The phylogeny of extant starfish (Asteroidea: Echinodermata) including *Xyloplax*, based on comparative transcriptomics

Gregorio V. Linchangco Jr.^{a,*}, David W. Foltz^b, Rob Reid^a, John Williams^a, Conor Nodzak^a, Alexander M. Kerr^c, Allison K. Miller^c, Rebecca Hunter^d, Nerida G. Wilson^{e,f}, William J. Nielsen^g, Christopher L. Mah^h, Greg W. Rouse^e, Gregory A. Wray^g, Daniel A. Janies^{a,*}

^a Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, Charlotte, NC, USA

^b Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA

^c Marine Laboratory, University of Guam, Mangilao, GU, USA

^d Department of Biology, Abilene Christian University, Abilene, TX, USA

^e Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA

^f Western Australian Museum, Locked Bag 49, Welshpool DC, Western Australia 6986, Australia

^g Department of Biology and Center for Genomic and Computational Biology, Duke University, Durham, NC, USA

^h Department of Invertebrate Zoology, Smithsonian Institution, Washington, District of Columbia, USA

ARTICLE INFO

Keywords:

Transcriptomics

Phylogeny

Asteroidea

Crown-group

Echinodermata

ABSTRACT

Multi-locus phylogenetic studies of echinoderms based on Sanger and RNA-seq technologies and the fossil record have provided evidence for the Asterozoa-Echinozoa hypothesis. This hypothesis posits a sister relationship between asterozoan classes (Asteroidea and Ophiuroidea) and a similar relationship between echinozoan classes (Echinoidea and Holothuroidea). Despite this consensus around Asterozoa-Echinozoa, phylogenetic relationships within the class Asteroidea (sea stars or starfish) have been controversial for over a century. Open questions include relationships within asteroids and the status of the enigmatic taxon *Xyloplax*. *Xyloplax* is thought by some to represent a newly discovered sixth class of echinoderms – and by others to be an asteroid. To address these questions, we applied a novel workflow to a large RNA-seq dataset that encompassed a broad taxonomic and genomic sample. This study included 15 species sampled from all extant orders and 13 families, plus four ophiuroid species as an outgroup. To expand the taxonomic coverage, the study also incorporated five previously published transcriptomes and one previously published expressed sequence tags (EST) dataset. We developed and applied methods that used a range of alignment parameters with increasing permissiveness in terms of gap characters present within an alignment. This procedure facilitated the selection of phylogenomic data subsets from large amounts of transcriptome data. The results included 19 nested data subsets that ranged from 37 to 4,281 loci. Tree searches on all data subsets reconstructed *Xyloplax* as a velatid asteroid rather than a new class. This result implies that asteroid morphology remains labile well beyond the establishment of the body plan of the group. In the phylogenetic tree with the highest average asteroid nodal support several monophyletic groups were recovered. In this tree, Forcipulatida and Velatida are monophyletic and form a clade that includes Brisingida as sister to Forcipulatida. *Xyloplax* is consistently recovered as sister to *Pteraster*. Paxillosida and Spinulosida are each monophyletic, with Notomyotida as sister to the Paxillosida. Valvatida is recovered as paraphyletic. The results from other data subsets are largely consistent with these results. Our results support the hypothesis that the earliest divergence event among extant asteroids separated Velatida and Forcipulatida from Valvatida and Spinulosida.

1. Introduction

Echinodermata are a unique animal phylum in that most species have bilaterally symmetrical larvae followed by pentaradial adult stages. However, echinoderm larvae and adults display great diversity

in form and life history (McEdward and Janies, 1997). Extant echinoderms include two clades, class Crinoidea (sea lilies) and superclass Eleutherozoa [classes Asteroidea (starfish or sea stars), Ophiuroidea (brittlestars), Echinoidea (sea urchins), and Holothuroidea (sea cucumbers)]. Historically echinoderm workers have considered two major

* Corresponding authors at: Department of Bioinformatics and Genomics, 9201 University City Blvd, Charlotte, NC 28223, USA.

E-mail addresses: glinchan@uncc.edu (G.V. Linchangco), djanies@uncc.edu (D.A. Janies).

<http://dx.doi.org/10.1016/j.ympev.2017.07.022>

Received 22 February 2017; Received in revised form 5 June 2017; Accepted 25 July 2017

Available online 27 July 2017

1055-7903/ © 2017 Elsevier Inc. All rights reserved.

competing hypotheses for the relationship of Asterozoa to other classes within Eleutherozoa (reviewed in Smith (1984) and Janies et al. (2011)). The first hypothesis is the cryptosyringid hypothesis, which posits that the classes Echinozoa, Holothurozoa, and Ophiurozoa form a clade known as Cryptosyringida, sister to the Asterozoa. The Cryptosyringid hypothesis is primarily based on a putative synapomorphy of an enclosed radial water vessel (Smith, 1984). An alternative hypothesis for the evolutionary relationships of extant Eleutherozoa is the Asterozoa-Echinozoa hypothesis. Asterozoa includes the stellate forms asteroids and ophiuroids. The monophyly of the Asterozoa is based on the putative synapomorphy of the adult body plan including stellate form and the construction of the mouth frame and ambulacral ossicles (Mooi and David, 2000). The Echinozoa includes holothuroids and echinoids. The monophyly of the Echinozoa is supported by the shared globose shape (Smith 1984). Janies (2001) used modest amounts of Sanger sequencing and morphology to show that there is more support for Asterozoa-Echinozoa than Cryptosyringida across an alignment parameter space. Later, Janies et al. (2011) used combined Sanger sequencing data and morphology to show that Asterozoa and Echinozoa are methods-dependent when alignment and optimality criteria are varied. Recent transcriptomic studies with limited taxonomic and locus sampling have supported Asterozoa-Echinozoa (Cannon et al., 2014; O'Hara et al., 2014; Telford et al., 2014; Reich et al., 2015; Simakov et al., 2015) and were reviewed by Peterson and Eernisse (2016). These studies also strongly supported a monophyletic Asterozoa, consistent with prior morphological analyses including fossils (Blake et al., 2000; Shackleton, 2005) and molecular studies (Janies, 2001; Janies et al., 2011).

All previous transcriptomic studies lack the enigmatic *Xyloplax*. In the 1980s, this small disc-shaped echinoderm was discovered on sunken wood in the deep sea off the coasts of the Bahamas and New Zealand (Baker et al., 1986). *Xyloplax* was initially described as having a circular water vascular system (Baker et al., 1986; Janies and McEdward, 1994a, 1994b) but later was re-described as having a derived pentaradial system with ambulacra splayed out to the periphery of the body (Janies and Mooi, 1998; Mooi et al., 1998; Janies et al., 2011). The small size and odd morphology of *Xyloplax* fueled the already fierce debate over echinoderm phylogeny (Smith, 1988; Pearse and Pearse, 1994; Janies and Mooi, 1998). A table of hypotheses for the placement of *Xyloplax* and their proponents is presented in Table 1. Some workers erected a new sixth class of extant echinoderms containing only *Xyloplax* (Baker et al., 1986). Subsequently, Rowe et al. (1988) proposed a working hypothesis that united *Xyloplax* and valvatinid asteroids. In doing so, these workers expressed a desire to keep *Xyloplax* as a class distinct from the asteroids – which was self-contradictory. In contrast, several researchers have argued that *Xyloplax* is an aberrant asteroid and that erecting a new class is not warranted (Smith, 1988; Janies and Mooi, 1998; Janies, 2001; Janies et al., 2011).

Another enduring quandary in asteroid phylogeny has concerned the earliest cladogenetic events giving rise to living asteroid lineages. This discussion was historically framed as the question of whether the

order Paxillosida (or one of its member families) diverged early or late during asteroid evolution. Evidence has been presented for (Mortensen, 1922, 1923; Fell, 1962a, 1962b, 1963; Gale, 1987; Lafay et al., 1995; Wada et al., 1996; Gale, 2013) and against (MacBride, 1921, 1923a, 1923b; Madsen, 1966; Blake, 1972, 1987, 1988; Janies et al., 2011; Mah and Blake, 2012; Blake and Mah, 2014) an early divergence of Paxillosida among asteroid lineages. Recent morphological and molecular-based hypotheses of phylogenetic relationships amongst traditional asteroid orders are summarized in Fig. 1.

The controversy illustrated in Fig. 1 underscores the need for a well-supported molecular phylogeny for asteroids, to permit the resolution of early divergence of asteroid orders as well as allow the placement of problematic and morphologically unusual taxa. Here, we used the recent demonstration, via molecular analyses of echinoderms (Janies, 2001; Cannon et al., 2014; O'Hara et al., 2014; Telford et al., 2014; Reich et al., 2015; Simakov et al., 2015) and our own broader analyses, that ophiuroids (brittlestars) are the sister group to asteroids and the outgroup of choice. However, recent transcriptomic analyses sampled asteroids poorly (Cannon et al., 2014; O'Hara et al., 2014; Telford et al., 2014; Reich et al., 2015; Simakov et al., 2015). Specifically, O'Hara et al. (2014) and Telford et al. (2014) each sampled two asteroids, Simakov et al. (2015) sampled one asteroid and Cannon et al. (2014) sampled four asteroids. Reich et al. (2015) sampled 11 asteroids, but only four families that represented four orders were studied. To better understand the phylogeny of extant asteroids and the placement of *Xyloplax*, we employed RNA-seq data to assemble thousands of orthologous loci for 15 species sampled from all extant asteroid orders and 13 families, plus four ophiuroid species as an outgroup (Table 2). To expand the taxonomic coverage, the study incorporated five previously published transcriptomes and one previously published expressed sequence tags (EST) dataset. Photographs of taxa of interest are shown in Fig. 2.

2. Materials and methods

2.1. Sampling

In terms of gene and taxon sampling, our sequence datasets are the largest assembled to date for asteroid phylogenetics (15 asteroid species from all orders and 13 families). We sampled families within orders to span the largest taxonomic divergences ascertained from earlier work. RNA-Seq data was obtained from four ophiuroid taxa for use as outgroups (*Ophiocoma wendtii*, *Ophiothrix spiculata*, *Astrophyton muricatum*, and *Ophioderma brevispinum*). We added data from published asteroid transcriptome sequences for the species *Asterias amurensis*, *Asterias forbesi*, *Asterias rubens*, *Marthasterias glacialis* and *Leptasterias* sp. (Reich et al., 2015). cDNA data for *Patiria pectinifera* was obtained from http://www.ncbi.nlm.nih.gov/biosample/LIBEST_019589, 45-h embryonic tissue. For quality control, one of the asteroid species served as a technical replicate (i.e. *Peribolaster* 1 and *Peribolaster* 2, two pieces of the same specimen used in separate library preparation and Illumina runs).

2.2. RNA extraction, sequencing and data pre-processing

We extracted RNA from muscle tissue samples (adult tube feet, pinnules or body wall) using a Qiagen miRNeasy kit. An Agilent Bioanalyzer 2100 ver. 2.6 was used for quality control prior to library preparation. Samples were then submitted to the Duke Center for Genomic and Computational Biology for library preparation with an Illumina TruSeq RNA kit, followed by RNA-Seq sequencing on an Illumina HiSeq 2000 platform (100 BP, paired end). Reads for each of the samples were filtered by quality score (cutoff threshold > Q20) by FASTXFILTER and filtered for adapters by way of cutadapt, both components of the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). *De novo* assembly of contigs was then performed using

Table 1
Various hypotheses for the placement of *Xyloplax*.

Hypothesis	Authors
New echinoderm class	Baker et al. (1986) Rowe et al. (1988)
Infraclass within the Asterozoa	Mah (2006)
Asterozoa (Velatida)	Smith (1988)
Asterozoa (Caymanostellidae)	Belyaev (1990)
Asterozoa (Forcipulatida)	Janies and Mooi (1998) Janies (2001)
Asteroid (Velatida)	Janies et al. (2011) Janies (2013) This paper

Fig. 1. Recent hypotheses of five asteroid orders based on morphological and/or molecular data. Following (Feuda and Smith, 2015), one to three exemplars of each order that are shared by the various studies are depicted. Two primarily deep-sea orders (Brisingida and Notomyotida) and the enigmatic *Xyloplax* have not been sampled in several other recent phylogenetic studies and are omitted to facilitate comparison among studies. The four studies summarized in this figure reached different conclusions as to which extant asteroid orders diverged earliest. Gale (2011) placed Paxillosida as sister to the rest of asteroids. Janies et al. (2011) placed Velatida as sister to the rest of the asteroids. The hypothesis labeled ‘Mah and Blake (2012)’ in Fig. 1 is based in part on molecular studies of Mah and Foltz (2011a, 2011b). Mah and Foltz (2011a, 2011b) did not attempt to locate the root of the asteroid tree, so relationships at the root were considered by them to be unresolved, contrary to a recent interpretation of their results by Feuda and Smith (2015). Knott and Wray (2000) placed Valvatida and Paxillosida as sister to the rest of the asteroids.

Trinity (Haas et al., 2013) on a high memory compute cluster using 500 GB of RAM and 24 CPUs. The resultant contigs for each sample was subsequently translated into amino acid space using Transdecoder (<http://transdecoder.github.io/>) and the PFAM-B protein family database (Finn et al., 2010), with a lower cutoff of 100 amino acids.

2.3. Taxonomic conventions

Taxonomic conventions for the sampled ingroup species followed recent nomenclatural changes reflected in <http://www.marinespecies.org/asteroidea>. Noteworthy changes included the change of *Porania antarctica* to *Glabraster antarctica*, following revisions to the Poraniidae by Mah and Foltz (2014). Sequences obtained from the *Xyloplax* in this study suggest that it may be a new species, which will be described elsewhere (Rouse, pers. comm.). To facilitate comparison to recent studies of asteroid relationships (Fig. 1) we describe our results using the conventional asteroid orders. This study is focused on high-level relationships within asteroids and, for simplicity, our selected vouchers are referred to by their currently assigned genus in our discussion, with our species identifications provided in our tables and figures.

2.4. Ortholog identification, alignment construction and phylogenetic analysis with paralogs

Each putative amino acid sequence was compared via a BLASTP search across all other contigs to discover orthologous clusters (orthoclusters) using OrthoMCL (Li, 2003). Following orthocluster discovery, a taxonomic filter of having at least eight asteroids and one ophiuroid species was applied to all orthoclusters to obtain the initial

dataset. Additional sequences from six species were then added to the RNA-Seq derived dataset via a BLASTP search on the longest asteroid sequence in the orthologous cluster versus sequence data from the species *Asterias amurensis*, *A. forbesi*, *A. rubens*, *Marthasterias glacialis*, *Leptasterias* sp. from Reich et al. (2015) and published cDNA data for *Patiria pectinifera* from http://www.ncbi.nlm.nih.gov/biosample/LIBEST_019589, 45-h embryonic tissue. Overall, the process of ortholog identification produced 5,868 orthoclusters for the initial dataset.

Multiple sequence alignment was then performed on each of the 5,868 orthoclusters using MAFFT 7.0130 (Katoh, 2002). To increase the quality of the aligned orthoclusters prior to tree construction, we used a two-step process including the program TrimAL (Capella-Gutiérrez et al., 2009) and an in-house Python script we call “BOXER” (<https://github.com/supramap/boxer>) (Fig. 3). First, TrimAL removed difficult to align sequences via a command line interface employing alignment statistics. BOXER then selected from aligned orthoclusters produced by TrimAL, preferring those aligned orthoclusters with the user-defined number of unique taxa and allowed percentage of gaps in the alignment. The difficult to align sequences were identified via the two parameters from TrimAL as described in the manual (http://trimal.cgenomics.org/_media/tutorial.pdf): (1) “residue overlap” which is a percentage of residues in an aligned orthocluster column that must be occupied with other residues (not gaps or missing data), and (2) “sequence overlap” which is a percentage of positions with observed residues (not gaps or missing data) that a row in an aligned orthocluster must have in order to be kept in the alignment. If a sequence did not fulfill both user thresholds for these parameters, it was removed from the orthocluster alignment. This process was performed for 36 different

Table 2

This table contains information for each species included in this study. Columns (left to right) include: Species, Voucher Nos., Number of Loci Included in the Analysis, Summary Statistics for Transcriptome Pre-Processing of Illumina Hi-Seq Read Counts Before (Raw) And After Quality Filtering and Adapter Removal, Bioproject Accession Nos.

Species	Voucher no. ^c	No. of loci	No. of raw reads	No. of reads removed	% Reads removed	Bioproject accession No. ^d
<i>Asteropsis carinifera</i>	KSORC 000276	1847	55,750,932	9,694,411	17.39	PRJNA 299419
<i>Astropecten duplicatus</i>	SIO-BIC E6745	1790	58,536,934	10,879,260	18.59	PRJNA 299417
<i>Astrophyton muricatum</i> ^a	SIO-BIC E6741	1285	52,762,224	9,218,837	17.47	PRJNA 299886
<i>Cheiraster</i> sp.	SIO-BIC E4758	55	30,190,658	4,898,857	16.23	PRJNA 299420
<i>Echinaster spinulosus</i>	— ^c	836	58,483,838	3,074,717	5.26	PRJNA 300370
<i>Glabaster antarctica</i>	SIO-BIC E5520	1517	54,906,620	9,703,154	17.67	PRJNA 299418
<i>Henricia leviuscula</i>	SIO-BIC E5597	1980	61,618,432	11,331,247	18.39	PRJNA 299415
<i>Labidiaster annulatus</i>	SIO-BIC E4756	2531	88,022,282	3,838,195	4.36	PRJNA 299411
<i>Luidia clathrata</i>	SIO-BIC E6744	1526	60,248,000	10,583,107	17.57	PRJNA 299414
<i>Odinella nutrix</i>	SIO-BIC E4241	38	40,650,604	7,334,900	18.04	PRJNA 299463
<i>Ophiocoma wendtii</i> ^a	— ^c	189	39,062,818	2,601,837	6.66	PRJNA 299897
<i>Ophioderma brevispina</i> ^a	SIO-BIC E6743	974	55,865,966	8,969,129	16.05	PRJNA 299887
<i>Ophiothrix spiculata</i> ^a	— ^c	364	47,255,758	2,588,148	5.48	PRJNA 299898
<i>Patiria pectinifera</i> ^b	— ^b	616	—	—	—	—
<i>Peribolaster folliculatus</i> 1	SIO-BIC E4754	1932	77,608,578	3,602,526	4.64	PRJNA 299409
<i>Peribolaster folliculatus</i> 2	SIO-BIC E4754	1621	40,810,648	33,914,958	16.89	PRJNA 299409
<i>Pisaster ochraceus</i>	SIO-BIC E6723	2531	88,987,394	3,962,217	4.45	PRJNA 299406
<i>Psilaster charcoti</i>	SIO-BIC E4755	2298	62,076,466	2,705,774	4.36	PRJNA 299410
<i>Pteraster tessellatus</i>	SIO-BIC E6727	2381	63,162,964	3,253,285	5.15	PRJNA 299398
<i>Remaster gourdoni</i>	SIO-BIC E4757	2164	55,504,406	2,071,104	3.73	PRJNA 299412
<i>Xyloplax</i> sp.	SIO-BIC E6809	1440	61,076,078	3,835,893	6.28	PRJNA 299326
<i>Asterias amurensis</i>	— ^f	1616	—	—	—	PRJNA 236087
<i>Asterias forbesi</i>	— ^f	2291	—	—	—	PRJNA 236087
<i>Asterias rubens</i>	— ^f	2279	—	—	—	PRJNA 236087
<i>Marthasterias glacialis</i>	— ^f	2257	—	—	—	PRJNA 236087
<i>Leptasterias</i> sp.	— ^f	2279	—	—	—	PRJNA 236087

No., number; sp., species; %, percentage.

^a Outgroup taxa (Class Ophiuroidea, brittlestars).

^b From published cDNA library, so no transcriptomic data or voucher available.

^c Specimen consumed in study.

^d <https://www.ncbi.nlm.nih.gov/bioproject/?term=Echinotol>.

^e KSORC, Korea South Pacific Ocean Research Center; SIO-BIC, Scripps Institution of Oceanography Benthic Invertebrate Collection.

^f From Reich et al. (2015) so no quality control data or voucher available.

parameter sets (See Fig. 3) of residue overlap and sequence overlap for each of the initial dataset's 5,868 aligned orthoclusters, potentially yielding 211,248 total aligned orthoclusters ($36 \times 5,868$). The result of this process was only a fraction of the total amount of potential aligned orthoclusters due to tens of thousands of aligned orthoclusters that failed to pass through more stringent filters. When too many sequences within the aligned orthocluster were deemed highly divergent, the remaining sequences could no longer be aligned and therefore the entire orthocluster was removed. With the identification and removal of highly divergent sequences from aligned orthoclusters, a total of 60,659 possible aligned orthocluster candidates remained from the 36 different parameter combinations. BOXER was then used to identify an optimal aligned orthocluster to represent each of the 5,868 original aligned orthoclusters, based on two user-defined parameters; number of unique taxa and percentage of gap characters present in the aligned orthocluster. Of the 5,868 original aligned orthoclusters, 4,281 that had at least 13 unique taxa (50% of the included species) and up to 95% gaps and were retained, to constitute a reduced dataset. 4,281 phylogenies were then estimated under Maximum likelihood analysis using RAXML (Stamatakis, 2014) using a CAT model of rate heterogeneity with a Jones Taylor Thornton (JTT) matrix of amino acid substitution, keeping computational efficiency in mind. 100 bootstrap replicates were performed for each retained aligned orthocluster and the tree with the highest likelihood was chosen.

2.5. Phylogenetic analysis without paralogs

The 4,281 aligned orthoclusters and associated gene trees were then assessed for the possible presence of paralogous sequences using Phylotreepruner (Kocot et al., 2013). After the removal of likely paralogous sequences, a locus selection test based on alignment occupancy was used to analyze each of the 4,281 alignments. This split the reduced

dataset into 19 nested subsets. Each nested subset is based on total allowable gap percentage in an alignment starting at 5% through 95% allowable gap characters at 5% increments. Each of the 19 nested subsets were then concatenated using FASconCAT (Kück and Meusemann, 2010). The resulting 19 concatenated (multi-locus) nested subsets were checked for all gap-only columns that were then removed using AliView (Larsson, 2014). The final nested subsets were analyzed under Maximum Likelihood using a single partition protein substitution model. The best protein substitution model for each nested subset was determined by RAXML based on the model with the highest likelihood score on the parsimony starting tree. The PROTAMMAUTO command determines the most appropriate protein substitution model among 21 models (Stamatakis, 2014). For each of the 19 nested subsets, 100 bootstrap replicates were performed and the tree with the highest likelihood was chosen. From the 19 topologies produced in this study, we highlight one phylogeny based on maximum average percentage bootstrap support values across asteroid nodes. The workflow used to reconstruct these phylogenies is graphically described in Fig. 4.

To derive basic details regarding the genetic composition of the included alignments, a functional analysis of the longest protein sequence from each of the 4,281 alignments was performed. To complete this task, a Biopython script was written to parse each alignment for the longest sequence, which was then used as input to InterProScan 5 (Jones et al., 2014). InterProScan 5 then returns annotations for each sequence by classifying the submitted protein sequence into families and predicting the presence of domains and important sites. Furthermore, an inquiry into the echinoderm genes included in the 4,281-locus reduced dataset was performed using BLASTP on the longest sequence per alignment as the query against the NR database. Alignments in which the longest sequence had a BLASTP hit to a protein that had not yet been described for an echinoderm were discarded.

Fig. 2. Representatives from the United States National Museum of Natural History (NMNH) dry collection of some species included in the analysis. All catalog numbers are NMNH except as noted. Except for E, scale bar = 10.0 mm. A. *Astropecten duplicatus* (Astropectinidae, Paxillosida) 39821. B. *Echinaster spinulosus* (Echinasteridae, Spinulosida) E40408. C. *Glabraster antarctica* (Poraniidae, “Valvatida”) E13647. D. *Pisaster ochraceus* (Asteriidae, Forcipulatida) E21485. E. *Xyloplax* n. sp. (Velatida) SIO-BIC E6809. Scale Bar = 5.0 mm. F. *Pteraster tessellatus* (Pterasteridae, Velatida) E1485.

3. Results and discussion

3.1. Raw data pre-processing and creation of initial and reduced datasets

RNA-seq produced a total of 1.11×10^9 raw reads. The sample from *Pisaster ochraceus* had the largest number of raw reads at 8.90×10^7 . The *Cheiraster* sp. sample had the least raw reads at 3.02×10^7 (see Table 2 for details). In the process of ortholog identification 93,908 orthoclusters that contained four or more taxa were detected. Taxonomic filters applied to the 93,908 orthoclusters (described in experimental procedures) yielded an initial dataset of 5,868 putative orthoclusters. Following the removal of highly divergent and paralogous sequences from the 5,868 initial aligned orthoclusters, a reduced dataset of 4,281 aligned orthoclusters was retained and later analyzed under an alignment occupancy method. The initial 5,868 aligned orthoclusters contained a total of 350,182 sequences; following the

removal of paralogous sequences the reduced dataset of 4,281 aligned orthoclusters contained 64,749 sequences.

3.2. Creation of nested data subsets

BOXER divided the 4,281 aligned orthoclusters into 19 nested data subsets of concatenated, aligned orthoclusters based on allowed gap characters, from 5% to 95% at 5% increments. The topology presented in all the 19 nested subsets placed *Xyloplax* as a velatid asteroid. These 19 data subsets and their corresponding topologies are available at <http://echinodb.uncc.edu/>. 18 of the 19 nested subsets (5–90% allowable gap characters) placed *Xyloplax* as sister to *Pteraster*, while the most permissive or inclusive subset (95% allowable gap characters) placed it as sister to the Velatida (represented by *Pteraster*, *Remaster*, and *Peribolaster*). Considering the technical replication strategy, *Peribolaster* 1 and *Peribolaster* 2 were consistently recovered as sister

Fig. 3. The process of selecting alignments using the TrimAL and BOXER programs.

taxa in all nested subsets. Of the 19 topologies derived from BOXER, 18 trees consistently recovered ordinal-level monophyly for Paxillosida, Forcipulatida, Velatida and Spinulosida (See Table 3). From these 18 topologies, we highlight the tree that resulted from the nested subset that allowed for 60% gaps and at least 13 unique taxa as it was the only one that produced 100% bootstrap support values for all asteroid nodes (Fig. 5). This topology recovered monophyly for many asteroid orders with 100% bootstrap support. Well-supported clades included Velatida, Paxillosida, Forcipulatida, Forcipulatida + Brisingida (i.e., the Forcipulatacea), Forcipulatacea sister to Velatida, Spinulosida and Paxillosida + Notomyotida. These results are consistent with the work of Janies et al. (2011) based on Sanger sequencing. One traditional order that was not monophyletic was Valvatida. The remaining trees resulting from the parameter variation are largely consistent with the highlighted tree (see Table 3).

The topology of the outgroup is incongruent with recent studies that

sampled brittle stars well (O'Hara et al., 2014; Hugall et al., 2016; O'Hara et al., 2017). These studies placed *Astrophyton* as sister to the three remaining ophiuroids. Our result is likely a function of too little sampling of ophiuroids, which we included only to form an outgroup.

3.3. Orthocluster alignment annotation

The 4,281 aligned orthoclusters were annotated using InterProScan 5 which generated InterProScan descriptions and Gene Ontology (GO) identifiers as output. These identifiers define concepts and classes that describe gene function and relationships between them. Of the 4,281 loci, 3,109 were associated with at least one GO ID and 3,935 were associated with an Interpro accession and description. This list of annotated orthoclusters and their descriptions are included in supplementary Table 1. The 4,281 aligned orthoclusters were also screened for described echinoderm genes. This was performed via a BLASTP search on the longest sequence in the aligned orthocluster against the RefSeq non-redundant protein database. This process removed 446 non-echinoderm aligned orthoclusters from the 4,281 aligned orthoclusters, leaving 3,833. The remaining 3,833 aligned orthoclusters were then concatenated and analyzed under maximum likelihood. The resulting maximum likelihood tree did not differ in topology from the 4,281 locus reduced dataset analysis (details not shown).

3.4. *Xyloplax*

As outlined earlier, since its discovery, the phylogenetic position of *Xyloplax* has been controversial, although molecular phylogenetic analyses have always placed it within the Asteroidea (Janies and Mooi, 1998; Janies, 2001; Janies et al., 2011; Janies, 2013). Affinities with velatid asteroids based on developmental models, especially based on comparisons with *Pteraster*, have been argued by Janies and McEdward (1994a, 1994b). The specifics of those ontogenetic inferences were revised with new embryological data on *Xyloplax* by Janies et al. (2011). Our highlighted tree (Fig. 5) placed *Xyloplax* as sister to *Pteraster*. Moreover, *Xyloplax* is recovered as a Velatid asteroid across parameter space (Table 3). An affinity between *Xyloplax* and the deep-sea *Caymanostella* as argued by Smith (1988) and Belyaev (1990) was not assessed here. In 2011, Janies et al. showed that *Caymanostella* was not closely related to *Pteraster* or *Xyloplax*.

3.5. The Velatida

The tree presented in Fig. 5 supported the monophyly of the Velatida, inclusive of *Xyloplax*, the Pterasteridae and the Korethrasteridae in agreement with several prior, but disparate accounts. The Velatida as originated by Perrier (1894) and Fisher (1930) included the Pterasteridae and the Myxasteridae. Blake's (1987) phylogenetic analysis of asteroid morphology returned the Velatida into usage. Blake's analysis showed Velatida as composed of the Pterasteridae, Korethrasteridae and Myxasteridae as well as the Solasteridae and Caymanostellidae. Molecular work by Mah and Foltz (2011b) further restricted the Velatida to the Pterasteridae, Korethrasteridae and the Myxasteridae and moved the Solasteridae to the Valvatida. Work by Janies et al. (2011) had the same result but lacked Myxasteridae. Although placed within the Velatida by Blake (1987), the Caymanostellidae displays affinities with other groups, such as the Asterinidae, and its phylogenetic position remains untested by phylogenomic methods. A morphological-fossil phylogenetic survey by Gale (2011) adopted a more traditional classification and placed *Remaster* and *Pteraster* as members of the Spinulosida, a paraphyletic grouping which has failed to find support from either morphology (e.g. Blake, 1987) or molecules (Mah and Foltz, 2011b). Janies et al. (2011) were the first to show *Xyloplax* as a member of the Velatida, as part of a clade that contained two pterasterids, *Pteraster* and *Hymenaster*, but lacked other velatids. In this study, we included the korethrasterids *Peribolaster* and *Remaster*.

Fig. 4. A simple graphical representation of phylogeny reconstruction from sample collection to tree creation.

Table 3

This table contains a topology summary for the nested data subsets included in this study. Columns (left to right) include: % gap criterion for inclusion in the nested data subset, number of loci in each dataset, orders recovered as monophyletic in the RAxML analysis, % gap characters in alignment, inserted N/X in the concatenated alignment, pairwise identity, and alignment length.

Nested subset [% gaps allowed]	No. of loci	Orders that are monophyletic ^a	Gaps [%]	Inserted N or X [%]	Pairwise identity [%]	Alignment length [Kbp] ^b
5	37	P ^c	0.10	38.96	36.1	7.360
10	48	P ^c , Ve, S	0.37	40.79	33.7	9.955
15	145	P, F, Ve, S	2.25	41.77	30.1	34.002
20	371	P, F, Ve, S	3.71	41.62	28.6	91.125
25	651	P, F, Ve, S	5.04	42.35	26.7	171.320
30	1012	P, F, Ve, S	6.36	42.84	24.9	285.890
35	1342	P, F ^d , Ve, S	7.53	42.97	23.7	402.754
40	1671	P, F ^d , Ve, S	8.78	42.99	22.6	531.031
45	1958	P, F, Ve, S	10.25	42.57	21.7	660.666
50	2233	P, F, Ve, S	11.80	42.18	20.7	794.603
55	2471	P, F, Ve, S	13.33	41.76	19.9	920.902
60 ^e	2683	P, F, Ve, S	14.68	41.48	19.2	1036.409
65	2929	P, F, Ve, S	16.11	41.38	18.3	1172.960
70	3198	P, F, Ve, S	18.00	41.24	17.2	1343.339
75	3523	P, F, Ve, S	19.95	41.41	16.0	1551.577
80	3984	P, F, Ve, S	22.07	41.48	14.7	1811.425
85	4163	P, F, Ve, S	23.70	41.45	13.8	2027.010
90	4249	P, F, Ve, S	24.29	41.44	13.4	2107.727
95	4281	P, F, Ve, S	24.58	41.36	13.3	2141.743

^a P = Paxillosida, Ve = Velatida, S = Spinulosida, F = Forcipulatida; Valvatida was never recovered as monophyletic.

^b This is the length of the concatenated alignment analyzed in RAxML.

^c Includes *Cheiraster* (Notomyotida).

^d Includes *Odinella* (Brisingida).

^e Indicates our highlighted data subset, for which we observed monophyletic orders with consensus to 18/19 datasets. This data subset is unique among all rows in having 100% bootstrap support at all asteroid nodes.

3.6. The Forcipulatacea

The monophyly of the forcipulataceans (Forcipulatida + Brisingida) has been the least controversial of the major asteroid groups. The distinctive morphology of forcipulates has always set them apart in traditional taxonomy (e.g. Fisher, 1928; Fisher, 1930), and they

have usually been supported as monophyletic in both morphology-based cladistic analyses (e.g. Blake, 1987; Gale, 1987) and molecular phylogenetic studies (e.g. Knott and Wray, 2000; Foltz et al., 2007; Janies et al., 2011; Mah and Foltz, 2011a). The position of the Forcipulatacea relative to other asteroids in Fig. 5 disagreed with the topology of Gale (2011) who embedded the forcipulates among other valvatidans within his “Tripedicularia” clade, a relationship which has never been observed in prior literature (Blake and Mah, 2014).

3.7. The Poraniidae

One phylogenetic result in Fig. 5 is the position of the *Glabraster antarctica*, a poraniid, as sister to the clade containing the Notomyotida, the Spinulosida, and the Paxillosida and an assemblage of taxa usually considered valvatids. Mah and Foltz (2011b) reported a similar position of the Poraniidae from their analysis of the Valvatacea. The traditional taxonomic placement of the Poraniidae has been among either the Valvatida (e.g. Blake, 1987) or the Spinulosida (Gale, 2011; Spencer and Wright, 1966). As part of his phylogenetic treatment of the Triassic poraniid, *Noriaster*, Blake et al. (2000) suggested that poraniids might lie outside these taxa, contrary to traditional placement. However, Blake et al. (2000) expressed uncertainty in morphological interpretation, due to the role of homeomorphy and the thick tissue overlying the skeleton in poraniids.

3.8. The Spinulosida, Patiria, and Asteropsis

Our highlighted tree shows *Patiria* and *Asteropsis* as sister to the Spinulosida (*Henricia* and *Echinaster*), a result similar to that seen in the recent phylogenomic study by Reich et al. (2015). We treated the Valvatida herein *sensu* Blake (1987) and Mah and Foltz (2011b), which verified the inclusion of the Asterinidae and the Asteropsidae within the Valvatida. Although non-monophyletic, in several recent molecular studies, Valvatida contains the highest number of species of all recent Asterozoa (Mah and Blake, 2012). Past treatments of Valvatida (e.g. Spencer and Wright, 1966) have included different taxa.

Many traditional classifications, such as Fisher (1911) and Spencer and Wright (1966), list the Echinasteridae and the Asterinidae as members of the Spinulosida, a group which has most recently been restricted to include only the Echinasteridae (Blake, 1987; Clark, 1996).

Fig. 5. RAxML maximum likelihood tree for seven asteroid orders, based on 2683 loci (60% gap characters permitted) and a PROTCATJTT model of amino acid substitution. Bootstrap values based on 100 pseudoreplicates are shown at nodes.

Gale's (1987) non-cladistic phylogeny embedded both the Echinasteridae and the Asterinidae on distant clades within the Valvatida. The molecular treatments of Wada et al. (1996) and Matsubara et al. (2004) included only *Henricia* in their analyses, a genus that was placed in varied positions on their trees. Wada et al. (1996) and Matsubara et al. (2004) also included several species of the Asterinidae which (although monophyletic) were consistently reconstructed as the sister clade to the Asteriidae (Forcipulatacea). This relationship has not been reproduced by other studies with comparable taxon sampling. LaFay et al. (1995) produced a combined morphological and molecular analysis (using a single gene) that supported *Echinaster* and *Henricia* as a clade that displayed highly variable relationships with other asteroid taxa. Knott and Wray's (2000) results based on Sanger sequencing showed a paraphyletic Spinulosida based on *Echinaster* and *Henricia*. Asterinids were recovered on distant branches away from the echinasterids in the Knott and Wray (2000) tree. The Gale (2011) morphological analysis

supported *Echinaster* as the sister clade to the Forcipulatida with *Asterina* as a member of a phylogenetically distant, non-valvatidan "Spinulosida." Trees from Janies et al. (2011) supported a monophyletic Spinulosida and a paraphyletic Valvatida.

3.9. The Paxillosida and Notomyotida

Our highlighted tree (Fig. 5) placed the Paxillosida as sister to *Cheiraster* sp. (representing the Benthoplectinidae); within the Paxillosida, *Luidia* was supported as the sister clade to *Astropecten* + *Psilaster*. This group was sister to a group that included the Spinulosida and two valvatids (Fig. 5). The tree exhibits a long terminal branch for *Patiria pectinifera*, which is likely a result of the different data source from which the *Patiria* sequences were derived (EST from embryonic tissue vs. *de novo* transcriptomes from adult tissue). Overall, Fig. 5 resembled Mah and Foltz's (2011b) two-locus tree, which included the

Paxillosida (Astropectinidae and Luidiidae) and Notomyotida as members of the same clade. The Mah and Foltz (2011b) tree included taxa traditionally considered as Paxillosida, including the Astropectinidae, Luidiidae, Gonioplectinidae and Ctenodiscidae, but also the Pseudarchasteridae, which had previously been classified within the Goniatsteridae.

As summarized in the introduction, Paxillosida is central to long-standing questions concerning the cladogenetic events that gave rise to extant asteroid lineages. Our tree represents one of the first well-sampled (for taxa and loci), well-supported phylogenomic evidence of crown-group asteroid phylogeny. In our result, the earliest cladogenetic event among extant asteroids separated Velatida + Forcipulatacea from the Spinulosida and Valvatacea.

Fig. 5 showed the Paxillosida and the Spinulosida as later diverging lineages within a Valvatacea + Spinulosida clade. If allowance is made for the position of the Velatida, the tree topology in Fig. 5 is nearly identical to that of Blake (1987), who has consistently argued for a late-diverging Paxillosida (e.g., Blake and Mah, 2014). Our results are similar to those of Mah and Foltz (2011b); however, their tree did not survey echinasterids, nor did they employ outgroup rooting (see Fig. 1). The phylogenetic results of Blake (1987), Mah and Foltz (2011b) and Fig. 5 are all consistent with a “Paxillosida is derived” scenario which counters the “Paxillosida is primitive” perspective of Mortensen (1922, 1923), Fell (1962a, 1962b), LaFay et al. (1995), Wada et al. (1996) and Gale (1987, 2011). The “Paxillosida is primitive” scenario was originally based on the assumption that the absence of brachiolaria larvae and suckered tube feet in the extant Paxillosida was the plesiomorphic condition for asteroids (Mortensen, 1922) and was later augmented with fossil data (Fell, 1962a, 1962b; Gale, 1987, 2011). The “Paxillosida is derived” scenario, as outlined by MacBride (1921, 1923a, 1923b) and Blake (1987, 1988) argued that larval morphology and fossil and living skeletal characters observed in the Paxillosida were adaptations for living on unconsolidated sediment, such as sand or mud.

4. Conclusions

The results presented in this study are robust to a wide spectrum of alignment parameter variation, concomitant locus selection variation, and technical replication of known velatids. Of the 19 topologies derived from BOXER, 18 trees consistently recovered ordinal-level monophyly for Paxillosida, Forcipulatida, Velatida and Spinulosida. We highlighted a phylogeny from the concatenated aligned orthoclusters that allowed for 60% gaps and at least 13 unique taxa on the basis of bootstrap support values of all asteroid nodes being 100%, a unique feature of this alignment with respect to the others (Fig. 5). This tree recovers monophyly for many asteroid taxa with nodes containing high bootstrap support from well-supported clades including: Velatida, Paxillosida, Forcipulatida, Forcipulatida + Brisingida, Forcipulatida + Brisingida sister to Velatida, Paxillosida + Notomyotida. The topology of the outgroup species however, was incongruent with recent studies that included ophiuroids (O'Hara et al., 2014; Hugall et al., 2016; O'Hara et al., 2017). This discrepancy can be best explained by the scope of the present study, which focused on relationships within the asteroid class. This study does not attempt to propose a new ophiuroid topology based on only four species. Furthermore, the loci included in this study were filtered based on the inclusion of at least 13 asteroids and only one ophiuroid, without regard for ophiuroid diversity. The ambiguity observed in this clade would benefit from further studies that include representatives from all extant echinoderm classes.

In regards to asteroid phylogeny, these results and the results of Janies et al. (2011) support the hypothesis of non-monophyly of Valvatida. This result implies that more work is warranted to investigate the diversity of groups assigned to Valvatida using transcriptomics and genomics technologies.

The results of this study support the inclusion of *Xyloplax* as a velatid asteroid. This result implies that asteroid morphology remains labile well beyond the establishment of the body plan of the group. Next steps require the means to understand the mechanistic underpinnings of drastic changes of morphology. *Xyloplax* is a rare deep-sea species and thus not a very tractable as a model organism. The challenge is to develop models among shallow-water echinoderm species with highly labile embryogenesis and life cycles (e.g. some members of the Pterasteridae).

Acknowledgments

We thank Ben Grupe, Brian Livingstone and Jim Nestler for collecting some of the specimens and Dan Blake, Professor Emeritus, the University of Illinois at Urbana-Champaign for useful discussions. We also thank the University of North Carolina Research Computing Team and the Department of Bioinformatics and Genomics for ongoing support.

Funding

This material is based upon work was supported by the National Science Foundation of the United States (www.nsf.gov) under Award Nos. DEB 1036416 to D.A.J., DEB 1036358 to D.W.F. and C.L.M., DEB 1036368 to G.W.R. and DEB 1036366 to G.A.W. and ANT 1043749 to N.G.W. and G.W.R. and Ron Burton. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2017.07.022>.

References

- Baker, A.N., Rowe, F.W.E., Clark, H.E.S., 1986. A new class of Echinodermata from New Zealand. *Nature* 321, 862–864. <http://dx.doi.org/10.1038/321862a0>.
- Belyaev, G.M., 1900. Is it valid to isolate the genus *Xyloplax* as an independent class of echinoderms? *Zool. Zhurnal* 83–96.
- Blake, D.B., 1972. Sea star *Platystrophia*: ossicle morphology and taxonomic position. *Science* 176, 306–307. <http://dx.doi.org/10.1126/science.176.4032.306>.
- Blake, D.B., 1987. A classification and phylogeny of post-Palaeozoic sea stars (Asteroidea: Echinodermata). *J. Nat. Hist.* 21, 481–528. <http://dx.doi.org/10.1080/00222938700771141>.
- Blake, D.B., Mah, C.L., 2014. Comments on “the phylogeny of post-Palaeozoic Asteroidea (Neoasteroidea, Echinodermata)” by A.S. Gale and perspectives on the systematics of the Asteroidea. *Zootaxa* 3779, 177–194.
- Blake, D.B., Tintori, A., Hagdorn, H., 2000. A Nee, early crown-group asteroid (Echinodermata) from the Norian (TRIASSIC) of Northern Italy. *Riv. Ital. di Paleontol. e Stratigr. (Research Paleontol. Stratigr.)* 106. <http://dx.doi.org/10.13130/2039-4942/5395>.
- Blake, D., 1988. Paxillosidans are not primitive asteroids: A hypothesis based on functional considerations. In: Burke, R., Mladenov, P., Lambert, P., R.L., P. (Eds.), *Echinoderm Biology*. Balkema, Rotterdam, pp. 309–314.
- Cannon, J.T., Kocot, K.M., Waits, D.S., Weese, D.A., Swalla, B.J., Santos, S.R., Halanych, K.M., 2014. Phylogenomic resolution of the hemichordate and echinoderm clade. *Curr. Biol.* 24, 2827–2832. <http://dx.doi.org/10.1016/j.cub.2014.10.016>.
- Capella-Gutiérrez, S., Silla-Martínez, J.M., Gabaldón, T., 2009. TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25, 1972–1973. <http://dx.doi.org/10.1093/bioinformatics/btp348>.
- Clark, A.M., 1996. An index of names of recent Asteroidea Part 3: Velatida and Spinulosida. *Echinoderm Studies* 5, 183–250.
- Fell, H.B., 1962a. A surviving somasteroid from the Eastern Pacific Ocean. *Science* 136 633 LP-636.
- Fell, H.B., 1962b. A living somasteroid, *platasterias latiradiata* Gray. *Univ. Kansas Paleontol. Contrib.* 1–16.
- Fell, H.B., 1963. The phylogeny of sea-stars. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 246 381 LP-435.
- Feuda, R., Smith, A.B., 2015. Phylogenetic signal dissection identifies the root of starfishes. *PLoS ONE* 10, e0123331.
- Finn, R.D., Mistry, J., Tate, J., Coghill, P., Heger, A., Pollington, J.E., Gavin, O.L., Gunasekaran, P., Ceric, G., Forslund, K., Holm, L., Sonnhammer, E.L.L., Eddy, S.R., Bateman, A., 2010. The Pfam protein families database. *Nucl. Acids Res.* 38, D211–D222.

- Fisher, W.K., 1911. Asteroidea of the North Pacific and adjacent waters. *Bull. United States Natl. Museum* 76, 1–420.
- Fisher, W.K., 1928. Asteroidea of the North Pacific and adjacent waters pt. 2: Forcipulata (part). *Bull. United States Natl. Museum* 76, 1–245. <http://dx.doi.org/10.5479/si.03629236.76.2>.
- Fisher, W.K., 1930. Asteroidea of the North Pacific and adjacent waters pt. 3: Forcipulata (concluded). *Bull. United States Natl. Museum* 1–356. <http://dx.doi.org/10.5479/si.03629236.76.3>.
- Foltz, D.W., Bolton, M.T., Kelley, S.P., Kelley, B.D., Nguyen, A.T., 2007. Combined mitochondrial and nuclear sequences support the monophyly of forcipulatacean sea stars. *Mol. Phylogenet. Evol.* 43, 627–634. <http://dx.doi.org/10.1016/j.ympev.2006.10.012>.
- Gale, A.S., 2013. Phylogeny of the Asteroidea. In: Lawrence, J.M. (Ed.), *Starfish: Biology and Ecology of the Asteroidea*. Johns Hopkins University Press, Baltimore, pp. 3–14.
- Gale, A.S., 1987. Phylogeny and classification of the Asteroidea (Echinodermata). *Zool. J. Linn. Soc.* 89, 107–132. <http://dx.doi.org/10.1111/j.1096-3642.1987.tb00652.x>.
- Gale, A.S., 2011. The Phylogeny of Post-Palaeozoic Asteroidea (Neoaeroidea, Echinodermata). *Spec. Pap. Palaeontol.* 5–112.
- Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M., Macmanes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., Leduc, R.D., Friedman, N., Regev, A., 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* 8, 1494–1512. <http://dx.doi.org/10.1038/nprot.2013.084>.
- Hugall, A.F., O'Hara, T.D., Hunjan, S., Nilsen, R., 2016. An exon-capture system for the entire class ophiuroidea. *Mol. Biol. Evol.* 33, 281–294. <http://dx.doi.org/10.1093/molbev/msv216>.
- Janies, D.A., McEdward, L.R., 1994a. A hypothesis for the evolution of the concentricycloid water-vascular system. In: *Reproduction and Development of Marine Invertebrates: Papers from a Symposium Held at Friday Harbor Laboratories of the University of Washington, June 9–11, pp. 246–257*.
- Janies, D.A., McEdward, L.R., 1994b. Heterotrophy, pelagic direct development, and new body plans in velatid asteroids. In: David, B., Guille, A., Feral, J.P., Roux, M. (Eds.), *Echinoderms Through Time*. Balkema, Rotterdam, pp. 319–324.
- Janies, D., 2001. Phylogenetic relationships of extant echinoderm classes. *Can. J. Zool.* 79, 1232–1250. <http://dx.doi.org/10.1139/z00-215>.
- Janies, D.A., Voight, J.R., Daly, M., 2011. Echinoderm phylogeny including *Xyloplax*, a progenetic asteroid. *Syst. Biol.* 60, 420–438. <http://dx.doi.org/10.1093/sysbio/syr044>.
- Janies, D.A., 2013. *Xyloplax*. In: *McGraw-Hill Yearbook of Science & Technology*. McGraw-Hill Education.
- Janies, D., Mooi, R., 1998. *Xyloplax* is an asteroid. *Echinoderm Res.* 311–316.
- Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A.F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S.-Y., Lopez, R., Hunter, S., 2014. InterProScan 5: genome-scale protein function classification. *Bioinformatics* 30, 1236–1240. <http://dx.doi.org/10.1093/bioinformatics/btu031>.
- Katoh, K., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucl. Acids Res.* 30, 3059–3066. <http://dx.doi.org/10.1093/nar/gkf436>.
- Knott, K.E., Wray, G.A., 2000. Controversy and consensus in asteroid systematics: new insights to ordinal and familial relationships. *Am. Zool.* 40, 382–392. <http://dx.doi.org/10.1093/icb/40.3.382>.
- Kocot, Moroz, L., Citarella, M., Halanych, K., 2013. PhyloTreePruner: a phylogenetic tree-based approach for selection of orthologous sequences for phylogenomics. *Evol. Bioinf.* 429. <http://dx.doi.org/10.4137/EBO.S12813>.
- Kück, P., Meusemann, K., 2010. FASconCAT: convenient handling of data matrices. *Mol. Phylogenet. Evol.* 56, 1115–1118. <http://dx.doi.org/10.1016/j.ympev.2010.04.024>.
- Lafay, B., Smith, A.B., Christen, R., 1995. A combined morphological and molecular approach to the phylogeny of asteroids (Asteroidea: Echinodermata). *Syst. Biol.* 44, 190–208. <http://dx.doi.org/10.1093/sysbio/44.2.190>.
- Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30, 3276–3278. <http://dx.doi.org/10.1093/bioinformatics/btu531>.
- Li, L., 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. *Genome Res.* 13, 2178–2189. <http://dx.doi.org/10.1101/gr.1224503>.
- Macbride, E.W., 1923a. Echinoderm larvae and their bearing on classification. *Nature* 111 <http://dx.doi.org/10.1038/111047a0>. 47–47.
- Macbride, E.W., 1923b. Echinoderm larvae and their bearing on classification (reply). *Nature* 111, 323–324. <http://dx.doi.org/10.1038/111323a0>.
- Macbride, E.W., 1921. Echinoderm larvae and their bearing on classification. *Nature* 108, 529–530. <http://dx.doi.org/10.1038/108529c0>.
- Madsen, F.J., 1966. The recent sea-star platasterias and the fossil Somasteroidea. *Nature* 209 <http://dx.doi.org/10.1038/2091367a0>. 1367–1367.
- Mah, C.L., 2006. A new species of *Xyloplax* (Echinodermata: Asteroidea: Concentricycloidea) from the northeast Pacific: comparative morphology and a re-assessment of phylogeny. *Invertebr. Biol.* 125, 136–153. <http://dx.doi.org/10.1111/j.1744-7410.2006.00048.x>.
- Mah, C.L., Blake, D.B., 2012. Global diversity and phylogeny of the asteroidea (Echinodermata). *PLoS ONE* 7, e35644. <http://dx.doi.org/10.1371/journal.pone.0035644>.
- Mah, C.L., Foltz, D.W., 2014. New taxa and taxonomic revisions to the Poraniidae (Valvatacea; Asteroidea) with comments on feeding biology. *Zootaxa* 3795, 327. <http://dx.doi.org/10.11646/zootaxa.3795.3.7>.
- Mah, C., Foltz, D., 2011a. Molecular phylogeny of the Forcipulatacea (Asteroidea: Echinodermata): systematics and biogeography. *Zool. J. Linn. Soc.* 162, 646–660. <http://dx.doi.org/10.1111/j.1096-3642.2010.00688.x>.
- Mah, C., Foltz, D., 2011b. Molecular phylogeny of the Valvatacea (Asteroidea: Echinodermata). *Zool. J. Linn. Soc.* 161, 769–788. <http://dx.doi.org/10.1111/j.1096-3642.2010.00659.x>.
- Matsubara, M., Komatsu, M., Wada, H., 2004. Close Relationship between Asterina and Solasteridae (Asteroidea) supported by both nuclear and mitochondrial gene molecular phylogenies. *Zool. Sci.* 21, 785–793. <http://dx.doi.org/10.2108/zsj.21.785>.
- McEdward, L.R., Janies, D.A., 1997. Relationships among development, ecology, and morphology in the evolution of Echinoderm larvae and life cycles. *Biol. J. Linn. Soc.* 60, 381–400. <http://dx.doi.org/10.1111/j.1095-8312.1997.tb01502.x>.
- Mooi, R., Rowe, F.W.E., David, B., 1998. Application of a theory of axial and extraxial skeletal homologies to concentricycloid morphology. In: Mooi, R., Telford, M. (Eds.), *Echinoderms*, San Francisco. Balkema, Rotterdam, pp. 61–62.
- Mooi, R., David, B., 2000. What a new model of the skeletal homologies tells us about asteroid evolution. *Am. Zool.* 40, 326–339. [http://dx.doi.org/10.1668/0003-1569\(2000\)040\[0326:WANMOS\]2.0.CO;2](http://dx.doi.org/10.1668/0003-1569(2000)040[0326:WANMOS]2.0.CO;2).
- Mortensen, T., 1922. Echinoderm larvae and their bearing on classification. *Nature* 110, 806–807.
- Mortensen, T., 1923. Echinoderm larvae and their bearing on classification. *Nature* 111, 322–323.
- O'Hara, T.D., Hugall, A.F., Thuy, B., Stöhr, S., Martynov, A.V., 2017. Restructuring higher taxonomy using broad-scale phylogenomics: the living Ophiuroidea. *Mol. Phylogenet. Evol.* 107, 415–430. <http://dx.doi.org/10.1016/j.ympev.2016.12.006>.
- O'Hara, T.D., Hugall, A.F., Thuy, B., Moussalli, A., 2014. Phylogenomic resolution of the class ophiuroidea unlocks a global microfossil record. *Curr. Biol.* 24, 1874–1879. <http://dx.doi.org/10.1016/j.cub.2014.06.060>.
- Pearse, V.B., Pearse, J.S., 1994. Echinoderm phylogeny and the place of concentricycloids. *Echinoderms through time* 121–126.
- Perrier, E., 1894. *Stellérides Expédit*. Sci. Trav. 3, 431.
- Peterson, K.J., Ernise, D.J., 2016. The phylogeny, evolutionary developmental biology, and paleobiology of the Deuterostomia: 25 years of new techniques, new discoveries, and new ideas. *Org. Divers. Evol.* 16, 401–418. <http://dx.doi.org/10.1007/s13127-016-0270-x>.
- Reich, A., Dunn, C., Akasaka, K., Wessel, G., 2015. Phylogenomic analyses of Echinodermata support the sister groups of Asterozoa and Echinozoa. *PLoS ONE* 10, e0119627. <http://dx.doi.org/10.1371/journal.pone.0119627>.
- Rowe, F.W.E., Baker, A.N., Clark, H.E.S., 1988. The morphology, development and taxonomic status of *Xyloplax* Baker, Rowe and Clark (1986) (Echinodermata: Concentricycloidea), with the description of a new species. *Proc. R. Soc. B Biol. Sci.* 233, 431–459. <http://dx.doi.org/10.1098/rspb.1988.0032>.
- Shackleton, J.D., 2005. Skeletal homologies, phylogeny and classification of the earliest asterozoan echinoderms. *J. Syst. Palaeontol.* 3, 29–114. <http://dx.doi.org/10.1017/S1477201905001525>.
- Simakov, O., Kawashima, T., Marlétaz, F., Jenkins, J., Koyanagi, R., Mitros, T., Hisata, K., Bredeson, J., Shoguchi, E., Gyoja, F., Yue, J.-X., Chen, Y.-C., Freeman, R.M., Sasaki, A., Hikosaka-Katayama, T., Sato, A., Fujie, M., Baughman, K.W., Levine, J., Gonzalez, P., Cameron, C., Fritzenwanker, J.H., Pani, A.M., Goto, H., Kanda, M., Arakaki, N., Yamasaki, S., Qu, J., Cree, A., Ding, Y., Dinh, H.H., Dugan, S., Holder, M., Jhangiani, S.N., Kovar, C.L., Lee, S.L., Lewis, L.R., Morton, D., Nazareth, L.V., Okwuonu, G., Santibanez, J., Chen, R., Richards, S., Muzny, D.M., Gillis, A., Peshkin, L., Wu, M., Humphreys, T., Su, Y.-H., Putnam, N.H., Schmutz, J., Fujiyama, A., Yu, J.-K., Tagawa, K., Worley, K.C., Gibbs, R.A., Kirschner, M.W., Lowe, C.J., Satoh, N., Rokhsar, D.S., Gerhart, J., 2015. Hemichordate genomes and deuterostome origins. *Nature* 527, 459–465. <http://dx.doi.org/10.1038/nature16150>.
- Smith, A.B., 1984. Classification of the Echinodermata. *Palaeontology* 27, 431–459.
- Smith, A., 1988. To group or not to group: the taxonomic position of *Xyloplax*. In: 6th Int. Echinoderm Conf. pp. 17–23.
- Spencer, W., Wright, C., 1966. Asterozoans, Part U. In: Moore, R.C. (Ed.), *Treatise on Invertebrate Paleontology*. Geological Society of America and University of Kansas Press, pp. U4–U107.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. <http://dx.doi.org/10.1093/bioinformatics/btu033>.
- Telford, M.J., Lowe, C.J., Cameron, C.B., Ortega-Martinez, O., Aronowicz, J., Oliveri, P., Copley, R.R., 2014. Phylogenomic analysis of echinoderm class relationships supports Asterozoa. *Proc. R. Soc. B Biol. Sci.* 281 <http://dx.doi.org/10.1098/rspb.2014.0479>. 20140479–20140479.
- Wada, H., Komatsu, M., Satoh, N., 1996. Mitochondrial rDNA phylogeny of the Asteroidea suggests the primitiveness of the Paxilloidea. *Mol. Phylogenet. Evol.* 6, 97–106. <http://dx.doi.org/10.1006/mpev.1996.0062>.

Glossary

Orthocluster: A group of molecular sequences joined by similarity under a user defined threshold.