family Arachnoididae
        - *Fellaster zelandiae* (Gray) [SSU6]
    - Suborder Scutellina
      - Family Fibulariidae
        - *Echinocyamus pusillus* (Müller) [LSU2]
      - Family Astriclypeidae
        - *Echinodiscus bisperforatus* [SSU6]
      - Family Mellitidae
        - *Encope aberrans* Martens [SSU6, LSU6]
  - Order Spatangoida
    - Family Spatangidae
      - *Spatangus purpureus* Müller [LSU2]
    - Family Loveniidae
      - *Echinocardium cordatum* (Pennant) [SSU6, LSU2]
    - Family Brissidae
      - *Meoma ventricosa* (Lamarck) [SSU6]
      - *Brissopsis lyrifera* (Forbes) [SSU6]
- Class Asteroidea
  - Order Forcipulatida
    - Family Asteriidae
      - *Asterias rubens* Linnaeus [LSU5]
      - *Asterias amurensis* (Lutleen) [SSU3]
      - *Coscinasterias tenuispina* (Lamarck) [LSU5]
      - *Marthasterias glacialis* (Linnaeus) [LSU5]
  - Order Paxillosida
    - Family Astropectinidae
      - *Astropecten irregularis* (Pennant) [SSU1, LSU5]
    - Family Luidiidae
      - *Luidia ciliaris* (Philippi) [LSU5]
  - Order Velatida
    - Family Solasteridae
      - *Crossaster papposus* (Linck) [LSU5]
  - Order Valvata
    - Family Poraniidae
      - *Porania pulvillus* (Müller) [SSU1, LSU1]
  - Order Spinulosida
    - Family Echinasteridae
      - *Henricia sanguinolenta* (Müller) [LSU5]
      - *Echinaster sepositus* Gray [LSU5]
- Class Ophiuroidea
  - Subclass Oegophiuridea
    - Family Ophiocanopidae
      - *Ophiocanops fugiens* Koehler [SSU1, LSU1]
  - Subclass Ophiuridea
    - Order Euryalida
      - Family Asteronychidae
        - *Asteronyx loveni* Müller & Troschel [LSU4]
      - Family Euryalidae
        - *Astrobrachion constrictum* (Farquhar) [SSU1]
    - Order Ophiurida
      - Suborder Ophiomyxina
        - Family Ophiomyxidae
          - *Ophiomyxa brevirima* Clark [SSU1]
      - Suborder Ophiurina
        - Family Ophiacanthidae
          - *Ophiacantha bidentata* (Retzius) [LSU4]
        - Infraorder Chilophiurina
          - Family Ophiurinae
            - *Ophiura ophiura* (Linnaeus) [LSU4]
            - *Ophiura albida* Forbes [LSU4]
        - Infraorder Gnathophiurina
          - Superfamily Gnathophiuridea
          - Family Amphiuridae
            - *Amphipholis squamata* (Delle Chiaje)
          - Family Ophiothricidae
            - *Ophiothrix fragilis* (Abildgard) [LSU4]
          - Family Ophiactidae
            - *Ophiopholis aculeta* Gray [SSU7, LSU4]

TABLE 1.—*continued*

Superfamily Ophiocomidea
Family Ophiocomidae
Ophiocomina nigra (Abildgaard) [LSU1]
Infraorder Ophiodermatina
Family Ophiochitonidae
Ophiochiton tenuispinus Lyman [LSU4]
Family Ophiodermatidae
Ophioderma longicauda (Retzius) [LSU4]
Infraorder Ophiolepidina
Family Ophiolepididae
Ophiomusium lymani Wyville Thomson [LSU4]
Ophioplocus japonicus Clark [SSU3]
Class Holothuroidea
Order Aspidochirota
Family Holothuriidae
Holothuria forskali Chiaje [LSU5]
Order Apoda
Family Synaptidae
Leptosynapta inhaerens (Müller) [LSU5]
Trochodota dunedinensis (Parker) [LSU1]
Order Dendrochirota
Family Cucumariidae
Cucumaria sykion Lampert [SSU1]
Pawsonia saxicola (Brady and Robertson) [LSU8]
Family Phyllophoridae
Lipotrapeza vestiens (Joshua) [SSU1]
Order Elasipodida
Family Psychropotidae
Psychropotes longicauda Théel [SSU1, LSU1]
Class Crinoidea
Order Comatulida
Family Antedonidae
Antedon bifida (Pennant) [LSU2]
Antedon serrata (A.H. Clark) [SSU3]
Order Isocrinida
Family Isocrinidae
Endoxocrinus parrae Gervais [SSU1]

Database Project (which has sequences aligned to recognized and published secondary structure models; Maidak *et al.*, 1996) and imported into the GDE editor file.

Terminal 5′ and 3′ regions of the alignment including incomplete sequences were discarded from the molecular data matrices. Also, those regions which could not be unambiguously aligned were discarded; unambiguously aligned regions were generally bordered by invariant nucleotides. The 18S rDNA alignment was 1957 bases long of which 1723 were judged to be unambiguously aligned and therefore included in the final matrix. The 28S rDNA alignment was 497 bases long of which 313 were judged to be unambiguously aligned and included in the final matrix. The full molecular data set and alignments of 18S and 28S rDNA data have been submitted to the European Molecular Biology Laboratory (accession no. D527564) and is available via anonymous FTP from FTP.EBI.AC.UK in directory/pub/databases/embl/align. Printed and electronic versions have also been deposited with The Librarian, The Linnean Society of London.

Phylogenetic analysis

All analyses were conducted using PAUP (Swofford, 1993). In all analyses only phylogenetically informative sites were included. With some modifications, outlined in the results, the analyses were planned and executed as follows:

Round 1. Search for most parsimonious tree(s) treating each data set independently. Branch-and-bound and heuristic search options were used for the morphological data sets. Where molecular data sets were too large for branch-and-bound, only heuristic search methods were employed. Data sets were bootstrapped with 1000 replicates; partial data sets, using selected representative taxa from each class of echinoderm, were bootstrapped for the molecular data as single searches took over 6 hours with complete data. Trees were rooted using selected outgroups and

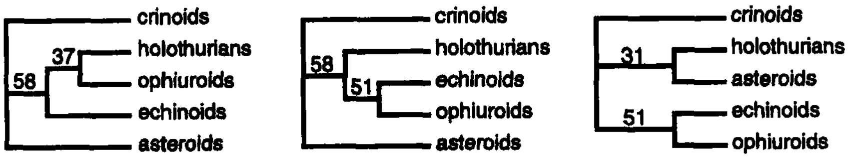


Figure 1. Three equally parsimonious trees based on larval morphology. Values on nodes are bootstrap percentages (1000 replicates). Tree length = 9; CI = 0.67; RI = 0.50.

character-states were optimised using the accelerated transformation optimization (ACCTRAN) option; i.e. reversals preferred over parallelisms (Kitching, 1992).

Round 2. Nucleotides in both 18S and 28S rDNA data sets were recoded simply as purines and pyrimidines so a phylogeny could be determined on the basis of transversions and insertion/deletion events only. These analyses were also bootstrapped as in Round 1 using the same reduced number of taxa. Transversions could not be weighted against transitions as the data sets were too large.

Round 3. Each molecular data set was reduced to echinoderm consensus sequences; see below for rationale and method.

Round 4. Consensus-sequence data sets from complete 18S and partial 28S rDNA data sets were combined to generate a total molecular data set and subsequently combined with the morphological data in order to search for a 'total evidence' phylogenetic solution.

Within-class relationships are not optimised in this analysis and consequently differ from previously published results. This is because we have had to exclude regions of the genes informative below class level, but which cannot be aligned amongst classes unambiguously.

We have also used maximum likelihood to analyse consensus sequence data (DNAML in PHYLIP, Felsenstein 1993); there were far too many ingroup taxa to perform maximum likelihood analyses on individual molecular data sets. Maximum likelihood analyses were performed with transition/transversion (Ti/Tv) ratios set to a value determined empirically (Kishino & Hasegawa, 1989) using all taxa (i.e. consensus sequences for each class and outgroup). The Ti/Tv ratio was applied to a global search using empirical base frequencies, one category of substitution rates and random input of taxa.

RESULTS AND ANALYSES

Morphological evidence

Larval data set

As pointed out by Strathmann (1988) the number of phylogenetically informative characters that can be derived from larval morphology is extremely limited. Although we have scored for 21 characters, only six of these are phylogenetically informative, the others being invariant within echinoderms, or autapomorphic. Parsimony analysis found three shortest trees (Fig. 1) whose strict consensus gave no resolution whatsoever. Reweighting characters according to their rescaled consistency index made no difference to the outcome.

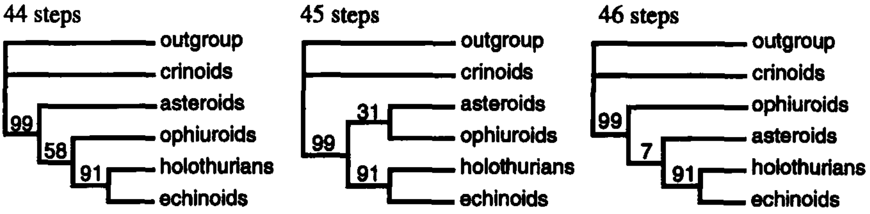


Figure 2. Single most parsimonious trees based on adult morphology (length = 44; CI = 0.70; RI = 0.5) and trees one and two steps longer. Values on nodes are bootstrap percentages (1000 replicates).

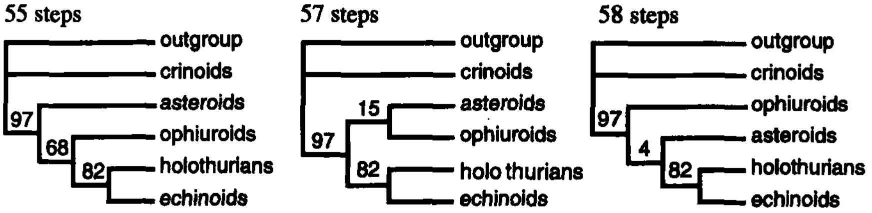


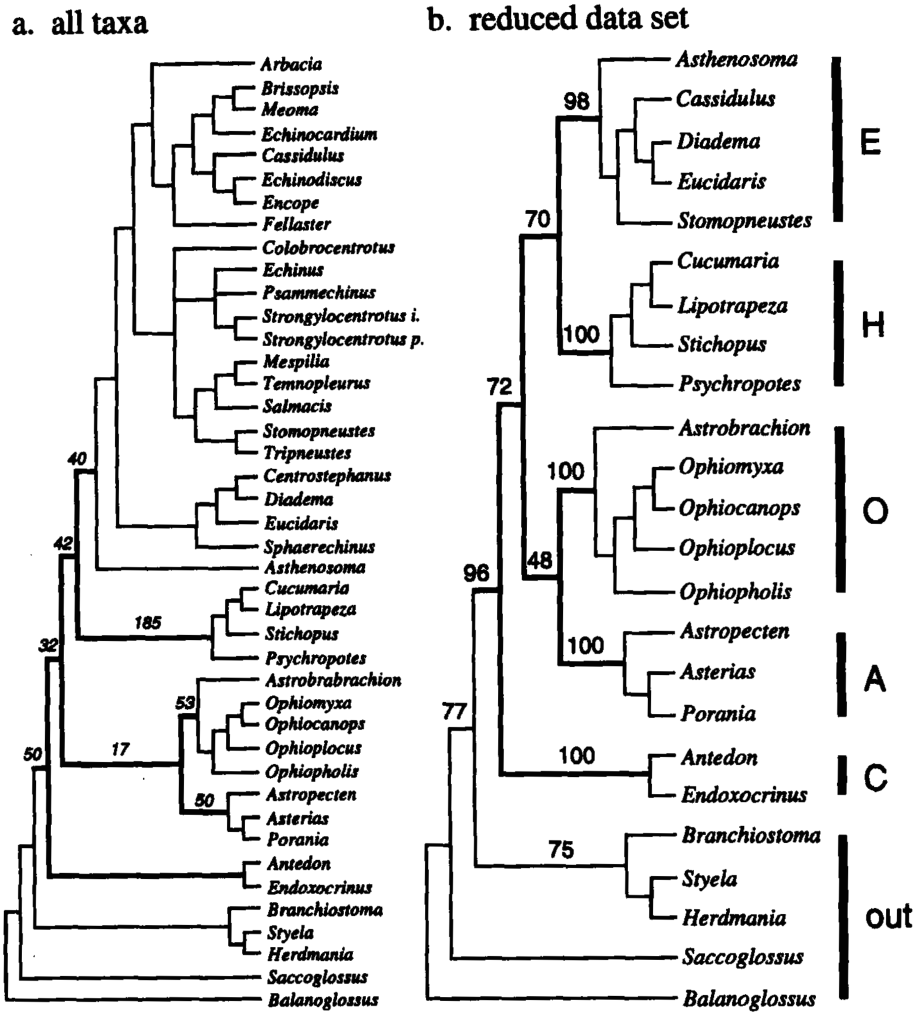
Figure 3. Single most parsimonious tree based on total (larval + adult) morphology (length = 82; CI = 0.68; RI = 0.53) and trees one and two steps longer. Values on nodes are bootstrap percentages (1000 replicates).

Adult morphology

Fifty characters were scored of which 29 were phylogenetically informative. Parsimony analysis identified a single most parsimonious tree at 44 steps (Fig. 2). Other trees were found at 1 and 2 steps longer. The single shortest tree identified crinoids as outgroup to the other classes, and echinoids and holothurians as sister taxa with high bootstrap support and the ophiuroids as sister group to the echinoid-holothuroid clade. The next most parsimonious solution placed asteroids and ophiuroids as sister taxa. The solution still one step longer (46 steps) paired asteroids with the echinoid-holothurians clade. Reweighting characters according to their rescaled consistency index made no difference to the outcome.

Combined adult and larval morphological data

In total, 35 phylogenetically informative characters were available from the morphological data. Parsimony analysis found one tree at 55 steps, a second at 57 and a third at 58 with subsequent topologies from 60 steps onwards. The most parsimonious solution placed ophiuroids as sister group to echinoids plus holothurians, and the tree two steps longer placed asteroids and ophiuroids as sister taxa. Again, bootstrapping gave high support for the pelmatozoan-eleutherozoan pairing and the echinoid-holothurian pairing and very low support for other branches (Fig. 3). Combining characters 41 and 60, which are probably scoring for the same morphological character in different parts of the body increased the bootstrap support for ophiuroids as sister group to echinoids and holothurians, but made no other difference. Reweighting characters according to their rescaled consistency index and rerunning the analysis found just one topology, that favouring ophiuroids as sister taxon to echinoids + holothurians.



a. all taxa

b. reduced data set

Figure 4. (a) strict consensus of six equally parsimonious trees based on SSU rRNA data using all available data, with branch lengths (italicized numbers) leading to echinoderm classes over thicker lines; tree length = 2610; CI = 0.56; RI = 0.72; (b) single most parsimonious tree based on SSU rRNA of a reduced data set with bootstrap percentages (1000 replicates); tree length = 2249; CI = 0.56; RI = 0.71. out = outgroup; E = echinoids; H = holothuroids; A = asteroids; O = ophiuroids; C = crinoids.

Molecular evidence

18S rDNA gene data

A total of 37 small subunit ribosomal RNA gene sequences of echinoderms and five outgroup taxa were available for analysis (new 18S rDNA sequences have been deposited with EMBL under accession numbers Z80947–56). The full alignment yielded 424 unambiguously aligned, phylogenetically informative sites. Parsimony analysis found six equally parsimonious trees which differed only in the placement of taxa within the echinoids. Each class of echinoderm was shown to be monophyletic with the echinoids + holothurians and ophiuorids + asteroids as sister taxa with crinoids as the outgroup; a strict consensus tree is shown in Figure 4a. Early divergent representatives of each clade, identified from previous studies on within-class phylogenies (echinoids: Littlewood & Smith, 1995; asteroids: Lafay, Smith & Christen, 1995; ophiuroids: Smith, Patterson & Lafay, 1995) were included in a reduced data set for bootstrap analysis ($n = 19$ taxa; Fig. 4b). By choosing two or three deep branches within each class we hoped to minimise the problem of long-branch attraction (Smith, 1994). Bootstrap resampling indicated strong support for the echinoid + holothurian clade and the eleutherozoan-pelmatozoan pairing but weak support for the ophiuroid + asteroid clade (48%).

The branch leading to the holothurian taxa (Fig. 4b) was relatively long (185 steps) in comparison to that leading to echinoids (40 steps), and in spite of a large region of the alignment, representing an insertion of at least 34bp and common to all the holothurians, being removed. Since they shared a common ancestor with the echinoids, the holothurians sampled in this study have accumulated approximately 4.5 times as many molecular changes than the echinoids. This has already been noted by Raff *et al.* (1988, p. 37).

Reanalysing the full and reduced 18S rDNA data sets with nucleotides recoded as simply purines and pyrimidines changed the topology of the most parsimonious solutions. Both the strict consensus of the eight shortest trees found with the full 37 echinoderm sequences and the consensus of the two shortest trees found with the reduced data set (trees not shown), gave a class topology of (C(A(O(E,H)))). However, as with the raw, reduced data set, although individual classes were strongly monophyletic no clade pairing any two classes was well supported with bootstrap resampling (<45%).

28S rDNA gene data

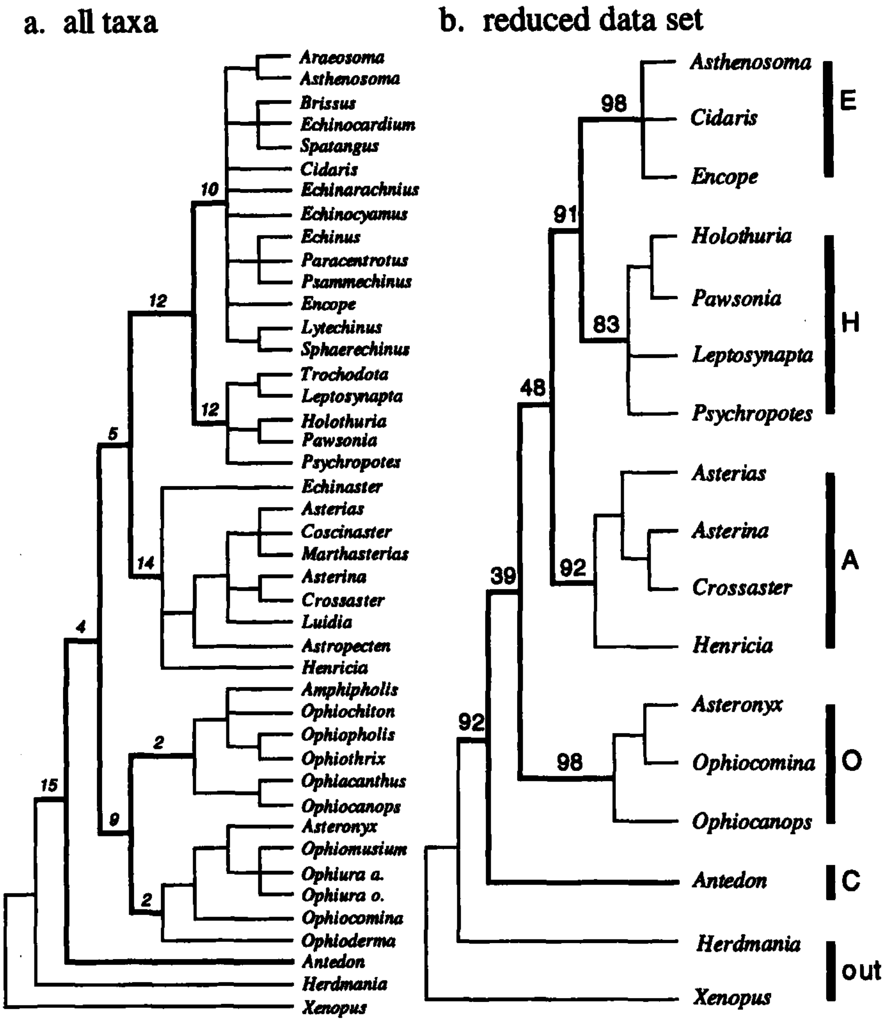
In total, 41 echinoderm partial 28S rRNA gene sequences were available for analysis; (new 28S rDNA sequences have been deposited with EMBL under accession numbers Z80943–6). The full alignment, including the two outgroup taxa, yielded 107 unambiguously aligned, phylogenetically informative sites. Parsimony analysis found 2746 equally parsimonious trees; the strict consensus of which indicated that the classes remained as monophyletic groups (Fig. 5a) with a class level phylogeny of (C(O(A(E,H)))). A taxonomically reduced data set retaining 16 echinoderm taxa was used for bootstrapping and yielded only four trees (which differed only amongst the relationships of the echinoids), but with identical relationships between the classes. Bootstrap support was high for an echinoid + holothurian clade and a pelmatozoan-eleutherozoan pairing but poor for all other possible class relationships; the next strongest support was for an ophiuroid-asteroid pairing. Bootstrap analysis on the reduced, recoded data set indicated strong support for the echinoids + holothurians clade (79%) and for asteroids as the sister taxa to ophiuroids + echinoids + holothurians (65%); Figure 5b.

Reanalysing the full 28S rDNA data set recoded as purines and pyrimidines still generated a large number of equally parsimonious trees ($n = 2012$), but the strict consensus tree was free of polytomies and gave a topology of (C(A(O(E,H)))).

Class consensus sequence analyses

Rationale

Within the 18S or 28S ribosomal genes there are some regions that have evolved more rapidly than others. This is obviously manifest from the way in which some


a. all taxa

b. reduced data set

Figure 5. (a) strict consensus of 2746 equally parsimonious trees based on LSU rRNA data using all available data, with branch lengths (italicized numbers) leading to echinoderm classes over thicker lines; tree length = 422; CI = 0.51; RI = 0.79; (b) single most parsimonious tree based on LSU rRNA of a reduced data set with bootstrap percentages (1000 replicates); tree length = 312; CI = 0.61; RI = 0.66. out = outgroup; E = echinoids; H = holothuroids; A = asteroids; O = ophiuroids; C = crinoids.

portions of the gene are conserved without change across all five echinoderm classes, while other regions may be alignable within a single class but difficult or impossible to align unambiguously across two or more classes. There are still other regions (those evolving most rapidly) that are impossible to align between members of the same echinoderm class; this is particularly noticeable with the LSU rRNA gene data. Clearly, if we are interested in the relationships of classes then changes that have occurred since the divergence of each crown group are of little interest and simply confuse the picture by overprinting the sequence present in the latest common ancestor to each class. One approach that can be taken is to construct consensus sequences for each class, which identify only those positions whose character state is unambiguous in the latest common ancestor to the extant taxa sampled for each class.

One of the problems of using sequences from individual taxa as representative of the entire class is that, in phylogenetics, choice of taxon can profoundly affect the topology found (Lanyon, 1985; Lecointre *et al.*, 1993). So that depending upon which taxa are chosen as representing echinoids, for example, we can come up with different hypotheses concerning class relationships. Further, although targeting key taxa and sampling densely are advocated as alternatives to choosing single taxa in molecular systematic studies (e.g. Baverstock & Moritz, 1996), there are limits to the number of nucleotide sequences which can be handled. Not only are there limitations imposed by computing time but, perhaps more importantly, the existence of only four nucleotide character states increases the chances that in any one lineage a homoplasious mutation can become fixed. At a higher taxonomic level, as with the echinoderm class relationships, this may weaken the signal in the data through mutational saturation. Using our consensus approach overcomes this problem, since it eradicates variation that is encountered within each class and constructs a phylogeny based solely on a class-wide character set.

Method

For every class, except crinoids, we had sequence data from four or more taxa. For each class we constructed a consensus sequence in the following way:

(1) Sequences of all taxa within a single class were aligned as best as possible.
(2) Any position where the base was invariant amongst sampled members of a class was included.
(3) Any obvious autapomorphic change was ignored. For example, if at one position all echinoderms have a 'C', except for one asteroid that has a 'T', 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.
(4) At sites where base composition was more varied, the following scores were used: R = A or G; Y = C or T; N = other combinations (transitions and transversions); ? = any base or gap.
(5) 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 aligned without ambiguity were included in the final analysis.

Results of consensus-sequence approach

Each gene sequence was treated independently, with and without outgroup and the two were then combined. Full exhaustive results are shown in Appendix 2.

SSU rDNA consensus

(a) Unrooted (no outgroup included). One tree, length 263 steps was found. The tree length distribution was strongly left-skewed (g_1, index = -0.91; exhaustive search option) showing that there was significant hierarchical signal in the data (Hillis &

Huelsenbeck, 1992). This gave the topology (C(A(O(E,H)))). The next closest topologies were two trees length 266 steps, with the topologies (C(O(A(E,H)))) and (C((O,A)(E,H)).
(b) Rooted. One tree, length 294 steps was found with the topology (out(C(A(O(E, H))))). One step longer a second tree was found, which had the same ingroup topology, but placed the root between the branch separating crinoids and asteroids. Three further topologies were found at length 296 steps; one of which was again identical to the most parsimonious topology for the ingroup, differing only in the position of the root. The other two topologies both placed crinoids as sister group to the other echinoderm classes, and were identical to the topologies identified as suboptimal in the unrooted analysis.

LSU rDNA consensus

(a) Unrooted. An exhaustive search produced a near symmetrical tree-length distribution ($g_1 = -0.45$) showing that there was little or no hierarchical signal in the data. One tree was found, length 57 steps, with the topology (C(O(A(E,H)))).
(b) Rooted. Again, an exhaustive search produced a tree-length distribution with little asymmetry ($g_1 = -0.58$). The same topology was found as in the unrooted analysis, with crinoids identified as sister group to other echinoderm classes.

Combined molecular data

SSU plus LSU rDNA consensus

(a) Unrooted. An exhaustive search produced a weakly asymmetric tree-length distribution ($g_1 = -0.60$) and identified two trees at length 318 steps. These have the topologies (C(A(O(E,H)))) and (C(O(A(E,H))))). The next most parsimonious topology was two steps longer (length 320 steps) with the topology (C((O,A)(E,H))).
(b) Rooted. Again this produced a left-skewed tree-length distribution ($g_1 = -0.75$). The most parsimonious solution, at 360 steps identified (out(C(O(A(E,H))))). However, at one step longer, the topology (out(C(A(O(E,H))))) was found and at a further step the topology (out(C((A,O)(E,H)))). All three topologies were rooted on crinoids.
(c) Maximum likelihood analysis resulted in a tree with the same topology as the most parsimonious solution with the same data set, namely (out(C(O(A(E,H))))); transition/transversion ratio set to 1.56 (empirically determined); ln likelihood = −4497.43.

Combined morphological and molecular data

We now have comparable data sets of morphological and molecular characters for the five classes of echinoderm plus outgroup. It is clear from analysing each data set independently that they are all suggesting very much the same phylogenetic solution, i.e. that either O or A is sister group to (E,H), or O is sister group to A; it is the position of the ophiuroids which is unresolved within the Eleutherozoa. In both molecular and morphological data, the same three alternatives appear as the best explanation of the character distributions. Clearly, there is only a weak

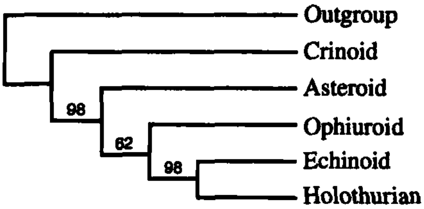


Figure 6. Single most parsimonious solution using all available evidence; combined larval + adult morphology and class consensus sequences for SSU and LSU rRNA data sets, with bootstrap percentages (1000 replicates); tree length = 446; CI = 0.81; RI = 0.48.

phylogenetic signal in each data set, and since both are in general agreement, our best estimate of the true phylogeny must come from combining the data sets together.

Total evidence

(1) No outgroup. Analysis of all evidence combined found a single unrooted topology of length 397 steps, (C(A(O(E,H)))). Tree length distribution was highly asymmetric at $g_1 = -1.00$. However, two other topologies were within three steps and well separated from other topologies. At 400 steps the tree (C(O(A(E,H)))) was found, while at 401 steps (C((A,O)(E,H) appeared. The next most parsimonious topologies came at 409 steps.

(2) Outgroup included. Analysis produced a strongly left-skewed tree-length distribution ($g_1 = -0.83$), with again the same most parsimonious solution rooted on crinoids at 443 steps. Bootstrap values, based on 1000 replicates are shown in Figure 6 and are generally high.

Levels of homoplasy

Adult versus larval homoplasy

Our new molecular and revised morphological data sets support the conclusion of Strathmann (1988), namely that considerable convergent evolution must have occurred in the evolution of echinoderm larvae. Smith, Littlewood & Wray (1996) tested this empirically amongst echinoids by comparing measures of homoplasy derived from adult and morphological data optimized on to the total evidence tree. We have repeated this approach with the full echinoderm class data set and have extended the procedure to compare levels of homoplasy within and between our class consensus molecular and morphological data sets. Excluding uninformative positions we have chosen consistency index (CI), retention index (RI) and rescaled consistency index (RC) as measures of homoplasy (Table 2). For morphology there were higher levels of homoplasy amongst the larval characters; indeed our estimates of larval homoplasy may be low as datasets with fewer characters tend to have higher CIs (Meier, Kores & Darwin, 1991).

Retention index expresses the amount of synapomorphy in a data set and has been promoted as a better means of 'evidential support' of groups than CI (see Siebert, 1992; Farris, 1988). The adult characters clearly exhibited higher RI values although the zero value scored for larval characters may be an artefact of the method used in its calculation. P.L. Forey (pers. comm.) has pointed out that the

TABLE 2. Consistency indices excluding uninformative positions (CI), retention indices (RI), and rescaled consistency indices (RC) for morphological and molecular characters optimized onto the total evidence tree. Total number of available characters (N) excluding unalignable regions of the molecular data set, number of variable positions (V) and number of informative sites (I) also shown. Molecular data are from the consensus sequences for each class (see text)

	N	V	I	CI	RI	RC
Larval characters	21	14	6	0.50	0.00^F	0.00
Adult characters	51	49	29	0.69	0.57^F	0.45
Total morphology	72	63	35	0.68	0.54	0.43
SSU rRNA	1696	352	154	0.82	0.44	0.39
LSU rRNA	310	90	59	0.90	0.50	0.46
Total molecular	2006	442	213	0.84	0.45	0.40
Total data	2078	505	248	0.81	0.48	0.42

F=with Hennig86 (Farris, 1988) RI values are 0.16 and 0.64 for larval and adult characters.

calculation of RI in PAUP can sometimes lead spuriously to values of zero; RIs calculated with Hennig86 (Farris, 1988) gave values of 0.16 and 0.64 for larval and adult characters mapped on to the total evidence tree.

Morphological versus molecular homoplasy

The value of our class consensus molecular approach is partly validated by the results in Table 2. Both SSU and LSU rRNA consensus data sets show remarkably low levels of homoplasy (high CI). Of course, as with morphology, our consensus approach is a reflection of characters having been sorted and variable sites within each class having been rejected. RI values were very similar and from this we can conclude that each data set was contributing similar levels of phylogenetic signal.

DISCUSSION

Morphological data generate a clear signal concerning certain parts of the echinoderm tree, and very little signal concerning other parts. They give clear and unambiguous support for a pelmatozoan-eleutherozoan division, and also provide surprisingly high support for the holothurians + echinoids pairing. The fact that it is very difficult to disentangle the relationships of asteroids, ophiuroids and echinozoans (holothurians and echinoids) suggests that the three groups may have arisen very close together in time. Indeed, estimates of divergence times gleaned from the appearance of each echinoderm class in the stratigraphic record (Smith, 1988) corroborate this notion; appearance of crinoids (530–500 Myr), asteroids, ophiuroids and echinozoans (*c.* 500 Myr). Individually, the molecular data sets also fail to resolve the relationships any further and our data support the conclusions of Philippe, Chenuil & Adoutte (1994) who, investigating the Cambrian radiation of metazoans, found that the 18S rDNA data could not "confidently resolve cladogenetic events separated by less than about 40Myr".

There has been much debate as to the merits of combining diverse, independent data sets in phylogenetic analysis (Barrett, Donoghue & Sober, 1991; Bull *et al.*, 1993; de Queiroz, Donoghue & Kim, 1995; Miyamoto & Fitch, 1995) and still further attention has been paid to the manner in which molecular data should, if at all, be combined or compared with morphological data (Hillis, 1987; Patterson, Williams & Humphries, 1993; Larson, 1994). Arguments between molecular and morphological approaches to systematics continue to be influenced by the development of methods of phylogenetic reconstruction, statistical tests of congruence, our understanding of molecular evolution, the limits of molecular phylogenetics (Felsenstein, 1988; Hillis, 1991; Hillis & Huelsenbeck, 1992; Huelsenbeck & Cunningham, 1994) and the levels of resolution each data set can afford particular taxonomic groups (e.g. amongst echinoids, Littlewood & Smith, 1995). However, when independent data sets suggest congruent phylogenetic solutions individually, we have confidence that our phylogeny represents evolutionary history faithfully. Indeed, on these grounds we believe we have achieved a faithful reconstruction of echinoderm interrelationships.

Class relationships

Throughout our analyses we were presented repeatedly with three competing phylogenetic solutions from the possible 105 evolutionary scenarios, namely (C(A(O(E,H)))), (C(O(A(E,H)))), and (C((A,O)(E,H))). We have also established that these topologies contain significant signal (by means of bootstrap proportions and distributions of tree length through exhaustive searches).

What other evidence can we invoke to choose between these trees?

Additional support for one topology over the other may be sought by mapping the morphological characters onto each tree, tracing character change and distribution, and assessing the biological implications of particular features arising independently throughout the Phylum.

If we examine the morphological characters, 18 differ in their distribution amongst the three rival topologies. Four of these (the presence of a doliolarian stage in larval development [C,O,H]; the retention of the larval mouth as the definitive adult mouth [O,H (?C)]; the presence of larval tube-feet developed as axocoel extensions and used for attachment [H,A] and the absence of batyl alcohol [A,H]) are homoplasious on all three trees and need not be considered further.

Only one of the remaining 14 characters directly supports the grouping [A,H,E], and that is the presence of internal ampullae. There is disagreement as to when asteroids evolved internal ampullae, but general consensus that all Ordovician asteroids lacked internal ampullae (Blake & Guensburg, 1988) so that asteroids must have independently evolved internal ampullae after they had diverged from echinoids and holothurians. There is therefore no morphological evidence of substance to support the asteroid-echinoid-holothurian clade. Scoring asteroids as primitively lacking internal ampullae improves the support for the [O,E,H] clade for both the morphological and combined data sets.

Three characters support the [E,O] grouping (the presence of a pluteus larva, the internalization of the radial water vessel and nerve by epineural folds during development, and the circumoral musculature (interradial and perradial) associated with the development of a jaw apparatus), all of which represent acquisitions of morphologically complex characters. They have either evolved independently in echinoids and ophiuroids, or have been subsequently lost in holothurians. Parallel evolution seems most likely to explain the pluteus form adopted by larvae of echinoids and ophiuroids. However, holothurians do begin spicule formation in the larva, a character they share with ophiuroids and echinoids and which is a pre-adaptation to pluteal formation. Similarly, although holothurians do not develop an internal radial water vessel through the formation of epineural folds (Smiley, 1988), it is internal as it is in echinoids and ophiuroids. Furthermore, in holothurians although the radial water vessel is initiated internally, it still retains an epineural sinus, which is very strong evidence that it originally formed by over-roofing of epineural tissue. However, at least one group of echinoids (cidaroids) internalize their radial water vessel without the formation of epineural folds (Emlet, 1988), so this character probably arose by convergence also. Finally, although all holothurians lack a jaw apparatus probable stem-group holothurians (Ophiocistoida) do have a fully homologous structure, again suggesting that it is secondary loss in holothurians that is creating homoplasy. Two additional characters support the [E,H,O] clade (major nerve extending into tube-feet; paroral ciliary bands developed). A third character, loss of anterior adhesive disc, may also support this grouping although one group of asteroids lacks this character, suggesting it may be plesiomorphic for eleutherozoans.

There are four characters that support the [A,O] clade. Two of these are closely related (the loss of an anus and the saccate form of the gut), but the fact that some asteroids do have an anus yet retain a saccate gut shows that the two characters can be treated as independent. Asteroids and ophiuroids also share a similar adambulacral system of skeletal plates and have multiple gonads. This last character, however, is known to be homoplasious within echinoids since Palaeozoic forms had only a single gonopore opening in contrast to all post-Palaeozoic forms. Whether primitive asteroids and ophiuroids had single or multiple gonads is unknown.

In conclusion, there is no support from morphological data for grouping ophiuroids as sister group to asteroids + echinoids + holothurians, leaving just two topologies as serious contenders. Both have some characters in their support, but evidence exists that some of the characters common to just echinoids and ophiuroids have been secondarily lost in holothurians, tipping the balance in favour of an Ophiuroid-Echinoid-Holothurian clade (also with the highest bootstrap support, Appendix 2) rather than an Asteroid-Ophiuroid clade.

What additional evidence do we need to choose between these trees?

Jacobs *et al.* (1988) and Smith *et al.* (1993) considered the phylogenetic significance of mitochondrial gene order in the echinoderms. Gene rearrangement is much slower than nucleotide substitution (Brown, 1985) and may be more informative (Boore *et al.*, 1995). Results show that in their mitochondrial DNA (mtDNA), asteroids and ophiuroids share a multigene inversion of 4.6 kb that is absent in echinoids and holothurians, thus grouping asteroids with ophiuroids and echinoids with holothurians in an unrooted network. However, it is not clear yet which condition is derived since the mitochondrial gene order of crinoids remains undocumented. In the vertebrate mitochondrial genome the sequence shows a third pattern which is slightly closer to that seen in echinoids and holothurians suggesting the monophyly of asteroids + ophiuroids. However, mitochondrial gene order in crinoids remains essential to solve which pattern is plesiomorphic. If crinoids shared the same inversion with echinoids and holothurians there would be strong evidence that the inversion is derived and plesiomorphic in asteroids and ophiuroids (suggested by Smith *et al.*, 1993), thereby favouring (out(C((A,O)(E,H)))).

In the meantime we have reduced 105 possible rooted trees to two plausible, well supported phylogenetic scenarios. It now remains for us to employ these topologies as the basis for investigating further the evolution of this highly diverse, successful and ancient phylum.

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APPENDIX 1

Morphological character definitions used to reconstruct phylogenetic relationships between the echinoderm classes. See main text for further explanation.

The following characters have been drawn from diverse sources, but primarily from Smith (1984), Strathmann (1988), Smiley (1988), and Pearse & Pearse (1994). Where individual characters have been used in previous analyses, the source and character number in the earlier analysis is given in square brackets (AS = Smith (1984); SS = Smiley (1988); RS = Strathmann (1988); PP = Pearse & Pearse (1994). Since Pearse & Pearse (1994) did not list character states some doubt exists as to precisely how they defined and scored their characters.

(1) Larval character set

[Characters 1–6 are common to all echinoderms, and are drawn from Strathmann (1988)]

1. Circumoral band of simple cilia that separates the larval surface into upstream and downstream fields: no = 0; yes = 1.
2. Anus formed at site of blastopore, mouth forms at new site: no = 0; yes = 1.

3. Coeloms formed by outpocketing from invaginated archenteron: no = 0; yes = 1.
4. Early formation of a single hydropore between the coelom and dorsal surface: no = 0; yes = 1.
5. Approximately triangular adoral band of cilia: no = 0; yes = 1.
6. Recurved gut with ventral anus in larva: no = 0; yes = 1.
7. Larval metamorphosis involves a strongly asymmetric phase in which left-hand coeloms come to dominate in adult structure: no = 0; yes = 1 [AS2, PP5].
8. Formation of calcitic larval skeleton: no = 0; yes = 1 [RS1].

[Smiley (1988) rejected the homology between the small calcitic deposits found posteriorly in some holothurians and the calcitic skeleton developed in echinoids and ophiuroids. Holothurian calcite deposits are single in some taxa but paired in others where they closely resemble in shape and position the reduced ossicles seen for example in vitellaria ophiuroid larvae (e.g. Hendler 1982, Fig. 1B). They also appear at about the same time in development from ingressed mesenchyme. They therefore pass the tests of similarity of position and origin and can be treated as putative homologous structures, as Strathmann (1988) has done].

9. Skeletal rods supporting arms that carry loops of the ciliary band [AS30, RS3, SS14, PP40].

[Some paxillosid asteroid larvae develop arms that carry loops of the ciliary band that are as extensive as those of echinoids and ophiuroids, but which never have supporting calcite rods. Therefore the development of arms and the development of supporting larval spicules are independent traits, as treated by Smith (1984). However, the possession of long arms as a trait independent of skeletal arm rods is not included in this analysis because it would have to be scored as both states present in asteroids, since long arms are restricted to only two families of extant asteroids].

10. Enterocoelom develops as: a single lateral pouch = 0; as a pair of lateral pouches (dorsal and ventral) = 1; by median constriction of the archenteron = 2 [AS16, RS2, SS8,9].

[Smiley (1988) scored crinoids as having a single dorsal structure, as described in Hyman (1955), whereas Strathmann (1988) scored crinoids as unknown. The descriptions of crinoid development make it quite clear that the archenteron separates into an anterior and posterior section through the formation of a median constriction whereas in holothurians (e.g. Hyman 1955: 182; Smiley, 1986, fig. 16) the initial enterocoelomic cavity is a laterally displaced (= dorsal) pouch from the end of the archenteron. This difference has also been emphasised by Balser, Ruppert & Jaeckle (1993). Consequently, the development of the single enterocoel in crinoids is significantly different from that seen in holothurians, and the pattern in holothurians resembles that seen in echinoids, ophiuroids and asteroids except that just one rather than a pair of lateral pouches forms. Note that in some asteroids, e.g. *Asterina*, *Henricia*, *Solaster*, there is a median constriction separating the entire anterior half of the archenteron, the anterior part then sending back right and left extensions. The absence of a right anterior coelom is directly related to the presence or absence of paired enterocoelomic lobes and is not scored as separate character (contra Smiley, 1988)].

11. Initial partitioning of archenteron: divides into anterior and posterior cavities, the posterior becoming the left and right somatocoels, the anterior becoming the axohydrocoel. A secondary connection between somatocoel and axohydrocoel forms subsequently = 0; forms distal cavity which is a primordium for the paired somatocoels, hydrocoel and axocoel. The somatocoel eventually separates from the axohydrocoel = 1.

[Balser *et al.* (1993) stressed this as a fundamental difference in the development of crinoids. They also demonstrated that holothurians have an axocoel, and that a stone canal, madreporite and rudimentary ampulla (axocoelic derivative) all form in holothurians, contrary to Smiley (1988)].

12. Paroral ciliary bands: absent = 0; present = 1 [RS4].

[Strathmann (1988) states that the distribution of this character amongst echinoderms is poorly known].

13. Doliolarian stage: unknown in any member = 0; present in at least some taxa = 1 [RS6].

[As discussed by Strathmann (1988) this character may be directly linked to character 14, and thus depend upon whether, during metamorphosis to adult, the larval mouth is retained or not].

14. Formation of adult mouth: larval mouth continues as adult structure = 0; adult rudiment forms on lower left side of larva and larval mouth is lost = 1 [AS 31, RS7, SS12].

[Smith (1984) gave an incorrect scoring for this character which was corrected by Strathmann (1988)].

15. Anterior adhesive pit or disc for attachment: no = 0; yes = 1 [AS18, RS5, SS13, PP32].

[This character has been scored either as present or absent in holothurians. Furthermore, only some asteroids have an anterior adhesive disc; it being wanting in all paxillosids. Smith (1984) and Smiley (1988) scored holothurians as having a larval attachment structure because holothurian larvae attach to the substratum by means of their primary podia (= buccal tube-feet). Strathmann, however, scored for the presence of an adhesive pit/disc in larvae, a structure which is absent in holothurians. Since asteroids have both an adhesive disc and larval primary podia, it seems best to score for these two features separately (characters 15 and 16). Hence crinoids have a pre-oral adhesive disc, asteroids have a pre-oral adhesive disc and primary podia that carry extensions of the axohydrocoel, and holothurians have just primary podia with extensions of the axohydrocoel].

16. Larval tube-feet developed as axohydrocoel extensions independent of radial water vessels and used for attachment: no = 0; yes = 1 [AS18, RS13].

[As Smiley (1988) argued, all asteroids except paxillosids possess three brachiolar arms that form as extensions from the axohydrocoel that are used for attachment. These must be treated as putative homologues of the holothurian buccal tube-feet because of their position, structure and function. However, unlike the buccal tube-feet of holothurians, they do not arise directly from the hydrocoel ring, thus their homology can be questioned].

17. Primary lobes from the hydrocoel ring: form tip of definitive radial water vessels = 0; form primary buccal podia = 1 [SS7].

[In holothurians the primary podia (buccal tentacles) arise as five buds from the circum-oral ring vessel prior to the development of radial water vessels. Ophiuroids also have their buccal tube-feet arising directly from the circum-oral ring, but they arise by lateral budding from the radial water vessels and do not represent independent branches of the circum-oral ring].

18. Torsion in development: absent = 0; present = 1.

[Torsion in development is seen in ophiuroids where the viscera migrate through 90° from the larval axis, and in echinoids and asteroids, where the rudiment forms at 90° from the larval axis. In crinoids there is a rotation of 90° of the internal organs from ventral to posterior. Only holothurians show little evidence of torsion (Smiley, 1988), although they, like crinoids, undergo a late stage rotation of the vestibule through 90° (Hyman, 1955:185) and also show an initial, short-lived, shift of the larval mouth away from the anterior-posterior axis. The axis of larval symmetry is thus retained in the transformation from larva to adult in holothurians, whereas this is not the case in echinoids, ophiuroids and asteroids. This character cannot be scored for crinoids because of their direct development].

19. Epineural folds form to enclose developing radial water vessel: no = 0; yes = 1.

[As demonstrated by Smiley (1986) the internal radial water vessel in holothurians forms in a different way to that of most echinoids and ophiuroids. Whereas the latter have external radial water vessels that become enclosed by the formation of epineural folds, the radial vessel in holothurians is initiated internally. This character therefore refers only to the way in which the radial water vessel is enclosed and not to the presence of an internalized water vascular system. Note, however, that in cidaroid echinoids epineural folds do not form and the radial water vessel is initiated internally, as in holothurians (Emlet, 1988)].

20. Larval hydropore: retained into adult = 0; does not persist through metamorphism = 1 [SS23].

[According to Balser *et al.* (1993) the undivided axohydrocoel and its union to the exterior are retained through metamorphism in crinoids, asteroids, ophiuroids and echinoids. Only in holothurians is the original external connection lost in at least the majority of taxa. (According to Hyman (1955), some species may retain an external hydropore connection as adults)].

21. Chambered organ formed as extension of right somatocoel: no = 0; yes = 1.

Larval character set:

Outgroup	?????	?????	?????	?????	?
Crinoids	??11?	?1??2	0???1	00?00	1
Asteroids	11111	11001	10011	10100	0
Holothurians	11111	11100	11100	11001	0
Ophiuroids	11111	11111	11100	00110	0
Echinoids	11111	11111	11010	00110	0

(2) *Adult character set*

22. Gill slits: absent = 0; present = 1 [PP1].

[Jefferies (1988) has argued that echinoderms differ from other deuterostomes in having lost their gill slits. Carpoids which are used as outgroup have a series of body openings that are best interpreted as gill slit openings].

23. High magnesium calcite endoskeleton with stereom microstructure: absent = 0; present = 1 [AS1, SS1, PP2].

[Carpoids have a skeletal microstructure identical to that of all radiate echinoderms].

24. Pentaradial symmetry to adult body plan: no = 0; yes = 1 [AS3, SS3, PP4].

[All extant echinoderms have a pentaradiate body plan that develops through an asymmetrical metamorphosis from a bilateral larva. Carpoids, however, lack any trace of pentaradial symmetry].

25. Water vascular system derived from axohydrocoel and giving rise to tube-feet: no = 0; yes = 1 [SS2, PP6].

[Solutes have a single ambulacrum developed as an arm with a hydropore at its base. It presumably carried a tubular extension of the water vascular system with tube-feet. This is equivalent to Smiley's character 2, which refers to ambulacra rather than the water vascular system specifically].

26. Attachment: stemmed for at least part of their post-larval development = 0; free-living after settlement = 1 [AS 7].

[Crinoids are either stemmed or pass through a pentacrinid stage where they are attached by a columnar stem and are thus clearly secondarily unattached; all other echinoderm classes develop as free-living juveniles. Solutes are attached by their stem as juveniles (Ubaghs & Robison, 1985; Daley, 1996)].

27. Ambulacral growth: radial creating stellate body form; oral and aboral surfaces equally developed = 0; ambulacral growth meridional, creating globular body form; aboral surface greatly reduced compared with oral surface = 1 [AS 12, 23, SS15, PP23].

[As argued in Smith, 1984, the genital and periproctal systems of echinoids is homologous to the entire aboral surface in asteroids, ophiuroids and crinoids; the reduction in aboral surface is intimately tied in with the development of a meridional growth pattern and the resulting globular rather than stellate body plan. Consequently the two characters are not independent and are here treated as a single character. Solutes have no radial symmetry but their single ambulacrum grows radially away from the centre like the ambulacra of crinoids].

28. Aboral (entoneural) nerve network: dominant = 0; subsidiary to ectoneural and hyponeural nerve nets = 1 [AS 13, PP7].

[The main nerve system in crinoids is the entoneural system. In all other groups the ectoneural and hyponeural systems dominate. Hyman (1955) accepted the suggestion that in asteroids the marginal nerve chord was a probable homologue of the entoneural system. However, Cobb (1987) states that at least part of this system is ectoneural, and Chia & Koss (1994) state that the part in the coelomic epithelium is part of the hyponeural system. It would appear therefore that asteroids lack an entoneural network. In the other groups the entoneural system is either wanting altogether (holothurians) or is restricted to a small ring around the periproct innervating the gonads (echinoids and ophiuroids)].

29. Ectoneural and hyponeural networks: separate, discrete systems = 0; combined into radial nerve with upper and lower sections separated by a thin membrane = 1.

[In echinoids the radial nerve is made solely from the ectoneural system; the hyponeural system being restricted to the lantern (Cobb, 1987)].

30. Ambulacral skeleton forming an integral part of the thecal wall = 0; ambulacral skeleton extending as free-standing structures = 1.

[Solutes have a single arm extending free of the theca, similar to the arms of crinoids. In all other extant echinoderms the ambulacra are part of the body wall and do not extend as free appendages].

31. Adult orientation: with mouth facing away from the substratum = 0; with mouth facing the substratum = 1.

[Holothurians have adopted both modes of life, with suspension filter-feeders living with their mouth facing away from the substratum, and deposit feeders with their mouth downwards. They are therefore scored as showing both conditions].

32. Plate addition terminal = 0; subterminal = 1. [AS8, SS16].

[During growth, ambulacral plates are either added terminally at the tip of the arm, as in crinoids, or the tip of the ambulacrum is permanently associated with a terminal plate (ocular plate) and new ambulacral plates are added behind this. Because the skeleton of holothurians is so reduced it is impossible to say which pattern holothurians follow].

33. Radial water vessel and nerve: basically external to mesoderm and skeleton; lacking epineural sinus = 0; basically internal, beneath skeletal elements; overlain by epineural sinus = 1. [AS 19, 21, SS11, PP27].

[Smiley (1988) argued that, although the radial water vessel of holothurians was internal, it developed in a different way to that of echinoids and ophiuroids. Specifically he argued that there was no epineural fold developed and that the outer sinus formed by cavitation *in situ*. Even if he is correct, the presence of an external cavity and the deep seated position of the radial water vessel and nerve are putative evidence for treating the holothurian system as subdermal rather than superficial. Furthermore, in at least some echinoids the radial water vessel develops internally without epineural folds (Emlet, 1988). The distinction in the developmental pathways between holothurian and echinoid/ophiuroid internalized water vascular systems has been recognized in the larval character set (character 18)].

34. Tiedeman's bodies: absent = 0; present = 1. [AS 42, SS17, PP39].
[Bachmann & Goldschmidt (1980) showed that the structures termed spongy bodies in echinoids by Hyman (1955) were Tiedeman's pouches. According to Hyman (1955) there may be possible homologous tubular structures in gorgonocephalid ophiuroids. However, because of the uncertainty, ophiuroids are scored as '?'; Pearse & Pearse (1994) scored echinoids as '?' for this character].
35. Polian vesicles: absent = 0; present = 1.
[Polian vesicles are restricted to holothurians, ophiuroids and asteroids. Polian vesicles reported in echinoids by some authors are now believed to be homologues of Tiedeman's bodies (Bachmann & Goldschmidt 1980)].
36. Ambulacra: composed of a biserial series of plates = 0; composed of a uniserial series of plates = 1.
[Although ophiuroids have a uniserial series of vertebral ossicles forming their ambulacra these are derived ontogenetically from fusion of opposite plates in a biseries—early Palaeozoic ophiuroids all had biserial ambulacra. By contrast the crinoid ambulacra appear to have been uniserial since the origin of the group. The ambulacra in solutes is composed of a biserial series of plates].
37. Haemal system: arranged as diffuse series of lacunae without radial canals = 0; arranged as discrete peripharangeal ring which sends out radial canals located between the radial nerve and hyponeural sinus = 1 [PP11].
38. Haemal system: with simple strands connected to gut = 0; with major dorsal and ventral sinuses developed along digestive system and connected by a complex network of fine vessels = 1 [AS34, PP26].
39. Gonads: single = 0; multiple = 1 [AS20, PP34].
[Early Palaeozoic echinoids have a single gonopore associated with the madreporite and thus presumably lacked multiple gonads. The same situation is seen today in extant holothurians. However, all extant echinoids have multiple gonopores. The condition in Palaeozoic asteroids and ophiuroids is unknown; none show clear evidence of gonopores and may therefore have had gonads associated with each arm in the same way that is seen in modern asteroids and the ophiuroid *Ophiocanops*. The solutes have a single gonopore opening associated with the hydropore and presumably therefore had a single internal gonad].
40. Genital rachis: originates from distal end of axial gland = 0; originates from the middle of the axial gland = 1.
[Erber (1983) noted this difference in his comparative analysis of axial organs of echinoderms].
41. Outer genital coelom surrounds the gonads: no = 0; yes = 1 [SS22].
[This character may not be independent of character 60. Like asteroids and ophiuroids the crinoids have a double-walled genital strand structure which includes an inner connective tissue space (genital lacuna) derived from the haemal system, lodged within a genital coelom (Heinzeller & Welsch, 1994: 25)].
42. Calcified madreporitic body forms around hydropore opening: no = 0; yes = 1 [AS5].
[This is well developed in early Palaeozoic ophiuroids although less so in modern ophiurine ophiuroids].
43. Stone canal calcified: no = 0; yes = 1.
[The internal structures of solutes and other carpoids are well known yet there has never been any report of their having a calcified tubular structure. By contrast, the calcified stone canals of fossil asteroids and ophiuroids are well documented. Holothurian and echinoid stone canals have dense spiculation. In crinoids the stone canals are thin tubes that have no skeletal support].
44. Hydropore opening: external in adults = 0; internalized and opening into body coelom = 1.
[The fact that solutes have an external opening interpreted as a hydropore and associated with a gonopore opening is strong evidence for the external hydropore opening being plesiomorphic. Furthermore, the sinus in which the stone canals of crinoids opens is almost certainly homologous to the ampulla in other echinoderm classes, thus the difference is solely in relative development of the sinus. Although most holothurians have internalized madreporites, there are some groups in which the madreporite remains external and open (Erber, 1983). The arrangement in holothurians is thus fundamentally similar to that in echinoids and asteroids and distinct from that of crinoids].
45. Perianal coelom: undifferentiated = 0; differentiated from the main body cavity = 1.
[Smiley (1988) combined the presence of a perianal and a peripharangeal coelom into one character. However, these two body coeloms are unrelated and are best treated as independent acquisitions. Both Smith (1984) and Pearse & Pearse (1994) scored them separately].
46. Peripharangeal coelom: undifferentiated = 0; differentiated from the main body cavity = 1.
[The peripharyngeal coelom surrounds pharynx enclosing the lantern in echinoids and immediately

adoral to the calcareous ring in holothurians. The coelom in echinoids is not partitioned, but in holothurians is crossed by horizontal septa, separating a peripharyngeal coelom from a peribuccal coelom. In ophiuroids a body coelom termed the peristomeal cavity occurs surrounding the pharynx (esophagus) immediately overlying the jaw apparatus (Jangoux & Lawrence 1982). This is partitioned by a horizontal septum. Because of its position, it can be treated as a putative homology of the coelom in holothurians and echinoids. No such coelom occurs in asteroids or crinoids].

47. Moveable articulated spines in dermis: no = 0; yes = 1.

[The reduction of holothurian skeleton to microscopic elements makes this character difficult to score. The plate and anchor elements of some groups are not strictly articulated nor under muscular control. The wheel-shaped ossicles of dendrochirotes are remarkably similar to the initial stages in spine formation seen in echinoids. However, larger plated holothurians such as psolids totally lack spines and no fossil holothurian has been reported with spines].

48. Tube-feet with calcified disc: no = 0; yes = 1 [AS25, PP19].

49. Tube-feet: forming as direct outpouch from the wide radial water vessel without valves = 0; forming from lateral side-branches of cylindrical radial water vessel with one-way valve = 1.

50. Tube-foot associated with internal ampulla: no = 0; yes = 1.

51. Circumoral ring water canal and nerve: adoral to first ambulacral ossicles and unassociated with skeletal elements = 0; intimately associated with first ambulacral ossicles or their derivatives = 1.

[In crinoids the circumoral water and haemal rings surround the oesophagus and lie adoral to the ambulacral skeleton. In asteroids and ophiuroids the nerve and water rings rest on the inner surface of the first ambulacral plates (mouth angle plates) forming distinct notches. In echinoids the rings are closely associated with the hemipyramids, which are homologues to the first ambulacrals (Smith, 1984). In holothurians the water and nerve rings run around the calcareous ring, which probably represents a highly derived ambulacral skeleton that has become internalized].

52. Internalized skeletal structure surrounding the oesophagus: no = 0; yes = 1.

[The homology between the echinoid lantern and the holothurian calcareous ring can certainly be questioned, since they are very different in appearance and construction. However, both lie internally, in intimate association with the circumoral water vessel and nerve; both are enclosed within the peripharangeal coelom. They are treated as possible homologies].

53. Anus: not present in adults = 0; present in adults = 1.

[Although most asteroids have an anus, certain paxillosids do not. Whether the absence of an anus is a late specialization, as argued by Blake (1988), or primitive, as argued by Lafay *et al.* (1995), is debatable. No definitive anal cone is known in any Palaeozoic asteroid, but then the presence of an anus in modern asteroids is extremely difficult to tell from aboral plating alone].

54. Anus: on same face as peristome = 0; on opposite pole to peristome = 1.

[In those asteroids with an anus it is positioned on the opposite face to the peristome, as is the case with echinoids and holothurians. In solutes the periproctal cone is at the opposite end of the theca to the base of the arm and presumed peristome position].

55. Saccate gut = 0; looped cylindrical gut = 1.

56. Microtubule cell in tube-feet: absent = 0; present = 1.

[First described by Holland (1970) and suggested as a phylogenetic character by McKenzie (1994:77).

57. Cuticular structure: microvillae-like structures arise from the lamellae that adorn the apical surfaces of support cells, penetrating the surface coats but covered by the glycocalyx = 0; layer beneath the glycocalyx covered with membrane-bound blebs of cytoplasm, that are derived from pinched-off tips of microvillae = 1.

[Autapomorphy of ophiuroids suggested by McKenzie (1988, 1994)].

58. Secretory cells in tube-feet: goblet cells only = 0; goblet and apical tuft cells = 1 [see McKenzie, 1988].

59. Sperm morphology in forms with external fertilization: spherical head with an invaginated acrosomal vesicle = 0; elongate head with an apically protruding acrosome = 1.

[Character from Afzelius 1977, Franzén, in Giese, Pearse & Pearse (1987:36). Forms with internal fertilization typically have highly atypical sperm and are ignored here].

60. Axial gland: within left axial sinus = 0; abuting the left axial sinus but not enclosed = 1.

[This character is probably not independent of character 41. Holland (1970) and Balser & Ruppert (1993) have shown that the crinoid axial organ is encased in an axocoelomic space, as in asteroids and ophiuroids. No such sinus is recorded from echinoids or holothurians. In echinoids the axial gland forms a major part of the boundary wall of the left axial sinus, but is not surrounded by it (Erber, 1983) and the same is true of holothurians. In ophiuroids, the axial gland abuts both the stone canal and the left axial coelom, but is not enclosed].

61. Axial gland: closely associated with stone canal (stone canal an integral part of the axial sinus wall) = 1; stone canal separated from axial sinus and axial gland = 0.

[Erber (1983) discusses the comparative structural relationships of the axial organ and stone canal in the different classes].

62. Axial structure with well-developed gland-like tubes (axial gland): present = 1; absent = 0.

[Heinzeller & Welsch (1994) recommend treating the tubular crinoidal 'axial gland' as a distinct organ, since the axial gland in other echinoderms may be homologus to Reichensperger's organ].

63. Right axial sinus: absent = 0; restricted to distal end of axial complex forming dorsal sac = 1; extending along most of the length of the axial complex = 2.

[In ophiuroids the right axial sinus is somewhat larger than in asteroids and echinoids, extending further down the axial complex and eventually enveloping the axial complex (Ferguson, 1995)].

64. Muscle-tendon attachment: attachment direct to calcite trabeculae = 0; attachment via tendons = 1 [PP10].

65. Tendons: composed of unstriated microfibrils = 0; composed of composite tendons with unstriated and striated fibrils = 1.

[For both 64 and 65, details are taken from Stauber & Märkel (1988)].

66. Adambulacral ossicles differentiated: no = 0; yes = 1 [PP13].

[The adambulacrals of asteroids and the lateral arm plates of ophiuroids are undoubtedly homologous plate systems which have no obviously expressed homologue in other groups].

67. Scleroblasts: single and do not form a syncytium = 0; forming a syncytium in which calcification occurs = 1.

[According to Heinzeller & Welsch (1994:54) crinoid scleroblasts remain single unlike those in other classes].

68. Discrete, well-defined nerve canal in tube-feet: no = 0; yes = 1 [PP18]

[Asteroid and crinoid tube-feet lack a distinct longitudinal nerve. Pearse & Pearse (1994) scored ophiuroids as lacking a distinct longitudinal podial nerve, though Hyman (1955) and and Byrne (1994) both report that one is typically present].

69. Batyl alcohol: no = 0; yes = 1; [SS30].

70. Gonads opening on oral surface: yes = 0; no = 1.

[Only *Ophiocanops*, amongst living ophiuroids, has gonads along the dorsal part of its arms opening adorally. Although possibly primitive, ophiuroids have been scored as having oral openings to the gonads].

71. Ambulacral plates surrounding mouth: more or less rigidly fixed = 0; specialized into a jaw apparatus with strongly developed circumoral ring of muscles = 1.

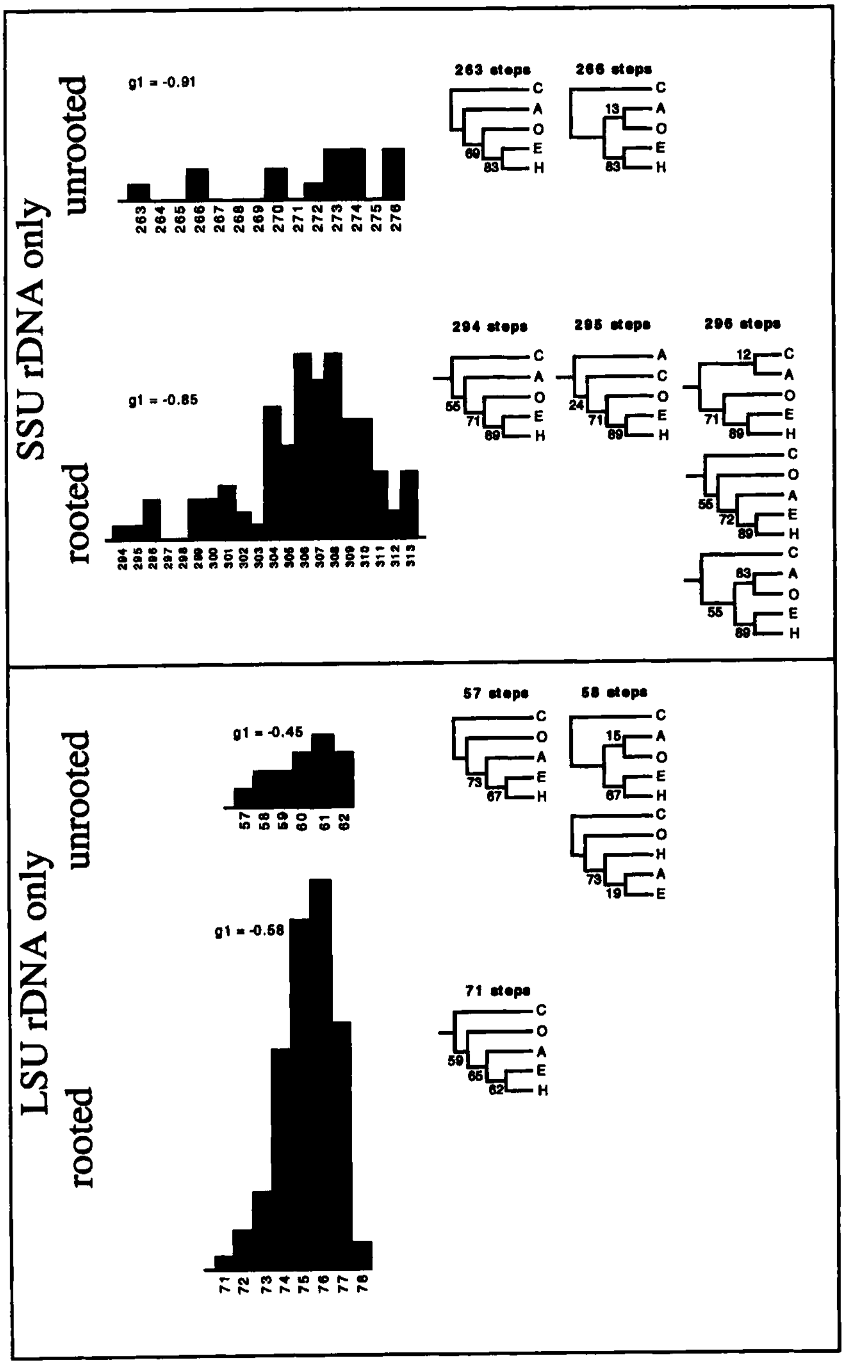
[The homology of the echinoid hemipyramids with the adoral ambulacral ossicles of ophiuroids and asteroids seems clear from embryological studies of Devanesen (1922). In asteroids the proximal ambulacral ossicles are united into a fairly inflexible peristomial ring, in ophiuroids the proximal ossicles are much more flexible, with a well-developed circumoral musculature. In echinoids the hemipyramids have well-developed circumoral musculature and are highly derived in comparison to the structure seen in ophiuroids. Finally, in holothurians the circumoesophageal ring may be homologous, in which case it is an inflexible ring, like that of asteroids. However, the structure is so derived that its homology is still open to question].

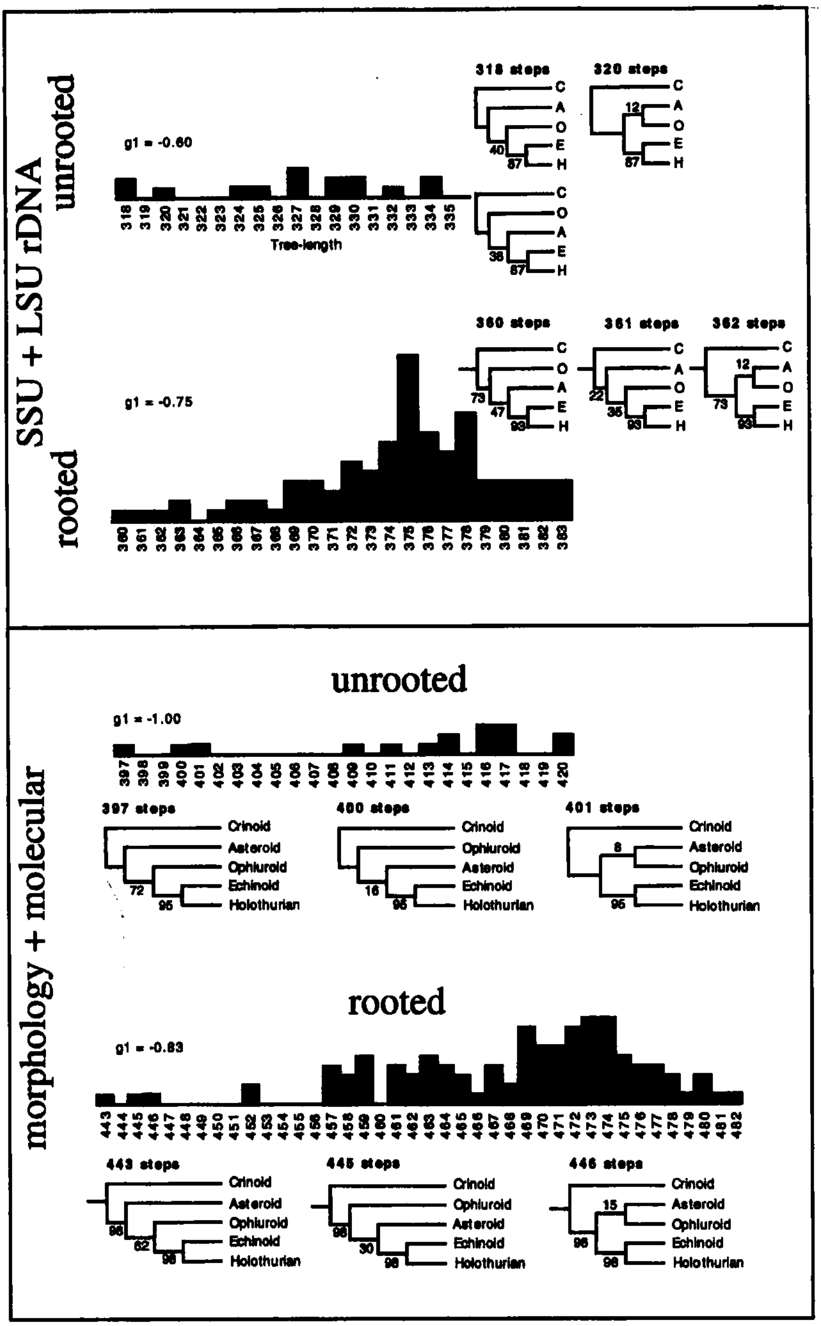
Adult character set:

Outgroup	11010	0??10	0???0	??0??	?00??	0???0	011??	?????	????0	????0
Crinoids	01110	00010	00001	00101	00100	00000	01011	00000	100?0	00110
Asteroids	01111	01101	10110	10111	11000	10111	0?100	00001	01101	10100
Holothurians	01111	1110?	?1010	11010	11011	01111	11110	01010	00??0	11110
Ophiuroids	01111	01101	11?10	10111	11001	10101	00?00	10001	02101	11011
Echinoids	01111	11001	11100	11010	11011	11111	11110	01111	01110	11001

APPENDIX 2

Results of exhaustive searches on consensus sequence data treated individually, as total molecular and combined with morphology (total evidence), unrooted and rooted against outgroup. Indications of skewness are given by g_1-values; percentage bootstrap support is given for each node on the most and near-most parsimonious solutions ($n = 1000$). SSU = small subunit (18S-like) and LSU = large subunit (28S-like) rDNA.




SSU + LSU rDNA
rooted
unrooted
g1 = -0.60
Tree-length
318 steps
320 steps
g1 = -0.75
360 steps
361 steps
362 steps
C
A
O
E
H
morphology + molecular
unrooted
g1 = -1.00
397 steps
400 steps
401 steps
rooted
g1 = -0.83
443 steps
445 steps
446 steps
Crinoid
Asteroid
Ophiuroid
Echinoid
Holothurian