

Echinoderm Phylogeny Including *Xyloplax*, a Progenetic Asteroid

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Abstract.—Reconstruction of the phylogeny of the five extant classes of the phylum Echinodermata has proven difficult. Results concerning higher-level taxonomic relationships among echinoderms are sensitive to the choice of analytical parameters and methods. Moreover, the proposal of a putative sixth class based on a small enigmatic disc-shaped echinoderm, *Xyloplax*, from the deep seas of the Bahamas and New Zealand in the 1980s further complicated the problem. Although clearly an echinoderm, *Xyloplax* did not have clear affinity among known groups. Using molecular sequence and developmental data from recently collected *Xyloplax* adults and embryos, we show that rather than representing an ancient distinct lineage as implied by its status as a class, *Xyloplax* is simply a starfish that is closely related to the asteroid family Pterasteridae. Many members of the Pterasteridae and all *Xyloplax* inhabit deep or polar seas and brood young. Brooding pterasterids and *Xyloplax* hold their young in specialized adult chambers until the young reach an advanced juvenile stage after which they are released as free-living individuals. We hypothesize that the unique morphology of *Xyloplax* evolved via progenesis—the truncation of somatic growth at a juvenile body plan but with gonadal growth to maturity. Although the overall phylogeny of extant echinoderms remains sensitive to the choice of analytical methods, the placement of *Xyloplax* as sister to pterasterid asteroids is unequivocal. Based on this, we argue that the proposed class and infraclass status of *Xyloplax* should be suppressed. [Asteroid; Asterozoa; Cryptosyringida; Echinodermata; Echinozoa; Pterasteridae; starfish; *Xyloplax*.]

The body plans of the extant members of the exclusively marine phylum Echinodermata include stalked flower-like sea lilies; ambulatory, stellate starfish and brittle stars; soft-bodied sea cucumbers; spiked, armored, and globose sea urchins; and flat sand dollars. The benthic adult forms of these diverse animals share a water-vascular system in which a single circumoral ring of coelom extends to form five, or occasionally more, radial canals that bear tube feet. Along with a pentaradial water-vascular design, features such as a calcite endoskeleton and mutable collagenous tissue unite the five extant classes of the phylum.

Baker et al. (1986) described the water-vascular system of *Xyloplax medusiformis* as having tube feet borne on dual circumoral rings rather than along radial canals in a pentaradial design. This description challenged the concept of uniform pentaradial water-vascular symmetry among extant echinoderms. *X. medusiformis* was considered so distinct from all other echinoderms that it required a new class of Echinoderm. Baker et al. (1986) and Rowe et al. (1988) created the class Concentricycloidea to contain only *Xyloplax*. Baker et al. (1986) named the class for the small disk shape and the described dual circumoral rings of the water-vascular system. The class-level status of Concentricycloidea rests on early descriptions of adult morphology of two species of *Xyloplax* and the perceived distinction of that morphology from other echinoderm classes (Baker et al. 1986; Rowe et al. 1988; Pearse V. and Pearse J. 1994).

Mah (2006) affixed morphological data from *X. janetae* to a preconstructed tree to place *Xyloplax* as a distinct

infraclass of extant asteroids. He placed *Xyloplax* as a sister taxon to Neoasteroidea, the subclass of all extant asteroids. In contrast, many others have placed *Xyloplax* within various extant asteroid groups based on the scant molecular and morphological character data available (Smith 1988; Belyaev 1990; Janies and McEdward 1994a, 1994b; Janies and Mooi 1998; Mooi et al. 1998; Janies 2001, 2004).

In addition to its taxonomic status as an asteroid, many hypothesize that *Xyloplax* is progenetic (Smith 1988; Janies and McEdward 1994b; Janies and Mooi 1998; Janies 2001). We use the term progenesis sensu Gould (1977) to describe *Xyloplax* because it has a life cycle with truncated somatic development producing a sexually mature organism with an overall juvenile body plan. Although progenesis and its encompassing concept of paedomorphosis are important in developmental and evolutionary theory, only a few natural examples have been studied (Garstang 1922; Gould 1977). Other forms of paedomorphosis, such as neoteny, are not applicable to *Xyloplax*. Neoteny means retention of juvenile structures (e.g., gills in the salamander *Ambystoma mexicanum*) in a sexually mature organism that has an overall adult body plan.

Before this study, molecular phylogenetic investigations of *Xyloplax* have been limited because specimens in the two previous collections were fixed in formalin, precluding extraction and sequencing of large amounts of DNA (Janies and Mooi 1998). In contrast, specimens of *X. janetae* recovered in 2004 in the Northeast Pacific were preserved in 95% ethanol (Voight 2005; Mah 2006)

and included several brooding females and juveniles. These specimens not only allowed us to sequence several genes from *X. janetae* but also provided an unprecedented view of its early development. We use confocal microscopy to illustrate the progenetic ontogeny of young *Xyloplax* in the brood chamber of adult females. We also demonstrate that the water-vascular system of *Xyloplax* has pentaradial symmetry but lacks dual circumoral rings.

In addition, we use a variety of alignment and tree search methods to construct phylogenies from large molecular and morphological character data sets that include exemplars of all echinoderm classes and several deuterostome outgroups. These analyses all show that *Xyloplax* is an asteroid. The best trees from all alignment parameters and tree search methods recover *Xyloplax* as sister to the asteroid family Pterasteridae.

Roadblocks to Reconstructing Echinoderm Phylogeny

Numerous phylogenetic studies of extant echinoderms have been undertaken, but class-level relationships remain controversial (reviewed in Smith et al. 2004). The crinoids, which are mostly sessile, are generally recognized as the sister group to the Eleutherozoa or mobile echinoderms. Eleutherozoa includes all other extant echinoderms: starfish (Asterozoa), sea urchins (Echinozoa), sea cucumbers (Holotheurozoa), and brittle stars (Ophiurozoa). Hypotheses of relationships within Eleutherozoa fall into two categories: the Asterozoa–Echinozoa hypothesis and the Cryptosyringida hypothesis (Fig. 1). The Asterozoa–Echinozoa hypothesis groups taxa based on adult body form (Fig. 1a). Asterozoa refers to the extant stellate forms, the asteroids and ophiuroids (Mooi and David 2000). Echinozoa includes globose forms such as holothuroids and echinoids (Smith 1984). In addition to general body form, similarities in the mouth frame and ambulacral ossicles have been interpreted to support Asterozoa (Mooi and David 2000).

In contrast to the Asterozoa–Echinozoa hypothesis, the Cryptosyringida hypothesis (Fig. 1b) groups ophiuroids, holothuroids, and echinoids based on the shared adult feature of enclosed radial elements of the water-vascular system (Smith 1984; Smith et al. 2004). The topology within Cryptosyringida is not often discussed. Smith (1984) pointed out the lack of morphological evidence for holothuroids and ophiuroids as sister taxa. Cryptosyringida typically implies Echinozoa (Holothuroids plus Echinoids in a clade) with Ophiuroidea sister to Echinozoa. Asteroids are assumed to be the sister group to Cryptosyringida (Fig. 1b; Smith 1984).

Studies attempting to resolve relationships among echinoderm classes using multilocus sequence and morphological data have found very similar optimality scores for trees supporting each of the two major competing hypotheses. Littlewood et al. (1997) narrowly supported the Cryptosyringida hypothesis, whereas Janies (2001) narrowly supported the Asterozoa–Echinozoa hypothesis. Moreover, both stud-

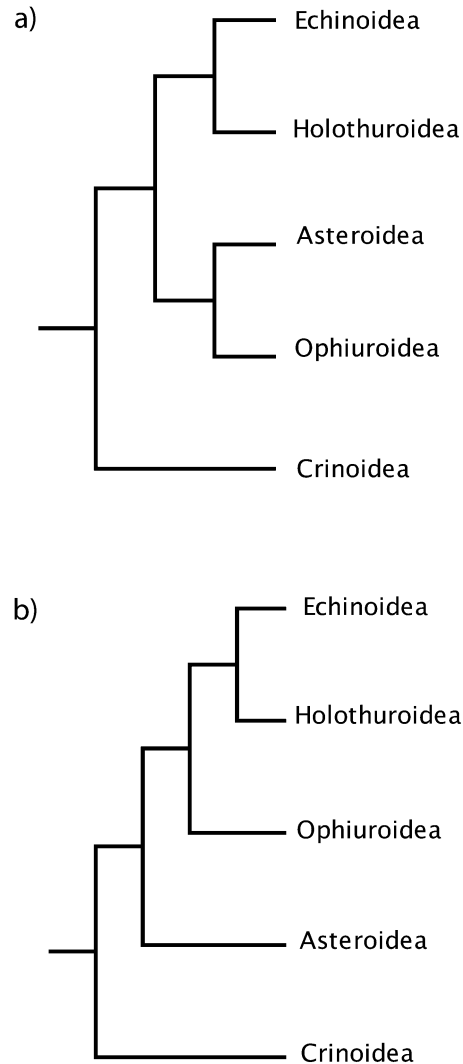


FIGURE 1. Competing hypotheses for the relationships of extant echinoderm classes: a) Asterozoa–Echinozoa hypothesis and b) Cryptosyringida hypothesis.

ies acknowledge near-optimal trees that challenge the best-supported hypotheses. In addition, the loci that have traditionally been used in animal phylogenetics (e.g., ribosomal genes) vary in sequence length among echinoderm classes. When compared with protein-coding loci, these length differences in orthologous ribosomal sequences widen the search space at both the levels of alignment and trees and thus require significant computational time and space to find satisfactory results. Studies of mitochondrial gene order sought to resolve class-level relationships among echinoderms (Smith et al. 1992). However, complex patterns of genomic rearrangements and low taxon sampling have thus far limited their ability to resolve relationships (Scouras et al. 2004). Recent studies of mitochondrial gene order have produced results supporting hypotheses for which there is little corroboration in morphology, development, or nuclear genes (Perseke et al. 2008, 2010).

MATERIALS AND METHODS

Taxon, Genome, and Phenotypic Sampling for Phylogenetic Analyses

We built a data set of 86 echinoderm exemplars and deuterostome outgroups. Vouchers are deposited at the American Museum Culture Collection (AMCC) and the Field Museum of Natural History (FMNH). Data were drawn from 1 urochordate, 4 hemichordates (including 1 pterobranch and 3 enteropneusts), 35 asteroids spanning 16 of 35 families, 12 ophiuroids spanning 8 of 15 families, 3 crinoids spanning 3 of 25 families, 22 echinoids spanning 11 of 45 families, and 9 holothuroids spanning 6 of 23 families. Percent of taxa sequenced for each locus is as follows: 18S: 96.51%, 28S: 52.33%, 16S: 79.07%, 12S: 38.37%, COI: 53.49%, tRNAs: 40.70%, and H3: 37.21%.

Specimens of *X. janetae* were collected in 2004 from experimental deployments of wood at 2675 m depth on Gorda Ridge (Voight 2005). Exemplars of other taxa were collected by dredges or divers. All specimens were preserved in 95% ethanol.

DNA was extracted from most specimens using the Qiagen DNAeasy kit. Polymerase chain reaction (PCR) was performed using Amersham Ready to Go PCR Beads. PCR reactions of 25 μ l final volume included: 2.0 μ l template DNA, 1 PCR bead, 21 μ l RNase free water, 1.0 μ l forward primer, and 1.0 μ l reverse primer. Primer sequences are available at <http://dx.doi.org/10.5061/dryad.1926>. Thermal cycling was conducted at 94°C, 49°C, and 72°C (annealing temperatures adjusted as necessary) for 40 cycles. PCR products were purified with a TeleChem kit. Sequencing was performed using Applied Biosystems Big Dye. Sequencing reactions were cleaned through isopropanol and EtOH precipitation and run on an Applied Biosystems 3700 analyzer. Chromatograms were analyzed and contiguous fragments were assembled in GeneCodes Sequencher.

The extraction, amplification, and DNA sequencing of *X. janetae* were performed in a laboratory in which no echinoderm samples had previously been processed, eliminating the possibility that *Xyloplax* samples were contaminated by DNA from other echinoderms. DNA from *X. janetae* was isolated and amplified using the protocols described above except that PCR and cycle sequencing products were cleaned using a magnetic plate and AmPure and SeqClean (Agencourt), and sequences were run on an Applied Biosystems Prism 3100/3100 analyzer.

Our nucleotide data set contains an average of 7.11 kb of DNA per taxon, including data from seven loci (18S rRNA, 28S rRNA, and histone H3 from the nucleus; 16S rRNA, 12S rRNA, cytochrome *c* oxidase subunit I, tRNA-Ala, tRNA-Leu, and tRNA-Pro of the mitochondrion). We drew from or deposited all nucleotide data in GenBank (<http://www.ncbi.nlm.nih.gov>). Approximately 59% (251 out of 422 GenBank records) of the nucleotide data analyzed are novel observations. We also incorporated 36 morphological characters drawn

from original observations and literature sources (e.g., Littlewood et al. 1997; Janies 2001). Among these characters are the main features that have been considered important in echinoderm evolution. Thirty-five characters were coded with binary states. One character was coded as a multistate. All character transformations were unordered. Accession numbers for sequences and data files in POY3 formats (molecular and morphological) are archived at <http://dx.doi.org/10.5061/dryad.1926>.

Microscopy

Young, in various developmental stages, were dissected from females of *X. janetae* that had been preserved in 95% ethanol (Field Museum of Natural History 12460), transferred to 100% EtOH, and cleared for 1 week in a 2:1 solution of benzyl alcohol to benzyl benzoate. Thus treated, some natural fluorescence is retained, obviating the need for dyes. Optical sections were created with laser light, generated by the 488 nm channel with a 10 \times microscope objective of a Zeiss 510 confocal laser scanning microscope and captured digitally. Light micrographs were captured digitally at 10 \times with a compound microscope.

Direct Optimization Searches

For POY3 (Wheeler 1996; Wheeler et al. 2006) analyses, we constructed data sets of: 1) all molecular data and 2) all molecular and morphological data. Each data set was run as a separate search across the sensitivity analysis parameter space (treated below). The gene coding for the H3 protein was treated as pre-aligned because its alignment was unambiguous. We used the command `-noleading` to avoid counting leading and trailing gaps in final tree length calculations. We created an alignment implied (IA; Wheeler 2003) by the best POY3 tree for each data set using the commands: `-diagnose`, `-impliedalignment`, and `-topofile`. Leading and trailing gaps in the IA were replaced with "?". IAs and resulting trees are archived at <http://dx.doi.org/10.5061/dryad.1926>.

Static Alignment

For each locus, we used progressive pairwise alignment (CLUSTALW; Thompson et al. 1994). We then made data sets of: 1) all molecular data and 2) all molecular and morphological data. To make combined data sets, static alignments for each locus, calculated under the same parameter set, were concatenated into NEXUS files. In data sets combining morphological and molecular data, phenotypic characters were added to NEXUS files. For all static alignments, leading and trailing gaps were replaced with "?". Static alignments and resulting trees are archived at <http://dx.doi.org/10.5061/dryad.1926>.

Sensitivity Analysis

For each method (i.e., direct optimization or static alignment), the edit costs among sequences were varied in a sensitivity analysis (Wheeler 1995). Edit costs for insertion–deletion events were 1 or 2 and the ratio of costs for transitions relative to transversions (ts/tv) was set at 0.5 or 1. This range of variation in edit costs produced a total of four parameter sets for nucleotide data. Results were compared for character congruence using the Mickevitch–Farris Extra Steps (MFES) index (Mickevitch and Farris 1981).

Edit costs for sequence data were set in POY3 using the command `-molecularmatrix`. Edit costs in morphological data were set at 1 using the `-weight` command. We performed CLUSTALW alignments for each locus with the commands `gapopen=`, `gapext=`, and `transweight=`. Tree search programs do not distinguish between an opening gap and an extension gap. Thus, to maintain consistency between treatment of edit costs during both alignment and tree search, the commands `gapopen=` and `gapext=` were set to the same value in CLUSTALW.

Choice of Models for Substitution and Insertion–Deletion Events

For direct optimization, we used a Sankoff matrix to define weighted models under parsimony, treating gaps as a fifth state. For POY3, tree lengths for each locus and combined data sets were compared using the MFES index. The trees resulting from the parameter set that were least incongruent (i.e., lowest MFES) for molecular and morphological data were used for further analysis.

We chose a model for MRBAYES version 3.1.2 (Huelsenbeck and Ronquist 2001) searches on each static alignment using MODELTEST version 3.8 (Posada 2006) and PAUP version 4.0b10 (Swofford 2002) to choose best-fit models of nucleotide substitution based on the Akaike information criterion (Posada and Buckley 2004), ignoring branch lengths. We used MODELTEST on all molecular data as well as on separate partitions of ribosomal data and protein-coding data. We explored two partitioned analyses in MRBAYES. In one analysis, we treated each gene as a partition. In the other analysis, we treated ribosomal genes and protein-coding genes as the partitions.

Tree Search

For each parameter set under direct optimization in POY3, we performed 100 replicates of direct optimization under parsimony and subsequent refinement with the following search commands: `-norandomizeoutgroup`, `-checkslop 5`, `-noleading`, `-random 100`, `-sprmaxtrees 2`, `-tbrmaxtrees 2`, `-fitchtrees`, `-holdmaxtrees 2`, `-buildmaxtrees 2`, `-treefuse`, `-fuselimit 10`, `-fusemingroup 5`, `-fusemaxtrees 10`, `-repintermediate`, `-iterativepass`, and `-exact`. When POY3 found multiple best trees,

each was examined for taxonomic implications and JACK2HEN (<http://research.amnh.org/scicomp/scripts/download.php>) was used to create a strict consensus for presentation.

We analyzed data sets of the static alignments of molecular data and phenotypic data in MRBAYES. For all runs, we used a general time-reversible model with gamma-distributed rate variation across sites and a proportion of invariable sites as indicated by MODELTEST. The MRBAYES runs in the sensitivity analysis consisted of 1,000,000 Markov chain Monte Carlo (MCMC) generations with a sampling frequency of every 1000th generation and a burn-in of 250 generations. The MRBAYES runs producing the maximum a posteriori (MAP) tree with the best likelihood scores were rerun with 10,000,000 MCMC generations with sampling frequency of every 1000th generation and a burn-in of 2500 generations. To assure that chains had converged, potential scale reduction factor ~ 1.0 was used as implemented with the `sump` command in MRBAYES. In MRBAYES analyses, the 50% majority rule consensus tree (CON) and MAP tree were both evaluated for taxonomic implications.

Support

POY3 views whole sequence fragments as characters. Although there are an average of ~ 7100 nucleotides per taxon in our data set, these are only 19 fragments—this limits the number of pseudoreplicates. Thus, for resampling searches, we used the IA in TNT with the “new technology” setting for tree search heuristics and “absolute frequencies” setting for bootstrap results (Goloboff et al. 2008). We also used the IA for MRBAYES analyses. For the CLUSTALW–MRBAYES and IA–MRBAYES analyses, we used the posterior probabilities on the CON trees to assess support.

RESULTS

Summary of Taxonomic Results

Regardless of alignment parameters, analytical methods, or whether molecular data are analyzed alone or in conjunction with morphological data, *Xyloplax* is closely related to the pterasterid asteroids. In this section, we summarize taxonomic results for best trees (Table 1). Sensitivity analyses, scores for unpartitioned analyses, and support values are provided in subsequent sections.

The best direct optimization trees (POY3) for combined molecular and morphological data (Fig. 2) and molecular data alone (Fig. 3) support the Cryptosyringida hypothesis. However, the arrangement within cryptosyringids does not include Echinozoa. Rather, we find support for a clade containing holothuroids and ophiuroids with the echinoids sister to that clade.

The MAP and CON from MRBAYES for the IA of the combined molecular and morphological data (Fig. 4) and the molecular data alone (Fig. 5) also support

TABLE 1. A summary of Echinoderm relationships as determined using various methods and data sets

Data type	Method	Echinoderm relationships	Topology of the Cryptosyringida
Molecular	DO ^a	Cryptosyringida	(OH)E ^b
Molecular	IA-MB ^c	Cryptosyringida	(EH)O ^d
Molecular	CL-MB ^e	Asterozoa	Not found
Molecular and morphological	DO	Cryptosyringida	(OH)E
Molecular and morphological	IA-MB	Cryptosyringida	(EH)O
Molecular and morphological	CL-MB	Asterozoa	Not found

Notes: *Xyloplax* is sister to Pterasteridae in each case.

^aDO = Direct optimization in POY3 (POY3 is a program for analysis of sequence data under dynamic homology; Wheeler 1996; Wheeler et al. 2006).

^b(OH)E = Ophiuroidea + Holothuroidea as a clade sister to Echinoidea.

^cIA-MB = Implied alignment (implied alignment creates a synapomorphy scheme akin to multiple sequence alignment based on a tree resulting from DO search; Wheeler 2003) by POY3 with tree search in MRBAYES (MRBAYES is a program for analysis of prealigned sequence data; Huelsenbeck and Ronquist 2001).

^d(EH)O = Echinoidea + Holothuroidea as a clade sister to Ophiuroidea.

^eCL-MB = Progressive pairwise sequence alignment by CLUSTALW (Thompson et al. 1994) followed by tree search in MRBAYES.

the Cryptosyringida hypothesis and *Xyloplax* as sister to the pterasterid asteroids. However, within Cryptosyringida, both IA and MRBAYES analyses find a more traditional Echinozoa (Holothuroidea plus Echinoidea; sensu; Smith 1984). Ophiuroidea is sister to the Echinozoa.

The MAP and CON trees from static alignment (CLUSTALW) followed by MRBAYES analysis for combined molecular with morphological (Fig. 6) and molecular data alone (Fig. 7) support the Asterozoa hypothesis and *Xyloplax* as sister to the pterasterid asteroids.

Sensitivity of Taxonomic Results to Alignment Parameter and Tree Search Variation

Under direct optimization (or resulting IA plus MRBAYES tree search) over all parameters investigated, *Xyloplax* is consistently recovered as sister to pterasterid asteroids. In addition, the five classes of echinoderms and Cryptosyringida are consistently found to be monophyletic.

CLUSTALW-MRBAYES runs are also consistent in their placement of *X. janetae* as sister to Pterasteridae and the recovery of the five classes of echinoderms and Asteroidea as monophyletic. However, other groups are more labile across parameter space under CLUSTALW-MRBAYES. In some cases, Cryptosyringida is not recovered or the consensus tree indicated that Cryptosyringida was unresolved. Asterozoa is recovered only under some conditions. Echinozoa is recovered consistently.

Partitioning schemes made no difference in taxonomic results from MRBAYES for any alignment.

Support and Scores for the Best Trees for Molecular and Morphological Data

Direct optimization.—For analyses conducted with POY3 on molecular plus morphological data, the lowest MFES (0.01303) was found at a gap cost of 1 and a ts/tv ratio of

0.5. Searches under this parameter set using the `-exact` command hit a minimum length of 19,834, producing eight unique trees (Fig. 2). The bootstrap values are 100% for all major groups of interest to the main hypothesis including *Xyloplax* plus Pterasteridae, Hemichordata, Echinodermata, Eleutherozoa, Cryptosyringida, Ophiuroidea plus Holothuroidea, Crinoidea, Asteroidea, Ophiuroidea, Holothuroidea, and Echinoidea (Fig. 2).

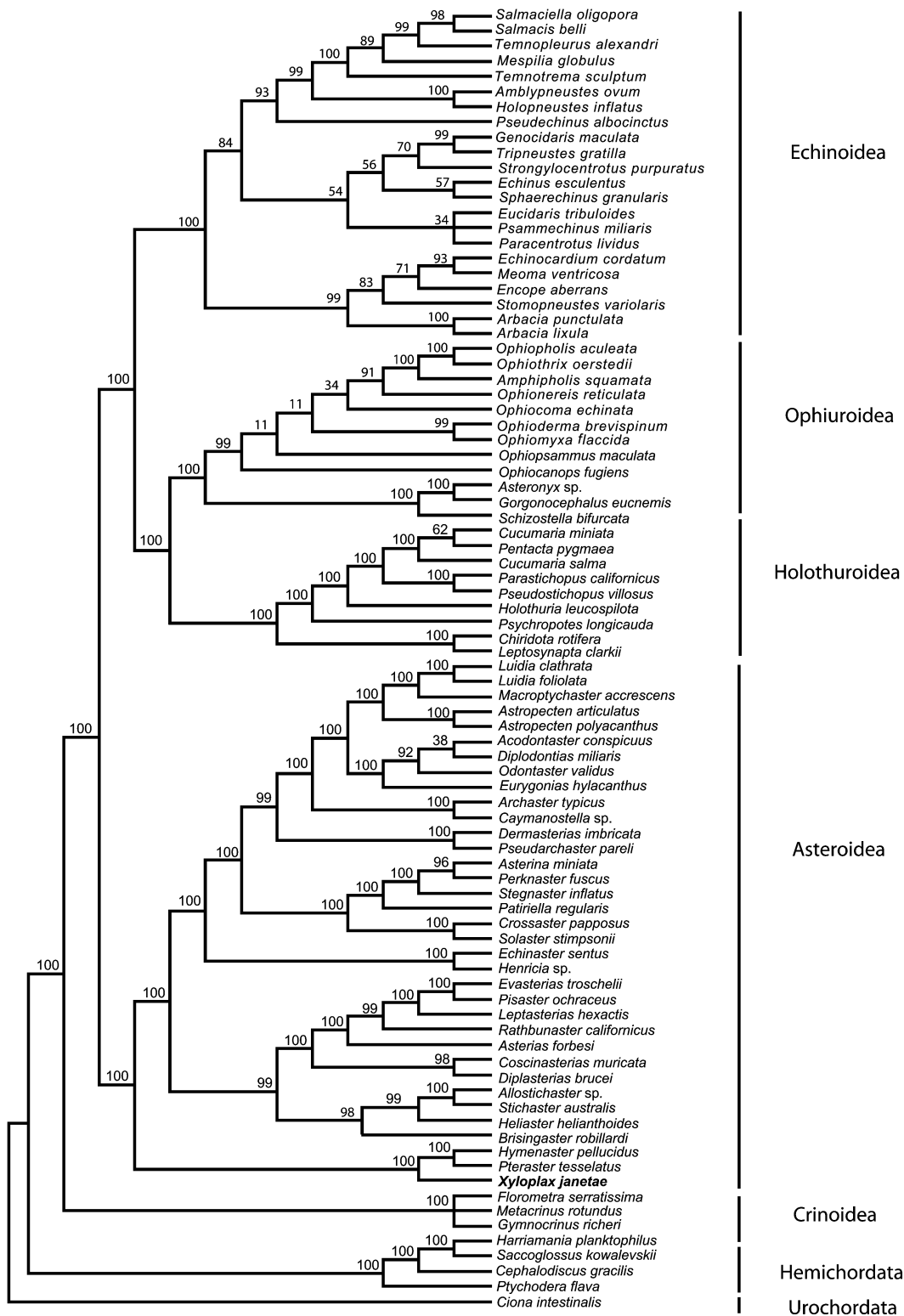
The MAP from MRBAYES analysis of the IA had a likelihood score of $-60,730.982$. The CON tree is presented in Fig. 4. Posterior probabilities in the CON are 1.0 for clades of interest, including *Xyloplax* plus Pterasteridae, Hemichordata, Echinodermata, Eleutherozoa, Cryptosyringida, Echinozoa, Crinoidea, Asteroidea, Ophiuroidea, Holothuroidea, and Echinoidea.

Static alignment.—For CLUSTALW alignment and MRBAYES tree search of molecular data combined with morphological data, the MAP tree (likelihood score of $\ln -60,721.81$) is found when the alignment parameters are set at a gap cost of 1 and a ts/tv ratio of 0.5. These edit cost parameters are the same as those associated with the best POY3 score for these data. The CON tree for this analysis is presented in Fig. 6. The posterior probabilities in the CON for major groups are 0.99–1.0 except for Eleutherozoa at 0.87 and Asterozoa at 0.85.

Support and Details for the Best Trees for Molecular Data

Direct optimization.—For analyses conducted with POY3 on molecular data alone the lowest MFES (0.02985) is found at a gap cost of 1 and a ts/tv ratio of 1. Searches under this parameter set using the `-exact` command analysis hit a minimum length of 16,295, producing one tree (Fig. 3). The bootstrap values for all major groups of interest to the main hypotheses ranged from 99% to 100%.

The MAP from MRBAYES analysis IA of the molecular data had a likelihood score of -66719.434 . The



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FIGURE 2. A phylogeny of extant echinoderms based on direct optimization of nuclear sequence data (18S rDNA, 28S rDNA, and histone H3), mitochondrial sequence data (S12 rDNA, S16 rDNA, tRNA cluster, and cytochrome c oxidase I), and character data for morphological features. Bootstrap values are placed next to each clade.

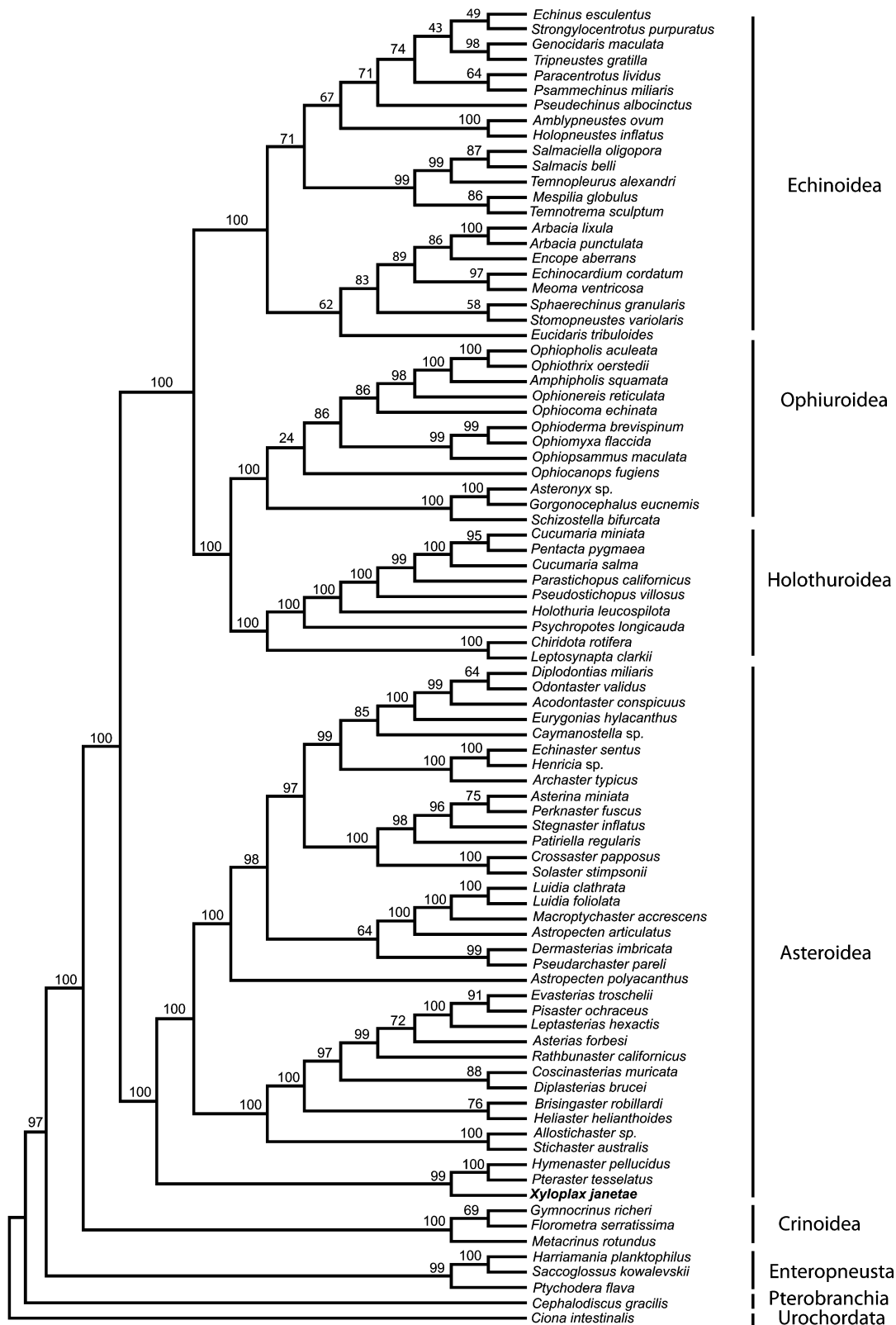


FIGURE 3. A phylogeny of extant echinoderms based on direct optimization of nuclear sequence data (18S rDNA, 28S rDNA, and histone H3) and mitochondrial sequence data (S12 rDNA, S16 rDNA, tRNA cluster, and cytochrome c oxidase I). Bootstrap values are placed next to each clade.

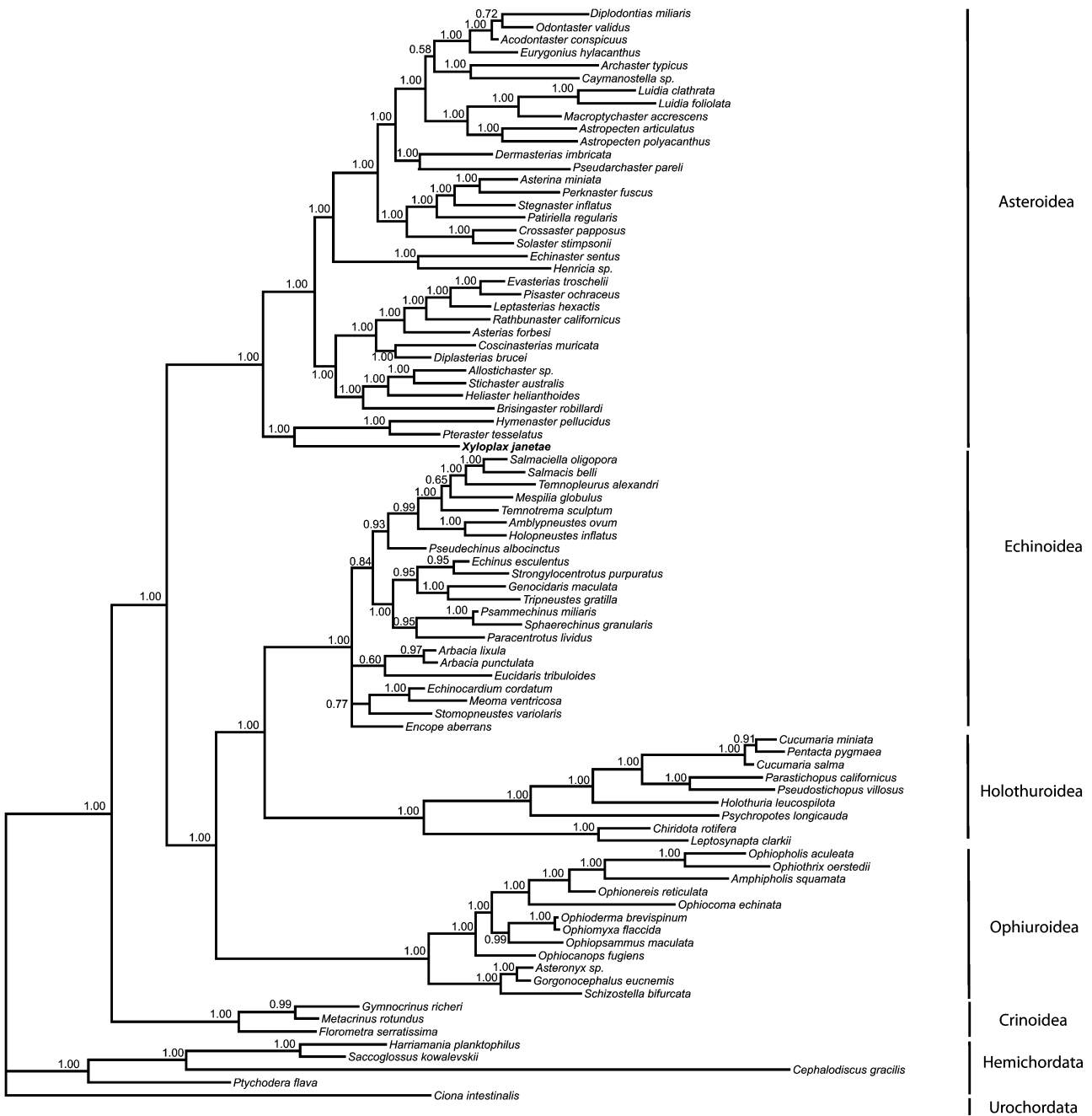


FIGURE 4. A 50% majority rule tree of extant echinoderms based on implied alignment followed by MRBAYES tree search of nuclear sequence data (18S rDNA, 28S rDNA, and histone H3), mitochondrial sequence data (S12 rDNA, S16 rDNA, tRNA cluster, and cytochrome c oxidase I), and character data for morphological features. The posterior probabilities are shown for each clade.

CON for this analysis is shown in Fig. 5. Posterior probabilities in the CON tree for major groups of interest to the hypotheses of the paper are 1.0.

groups of interest to the hypotheses of the paper are 0.98 to 1.0 except for Eleutherozoa at 0.77 and Asterozoa at 0.73.

Static alignment.—In this analysis of molecular data, the alignment parameters for CLUSTALW were set at a gap cost of 1 and a ts/tv ratio of 0.5. The MAP had a likelihood score of $-60,372.177$. The CON tree is shown in Fig. 7. Posterior probabilities in the CON for major

Microscopy

Embryos of *X. janetae* at $\sim 100 \mu\text{m}$ in diameter are bilaterally symmetric and have a juvenile rudiment that is oriented sagittal to the anterior posterior axis (Figs. 8a,b). When embryos of *Xyloplax* reach $\sim 200 \mu\text{m}$

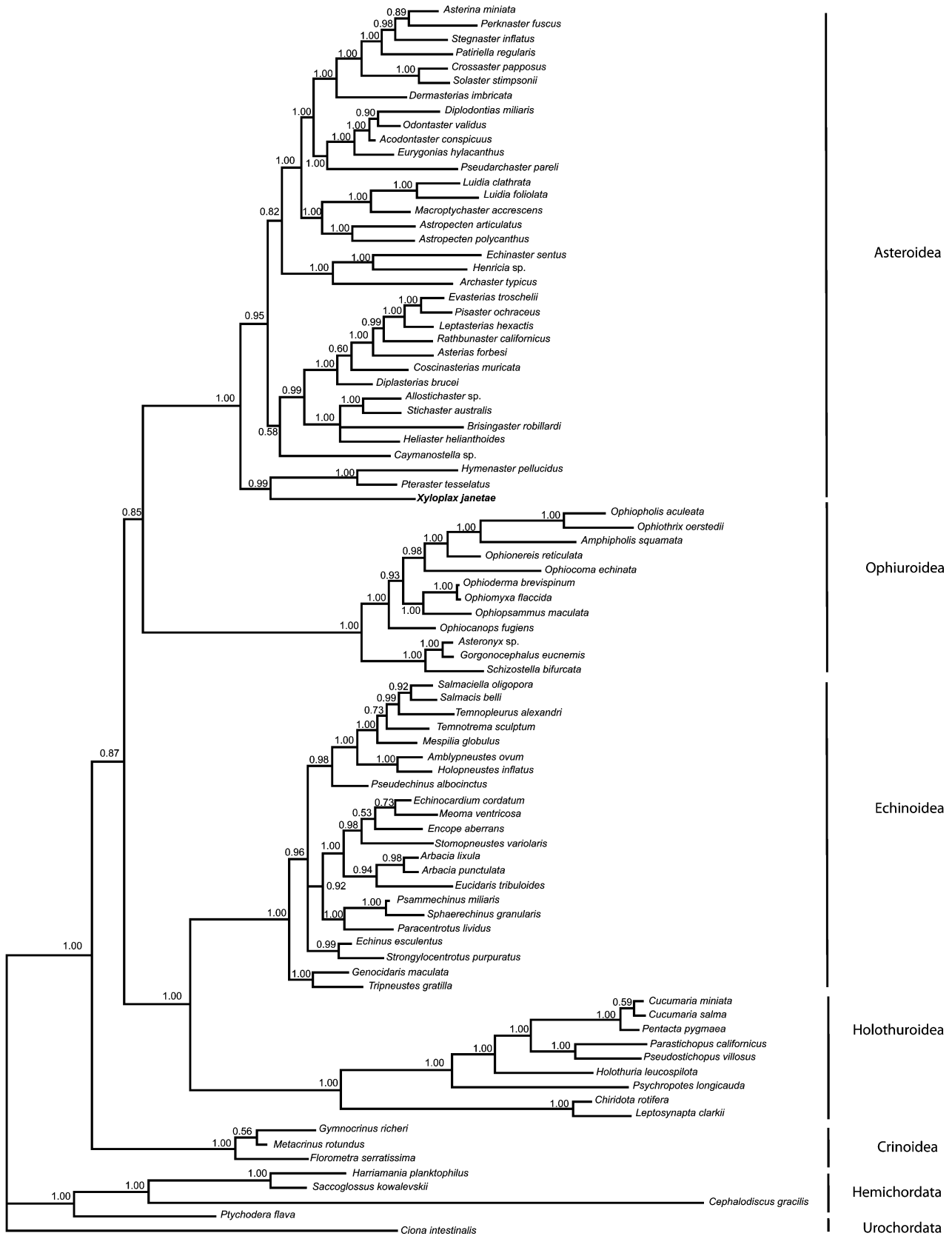
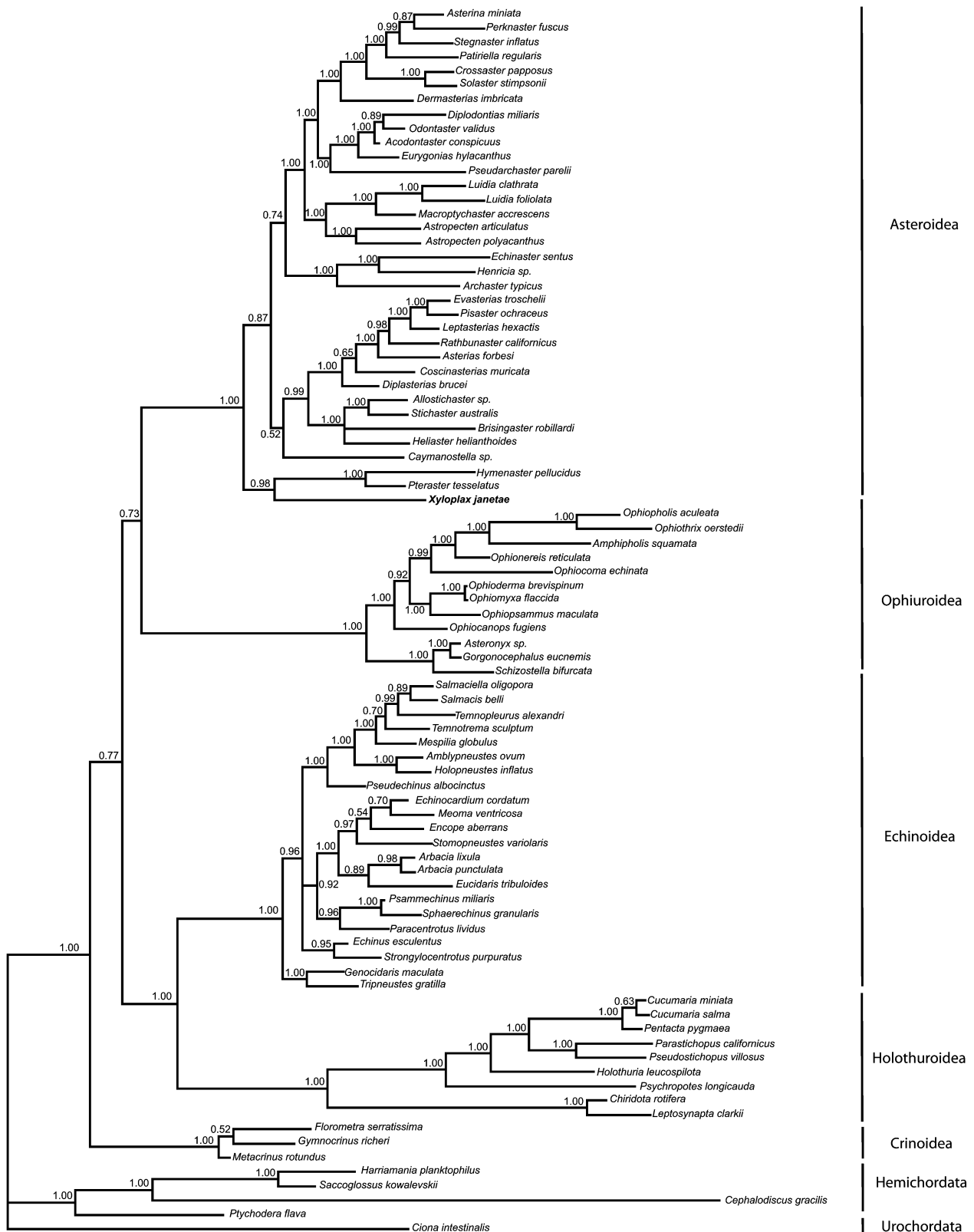


FIGURE 6. A 50% majority rule tree of extant echinoderms based on static alignment followed by MRBAYES tree search of nuclear sequence data (18S rDNA, 28S rDNA, and histone H3), mitochondrial sequence data (S12 rDNA, S16 rDNA, tRNA cluster, and cytochrome *c* oxidase I), and character data for morphological features. The posterior probabilities are shown for each clade.



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FIGURE 7. A 50% majority rule tree of extant echinoderms based on static alignment followed by MRBAYES tree search of nuclear sequence data (18S rDNA, 28S rDNA, and histone H3) and mitochondrial sequence data (S12 rDNA, S16 rDNA, tRNA cluster, and cytochrome c oxidase I). The posterior probabilities are shown for each clade.

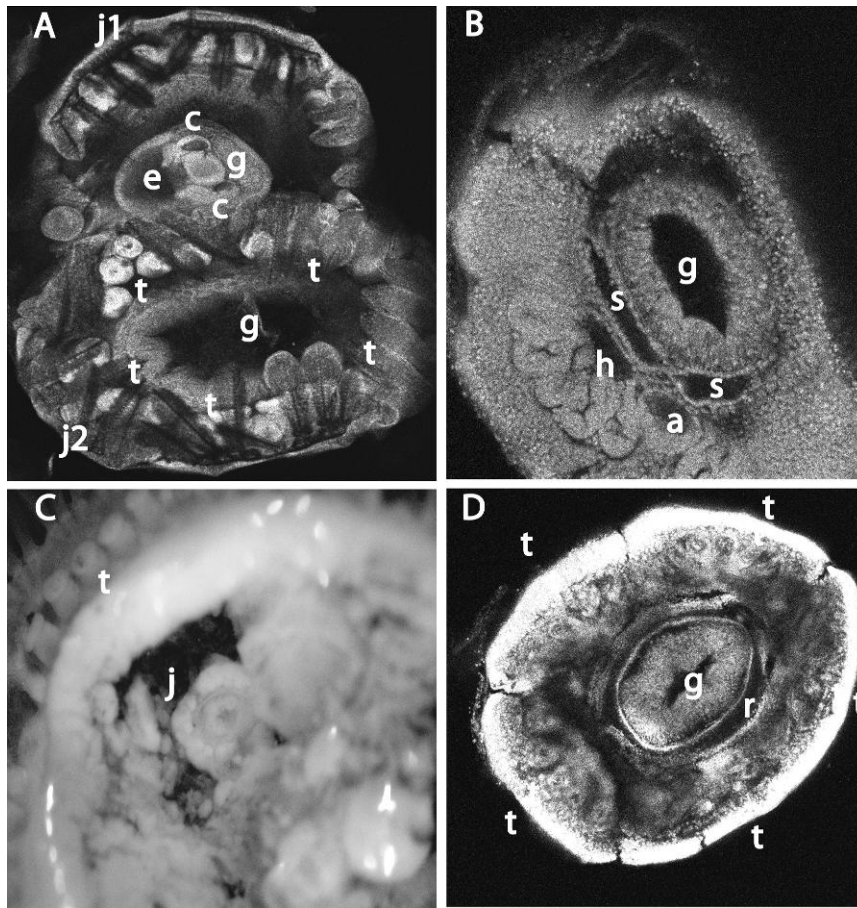


FIGURE 8. Internal and external anatomy of *Xyloplax janetae* (FMNH 12460). a) Optical section of three young of *X. janetae* in various stages of development removed from a brood of an adult. The embryo (e) in the middle of the frame shows the bilateral symmetry of the coeloms (c). The other young (j1 and j2) at the top and bottom of the frame are late-stage juveniles showing clusters of tube feet (t) in pentaradial symmetry around the gut (g). b) Optical section of an embryo of *X. janetae*, transverse to the anterior posterior axis of the embryo, showing the hydrocoel (h) in a sagittal orientation in the embryo and the gut (g). Also visible are the axocoel (a) and somatocoels (s). c) Light micrograph of partially dissected adult *X. janetae* showing tube feet (t) in the circular symmetry of the adult. A brooded juvenile (j) is also visible. d) Optical section of juvenile *X. janetae* showing internal anatomy of water-vascular system and gut. Five clusters of tube feet (t) forming in pentaradial symmetry around the single circumoral ring (r) and gut (g) are visible.

in diameter they undergo a truncated metamorphosis. At this stage, embryos begin to take on the terminal body plan of juvenile asteroids (Fig. 8c). The juvenile rudiment encircles the gut and fuses to form a single circumoral water-vascular ring (Fig. 8d) while clusters of tube feet begin to grow radially (Fig. 8d), only later growing along the meridian of the adult *Xyloplax* (Figs. 8c, 9a). This last step in water-vascular development is the refinement of the body of the young *Xyloplax* to the ultimate disk-shaped form (Fig. 9). Concomitant skeletogenesis is described in Mooi et al. (1998).

DISCUSSION

Irrespective of method or data sampling scheme, phylogenetic analyses of large and diverse data sets place *Xyloplax* as sister to pterasterids among asteroids with high support values. *Xyloplax* is an asteroid—thus resolving the question of whether there are five or six

classes of extant echinoderms. We also resolve questions on water-vascular anatomy and developmental evolution of *Xyloplax* based on confocal microscopy of embryos. However, results for the relationships among echinoderm classes are dependent on choice of parameters and methods.

Water-Vascular Anatomy and Developmental Evolution of Xyloplax

New embryological data from brooded embryos of *X. janetae* (Fig. 8) further support the hypothesis that *Xyloplax* is a progenetic asteroid (Smith 1988; Janies and McEdward 1994a). The coelomogenesis of *Xyloplax* appears typical of asteroids. These observations offer evidence that a juvenile asteroid body plan, but not an adult asteroid body plan, is achieved in *Xyloplax*. Thus, no pattern describes the developmental evolution of *Xyloplax* better than progenesis.

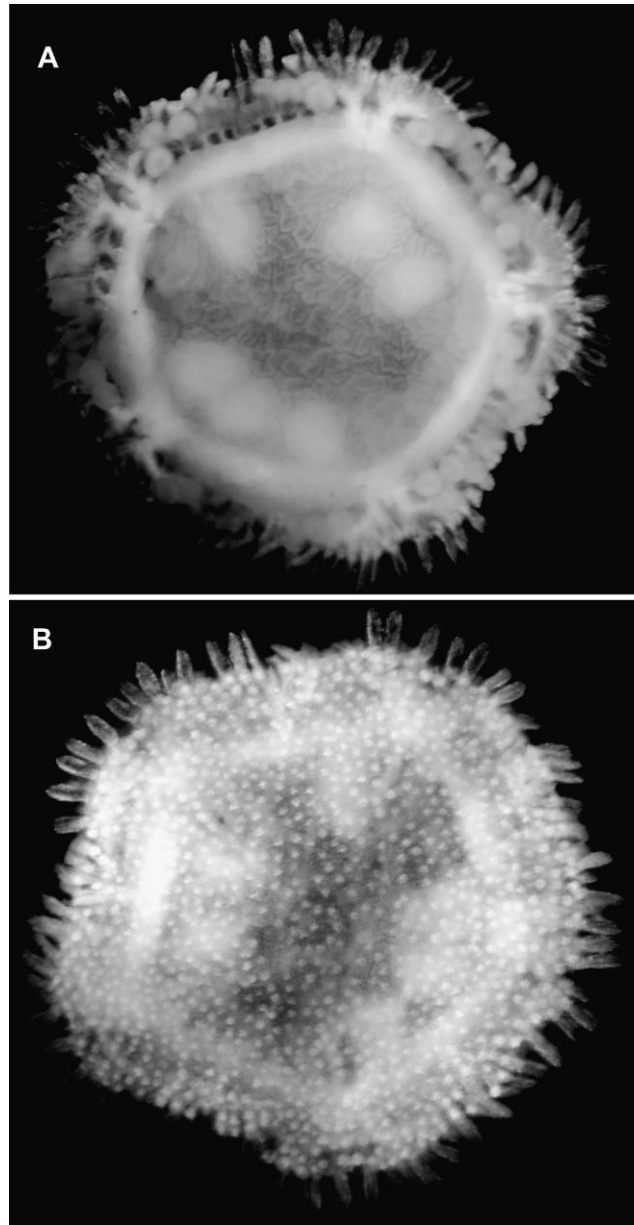


FIGURE 9. Light micrographs of specimens of *Xyloplax* sp. a) Oral view (about 4 mm in diameter). b) Aboral view (about 4 mm in diameter). These specimens were collected in 2010 at a depth of 2202 m along the Juan de Fuca ridge in the Eastern Pacific. These are females that show brooded embryos. (Images courtesy of Ben Grupe, Scripps Institution of Oceanography, University of California, San Diego.)

Our confocal results for soft embryological anatomy and the observations on skeletal anatomy in Mooi et al. (1998) refute the model of coelomogenesis for *Xyloplax* predicted in Janies and McEdward (1994b). This prediction was based on the early description of dual circumoral rings (Baker et al. 1986; Rowe et al. 1988), which we can now refute by observation of newly discovered embryos. This being said, the refutations of the descriptions and prediction do not alter the systematic conclusions of this paper. The ontogenetic results provided herein uncover even more diversity of coelom formation among the clade formed by Pterasteridae and

Xyloplax than previously observed (Janies 1995) and add more asteroid features to what we know about *Xyloplax*.

Taxonomic Recommendations

As *Xyloplax* is nested within asteroids under a variety of optimality criteria and under a wide alignment parameter space, we consider the class Concentricycloidea to be a synonym of class Asteroidea. Similarly, these results contradict the concept of an Infraclass Concentricycloidea (erected by Mah in 2006 for *Xyloplax*) as a sister taxon to Neoasteroidea.

TABLE 2. *Continued*

Phylum	Subphylum	Class	Order	Family	Genus	Species	Voucher	18S rDNA	28S rDNA	16S rDNA	12S rDNA	COI DNA	tRNAs rDNA	H3 DNA
	Asterinidae			<i>Asterina</i>	<i>miniata</i>	AMCC 113402	DQ060777	DQ060004	DQ297074	DQ273727	U50056	U50056	DQ676897	
				<i>Patiriella</i>	<i>regularis</i>	AMCC 113390	DQ060811	DQ273717 and DQ297115	AY370734	DQ273733	U50045	U50045	DQ676925	
				<i>Stegnaster</i>	<i>inflatus</i>	AMCC 113381	DQ060820	DQ273724 and DQ297116	AY370719	DQ273734		DQ377821	DQ676931	
	Caymanostellidae			<i>Caymanostella</i>	sp.	FMNH 5167			DQ297082					
	Astropectinidae			<i>Astropecten</i>	<i>artificialatus</i>	AMCC 113394	DQ060781	AF088827 and DQ273699	DQ297079	DQ273741	DQ380242	DQ377823	DQ676901	
				<i>Astropecten</i>	<i>polyacanthus</i>	AMCC 113389	DQ060782	DQ060007	DQ297080	DQ273735			DQ676902	
				<i>Macroptychaster</i>	<i>accrescens</i>	AMCC 113410	DQ060797		DQ297098	DQ273742		DQ377822	DQ676915	
	Luidiidae			<i>Luidia</i>	<i>clathrata</i>	AMCC 113393	DQ060795		DQ297096	DQ273743	AF217377	AF217327 and AF217331	DQ676913	
				<i>Luidia</i>	<i>foliolata</i>	AMCC 113401	DQ060796	AF088828 and DQ273702	DQ297097	DQ273744	DQ380243	AF217336	DQ676914	
	Solasteridae			<i>Solaster</i>	<i>stimpsoni</i>	AMCC 113400	DQ060819	DQ060034	DQ297113	DQ273726	AF217382	AF217341	DQ676930	
				<i>Crossaster</i>	<i>papposus</i>	AMCC 113349	AB084556	AJ225842 and AF088830	D63731 and DQ297084	D63730 and DQ273725	AF217383	AF217342	DQ676904	
	Pterasteridae			<i>Hymenaster</i>	<i>pellucidus</i>	AMCC 113310	DQ060792	DQ297131 and DQ029081		DQ273745				
				<i>Pteraster</i>	<i>tesselatus</i>	AMCC 113396	DQ060817	DQ273722 and DQ060016	DQ297111		AF217373	AF217323	DQ676928	
				<i>Xyloplax</i>	<i>janetae</i>	FMNH 12460	DQ380245	DQ380246 and DQ380247	DQ777080	DQ377828	DQ377828			
	Echinasteridae			<i>Echinaster</i>	<i>sentus</i>	AMCC 114323	DQ060786	DQ060012	DQ297088	DQ273746	AF217376	AF217326 and DQ377807	DQ676907	
				<i>Henricia</i>	sp.	AMCC 113385	DQ060791	DQ273707 and DQ297118	DQ297093	DQ273728			DQ676911	
	Ophiuroidea			Ophiurina	Ophiurina		DQ060803	DQ060015 and DQ273710	DQ297103				DQ676920	
				Ophiodermatidae	Ophioderma		DQ297125 and DQ060021							

Continued

TABLE 2. *Continued*

Phylum	Subphylum	Class	Order	Family	Genus	Species	Voucher	18S rDNA	28S rDNA	16S rDNA	12S rDNA	COI DNA	rRNAs rDNA	H3 DNA
					<i>Ophiopsammus</i>	<i>maculata</i>	AMCC 114319	DQ060807	DQ029076 and DQ273714 and DQ297126	DQ297106				DQ676923
				Amphiuridae	<i>Ophiomeris</i>	<i>reticulata</i>		DQ060805	DQ029075 and DQ273712 and DQ297129	DQ297108				
					<i>Amphipholis</i>	<i>squamata</i>		X97156	DQ060023					
				Ophiomyxina	<i>Ophiomyxa</i>	<i>flaccida</i>		DQ060804	AJ25848 and DQ029077 and DQ273711 and DQ297123	DQ297104				DQ676921
					<i>Gorgonocephalus</i>	<i>eucnemis</i>		DQ060790	DQ060022 and DQ029072 and AF088835 and DQ297119	DQ297092				DQ676910
					<i>Schizostella</i>	<i>bifurcata</i>		DQ060818	DQ060014 and DQ029073 and DQ029074 and DQ273723 and DQ297120	DQ297112				DQ676929
					<i>Asteromyx</i>	sp.	AMCC 113420	DQ060809	DQ060033 and DQ029071 and DQ273716 and DQ060005	DQ297107				DQ676924
				Gnathophiurina	<i>Ophiocoma</i>	<i>echinata</i>		DQ060802	DQ029074 and DQ273709 and DQ297122 and DQ060020	DQ297102				DQ676919

Continued

TABLE 2. Continued

Phylum	Subphylum	Class	Order	Family	Genus	Species	Voucher	18S rDNA	28S rDNA	16S rDNA	12S rDNA	COI DNA	tRNAs rDNA	H3 DNA	
				Ophiactidae	<i>Ophiopholis</i>	<i>aculeata</i>		DQ060806	DQ29078 and AF088826 and DQ273713	U60198 and DQ297105	AF314589	AF314589	564538	DQ676922	
				Ophiotrichidae	<i>Ophiotrix</i>	<i>oerstadii</i>		DQ060808	DQ060024 and DQ029079 and DQ273715 and DQ297127 and DQ297128 and DQ060026						
			Oegophiuridea	Ophiocanopidae	<i>Ophiocanops</i>	<i>fugiens</i>	AMCC	Z80954	DQ060026						
			Apodida	Chiridotidae	<i>Chiridota</i>	<i>rotifera</i>	113293	DQ777081	DQ777090						
				Synaptidae	<i>Leptosynapta</i>	<i>clarkii</i>	AMCC	DQ777083	DQ777089						
			Aspidochirotida	Stichopodidae	<i>Parastichopus</i>	<i>californicus</i>	AMCC	DQ777084	DQ777087	DQ777096					
					<i>Pseudostichopus</i>	<i>villosus</i>	113282	DQ777086	DQ777088	DQ777098					
			Elaeipodida	Psychropotidae	<i>Psychropotes</i>	<i>longicauda</i>	AMCC	Z80956	DQ777094	DQ777099					
			Aspidochirotida	Holothuriidae	<i>Holothuria</i>	<i>leucospilota</i>	113297	AY133472	DQ777093	AY338419	DQ777101				
			Dendrochirotida	Cucumariidae	<i>Cucumaria</i>	<i>miniata</i>	113269	DQ777082	DQ777092	AY182376					
					<i>Cucumaria</i>	<i>salma</i>	113300	AF212170							
					<i>Pentacta</i>	<i>pigmaea</i>	AMCC	DQ777085	DQ777091	DQ777097	DQ777100				
			Echinoidea	Genocidaridae	<i>Genocidaris</i>	<i>maculata</i>	AMCC	AF279199		AF279148		AF279174			
				Strongylocentrotidae	<i>Strongylocentrotus</i>	<i>purpuratus</i>	113256	L28055	AF212171	X12631	X12631	X12631	X12631	NM_214547	
				Echinidae	<i>Echinus</i>	<i>esculentus</i>		Z37125	AJ225814			U21669			
					<i>Psammochinus</i>	<i>miliaris</i>		AF279215	AJ225813	AF279170	AF279196	AY221004	AY221021		
			Phymosomatoida	Stomechinidae	<i>Paracentrotus</i>	<i>lividus</i>		AY428816	DQ073766	AF279169		AF279195			
			Clypeasteroida	Mellitidae	<i>Stomopneustes</i>	<i>variolaris</i>		AF279214	DQ073773						
			Spatangoida	Brissidae	<i>Encype</i>	<i>aberrans</i>		Z37126	Z37117						
				Loveniidae	<i>Meoma</i>	<i>ventricosa</i>		Z37129	AJ639796	AJ639820		AJ639919			
			Cidaroida	Cidaridae	<i>Echinocardium</i>	<i>cordatum</i>		Z37123	AJ225812	DQ073742					
			Arbacoidea	Arbaciidae	<i>Eucidaris</i>	<i>tribuloides</i>		Z37127							
					<i>Arbacia</i>	<i>punctulata</i>		M20050	AY026367	DQ073733					
			Tennopleuroidea	Tennopleuridae	<i>Arbacia</i>	<i>livula</i>		Z37514	DQ073753	X80396	X80396	X80396	X53726		
					<i>Holopneustes</i>	<i>inflatus</i>		AF279209		AF279161		AF279187			
					<i>Amblypneustes</i>	<i>ocum</i>		AF279207		AF279157		AF279183			
					<i>Salmacis</i>	<i>belli</i>		AF279213		AF279167		AF279193			
					<i>Salmaciella</i>	<i>oligopora</i>		AF279211		AF279163		AF279189			
					<i>Tennopleurus</i>	<i>alexandri</i>		AF279206		AF279156		AF279182			
					<i>Tennotrema</i>	<i>sculptum</i>		AF279201		AF279150		AF279176			
					<i>Mespilia</i>	<i>globulus</i>		AF279203		AF279152		AF279178			
					<i>Pseudoechinus</i>	<i>albocinctus</i>		AF279204		AF279153		AF279179			
					<i>Tripaneustes</i>	<i>gratilla</i>		Z37134		AB154279		AY205373			
					<i>Sphaerechinus</i>	<i>granularis</i>		Z37132	AJ225817	DQ073749		AY183329			

At lower taxonomic levels, our results suggest that *Xyloplax* is sister to or within Pterasteridae. Belyaev (1990) and Smith (1988) suggested alternative familial classifications for *Xyloplax*. Belyaev (1990) proposed that *Xyloplax* was closely related to *Caymanostella*, another wood-dwelling deep-sea asteroid. Smith's proposal to group *Xyloplax* among Velatida was based on taxonomy of Blake (1987). In Smith (1984) and Blake (1987), Myxasteridae, Korethrasteridae, Caymanostellidae, Pythonasteridae, and Solasteridae were united with Pterasteridae in the order Velatida. Our analyses show that Solasteridae and Caymanostellidae are not closely related to Pterasteridae or *Xyloplax*. The other taxa in Blake's (1987) Velatida live in abyssal seas and are as rare as *Xyloplax*. As such, collection of more data for these taxa will take some time and luck. However, because we do not yet have data from Myxasteridae, Korethrasteridae, or Pythonasteridae, we cannot test a relationship between any of these families and *Xyloplax*.

Results for Relationships among the Five Classes are Dependent on Methods

Despite our confidence in identifying *Xyloplax* as a progenetic asteroid closely related to pterasterids, unraveling the relationships among extant classes of echinoderms remains difficult. Results of analyses of echinoderm-wide multilocus data sets are very sensitive to the choice of alignment and tree search parameters and methods (Table 2). We expect these results to be updated with new data. One reason is the plummeting cost of nucleotide sequencing which will soon provide very large data sets for extant echinoderms and outgroups. Moreover, the history of echinoderms is replete with extinction events: The phylum includes 21 classes of which the extant echinoderms are but a small fraction. Thus, another avenue to address the problem of relationships among major lineages of extant echinoderms is to incorporate fossil taxa. For example, a contentious issue is the position of Crinoidea and other stemmed echinoderms such as extinct Blastozoa. Crinoidea may be sister to the Eleutherozoa as many assume (David et al. 2000) or nested within stemmed forms, such as the Blastozoa (Sumrall 1997; Ausich 1998). If crinoids are nested within Blastozoa, the sister group of Eleutherozoa remains unknown and must be searched for among fossil lineages (Sumrall and Zamora 2010; Zhao et al. 2010). A concerted tree-of-life scale effort, bringing together paleontological, morphological, developmental, genomic, and analytical disciplines, is required to better understand the major radiations within Echinodermata.

SUPPLEMENTARY MATERIAL

Supplementary material, including data files and/or online-only appendices, can be found at <http://dx.doi.org/10.5061/dryad.1926>.

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