

# Phylogenetic relationships of extant echinoderm classes<sup>1</sup>

Daniel Janies

**Abstract:** A well-supported phylogeny of echinoderm classes has eluded morphological, embryological, molecular, and combined analyses. From this body of work it is apparent that (i) echinoids (sea urchins) and holothuroids (sea cucumbers) are related, and (ii) crinoids (sea lilies) are the sister taxon to extant eleutherozoan classes (asteroids, ophiuroids, echinoids, and holothuroids). However, the relationships of asteroids and ophiuroids to other echinoderm classes have been difficult to recover. To address relationships between the asteroids and ophiuroids and other echinoderms, I have sequenced additional nuclear loci and taxa and used novel computational approaches for co-optimizing morphological with molecular evidence at the level of sequence alignment. Support for the monophyly of each class is strong. Support for a monophyletic Asterozoa + *Xyloplax* is as strong as for Asterozoa. Support for Asterozoa (Asterozoa + Ophiurozoa) is apparent, albeit not as strong as for other clades (e.g., Echinozoa, Eleutherozoa, and Echinodermata). I also present detailed sensitivity analyses to provide (i) a test of the monophyly of groups under a variety of evolutionary models and (ii) a statement of the evidential value of various character systems.

**Résumé :** Les analyses morphologiques, embryologiques, moléculaires et combinée n'ont pas encore permis d'établir une phylogénie solide des classes d'échinodermes. D'après le bilan de ces travaux, il est évident que (i) les échinoides (oursins de mer) et les holothuroïdes (concombres de mer) sont des groupes apparentés et (ii) que les crinoïdes (lys de mer) forment le taxon soeur des classes d'éleuthérozoaires actuels (astéroïdes, ophiuroïdes, échinoides et holothuroïdes). Cependant, la relation entre les astéroïdes et les ophiuroïdes et les autres classes d'échinodermes est difficile à établir. Pour examiner cette relation, j'ai procédé au séquençage de nouveaux locus nucléaires et examiné d'autres taxons et j'ai utilisé de nouvelles approches informatiques pour cooptimiser les données morphologiques et moléculaires à l'alignement des séquences. Tous les indices appuient l'hypothèse du monophylétisme de chacune des classes. Le monophylétisme des Asterozoa + *Xyloplax* est aussi corroboré que celui des Asterozoa. L'appuie en faveur des Asterozoa (Asterozoa + Ophiurozoa) est également corroboré, mais pas aussi fortement que pour les autres clades (p. ex., Echinozoa, Eleutherozoa et Echinodermata). Je présente également ici des analyses détaillées de sensibilité : (i) un test du monophylétisme des groupes à la lumière de plusieurs modèles évolutifs et (ii) un jugement sur la valeur indicatrice de différents systèmes de caractères.

[Traduit par la Rédaction]

## Introduction

Echinoderms are unique among metazoans because of their pentaradial body plan. Fundamental to this design is the water-vascular system, a circular coelomic tube surrounding the mouth with five radial canals that bear rows of lateral canals and tube feet. Extant echinoderm classes are distinctive: it is not difficult to recognize a starfish and its similarities to and differences from a sea urchin. However, in the mid-1980s, two species of *Xyloplax*, a small disk-shaped echinoderm, were discovered on sunken wood. *Xyloplax medusiformis* occurs in the South Pacific Ocean and Tasman

Sea (1057–1208 m depth) off the North Island and South Island of New Zealand (Baker et al. 1986). *Xyloplax turnerae* occurs in the Tongue of the Ocean (2066 m depth) off Andros Island, Bahamas (Rowe et al. 1988). The water-vascular system of *Xyloplax* is circular rather than pentaradial as is characteristic of echinoderms. Because of this unusual body organization, a new class of the phylum Echinodermata, Concentricyclozoa, was erected. Smith (1984, 1988) argued that echinoderms have been classified in an arbitrary manner and criticized the practice of assigning class status to enigmatic fossils and newly discovered species on the basis of “distinctiveness” rather than character evidence. Even when character evidence has been employed, classifications or phylogenies are sometimes built on the basis of a single character system (e.g., embryology, Macbride 1896; Smiley 1988; skeletal characters, Fell 1963; DNA sequence from one gene and only one species of each class, Wada and Satoh 1994).

Combined-analysis approaches to echinoderm phylogeny can overcome this sort of subjectivity in classification by (i) explicitly defining clades supported by synapomorphies and (ii) using all data at the disposal of the investigator (e.g., molecular and morphological characters). In the first com-

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<sup>1</sup>This review is one of a series dealing with aspects of the biology of the phylum Echinodermata. This series is one of several virtual symposia on the biology of neglected groups that will be published in the Journal from time to time.

bined molecular and morphological phylogeny of echinoderm classes, Littlewood et al. (1997) made great strides in increasing taxonomic sampling among all taxa plus reviewing and coding many morphological characters. However, they undersampled the taxonomic diversity within classes of echinoderms.

In the study presented in this paper, I increased taxonomic sampling of asteroids, ophiuroids, and crinoids for 18S and 28S ribosomal DNA (rDNA). The taxonomic diversity of holothuroids remains poorly sampled; however, like echinoids, their monophyly and position in the crown group of Eleutherozoa remain stable under a variety of analytical conditions. Furthermore, I used a combination of direct-optimization and sensitivity-analysis techniques in order to avoid biases in phylogenetic results due to alignment ambiguity and choice of a single evolutionary model.

## Methods

### Molecular and taxonomic sampling

Forty-four partial and complete 18S sequences and 30 partial 28S sequences were analyzed. Species were chosen from sequence data bases or sequenced to represent almost all echinoderm orders. Hemichordates are represented by three full 18S rDNA sequences from Enteropneusta and one partial 18S rDNA sequence for the Pterobranchia, *Rhabdopleura*. Taxonomic coverage includes 10 of 12 orders of the class Echinozoa, 2 of 2 orders or 7 of 17 families of the class Ophiurozoa, 6 of 7 orders of the class Asterozoa, and 2 of 4 orders of the class Crinozoa (see Table 1 for species and accession numbers). Tissues were obtained from a variety of museum and field sources and preserved in ethanol, frozen, or in the case of *Xyloplax*, aldehyde-fixed.

DNA was amplified with the polymerase chain reaction (PCR) and sequenced on an ABI 373 automated DNA sequencer using the methods described in manufacturer's protocols (Perkin-Elmer/Applied Biosystems Prism kit). After sequences were obtained from *X. turnerae*, DNA was extracted and amplified with the same protocols and 18S and (or) 28S rDNA were (was) sequenced for the following species: *Amphipholis squamata*, *Gorgonocephalus eucnemis*, *Cucumaria pseudocurata*, *Brisingaster robillardii*, *Asterias forbesi*, *Pteraster obscurus*, *Pseudarchaster parelli*, *Rathbunaster californicus*, *Dermasterias imbricata*, *Echinaster sepositus*, *Solaster dawsonii*, *Astropecten articulatus*, *Asterina gibbosa*, *Luidia foliolata*, *Heliaster helianthoides*, *Dorometra aegyptica*, *Capillaster multiradiatus*, and *Antedon mediterranea*. An unpublished 18S rDNA *Glossobalanus minutus* sequence was provided by Gonzalo Giribet (Harvard University).

### Morphological and other character data

Morphological and nonsequence characters, such as gene order, relevant to relationships among extant echinoderm classes and the relationships of *Xyloplax* were reviewed. This analysis draws characters from the following: Fell (1941, 1963); Olsen (1942); Dawydoff (1948); Choe (1963); Patent (1970, 1976); Hendler (1982); Blake (1987, 1998); Healy et al. (1988); Lester (1988); Rowe et al. (1988, 1994); Smiley (1988); Strathmann (1988); Pearce and Pearce (1994); Emler (1995); Neilsen (1995); Peterson (1995); Lacalli (1996); Littlewood et al. (1997); David and Mooi (1998); Mooi et al. (1998); Scouras and Smith (2001); Smiley et al. (1991) (Table 2 is the character matrix; character descriptions are in Table 3). One significant difference between the dataset herein and others is that, where applicable, each character was recoded or originally coded for each terminal taxon represented by DNA sequences.

A total of 62 characters were coded (Table 2). Eight characters are multistate, 54 are binary, and all are unordered. All characters are documented with respect to source and justification for coding in Table 3.

## Analyses

### Static homology

Sequences of 18S and 28S rDNA were aligned using CLUSTAL X (Higgins and Sharp 1988) under different conditions and then primer regions were removed from each alignment before tree search. Analyses of static alignments were performed in PAUP\*4.0 (Swofford 1999). These searches were for comparison with the dynamic homology searches described in the next section. Two analyses under different parameter sets were conducted. (1) CLUSTAL X was used to perform multiple alignment with default settings (i.e., gap opening cost = 15, gap extension cost = 6.66, transitions weighted 0.5 transversions). This alignment was subjected to heuristic tree searches under the parsimony criterion with PAUP\*4.0 with all characters equally weighted (gap, transversions, transitions, and all character data cost = 1, gaps treated as "fifth base"). (2) CLUSTAL X was used with gap opening cost = 2, gap extension cost = 2, and transitions weighted 0.5 transversions. PAUP\*4.0 searches were conducted similarly to the above search except that a step matrix was used to incorporate biases between gaps, transversions, and transitions in the tree search. Character data were equally weighted at 1. All searches included 10 random addition replicates and tree bisection-reconnection (TBR) branch swapping.

### Dynamic homology

The CLUSTAL X multiple alignment obtained using default settings was imported into Genetic Data Environment software (Smith 1994) and prepared for direct optimization in POY software as follows. The multiple alignment was separated along columns and cut into several regions flanked by the primer sequences and gaps were removed. Eleven regions were created for the 18S rDNA data and 5 for the 28S rDNA data. Dividing the sequence into several regions abates the severe memory and computational demands of direct optimization of large numbers of long sequences. Furthermore, there are strong biological bases for delimiting regions of DNA flanked by primers as multibase characters for POY. First, this practice reflects the fact that the primary homology observations on DNA in the taxa under study are made in the laboratory at the time of PCR amplification. Second, the use of regions of DNA flanked by primers as characters is similar to the use of 3D structure prediction to delimit comparable genetic regions of RNA molecules for alignment preceding tree search.

Direct-optimization analysis was done with POY software (Gladstein and Wheeler 2000) in parallel on a cluster of 23 UNIX workstations of heterogeneous architectures integrated into a parallel virtual machine (PVM) (Geist et al. 1993). Direct optimization is a novel method of comparing putatively homologous sequence residues during cladogram diagnosis, thus obviating multiple alignment (Wheeler 1996). Alignment algorithms create correspondences between sequence strings of various lengths by inserting gaps. In multiple alignment the relative costs of insertion-deletion and substitution events determine the number and position of gap characters inserted in sequences. Direct optimization works by creating parsimonious hypothetical ancestral sequences at internal cladogram nodes. The key difference between direct optimization and multiple alignment is that evolutionary differences in sequence length are accommodated not by the use of gap characters but by allowing insertion-deletion events between ancestral and descendent sequences. Evolutionary base substitution and insertion-deletion events between ancestor and descendent sequences are

**Table 1.** Species and gene regions analyzed.

| Class         | Higher taxon  | Family   | Species  | Accession No.                           |                    |                    |
|---------------|---|--|--|---|--------------------|--------------------|
|               |   |  |  | 18S rDNA                                | 28S rDNA<br>5' end | 28S rDNA<br>3' end |
| Pterobranchia | Order Rhabdopleurida  | 3 genera (Hyman 1955):<br><i>Cephalodiscus</i><br><i>Rhabdopleura</i><br><i>Atubaria</i> | <i>Rhabdopleura normani</i>  | U15664                                  |                    |                    |
| Enteropneusta |   | 2 of 3 families<br>(Hyman 1955):<br>Ptychoderidae<br>Ptychoderidae<br>Harrimaniidae      | <i>Balanoglossus carnosus</i><br><i>Glossobalanus minutus</i><br><i>Saccoglossus kowalevskii</i> | D14359<br>Courtesy G. Giribet<br>L28054 |                    |                    |
| Crinoidea     | 2 of 4 orders (Simms 1988):   | 3 of 25 families<br>(Mah and Mooi 1997):   |  |   |                    |                    |
|               | Comatulida  | Antedonidae  | <i>Dorometra aegyptica</i>   | AF088803                                |                    | AF088840           |
|               | Comatulida  | Comasteridae   | <i>Capillaster multiradiatus</i>   | AH008328                                |                    | AF088842           |
|               | Isocrinida  | Isocrinidae  | <i>Endoxocrinus parrae</i>   | Z80951                                  |                    |                    |
|               | Comatulida  | Antedonidae  | <i>Antedon serrata</i>   | D14357                                  |                    |                    |
|               | Comatulida  | Antedonidae  | <i>Antedon bifida</i>  |   | AJ225818           |                    |
|               | Comatulida  | Antedonidae  | <i>Antedon mediterranea</i>  |   |                    | AF088832           |
| Holothuroidea | 3 of 6 orders (Pawson and Fell 1965):   | 4 of 25 families<br>(Pawson 1982):   |  |   |                    |                    |
|               | Elasipodida   | Psychropotidae   | <i>Psychropotes longicauda</i>   | Z80956                                  | Z80946             |                    |
|               | Dendrochirotida   | Cucumariidae   | <i>Cucumaria sykion</i>  | Z80950                                  |                    |                    |
|               | Dendrochirotida   | Cucumariidae   | <i>Cucumaria pseudocurata</i>  |   |                    | AF088837           |
|               | Aspidochirotida   | Stichopodidae  | <i>Stichopus japonicus</i>   | D14364                                  |                    |                    |
|               | Dendrochirotida   | Phyllophoridae   | <i>Lipotrabeza vestiens</i>  | Z80952                                  |                    |                    |
| Echinoidea    | 10 of 12 orders (Littlewood et al. 1997):                                       | 10 of 46 families<br>(Mah and Mooi 1997):  |  |   |                    |                    |
|               | Arbacioidea   | Arbaciidae   | <i>Arbacia lixula</i>  | Z37514                                  | AJ225811           |                    |
|               | Echinothuroidea   | Echinothuriidae  | <i>Asthenosoma owstoni</i>   | Z37118                                  | Z37507             | Z37507             |
|               | Spatangoida   | Loveniidae   | <i>Echinocardium cordatum</i>  | Z37123                                  | AJ225812           |                    |
|               | Cassiduloida  | Cassidulidae   | <i>Cassidulis mitis</i>  | Z37148                                  |                    |                    |
|               | Diadematoidea   | Diadematidae   | <i>Diadema setosum</i>   | Z37122                                  |                    |                    |
|               | Clypeasteroidea   | Mellitidae   | <i>Encope aberrans</i>   | Z37126                                  | Z37117             | Z37117             |
|               | Cidaroida   | Cidaridae  | <i>Eucidaris tribuloides</i>   | Z37127                                  |                    |                    |
|               | Temnopleuroidea   | Temnopleuriidae  | <i>Mespilia globulus</i>   | Z37130                                  |                    |                    |
|               | Echinoidea  | Echninidae   | <i>Psammechinus miliaris</i>   | Z37149                                  | AJ225811           |                    |
|               | Phymosomatoida  | Stomopneustidae  | <i>Stomopneustes variolaris</i>  | Z37133                                  |                    |                    |
| Ophiuroidea   | 2 of 2 subclasses, 2 of 2 orders, 2 of 2 suborders (Smith et al. 1995, p. 230): | 7 of 17 families:  |  |   |                    |                    |
|               | Subclass Ophiuridea   | Amphiuridae  | <i>Amphipholis squamata</i>  | X97156                                  | AJ225848           | AF088825           |
|               | Order Ophiurida   |  |  |   |                    |                    |
|               | Suborder Ophiurina  |  |  |   |                    |                    |

|            |  |  |                                  |          |          |          |
|------------|--|--|----------------------------------|----------|----------|----------|
|            | Subclass Ophiuridea  | Asteronychidae   | <i>Astrobrachion constrictum</i> | Z80948   |          |          |
|            | Order Euryalida  |  |                                  |          |          |          |
|            | Subclass Ophiuridea  | Gorgonacephalidae  | <i>Gorgonacephalus eucnemis</i>  | AH008331 |          | AF088835 |
|            | Order Euryalida  |  |                                  |          |          |          |
|            | Subclass Oegophiurida  | Ophiocanopidae   | <i>Ophiocanops fugiens</i>       | Z80954   | Z80943   |          |
|            | Subclass Ophiuridea  | Ophiomyxidae   | <i>Ophiomyxa brevirima</i>       | Z80953   |          |          |
|            | Order Ophiurida  |  |                                  |          |          |          |
|            | Suborder Ophiomyxina   |  |                                  |          |          |          |
|            | Subclass Ophiuridea  | Ophiactidae  | <i>Ophiopholis aculeata</i>      | L28056*  | AJ225836 | AF088826 |
|            | Order Ophiurida  |  |                                  |          |          |          |
|            | Suborder Ophiurina   |  |                                  |          |          |          |
|            | Order Ophiurida  | Ophiuridae   | <i>Ophioplocus japonicus</i>     | D14361   |          |          |
|            | Suborder Ophiurina   |  |                                  |          |          |          |
| Asteroidea | 6 of 8 orders (Blake 1987; plus Peripodida to accommodate <i>Xyloplax</i> ): | 13 of 32 families (Blake 1987; plus Xyloplacidae to accommodate <i>Xyloplax</i> ): |                                  |          |          |          |
|            | Brisingida   | Brisingidae  | <i>Brisingaster robillardi</i>   | AF088802 |          | AF088836 |
|            | Forcipulatida  | Heliasteridae  | <i>Heliaster heliantoides</i>    | AF088804 |          | AF088844 |
|            | Forcipulatida  | Asteriidae   | <i>Asterias amurensis</i>        | D14358   |          |          |
|            | Forcipulatida  | Asteriidae   | <i>Asterias rubens</i>           |          | AJ225843 |          |
|            | Forcipulatida  | Asteriidae   | <i>Asterias forbesi</i>          |          |          | AF088829 |
|            | Forcipulatida  | Labidasteridae   | <i>Rathbunaster californicus</i> | AF088807 |          | AF088833 |
|            | Valvatida  | Poraniidae   | <i>Dermasterias imbricata</i>    | AH008329 |          | AF088843 |
|            | Valvatida  | Goniasteridae or Pseudarchasterinidae (uncertain; see Blake 1987)                  | <i>Pseudarchaster parelli</i>    | AF088806 |          | AF088845 |
|            | Valvatida  | Poraniidae   | <i>Porania pullvillus</i>        | Z80955   | Z80945   |          |
|            | Valvatida  | Asterinidae  | <i>Asterina gibbosa</i>          | AF088801 | AJ225840 | AF088839 |
|            | Velatida   | Pterasteridae  | <i>Pteraster tesselatus</i>      | AF088808 |          |          |
|            | Velatida   | Pterasteridae  | <i>Pteraster obscurus</i>        |          |          | AF088838 |
|            | Velatida   | Solasteridae   | <i>Crossaster papposus</i>       |          | AJ225842 | AF088830 |
|            | Velatida   | Solasteridae   | <i>Solaster dawsonii</i>         | AH008332 |          | AF088841 |
|            | Spinulosida  | Echinasteridae   | <i>Echinaster sepositus</i>      | AH008330 | AJ225844 | AF088831 |
|            | Paxillosida  | Astropectinidae  | <i>Astropecten articulatus</i>   |          |          | AF088827 |
|            | Paxillosida  | Astropectinidae  | <i>Astropecten irregularis</i>   | Z80949   | AJ225837 |          |
|            | Paxillosida  | Luidiidae  | <i>Luidia ciliaris</i>           | AJ225825 |          |          |
|            | Paxillosida  | Luidiidae  | <i>Luidia foliolata</i>          | AF088805 |          | AF088828 |
|            | Peripodida   | Xyloplacidae   | <i>Xyloplax turnerae</i>         | AH008333 |          |          |

**Note:** Sequences and detailed information can be cross-referenced by GenBank accession numbers (available at <http://ncbi.nlm.nih.gov>).

\*GenBank label is wrong.

**Table 2.** Matrix of characters based on morphological and other nonsequence data.

| xread              |  |
|--------------------|--|
| 'character numbers | 0123456789111111112222222233333333444444445555555566<br>0123456789012345678901234567890123456789012345678901 |
| 62 44              |  |
| BALANOGLOSSUS      | ?100?00010000??????11??00??0?????11?0011?????2??1?????   |
| SACCOGLOSSUS       | ?100?00010000??????11??00??0?????11?0011?????2??1?????   |
| GLOSSOBALANUS      | ?100?00010000??????11??00??0?????11?0011?????2??1?????   |
| RHABDOPLEURA       | ?1??0000?000??????11??00??0?????101?0011?????0?0?????  |
| XYLOPLAX           | ??????011110101010?10?1?00000?011011100?0??????11?0011?????  |
| ASTROPECTEN        | 0?10?001011110101011011111001011011100?000011101101201000????  |
| HELIASTER          | 0????0?0111101010110111110010110111011000011101101101000????   |
| LUIDIA             | 0?10?001011110101011011111001011011100?000011101101201000????  |
| CROSSASTER         | ?1??00110111101010110111110010110111011000011101101101000????  |
| BRISINGASTER       | ??????0111101010110111110010110111011000011101101101000????  |
| ASTERIAS           | 0?10?011011110101011011111001011011101100001110110110100011?1  |
| RATHBUNASTER       | ??????0111101010110111110010110111011000011101101101000????  |
| PSEUDARCHASTER     | ??????0111101010110111110010110111011000011101101101000????  |
| PTERASTER          | ?1??00000111101010110111110010110111011000011101101101000????  |
| SOLASTER           | ?1??00110111101010110111110010110111011000011101101101000????  |
| ECHINASTER         | ?1??00110111101010110111110010110111011000011101101101000????  |
| ASTERINA           | ?0??0011011110101011011111001011011101100001110110110100011?1  |
| DERMANETERIAS      | 0?10?0110111101010110111110010110111011000011101101101000????  |
| PORANIA            | 0?10?0110111101010110111110010110111011000011101101101000????  |
| STOMOPNEUSTES      | ??????01111101110001101101111111111111111111111111110110110000????   |
| PSAMMECHINUS       | 0?11?101011111011100011011011111111111111111111111110110110000??02   |
| ENCOPE             | 0?11?101011111011100011011011111111111111111111111110110110000????   |
| MESPILIA           | 0????0?011111011100011011011111111111111111111111110110110000????  |
| EUCIDARIS          | 0?11?1010111110111000110110111111111111111111111111110110110000????  |
| ASTHENOSOMA        | ?0??01010111110111000110110111111111111111111111111110110110000????  |
| ECHINOCARDIUM      | 0?110101011111011100011011011111111111111111111111110110110000????   |
| CASSIDULIS         | ???0?0101011111011100011011011111111111111111111111110110110000????  |
| ARBACIA            | 1?11?10101111101110001101101111111111111111111111111101101100001?02  |
| DIADEMA            | 1?11?10101111101110001101101111111111111111111111111110110110000????   |
| OPHIOPHOLIS        | 0?11?1000111101011?10111111001011010100?0000121011100100003313   |
| AMPHIPHOLIS        | ?2??00000111101011?10111111001011010100?000012101110010000????   |
| OPHIOPLOCUS        | ?0?????0111101011?10111111001011010100?000012101110010000????  |
| GORGONACEPHALUS    | ?2??00000111101011?10111111001011010100?000012101110210000????   |
| OPHIOCANOPS        | ??????0111101011?10111111001011010100?000012101110210000????   |
| ASTROBRACHION      | ??????0111101011?10111111001011010100?000012101110010000????   |
| OPHIOMYXA          | ?2??0?000111101011?10111111001011010100?000012101110010000????   |
| PSYCHROPOTES       | ??????01111110?1010001011011101111111111110100??0111000000????   |
| STICHOPUS          | ?0?11?1000111110?10100010110111011111111110100??0111000000??02   |
| CUCUMARIA          | ?0??11000111110?101000101101110111111111110100??0111000000????   |
| LIPOTRAPEZA        | ??????01111110?10100010110111011111111110100??0111000000????   |
| ANTEDON            | ?1??1100111000100001110100100000000010100000?0001000011221?  |
| DOROMETRA          | ??????0111000100001110100100000000010100000?0001000011????   |
| ENDOXOCRINUS       | ??????0111000100001110100100000000010100000?0001000011????   |
| CAPILLASTER        | ??????0111000100001110100100000000010100000?0001000011????   |
| ;                  |  |
| cc - 0.61;         |  |
| proc/;             |  |

treated with the same cost functions (e.g., step matrices) in POY as multiple alignment and tree search.

The basic strength of the combined-analysis approach lies in the ability of synapomorphies from different types of data to provide additive support for related groups. Dynamic homology takes combined analysis one step further by allowing co-optimization of molecules and morphology at the level of sequence alignment. Putative sequence homologies are tested and revised via optimization of their congruence with morphological synapomorphies. This contrasts sharply with standard combined analyses in which prealigned sequences are attached to morphological characters. Standard analysis is restricted to searching for congruent trees from the limited common phylogenetic signal that can be found between a static alignment of sequence and a morphological character matrix. It has been demonstrated that in terms of character congruence and topo-

logical congruence, tree searching on statically aligned sequence datasets combined with morphological characters produces cladograms that are suboptimal to those produced when the same raw data are analyzed with direct optimization (Wheeler 1998). Direct optimization produces more congruent cladograms because the putative homologies among sequence data are realigned and co-optimized with the morphological data every search replicate.

Bremer support values were calculated in POY via a TBR search rather than searching for trees of additional length and creating consensus trees. Hence, these values may overestimate group support.

*Sensitivity analysis*

The results of multiple alignment and phylogenetic analysis, regardless of the algorithms, are sensitive to choice of evolutionary

**Table 3.** Descriptions of characters based on morphological and other nonsequence data (cross-reference to character matrix (Table 2)).

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|   |                           |
|---|---------------------------|
| Character 0. Origin of oral somatocoel in feeding larvae (Note: For <i>Ophiopholis</i> see Olsen (1942)):   |                           |
| 0   | left anterior enterocoel  |
| 1   | posterior enterocoel      |
| 2   | schizocoely               |
| Character 1. Origin of oral somatocoel in nonfeeding larvae:  |                           |
| 0   | anterior enterocoel       |
| 1   | posterior enterocoel      |
| 2   | schizocoely               |
| Character 2. Post-oral, dorsal, and lateral ciliated band in feeding larvae (Lacalli 1996):   |                           |
| 0   | absent                    |
| 1   | present                   |
| Character 3. Paroral ciliated bands in feeding larvae:  |                           |
| 0   | absent                    |
| 1   | present                   |
| Character 4. Cilia in nonfeeding larvae (Strathmann (1988), Hendler (1982), Smiley et al. (1991), and Littlewood et al. (1997)):  |                           |
| 0   | uniform cilia             |
| 1   | transverse bands of cilia |
| Character 5. Larval skeleton (this character can be coded in both feeding and nonfeeding larvae, e.g., Emler (1995) and Hendler (1982). Cross-reference character 8 in Littlewood et al. (1997) and characters 1 and 3 in Strathmann (1988)): |                           |
| 0   | absent                    |
| 1   | present                   |
| Character 6. Anterior adhesive pit in larva:  |                           |
| 0   | absent                    |
| 1   | present                   |
| Character 7. Adult mouth forms from larval left:  |                           |
| 0   | no                        |
| 1   | yes                       |
| Character 8. Gill slits (cross-reference character 22 in Littlewood et al. (1997)):   |                           |
| 0   | absent                    |
| 1   | present                   |
| Character 9. Calcitic endoskeleton (cross-reference character 23 in Littlewood et al. (1997)):  |                           |
| 0   | absent                    |
| 1   | present                   |
| Character 10. Pentaradial symmetry in adults (cross-reference character 24 in Littlewood et al. (1997)):  |                           |
| 0   | absent                    |
| 1   | present                   |
| Character 11. Water-vascular system (cross-reference character 25 in Littlewood et al. (1997)):   |                           |
| 0   | absent                    |
| 1   | present                   |
| Character 12. Free-living (cross-reference character 26 in Littlewood et al. (1997)):   |                           |
| 0   | stemmed                   |
| 1   | free-living               |
| Character 13. Ambulacral growth (cross-reference character 27 in Littlewood et al. (1997)):   |                           |
| 0   | radial                    |
| 1   | meridional                |
| Character 14. Nervous system (cross-reference character 28 in Littlewood et al. (1997); also Rowe et al. (1988, 1994) describe a circumferential “radial” ectoneural nerve for <i>Xyloplax</i> but provide little detail):                    |                           |
| 0   | entoneural predominant    |
| 1   | ectoneural predominant    |
| Character 15. Ambulacral skeleton (cross-reference character 30 in Littlewood et al. (1997)):   |                           |
| 0   | integral to body          |
| 1   | appendage                 |
| Character 16. Ambulacral plate addition (cross-reference character 32 in Littlewood et al. (1997) and Blake (1998)):  |                           |
| 0   | terminal                  |
| 1   | subterminal               |

---

**Table 3** (*continued*).

---

|  |   |
|--|---|
| Character 17. Epineural sinus over radial nerves (cross-reference character 33 in Littlewood et al. (1997) and characters 19 and 21 in Smith (1984)):  |   |
| 0  | absent  |
| 1  | present   |
| Character 18. Tiedemann's bodies (cross-reference character 34 in Littlewood et al. (1997); Smiley (1988) coded this character as present in ophiuroids):  |   |
| 0  | absent  |
| 1  | present   |
| Character 19. Polian vesicles (cross-reference character 35 in Littlewood et al. (1997)):  |   |
| 0  | absent  |
| 1  | present   |
| Character 20. Ambulacral plates (cross-reference character 36 in Littlewood et al. (1997)):  |   |
| 0  | biserial  |
| 1  | uniserial   |
| Character 21. Hemal system (cross-reference character 37 in Littlewood et al. (1997)):   |   |
| 0  | diffuse lacunae   |
| 1  | discrete canals   |
| Character 22. Gonads (cross-reference character 39 in Littlewood et al. (1997)):   |   |
| 0  | single  |
| 1  | multiple  |
| Character 23. Genital rachis (cross-reference character 40 in Littlewood et al. (1997)):   |   |
| 0  | origin at distal end of axial gland   |
| 1  | origin at middle of axial gland   |
| Character 24. Outer genital coelom surrounds gonad (cross-reference character 41 in Littlewood et al. (1997); see also Rowe et al. (1994), where text on page 158 says that <i>Xyloplax</i> lacks a genital coelom, but see figures 8A and 10A): |   |
| 0  | no  |
| 1  | yes   |
| Character 25. Madreporite (cross-reference character 42 in Littlewood et al. (1997)):  |   |
| 0  | absent  |
| 1  | present   |
| Character 26. Stone canal calcified (cross-reference character 43 in Littlewood et al. (1997)):  |   |
| 0  | no  |
| 1  | yes   |
| Character 27. Hydropore (cross-reference character 44 in Littlewood et al. (1997)):  |   |
| 0  | external  |
| 1  | internal  |
| Character 28. Perianal coelom (cross-reference character 45 in Littlewood et al. (1997)):  |   |
| 0  | undifferentiated from main body coelom  |
| 1  | differentiated from main body coelom  |
| Character 29. Perihemal diverticula (modified from character 46 in Littlewood et al. (1997) and David and Mooi (1997)):  |   |
| 0  | undifferentiated  |
| 1  | a separate coelom is created by interradial pocketing of the left somatocoel; however, this coelom has various fates in different classes |
| Character 30. Expansion of lantern coelom (David and Mooi 1998):   |   |
| 0  | absent  |
| 1  | present   |
| Character 31. Moveable articulated spines in adult (cross-reference character 47 in Littlewood et al. (1997)):   |   |
| 0  | absent  |
| 1  | present   |
| Character 32. Ambulacral growth in adults (see character 16 in Smiley (1988); see also Fell (1963)):   |   |
| 0  | terminal  |
| 1  | subterminal   |
| Character 33. Tube feet with calcified disk (cross-reference character 48 in Littlewood et al. (1997)):  |   |
| 0  | no  |
| 1  | yes   |

---

**Table 3** (*continued*).

---

|  |   |
|--|---|
| Character 34. Tube feet (cross-reference character 49 in Littlewood et al. (1997)):  |   |
| 0  | direct outpouch from wide radial water canal; without valves        |
| 1  | lateral side branch of cylindrical water canal; with one-way valves |
| Character 35. Tube foot with internal ampulla (cross-reference character 50 in Littlewood et al. (1997)):  |   |
| 0  | no  |
| 1  | yes   |
| Character 36. Circumoral water-vascular ring and nerve (cross-reference character 51 in Littlewood et al. (1997)):   |   |
| 0  | adoral to ambulacral skeleton                                       |
| 1  | associated with first ambulacrum                                    |
| Character 37. Internal skeleton on esophagus (cross-reference character 52 in Littlewood et al. (1997)):   |   |
| 0  | absent  |
| 1  | present   |
| Character 38. Anus in adult (cross-reference character 53 in Littlewood et al. (1997)):  |   |
| 0  | absent  |
| 1  | present   |
| Character 39. Position of anus with respect to peristome (cross-reference character 54 in Littlewood et al. (1997)):   |   |
| 0  | same face   |
| 1  | opposite  |
| Character 40. Gut (cross-reference character 55 in Littlewood et al. (1997)):  |   |
| 0  | saccate   |
| 1  | looped and cylindrical  |
| Character 41. Secretory cells in tube feet (cross-reference character 58 in Littlewood et al. (1997)):   |   |
| 0  | goblet cells only   |
| 1  | goblet and apical tuft cells  |
| Character 42. Sperm morphology in species with external fertilization (cross-reference character 59 in Littlewood et al. (1997); <i>Xyloplax</i> likely has internal fertilization (Healy et al. 1988)): |   |
| 0  | spherical   |
| 1  | elongate  |
| Character 43. Axial gland (cross-reference character 60 in Littlewood et al. (1997)):  |   |
| 0  | within axial sinus  |
| 1  | abutting left axial sinus but not enclosed                          |
| Character 44. Axial complex (cross-reference character 61 in Littlewood et al. (1997)):  |   |
| 0  | stone canal separated from axial sinus and gland                    |
| 1  | stone canal in axial-sinus wall                                     |
| Character 45. Right axial sinus (cross-reference character 63 in Littlewood et al. (1997)):  |   |
| 0  | absent  |
| 1  | restricted to distal end of complex, forming dorsal sac             |
| 2  | extends along length of axial complex                               |
| Character 46. Muscle–tendon attachment (cross-reference character 64 in Littlewood et al. (1997)):   |   |
| 0  | directly to calcite trabeculae                                      |
| 1  | via tendons   |
| Character 47. Tendons (cross-reference character 65 in Littlewood et al. (1997)):  |   |
| 0  | composed of unstriated microfibrils                                 |
| 1  | composed of striated and unstriated microfibrils                    |
| Character 48. Adambulacral ossicles differentiated (cross-reference character 66 in Littlewood et al. (1997)):   |   |
| 0  | no  |
| 1  | yes   |
| Character 49. Scleroblasts (cross-reference character 67 in Littlewood et al. (1997)):   |   |
| 0  | single and do not form a syncytium                                  |
| 1  | form a syncytium that calcifies                                     |
| Character 50. Longitudinal nerve in podia (cross-reference character 68 in Littlewood et al. (1997)):  |   |
| 0  | absent  |
| 1  | present   |
| Character 51. Batyl alcohol (cross-reference character 69 in Littlewood et al. (1997)):  |   |
| 0  | absent  |
| 1  | present   |

---

**Table 3** (concluded).

---

|   |                 |
|---|-----------------|
| Character 52. Gonopores (cross-reference character 70 in Littlewood et al. (1997)):   |                 |
| 0   | oral            |
| 1   | aboral          |
| 2   | serial          |
| Character 53. Mouth plates (cross-reference character 71 in Littlewood et al. (1997)):  |                 |
| 0   | rigid           |
| 1   | specialized jaw |
| Character 54. Odontophore:  |                 |
| 0   | absent          |
| 1   | present         |
| Character 55. Dorsal hollow nerve cord:   |                 |
| 0   | absent          |
| 1   | present         |
| Character 56. Imperforate extraxial skeleton:   |                 |
| 0   | absent          |
| 1   | present         |
| Character 57. Pinnules:   |                 |
| 0   | absent          |
| 1   | present         |
| Character 58. y tRNA is adjacent to the following gene regions in mitochondrial gene order (Note: Family Antedonidae coded from Scouras and Smith (2001); GenBank accession No. AF049132):  |                 |
| 0   | g tRNA-5'       |
| 1   | lrRNA-5'        |
| 2   | v tRNA-5'       |
| Character 59. p tRNA is adjacent to the following gene region in mitochondrial gene order (Note: Family Antedonidae coded from Scouras and Smith (2001); GenBank accession No. AF049132):   |                 |
| 0   | COI-5'          |
| 1   | q tRNA-3'       |
| 2   | srRNA-5'        |
| Character 60. lrRNA is adjacent to the following gene regions in mitochondrial gene order (Note: Family Antedonidae coded from Scouras and Smith (2001); GenBank accession No. AF049132; for <i>Arbacia lixula</i> see GenBank accession No. X80396): |                 |
| 0   | COI-5'          |
| 1   | g tRNA-5'       |
| Character 61. COI is adjacent to the following gene regions in mitochondrial gene order (Note: Family Antedonidae coded from Scouras and Smith (2001); GenBank accession No. AF049132):   |                 |
| 0   | p tRNA-5'       |
| 1   | lrRNA-3'        |
| 2   | w tRNA-3'       |

---

model. For example, various weights must be assigned to parameters such as transitions, transversions, and insertion–deletion events. There are no known means of determining a priori which alignment parameters are appropriate for recovering evolutionary relationships. Superior methods and parameters produce a phylogeny that minimizes incongruence among datasets. Sensitivity analysis allows one to limit assumptions about models of evolution by testing many parameters (Wheeler 1995). Various analyses produced by different parameter sets can be examined with congruence measures to understand the explanatory limitations of the datasets.

A wide range of parameters for costs of insertion–deletion events (indels), nucleotide base change events (transversions and transitions), and character data steps were specified to explore the sensitivity of phylogenetic results to parameter choice and inclusion of various data partitions. Twenty parameter sets were explored. The ratio of weights between indels and the greater of transversion or transition weights ranged from 1 to 8. Transversion:transition ratios ranged between 0.5 and 4. In addition, transitions were set at 0 cost (transversion parsimony), yielding a

transversion:transition ratio of  $\infty$ . DNA sequence data were analyzed with character data weighted at 1 and with character data assigned the cost of indels (upweighted). Sequence data were also analyzed without character data as 18S and 28S rDNA, 18S only, and 28S only. Nonsequence character data were also analyzed as a single partition (Fig. 3).

#### *Congruence metrics*

Topologies resulting from phylogenetic searches were scored using two metrics: taxonomic congruence and character congruence (Figs. 3, 4, 5, 6).

*Taxonomic congruence*—Strict consensus was used to summarize multiple equally parsimonious trees. Monophyly of a group was scored as 1; nonmonophyly was scored as 0. (Each tree was examined for cases in which lack of resolution was potentially consistent with monophyly; however, no such cases were discovered.) This information is reported graphically in two forms: (1) interpolated Cartesian graphs of continuous quantitative measures, percent recovery of monophyletic groups in a search under a parameter set

(Fig. 3), and (2) non-interpolated Cartesian graphs of binary notation of areas of the parameter space in which the analysis recovered or did not recover a monophyletic group (Figs. 4, 5, 6) (Wheeler 1995). Taxonomic congruence was assayed and plotted by the relative percent recovery (high percent recovery = high taxonomic congruence) of monophyletic groups among seven traditionally recognized groups: Asterozoa, Echinozoa, Ophiurozoa, Crinozoa, Holothurozoa, Hemichordata, and Echinodermata (Fig. 3). In all the analyses of more than a single data partition, the recovery of the groups mentioned above and additional groups proposed by various workers as natural was noted in a binary Cartesian graph (Figs. 4, 5, 6). These groups included

Asterozoa = (Asterozoa inclusive of *Xyloplax* + Ophiurozoa)  
 Echinozoa = (Echinozoa + Holothurozoa)  
 Cryptosyringida = ((Echinozoa + Holothurozoa) Ophiurozoa)  
 Eleutherozoa = (Echinozoa + Holothurozoa + Ophiurozoa + Asterozoa)

$$\text{MFES} = \frac{\text{treelength}_{\text{combined}} - \text{treelength}_{18\text{S}} - \text{treelength}_{28\text{S}} - \text{treelength}_{\text{character}}}{\text{treelength}_{\text{combined}}}$$

## Results

Analysis of morphological and other character datasets in NONA (Goloboff 2000) yielded 5041 equally parsimonious topologies of length 92. A strict-consensus tree of these topologies (Fig. 1a) can be summarized as follows: (Enteropneusta (*Rhabdopleura* (Crinozoa ((Asterozoa including *Xyloplax*) (Ophiurozoa (Echinozoa Holothurozoa)))))).

This topology supports a monophyletic Echinodermata as sister taxon to *Rhabdopleura* (a pterobranch hemichordate), a monophyletic clade of enteropneust hemichordates as sister taxon to (Echinodermata + *Rhabdopleura*), and the following groups within Echinodermata:

Eleutherozoa = (Ophiurozoa + Asterozoa + Echinozoa + Holothurozoa)  
 Cryptosyringida = ((Echinozoa + Holothurozoa) Ophiurozoa)  
 Echinozoa = (Echinozoa + Holothurozoa)  
 Asterozoa including *Xyloplax*  
 Crinozoa

However, the morphological data provide very little resolution within classes. One synapomorphy, the presence of the odontophore, supports Asterozoa (including *Xyloplax*) as a monophyletic group. One synapomorphy, the absence of an anus in adults, supports the clade (*Xyloplax* (*Astropecten* + *Luidia*)).

### Analysis of DNA sequence data

Combined analysis of the 18S and 28S DNA sequences across 20 parameter sets in POY yielded a single most congruent topology (MFES = 0.0174) when indels, transversions, and transitions were weighted at a cost of 1. This tree (Fig. 1b) can be summarized as (((((Echinozoa + Holothurozoa) (Asterozoa including *Xyloplax*)) Ophiurozoa) Crinozoa).

This topology supports a monophyletic Echinodermata as sister taxon to Hemichordata and the following groups within Echinodermata:

Eleutherozoa  
 Echinozoa  
 Asterozoa including *Xyloplax*  
 Ophiurozoa  
 Crinozoa

The recovery of these groups was not used in the quantitative metric of taxonomic congruence because many of these groups are mutually exclusive.

**Character congruence**—Character congruence, an extension of parsimony, was used as the optimality criterion for choosing among topologies that are produced under various parameter sets. The Mickevitch–Farris extra-steps (MFES) index measures the number of extra steps that occur in an analysis of combined data versus separate analyses of individual partitions (Mickevitch and Farris 1981). As character incongruence among data partitions increases, the MFES index increases. When parameter-sensitivity analyses are conducted on the same data partitions, MFES scores are comparable despite different weighting schemes. Therefore, minimal incongruence is used to choose the most parsimonious topologies in various analyses and compare the efficacy of parameter sets when analyses were conducted on the same sets of data (Fig. 3).

In this study the MFES index was measured for sequence and character data with the following equation:

However, two next most congruent topologies (MFES = 0.0178) were found when indels and transversions were weighted at 2 and transitions at 1. These two topologies can be summarized as (((((Echinozoa + Holothurozoa) Ophiurozoa) (Asterozoa including *Xyloplax*)) Crinozoa).

In summary, the most congruent tree for molecular data does not support Cryptosyringida (Fig. 1b). The recovery of Cryptosyringida does occur in near-suboptimal trees that are 2.21% less congruent than the most congruent tree. (Note that the character-congruence values in this section are not comparable to values derived from the combined analyses given below because they do not contain the same sets of data.)

### Analysis of DNA sequence data plus morphological and other character data

#### Most congruent trees

Combined analyses of the 18S and 28S DNA sequences plus character data across 20 parameter sets in POY yielded six shortest topologies (weighted length = 5265; MFES = 0.0186) when indels and transversions were weighted at 2, transitions at 1, and character data at 1. The strict consensus of these topologies (Fig. 2) supports a monophyletic Echinodermata as sister taxon to Hemichordata, Eleutherozoa, and the following groups within Eleutherozoa:

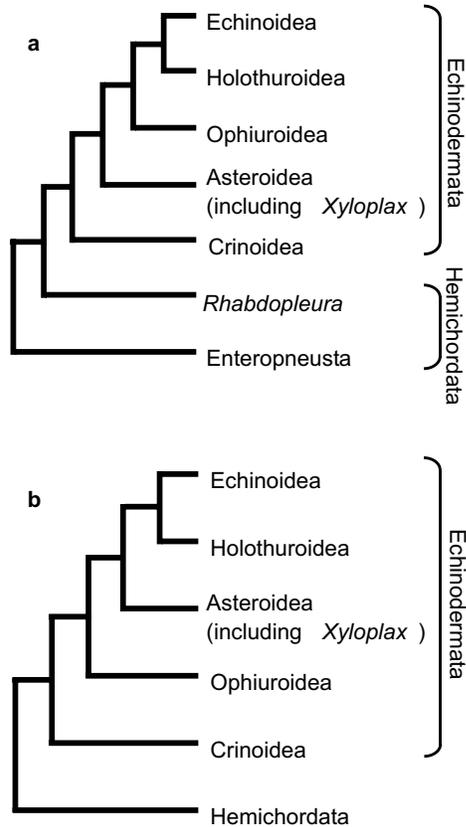
Asterozoa = ((Asterozoa including *Xyloplax*) Ophiurozoa)  
 Echinozoa  
 Crinozoa

In one highly suboptimal tree (MFES = 0.0418), *Xyloplax* is recovered as a sister taxon to other echinoderm classes.

### Support in combined and partitioned analyses

A summary of apomorphies for each branch of the best combined-analysis tree (Fig. 2) is presented in Table 4. Combined-analysis, relative, and partitioned Bremer support values are provided in Table 5. Definite character-state changes include insertions, deletions, transversions, and transitions in DNA sequence data and state changes in morphological or

**Fig. 1.** Results of analyses of various data types (Janies and Mooi 1999). (a) Summary of most parsimonious topology supported by nonsequence character data (92 steps). (b) Summary of most congruent topology supported by 18S and 28S rDNA data (Mickeyvitch–Farris extra-steps (MFES) index = 0.0174). In both analyses *Xyloplax* is nested within the class Asterozoa.



other character data. Optimization-dependent changes include instances where a change may have occurred but the ancestor and descendants have some but not all states in common.

The changes along branches represent a hierarchical map of features that evolved in the ancestry of natural groups of echinoderms. Most changes in 18S and 28S rDNA and all changes in morphology or other character data occurred during the evolution of subphyla or classes, as described in the following paragraphs. Some character transformations occur in more than one group. These are synapomorphies nevertheless, but diagnosis of the group requires a suite of synapomorphies rather than a single character. Synapomorphies in molecular characters cannot be distinguished as unique or shared because the position number in a sequence changes as nodal sequence length changes throughout the tree. Character numbers in the matrix used in this study are denoted with brackets, [ ].

Eleutherozoa (HTU 21) has a Bremer support of 10 weighted steps (of a total weighted length of 5265) for combined data analysis. Bremer values for partitioned analysis in this clade include 1 step for 18S rDNA, –20 steps for 28S rDNA, and –2 steps for other character data. Eleutherozoa is supported by the following synapomorphies: free-living rather than stalked morphology [12], the presence of a stone

canal [26], and 1 deletion, 16 transversions, and 11 transitions in 18S and 28S rDNA.

Asterozoa (HTU 14) has a Bremer support of 1 weighted step for combined data analysis. Bremer values for partitioned analysis include –7 steps in 18S rDNA, –38 steps in 28S rDNA, and –3 steps in other character data. Asterozoa is supported by the following synapomorphies: a saccate gut [40], undifferentiated ambulacral ossicles [48], and 20 insertions, 20 transversions, and 5 transitions in 18S and 28S rDNA.

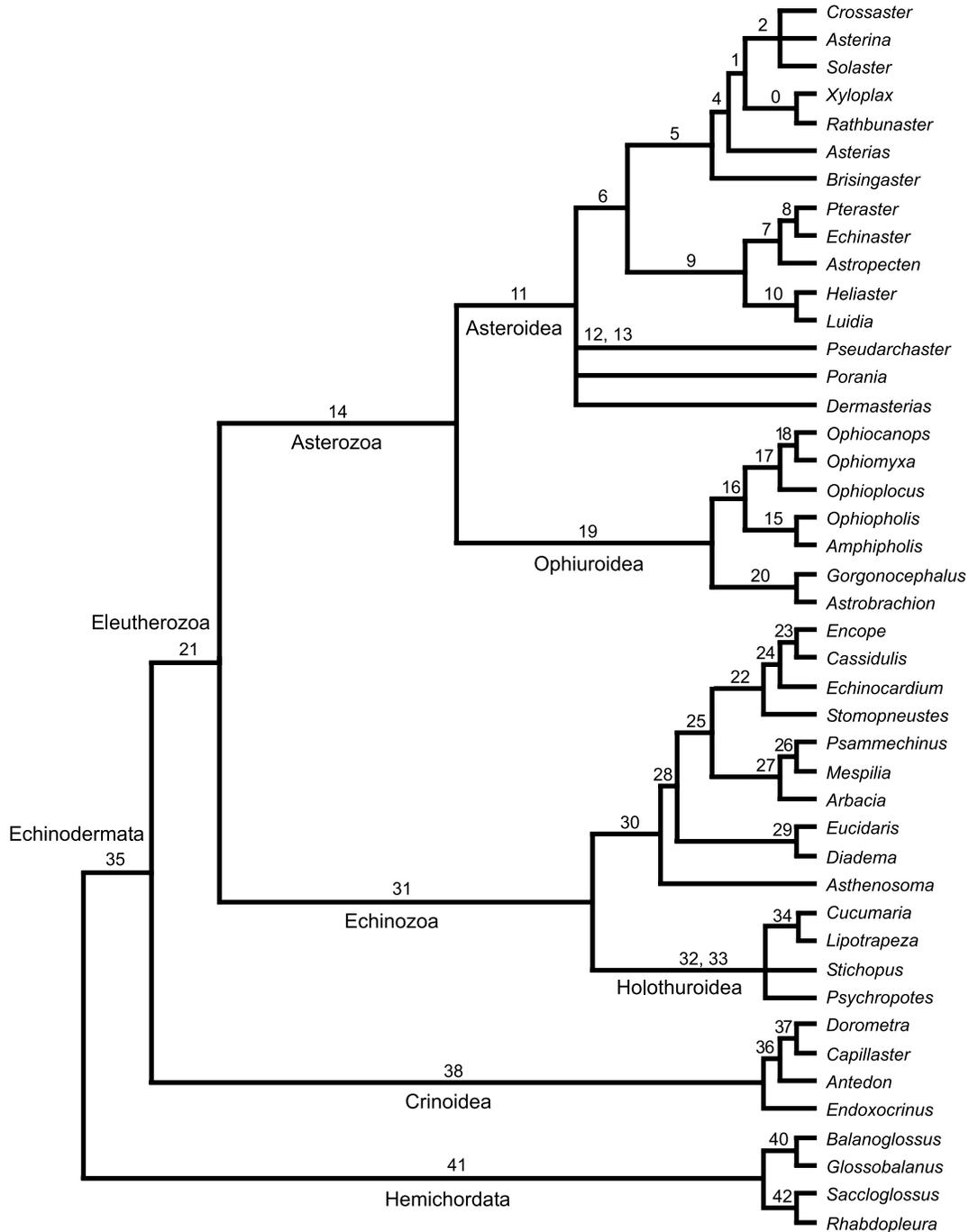
Echinozoa (HTU 31) is united by many synapomorphies: 17 insertions, 4 deletions, 23 transversions, and 18 transitions in 18S and 28S rDNA, origin of the oral somatocoel from the anterior enterocoel in nonfeeding larvae [1], meridional ambulacral growth [13], hemal system with diffuse lacunae [21], lack of an outer genital coelom surrounding the gonad [24], the perianal coelom differentiated from the main body coelom [28], expansion of the lantern coelom [30], tube feet with a calcified disk [33], internal skeleton in the esophagus [37], goblet and apical tuft secretory cells in tube feet [41], axial gland abutting the left axial sinus but not enclosed [43], and cytochrome C oxidase subunit I trails the 3' end of the 16S rDNA in the mitochondrial genome [60]. The Bremer support for Echinozoa includes 21 weighted steps for combined data analysis. The Bremer values of partitioned analyses include 10 steps for 18S rDNA, –38 steps for 28S rDNA, and 2 steps for other character data.

The presence of the odontophore [54] is the only unique morphological synapomorphy for Asterozoa (HTU 11) in this analysis (see also Dean 1998; Janies and Mooi 1999). Two synapomorphies among Asterozoa also occur with Echinozoa in the results of the combined data analysis. These features include formation of the adult mouth on the left side of the larva [7] (see also Fig. 6 and the Discussion) and aborally opening gonopores [52]. In addition, another state of character 52, serially arranged gonopores, evolved in the asteroids *Astropecten* and *Luidia*. Asterozoa is supported by the many synapomorphies in 18S and 28S rDNA: 9 insertions, 1 deletion, 23 transversions, and 31 transitions. Bremer support for Asterozoa includes 43 weighted steps in combined data analyses. Values for partitioned data analyses include 26 weighted steps in 18S rDNA, –36 weighted steps in 28S rDNA, and 0 steps in other character data.

Ophiurozoa (HTU 19) is supported by two unique morphological synapomorphies: schizocoelous origin of the oral somatocoel in nonfeeding larvae [1], and the extension of the right axial sinus along the length of the axial complex [45]. Ophiurozoa shares the absence of an anus in adults [38] with some asteroids, e.g., *Xyloplax*, *Astropecten*, and *Luidia*. The absence of batyl alcohol [51] and the evolution of a specialized jaw [53] are synapomorphies shared with echinoids. The Bremer support for Ophiurozoa in combined analysis is 52 weighted steps. Bremer values for partitioned analysis are 43 weighted steps for 18S rDNA, –24 weighted steps for 28S rDNA, and 0 steps for other character data. Ophiurozoa has many molecular synapomorphies: 6 insertions, 6 deletions, 27 transversions, and 30 transitions in 18S and 28S rDNA.

Echinoidea (HTU 30) is supported by one unique synapomorphy, elongate sperm morphology in species with external fertilization [42]. However, as discussed above, several fea-

**Fig. 2.** The best total evidence tree of all analyses, consensus of two trees at length = 5265 (MFES index = 1.86). This tree resulted from analysis of DNA sequence data plus morphological and other character data when gaps and transversions cost 2; transitions and changes in morphological or other character data cost 1. Hypothetical taxonomic units (HTUs) are marked with numerals to be used in referencing data on changes along branches in Table 4 and support of groups in Table 5.

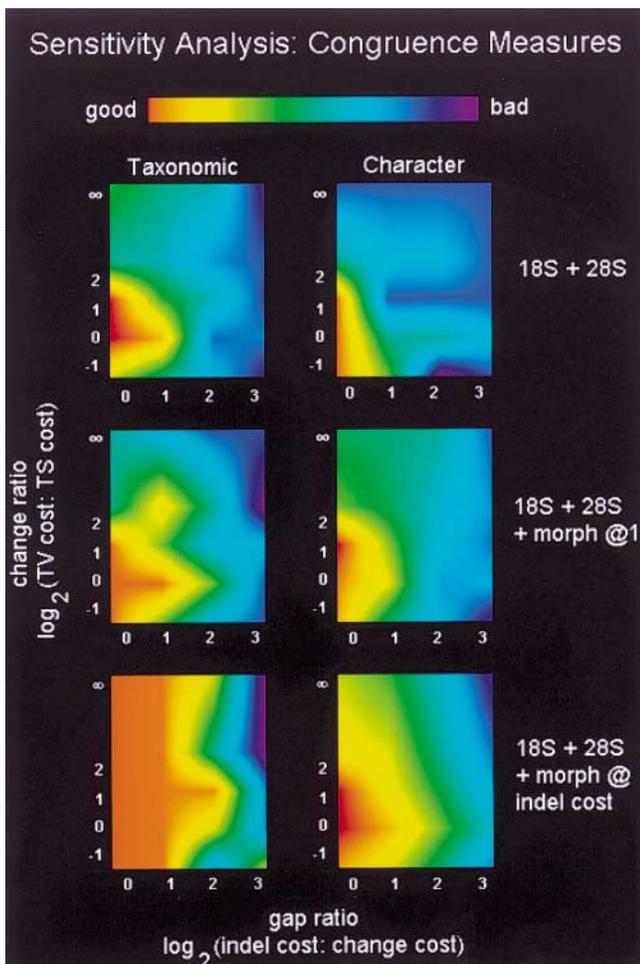


tures that unite Echinozoa are shared with other taxa. The absence of batyl alcohol [51] and the evolution of a specialized jaw [53] are shared with ophiuroids. Formation of the adult mouth on the left side of the larva [7] and aborally opening gonopores [52] are shared with asteroids. There is strong support for Echinozoa in molecular synapomorphies: 6 insertions, 25 deletions, 34 transversions, and 20 transitions. The Bremer support for Echinozoa in combined analysis is 61 weighted steps. Bremer values for Echinozoa in

partitioned analysis include 22 weighted steps for 18S rDNA, -17 weighted steps for 28S rDNA, and 3 weighted steps for other character data.

The Bremer support for Holothuroidea (HTU 32, 33) in combined analysis is 188 weighted steps. Partitioned analysis reveals Bremer values of 185 weighted steps for 18S rDNA, -38 weighted steps for 28S rDNA, and 2 weighted steps for other character data in Holothuroidea. Holothuroidea is supported by one unique morphological synapomorphy, a

**Fig. 3.** Congruence surfaces for sensitivity to parameter choice and data sets. Row 1 is sequence data only, row 2 is sequence data and other character data (weighted at 1), and row 3 is sequence data and other character data (weighted variably, at the cost of an indel, in each parameter set). “Good” denotes most congruent scores; “bad” denotes least congruent scores. The left-hand column shows taxonomic congruence measured as percent recovery of monophyletic groups across parameter sets. Recovery of seven traditionally recognized groups, Asterozoa, Echinozoa, Ophiurozoa, Crinozoa, Holothurozoa, Hemichordata, and Echinodermata, was recorded. For example, if the consensus of all the trees produced in analysis under a certain parameter set recovered each of these groups as monophyletic, that parameter set was scored at 7/7, or 100% recovery. Similarly, if a parameter set recovered 3 of 7 groups, it was scored at 43% recovery. The right-hand column shows character congruence measured by the MFES index (Mickey and Farris 1981). This index measures the number of extra steps that occur in an analysis of combined data versus separate analysis of individual data sets; see the MFES equation. Surfaces are interpolated by NCSA Datascope 2.0.3, which is available anonymously at <ftp://ftp.ncsa.uiuc.edu/Visualization/DataScope/Mac>.



single gonad [22]. The synapomorphies, separation of the stone canal from the axial sinus and gland [44] and the absence of a right axial sinus [45], are shared with Crinozoa. Holothurozoa is supported by the following molecular

synapomorphies in 18S and 28S rDNA: 41 insertions, 11 deletions, 51 transversions, and 66 transitions.

Crinozoa (HTU 38) is supported by two synapomorphies: an internal hydropore [27] and the anus on the same face as the peristome [39]. Two features are shared with the Holothurozoa (as discussed above): separation of the stone canal from the axial sinus and gland [44] and the absence of a right axial sinus [45]. There are no optimization-independent character-state changes in 18S and 28S rDNA for this group (yet there are 87 possible changes).

Six morphological characters are shared by the Echinodermata (HTU 7), but the polarity of change in these characters cannot be determined with the rooting and sampling used in this analysis (i.e., Hemichordata is represented in this study by one of three genera of the order Pterobranchia, *Rhabdopleura*, and two of three families of the order Enteropneusta, Ptychoderidae and Harrimaniidae). Echinodermata lacks gills slits [8] and a hollow dorsal nerve tube [55]; however, the polarities of these character-state transformations are uncertain, as these features are absent in *Rhabdopleura* but present in enteropneust hemichordates. The presence of a calcitic endoskeleton [9], pentaradial symmetry [10], and a water-vascular system [11] and the absence of a dorsal hollow nerve tube and gill slits are widely regarded as synapomorphies of extant echinoderms. The questions left open in this area are being addressed in a more comprehensive study of deuterostome relationships, including fossil lineages. For example, the ancestral state of the dorsal hollow nerve tube character is not explicit in the present analysis, owing to the limited number of outgroups. The branch from the common ancestor of echinoderms and hemichordates to echinoderms contains only optimization-dependent character-state changes.

#### Nearest suboptimal trees

**POY**—Combined analyses of the 18S and 28S DNA sequences plus character data pegged at the variable for the cost indel were conducted across 20 parameter sets in POY. This sensitivity analysis yielded two near-suboptimal topologies (MFES = 0.0199) when analysis parameter weights for all character transformations (DNA or morphology or gene order) were equally weighted at 1. The strict consensus of these topologies is (((((Echinozoa Holothurozoa) (Asterozoa including *Xyloplax*)) Ophiurozoa) Crinozoa) Hemichordata).

This topology supports a monophyletic Echinodermata as sister taxon to the Hemichordata, the Eleutherozoa, and following groups within the Echinodermata:

- Echinozoa
- Asterozoa including *Xyloplax*
- Ophiurozoa
- Crinozoa

In summary, the nearest suboptimal topologies for combined analysis in POY are 6.68% less congruent than the most congruent combined-analysis tree and do not support the monophyly of Asterozoa or Cryptosyringida.

**CLUSTAL** → **PAUP**—Combined analyses of the 18S and 28S DNA sequences, plus character data weighted at the indel cost across two parameter sets in CLUSTAL X → PAUP\*4.0, yielded two most congruent topologies when gap opening and extension cost were set at 2 and transitions at

**Table 4.** Summary list of apomorphies resulting from POY diagnosis of total evidence tree (Fig. 2). Match hypothetical taxonomic unit (HTU) numerals to branches in Fig. 2.

| Taxon         | Branch                       | Insertions | Deletions | Transversions | Transitions | Morphological and non-sequence characters with definite changes along branch |
|---------------|------------------------------|------------|-----------|---------------|-------------|--|
|               | HTU 1 → 0                    | 15         | 0         | 6             | 15          |  |
|               | HTU 1 → 2                    | 7          | 4         | 2             | 10          |  |
|               | HTU 2 → 3 <sup>a</sup>       |            |           |               |             |  |
|               | HTU 4 → 1                    | 2          | 1         | 10            | 6           |  |
|               | HTU 5 → 4                    | 2          | 1         | 10            | 6           |  |
|               | HTU 6 → 5                    | 1          | 2         | 3             | 2           |  |
|               | HTU 6 → 9                    | 7          | 4         | 6             | 8           |  |
|               | HTU 7 → 8                    | 8          | 3         | 4             | 8           |  |
|               | HTU 9 → 7                    | 5          | 1         | 9             | 3           |  |
|               | HTU 9 → 10                   | 0          | 0         | 4             | 4           |  |
|               | HTU 11 → 6                   | 10         | 2         | 15            | 18          |  |
|               | HTU 11 → 12, 13 <sup>b</sup> | 0          | 0         | 4             | 1           |  |
| Asteroidea    | HTU 14 → 11                  | 9          | 1         | 23            | 31          | 7, 52, 54  |
| Ophiuroidea   | HTU 14 → 19                  | 6          | 6         | 27            | 30          | 1, 38, 45, 51, 53  |
|               | HTU 16 → 15                  | 1          | 1         | 3             | 7           |  |
|               | HTU 16 → 17                  | 1          | 0         | 3             | 2           |  |
|               | HTU 17 → 18                  | 0          | 0         | 0             | 3           |  |
|               | HTU 19 → 20                  | 3          | 1         | 18            | 18          |  |
| Asterozoa     | HTU 21 → 14                  | 20         | 0         | 20            | 5           | 40, 48   |
| Echinozoa     | HTU 21 → 31                  | 17         | 4         | 23            | 18          | 1, 13, 21, 24, 28, 30, 33, 37, 41, 43, 60                                    |
|               | HTU 22 → 24                  | 1          | 2         | 0             | 2           |  |
|               | HTU 24 → 23                  | 0          | 0         | 3             | 2           |  |
|               | HTU 25 → 22                  | 0          | 0         | 2             | 3           |  |
|               | HTU 25 → 27                  | 0          | 1         | 1             | 2           |  |
|               | HTU 27 → 26                  | 0          | 1         | 2             | 1           |  |
|               | HTU 28 → 29                  | 2          | 1         | 0             | 4           |  |
|               | HTU 30 → 28                  | 5          | 8         | 11            | 13          |  |
| Echinoidea    | HTU 31 → 30                  | 6          | 25        | 34            | 20          | 7, 42, 51, 52, 53  |
| Holothuroidea | HTU 31 → 32, 33 <sup>c</sup> | 41         | 11        | 51            | 66          | 22, 44, 45   |
|               | HTU 32, 33 → 34              | 9          | 1         | 9             | 10          |  |
| Eleutherozoa  | HTU 35 → 21                  | 0          | 1         | 16            | 11          | 12, 26   |
| Crinoidea     | HTU 35 → 38 <sup>d</sup>     |            |           |               |             | 27, 39, 44, 45   |
|               | HTU 36 → 37                  | 0          | 0         | 1             | 3           |  |
|               | HTU 38 → 36                  | 0          | 1         | 1             | 8           |  |
| Echinodermata | HTU (root) → 35 <sup>e</sup> | 1          | 11        | 30            | 24          |  |
| Hemichordata  | HTU (root) → 41 <sup>f</sup> |            |           |               |             |  |
|               | HTU 41 → 40                  | 2          | 1         | 7             | 9           |  |
|               | HTU 41 → 42                  | 3          | 1         | 4             | 8           |  |

**Note:** Insertions equals the number of insertions on each branch. Deletions equals the number of deletions on each branch. Transversions equals the number of transversions on each branch. Morphological and nonsequence characters with definite changes along the branch correspond to the character number in the Hennig matrix (Table 2) that changes state. POY reports both definite and optimization-dependent character-state changes for all branches, but Table 1 summarizes only definite changes for internal nodes; the complete apomorphy list is available from the author.

<sup>a</sup>Collapsed in consensus; 106 optimization-dependent changes in molecular characters.

<sup>b</sup>Collapsed in consensus.

<sup>c</sup>Collapsed in consensus.

<sup>d</sup>Eighty-seven optimization-dependent molecular changes.

<sup>e</sup>Six optimization-dependent changes in morphological characters; see the Results section.

<sup>f</sup>Twenty-two optimization-dependent molecular character changes and 6 optimization-dependent changes in morphological characters; see the Results section.

0.5 (MFES = 0.0203). The strict consensus of these topologies is ((Echinoidea + Holothuroidea) (Ophiuroidea + Crinoidea)) (Asteroidea including *Xyloplax*) Hemichordata). This topology supports a monophyletic Echinodermata as sister taxon to Hemichordata and the following groups within Echinodermata:

Echinozoa  
Asteroidea (including *Xyloplax*)  
Crinoidea  
Ophiuroidea  
Thus, the CLUSTAL X → PAUP\*4.0 analyses do not

**Table 5.** Partitioned Bremer support values.

|               | HTU (see Fig. 2) | Combined-analysis Bremer support | Relative intensity of Bremer support for combined analysis (ranges from 0 to 1) | Bremer support by partitions |     |            |
|---------------|------------------|----------------------------------|---|------------------------------|-----|------------|
|               |                  |                                  |   | 18S                          | 28S | Morphology |
| Echinodermata | 35               | 52                               | 0.28  | 30                           | -20 | 2          |
| Eleutherozoa  | 21               | 10                               | 0.05  | 1                            | -20 | -2         |
| Asterozoa     | 14               | 1                                | 0.01  | -7                           | -38 | -3         |
| Asteroidea    | 11               | 43                               | 0.23  | 26                           | -36 | 0          |
|               | 6                | 2                                | 0.01  | -20                          | -10 | -3         |
|               | 5                | 6                                | 0.03  | -20                          | -10 | -1         |
|               | 4                | 14                               | 0.07  | -20                          | -8  | -1         |
|               | 1                | 26                               | 0.14  | -20                          | 0   | -1         |
|               | 0                | 10                               | 0.05  | -5                           | -1  | -1         |
|               | 2                | 12                               | 0.06  | -14                          | -9  | -1         |
|               | 9                | 0                                | 0.00  | -17                          | -5  | -3         |
|               | 7                | 15                               | 0.08  | -9                           | -2  | -3         |
|               | 8                | 12                               | 0.06  | 1                            | -4  | -3         |
|               | 10               | 9                                | 0.05  | -12                          | -5  | -2         |
| Ophiuroidea   | 19               | 52                               | 0.28  | 43                           | -24 | 0          |
|               | 16               | 2                                | 0.01  | -1                           | -32 | -2         |
|               | 15               | 3                                | 0.02  | 1                            | -29 | -1         |
|               | 17               | 4                                | 0.02  | 1                            | -32 | -2         |
|               | 18               | 4                                | 0.02  | 2                            | -32 | -1         |
|               | 19               | 52                               | 0.28  | 49                           | -32 | -1         |
| Echinozoa     | 31               | 21                               | 0.11  | 10                           | -38 | 2          |
| Echinoidea    | 30               | 61                               | 0.32  | 22                           | -17 | 3          |
|               | 28               | 5                                | 0.03  | 5                            | -24 | 0          |
|               | 25               | 7                                | 0.04  | 5                            | -17 | 0          |
|               | 22               | 7                                | 0.04  | 5                            | -3  | 0          |
|               | 24               | 7                                | 0.04  | 5                            | -3  | 0          |
|               | 23               | 9                                | 0.05  | 7                            | -15 | 0          |
|               | 27               | 5                                | 0.03  | -2                           | -3  | 0          |
|               | 26               | 7                                | 0.04  | 5                            | -3  | 0          |
|               | 29               | 5                                | 0.03  | -2                           | -24 | 0          |
| Holothuroidea | 32               | 188                              | 1.00  | 185                          | -38 | 2          |
|               | 33               | 55                               | 0.29  | 33                           | -38 | 2          |
|               | 34               | 85                               | 0.45  | 45                           | -38 | 0          |
| Crinoidea     | 38               | 49                               | 0.26  | 49                           | -20 | 0          |
|               | 36               | 7                                | 0.04  | 7                            | -20 | 0          |
|               | 37               | 6                                | 0.03  | 6                            | -20 | 0          |
| Hemichordata  | 41               | 52                               | 0.28  | 30                           | -20 | 2          |
|               | 40               | 23                               | 0.12  | 22                           | -20 | 0          |
|               | 42               | 25                               | 0.13  | 25                           | -20 | -3         |

support Asterozoa or Cryptosyringida, yet they are 8.83% less congruent than the most congruent combined-analysis tree.

#### Congruence metrics

In Fig. 4, taxonomic congruence was recorded among five traditionally recognized classes of echinoderms, Asteroidea, Echinoidea, Ophiuroidea, Crinoidea, and Holothuroidea, and two traditionally recognized phyla, Hemichordata and Echinodermata. In Figs. 5 and 6, taxonomic congruence was recorded for several groups proposed by various workers:

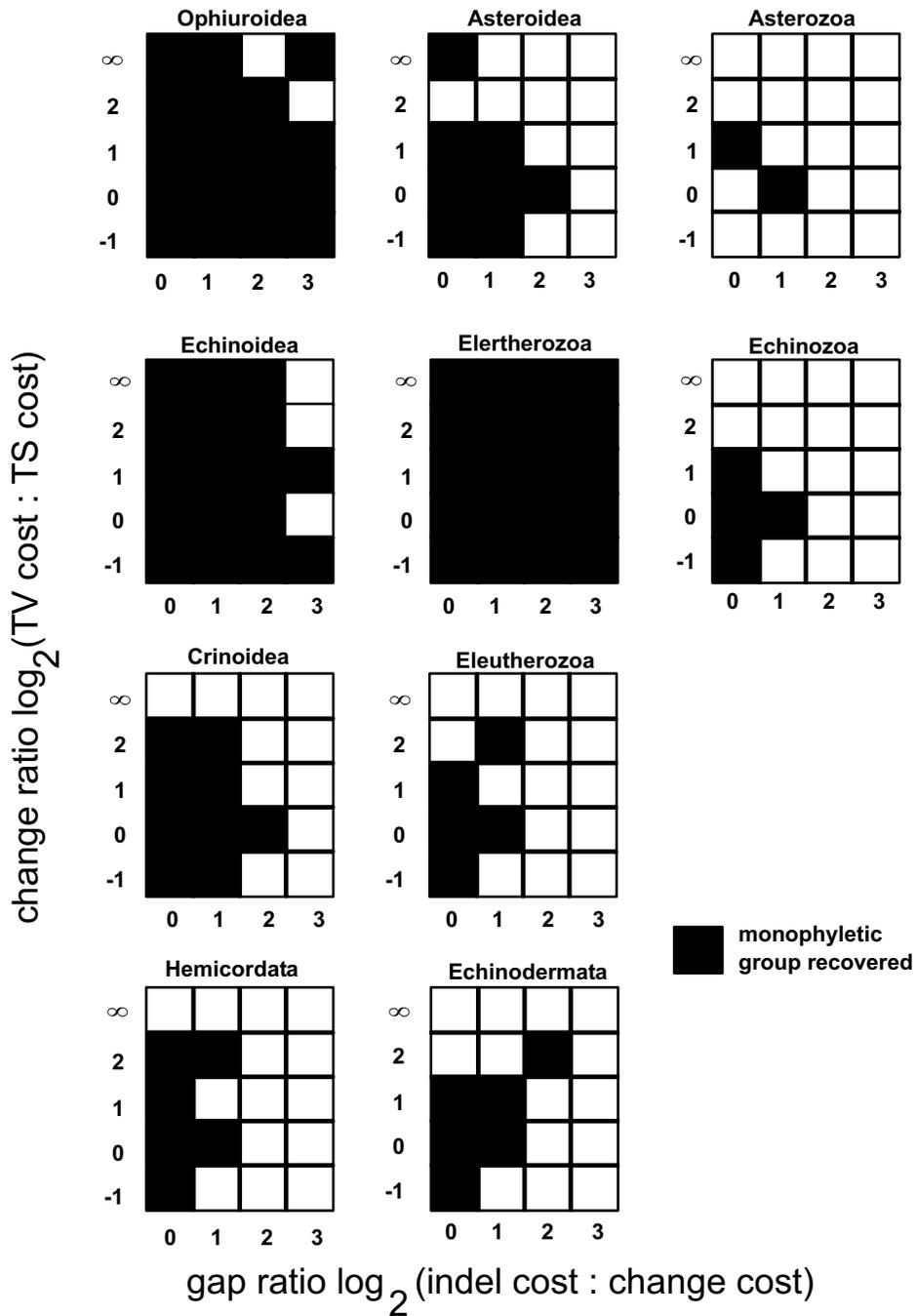
Asterozoa = (Asteroidea including *Xyloplax* + Ophiuroidea)  
 Echinozoa = (Echinoidea + Holothuroidea)  
 Cryptosyringida = (Ophiuroidea (Echinoidea + Holothuroidea))

Eleutherozoa = (Echinoidea + Holothuroidea + Ophiuroidea + Asteroidea).

Figure 5 illustrates the fact that although recovery of a monophyletic Echinoidea, Holothuroidea, and Ophiuroidea is largely unaffected by parameter variation, the hypothesized subphylum, Cryptosyringida (Smith 1984), is never recovered.

In Fig. 6, the placement of *Xyloplax* among asteroids and ophiuroids in the combined analysis under various analytical conditions is summarized. Although a monophyletic Ophiuroidea is recovered under most conditions, *Xyloplax* was never placed among this class. *Xyloplax* is, however, recovered among the Asteroidea in most of the conditions under which a monophyletic Asteroidea is recovered, including the parameter that yielded the six most congruent topologies (weighted length = 5265; MFES = 1.86) when indels and

**Fig. 4.** Summary of higher taxonomic groups (class and above) recovered under various analytical conditions. The recovery of a monophyletic group is designated by a black square.



transversions were weighted at 2, transitions were weighted at 1, and character data were weighted at 1.

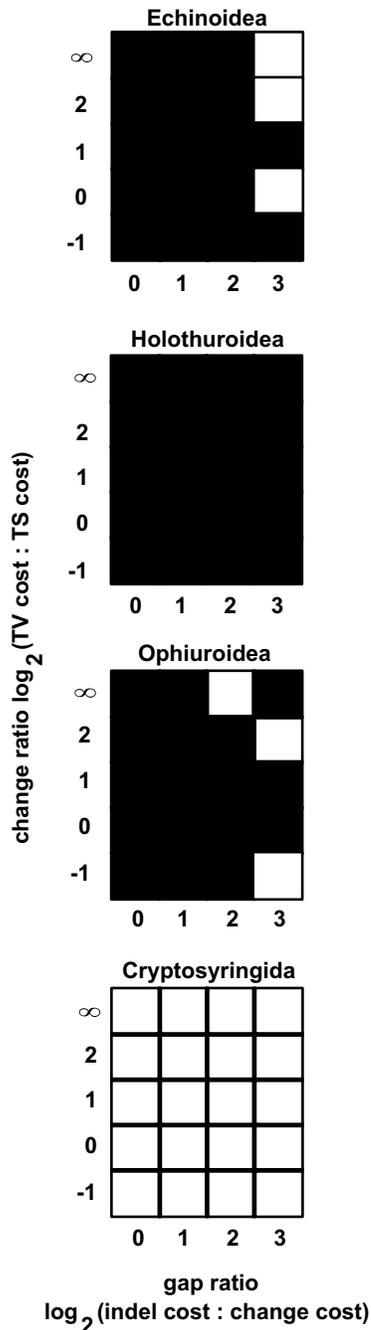
### Discussion

The sensitivity analyses presented have pinpointed areas of weakness in our understanding of echinoderm relationships. Many clades, such as the Echinoidea, Holothuroidea, and Ophiuroidea, are stable despite varying analysis parameters. Furthermore, several clades, including Asteroidea, Hemicordata, Echinodermata, Crinoidea, and Eleutherozoa, were

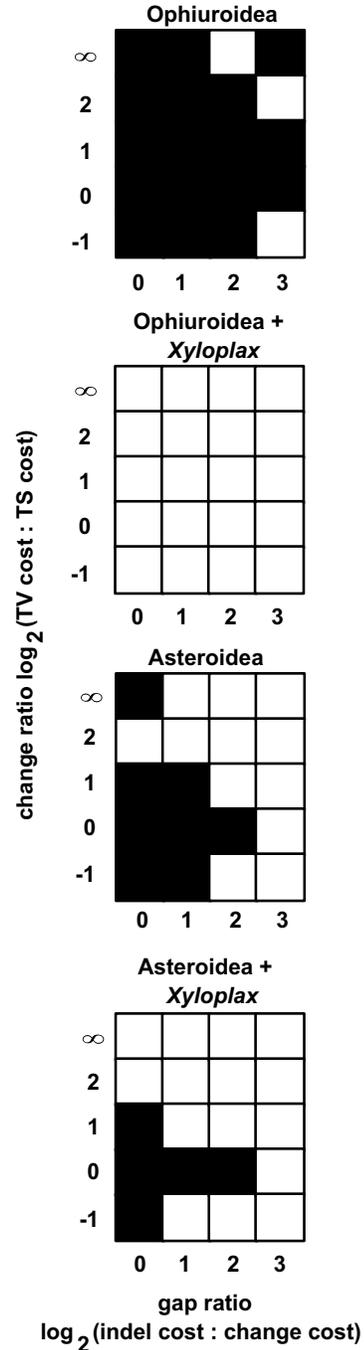
recovered under many analytical conditions. Areas of weakness in our understanding of echinoderms include the comparatively little support available for such groups as Eleutherozoa, Asterozoa, and Echinozoa and the relationship of *Xyloplax* within Asteroidea. These results do not mean that support is nonexistent for these groups, only that much of the available character evidence is equivocal. New data in terms of loci, morphology, and taxonomic sampling are needed.

The above statement is certainly true for stellate forms, whose history is not well understood because it is replete

**Fig. 5.** Summary of groups recovered under various analytical conditions. Recovery of a monophyletic group is indicated by a black square. Although each of the component classes (Echinoidea, Holothuroidea, and Ophiuroidea) of the hypothesized subphylum Cryptosyringida (Smith 1984) was recovered as monophyletic under most analytical conditions, Cryptosyringida is never recovered in the parameter space explored in this paper.



**Fig. 6.** Summary of groups recovered under various analytical conditions. Recovery of a monophyletic group is indicated by a black square. Although monophyletic Ophiuroidea is recovered under many conditions, *Xyloplax* is never among this class. *Xyloplax* is recovered as an asteroid in most of the conditions under which monophyletic Asterozoa is recovered.



with extinctions. Although asteroids originated in the Ordovician Period (510 million years ago (mya)), the subset of lineages of extant starfish that we recognize as modern orders dates only as far back as the Middle Jurassic Period, (between 144 and 208 mya) (Blake 1987). Addressing the entire history of stellate echinoderms, therefore, may be es-

sential for understanding the relationships among extant taxa. A robust and well-corroborated asterozoan phylogeny has not yet been constructed. Despite increased sequencing of loci and taxa, relationships within the stellate forms (starfish and brittle stars) and between stellate forms and other echinoderms remain difficult to recover. Certainly, much of the confusion about echinoderm and asteroid phylogeny is

related to the fact that no single study has yet taken into account all of the rich character systems available. The data upon which studies have been conducted almost certainly represent an undersampling of asteroid diversity in terms of the taxa sampled, the loci sequenced, and the incorporation of morphological or other character data. Thus far, molecular phylogenies have been based on exemplars from only a handful of families that are available in restricted geographic areas (e.g., shallow waters off Europe or Japan). Two nuclear loci (18S and 28S rDNA) have been sequenced from representatives of seven of eight asteroid orders and two mitochondrial loci (12S and 16S rDNA) have been sequenced from five orders. None of these studies have incorporated fossil taxa or co-optimized morphology and sequences. The next important steps in echinoderm and asteroid phylogeny will be to collect fresh tissues and combine data from a wide variety of loci from as many lineages as possible, incorporating fossil taxa and genomic-level characters.

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