Supplementary Figures



Supplementary Figure 1 Estimation of genome size by 17-mer analysis.

Kmer analysis has been done using similar method previously described⁶⁴. The homozygous peak (around depth = 150 for *A. japonica* and 120 for *L. variegatus*) of the 17-mer frequency (M) is known to correlate with the real sequencing depth (N), read length (L), and kmer length (K), and their relations can be expressed in an experienced formula: M = N * (L - K + 1) / L. Estimated genome sizes of the feather star and green sea urchin were ~ 553 Mb and ~ 952 Mb respectively.



Supplementary Figure 2 GC content in genomes.

The x-axis represents GC content and the y-axis represent the proportion of the windows number divided by the total windows. The GC content was calculated using a 500bp sliding window (250 bp stepwise). Note that Feather star genome showed slightly higher G + C content compared to other echinoderms.



Supplementary Figure 3 The statistics of repetitive sequences. Repetitive sequences (*de novo* predicted and known sequences) in the genomes of the feather star genome (a) and the green sea urchin (b) are shown. LINE: Long interspersed elements, LTR: Long terminal repeat, SINE: Short interspersed elements.



Supplementary Figure 4 Basic statistics of predicted genes for feather star and green sea urchin. Features of predicted (by Evidence Modeler, EVM) gene sets of zebrafish, purple sea urchin, sea star, feather star, and green sea urchin are illustrated. The x-axis indicates length (bp) of each genetic feature and the y-axis indicates the percentage of genes that have the corresponding length.





Left panel: identified number of genes in each genome were categorized by their copy number of paralogs and homologs in the other species. Right panel: Relative ratio of categorized genes. *Unique gene* (species-specific, single copy gene): species-specific gene (no homologs in other species) without paralogs; *Species-specific paralogs*: species-specific genes (no homologs in other species) with paralogs in each species; *Single-copy ortholog*: gene that have only one copy in each species, together with homologs in other species; *Multi-copy homolog*: orthologs that do not belong to any type of the above orthologs/genes. The x-axis represents species used in this analysis, and y-axis represents the number of orthologs/genes in each category.



Supplementary Figure 6 Gene families shared amomg echinoderms, chordates and hemichordate Conserved protein coding genes analyzed by analyzed by orthomcl⁵⁴. (a) conserved protein coding genes among 5 echinoderm species, and (b) those among mouse, acon worm and feather star. Venn diagrams were drawn by R package "venn". Note that numbers indicate gene families (ortholog groups) defined by orthomcl, and do not indicate the actual numbers of coding genes.



Supplementary Figure 7 Phylogeny and estimated divergence time

The tree was constructed using the 4 fold degenerate codon site of 1196 one-to-one ortholog genes. Divergence time was estimated by MCMCtree software with calibration points indicated in red points. Numbers in blue represent bootstrap values of each node.





Protein sequences of 1196 one-to-one ortholog genes (identified by RBBH) were analyzed by LINTRE software (a) and MEGA with Tajima's Relative Ratio Test (b). Y axis represents relative branch length of protein sequences (compared to that of *A. japonica* from the common ancestor of echinoderms/hemichordates/chordates). While *C. intestinalis* shows the longest branch length, those of echinoderm species were relatively longer than those of most vertebrates.



Supplementary Figure 9 Relative evolutionary rate of protein sequences analyzed by APE package. The data used in this analysis was produced with 1,196 one-to-one genes (identified by RBBH) and analyzed by APE R-package.





Numbers in red represent numbers of expanded gene families (ortholog groups) defined by orthomcl, and numbers in blue represent those contracted gene families (ortholog groups). The pie figures on each node were drawn according to the ratio of expanded and contracted families. See Supplementary Figure 11 for GO analyses of gene families expanded / contracted in the common ancestor of echinoderms (marked with * in the figure).

a GO term enrichment of Echinoderm-contracted genes



b GO term enrichment of Echinoderm-expanded genes



Supplementary Figure 11 GO enrichment analysis of genes expanded/contracted in echinoderm lineage

Genes predicted to be contracted (**a**) and expanded (**b**) in the hypothetical common ancestor of echinoderms (see "*" position in Supplementary Figure 10 for the gene set analyzed in this figure) were analyzed for their GO term enrichment. Amphioxus (*B. floridae*) gene counterparts of the echinoderm contracted/expanded genes were first identified, and further analyzed for their GO slim term enrichment against the amphioxus genomic background using CateGOrizer (https://www.animalgenome.org/tools/catego/). Enrichment represents relative ratio of the GO terms of echinoderm-contracted/expanded genes against the genomic background. Only statistically significant results are shown here.

Average numbers in hemi-chordate/chordates





Supplementary Figure 12 Domains lost / specifically found in echinoderms

Pfam defined protein domains lost (a) or detected only in echinoderms (b) are shown. Genomes of five echinoderm species (A. japonica, O. spiculata, L. variegatus, A. planci, A. japonicus), and 8 other species (S. kowalevskii, B. floridae, D. rerio, G. gallus, A. mississippiensis, M. musculus, P. marinus, X. laevis) were used for this analysis. a. Protein domains not detected in any of the echinoderm speices, but were detected in the hemichordates or chordate species were defined as lost. b. Protein domains detected only in the echinoderms.



Supplementary Figure 13 Mapping of Hox genes onto scaffolds and chromosomes in feather star a. Chromosome walking using 23 BAC clones yielded four BAC-contigs which contained hox1 and hox2 [1], hox4 and hox5 [2], hox7, hox8, hox9/10 and hox11/13a; [3] and hox11/13c [4], respectively. The 4 contigs were turned out to be included by 2 scaffolds, 288292 and 287987. b. Two color-chromosomal FISH. Metaphase chromosome spreads were prepared from feather star embryos and hybridized with two probes labeled with digoxigenin (red) or biotin (green) for genes indicated at the bottom of each panel. Chromosomes were stained with DAPI. Red and green spots indicate signals for the gene of the same color code. FISH analysis revealed that all of the 12 Hox genes contained in the 2 scaffolds are localized closely together on the chromosome.

iox7 & hox8

hox8 & hox9/10

5μm

ox4 & hox5



Supplementary Figure 14 Genomic organization of ambulacrarian Hox gene clusters

The ambulacrarian phylogenetic tree based on the present study and genomic organization of ambulacrarian Hox gene clusters are indicated. Arrows and solid lines represent Hox gene CDSs and scaffolds, respectively. As regards *A. japonicus hox2*, only partial CDS was identified, hence represented by the dashed arrow. In *L. variegatus*, 11 Hox genes were located on 2 scaffolds. Similarly, in *A. japonica*, 10 genes (*hox1* through *hox11/13a*) and 2 genes (*hox11/13b* and *hox11/13c*) were on 2 scaffolds, both of which were, however, on the same chromosome according to the FISH analysis (see Supplementary Fig.13). Note the (nucleotide) length from *hox11/13b* of *L. variegatus* and *hox11/13a* of *A. japonica* to the end of respective scaffolds was more than 100 kb. Previous studies were refereed for the Hox cluster structures of *P. flava*⁹, *A. japonicus*³³, *S. purpuratus*⁷ and *A. planci*⁸.











Supplementary Figure 15 Developmental stages of green sea urchin, sea cucumber and feather star Representative images of collected echinoderm embryos for (a) green sea urchin (L. variegatus), (b) sea cucumber (A. japonicus), and (c) feather star (Anneissia japonica). (a) Images were modified from manuscript under preparation (Hogan et al.⁵⁸). Images of the remaining stages; 7 weeks post fertilization (wpf), 8 wpf, 8 wpf Larva (without rudiment), 8 wpf rudiment, 8.1wpf, 1 day post-metamorphosis, 9wpf (1 week postmetamorphosis), are not shown here. Adult stage here corresponds to 20wpf. (b) Images were modified from previously published work²³. (c) An arrow in the arm branching stage indicates the position of the branching arm (magnified image on it's top right). Arrows in the magnified image indicate the branching arm buds. Scale bars in unfertilized egg to early cystidean indicate 100 µm.



Supplementary Figure 16 Overview of gene expression patterns in echinoderm species

a. Gene expression patterns estimated from RNAseq data for each echinoderm species. For each gene, expression levels (TPM values) were z-score normalized along developmental stages. (**b**-**e**). Numbers of genes detected during echinoderm embryogenesis. Increasing number of coding genes detected during embryogenesis of *Lytechinus variegatus* (**b**), *Strongylocentrotus purpuratus* (**c**), *Apostichopus japonicus* (**d**), and *Anneissia japonica* (**e**). TPM higher than 1 was utilized as detected at the stages indicated in Y-axis, and error bars indicate S.D. of numbers of detected coding genes in the biological replicate samples. Results for *S. purpuratus* do not have error bars, as the dataset (PRJNA81157) did not contain biological replicates for each stage.



Supplementary Figure 17 Calculation of expDists for estimating conserved stages

In calculating expression <u>Distance</u> (expDist) of species in a single clade (as in Fig.2), phylogenetic relationship was taken into consideration to avoid unwanted bias arising from simple pair-wise comparisons of species⁶². **a**. For example, expDists in clade (A,B)C)D) was calculated as (AD + BD)/2 + CD)/2 and did not include species-pair such as BC or AC, as these do not reflect phylogenetic scale of interest. This calculation slightly differs from the previously reported method [(AD)/3 + (BD)/3 + (CD)/3 for the above case], as the previous method could be biased by distances among the species in a sub-clade (A,B)C). **b**. Hypothetical clade ((A,B),(C,D)) could be calculated as (((AD + BD)/2 + (AC + BC)/2)/2. However, this kind of phylogenetic relationship was not found in the echinoderm species we analyzed in this study, and thus was not utilized in estimating conserved stages of echinoderms.



Supplementary Figure 18 Estimation of most conserved developmental stages in echinoderms without purple sea urchin.

Vertical axis represents percentages of stage being included in the most (top 1%) conserved stage-combinations¹³ (Ptop). Changes of the conservation score (Ptop scores, %) were significant among stages (Friedman test). Error bars represent S.D. of Ptop values. Similar stages as in Figure 2 were found to be conserved for species without purple sea urchin (obtained from public database [PRJNA81157] sequenced by Tu Q *et al*^{21,22}). In each species, developmental phase when pentameral body plan establishment begins were colored in gray.



Supplementary Figure 19 Pair-wise comparisons of echinoderm transcriptome based on 1:1 orthologs Whole embryonic transcriptome similarities were calculated for all the combinations of echinoderm stages in pairwise manner. Expression levels (TPM) of 1:1 orthologs (defined by reciprocal best blast hits, RBBH) for Lv-Sp (11967 orthologs), Lv-Sp-Apj (5792 orthologs), and Lv-Sp-Apj-Anj (4225 orthologs) were used to calculate transcriptomic dissimilarities (1 – Spearman correlation coefficiency).



Supplementary Figure 20 Stage specific genes during echinoderm embryogenesis

Numbers of stage-specific genes were defined by tau index (tau > 0.5). Relative ratio of the stage-specific genes over the total numbers of expressed (> 1tpm) genes at each stage was also calculated (red plots, right axis in red). Error bars in panel a, c and d indicate S.D. of biological replicates calculated with the 100 random combination of expression data (BRI-exp data). The changes of these values along development were statistically significant (ANOVA without equal variance, p < 2.2 ' 10-16).



Supplementary Figure 21 Stages higher ratio of pleiotropic genes tend to be evolutionarily conserved in smaller phylogenetic group

Relationships between the ratios of pleiotropic genes (defined as genes expressed in more than 50% of staged analyzed, as in Supplementary Figure 20) and transcriptome similarity (expDsit). Each dot represent a combination of stages in the phylogenetic category of interest. For each dot, the average ratio of pleiotropic genes was calculated, and further analyzed for the relationship against their expDists (expDists). Pearson correlation coefficients and p-values (test of no correlation) are shown for each panel. Weak negative correlation was detected for Lv-Sp and Lv-Sp-Apj-Anj but not for clades of larger evolutionary scale (Lv-Apj-Anj and Lv-Sp-Apj-Anj).



Supplementary Figure 22. Expression dynamics of left-right patterning and axis-forming genes during the feather star development.

Temporal expression profiles of the left-right (LR) patterning and axis-forming during *Anneissia japonica* embryogenesis. The horizontal and vertical axes indicate developmental stages and relative expression levels (transcripts per kilobase million, TPM, mean values of two biological replicates), respectively. Error bars are not shown according to the guideline of the journal. Abbreviations of the developmental stages indicates as follows: EGG, unfertilized egg; 2C, two cells stage; 8C, eight cells stage; 32C, 32 cells stage; GA, gastrula; HA, hatching stage; ED, early doliolaria; LD, late doliolaria; AT, attachment stage; EC, early cystidean; LC, late cystidean; EP, early pentacrinoid; LP, late pentacrinoid; JUV, juvenile; AB, arm branching stage; AD, adult. Error bars represent S.D.



Supplementary Figure 23. Spatial expression of the left-right patterning and axis-forming in the attached larvae of feather star (*A. japonica*).

Left-right patterning related and axis-forming genes were analyzed for their expression patterns by WISH experiments. Shown are expression of bmp2/4 (**a**, **g**, **m**), chordin (**b**, **h**, **n**), nodal (**c**, **l**, **o**), lefty (**d**, **j**, **p**), pitx (**e**, **k**, **q**), and not (**f**, **l**) in larvae at the attachment stage (4 days after fertilization) (**a**-**f**) and at the cystidean stage (6 days after fertilization) (**g**-**q**). Lateral views are shown for **a**-**l**. **m**-**q** are the sections of WISH samples. In the attachment stage, pitx was expressed in the inner tissue of the calyx (an arrow in **e**) and the forming stalk (an arrowhead in **e**), while no other genes were expressed in these tissues. In the cystidean, chordin was expressed in the two spots in the calyx (arrows in **h**, **n**) and the inner tissue of the stalk (arrowheads in **h**, **n**), and pitx was expressed in the tissues around the gut (arrows in **k**, **q**) and the inner tissue of the whole stalk (arrowheads in **k**, **q**). No other genes were expressed in the calyx. Asterisks on each figure (except **l**) indicate the staining in the outer layer of basal stalk, which may be caused by the mucus that are secreted around the attachment disk. Scale bars: 100 µm.



Supplementary Figure 24. Alignment of MSP130 proteins from *Strongylocentrotus purpuratus (SPU), Patiria miniata (PMI) and Oxymocanthus japonicas (Oj).*

Proteins with a *following the name are present in the genomes but not found in the skeletal proteomes of the indicated species, while the others are found in the their respective skeletal proteomes.







Supplementary Figure 25. Alignment of C-type lectin proteins found in the skeletal proteomes of the sea urchin *Strongylocentrotus purpuratus (SPU)*, Brittle stars *Ophiocoma wendtii (Ow) and Ophothrix spiculata (Os) and* the feather star *Oxymocanthus japonicas (Oj)*.

Supplementary Tables

Library	Library	Insert	Read length	Sequencing	Data	Sequencing
type	name	length	(bp)	strategy	(Gb)	depth (X)
	Lv-1	394 bp	150	PE150	71.1	74.7
Short	Lv-2	424 bp	150	PE150	43.2	45.4
insert	Lv-3	479 bp	150	PE150	42.6	44.7
library	Lv-4	496 bp	150	PE150	75.8	79.6
	Lv-5	522 bp	150	PE150	13.5	14.2
Long	Lv-6	2-5 K	150	PE150	24.2	25.4
Long	Lv-7	5-9 K	150	PE150	43.7	45.9
librory	Lv-8	9-14 K	150	PE150	37.1	39.0
library	Lv-9	14-18 K	150	PE150	29.7	31.2
		Total			380.9	400.1

Supplementary Table 1. Paired-end DNA libraries sequenced for green sea urchin assembly. Sequencing depths were calculated for high-quality clean data based on genome size (952 Mb for sea urchin). The basic statistics were performed on cleaned sequencing data.

Library	Library	Insert	Read	Sequencing	Data	Sequencing
type	name	length	length (bp)	strategy	(G)	depth (X)
	Anj-1	277 bp	150	PE150	32.7	59.1
Chart	Anj -2	324 bp	150	PE150	26.9	48.6
Short	Anj -3	381 bp	150	PE150	14.4	26.0
librory	Anj -4	450 bp	150	PE150	24.2	43.8
norary	Anj -5	450 bp	250	PE250	36.7	66.4
	Anj -6	477 bp	150	PE150	18.8	34.0
	Anj -7	2-5 Kb	150	PE150	6.2	11.2
Long	Anj -8	5-9 Kb	150	PE150	9.9	17.9
Long	Anj -9	14-18 Kb	150	PE150	11.0	19.9
insert	Anj -10	2.5 Kb	45-80	PE150	19.6	35.4
library	Anj -11	3.6 Kb	45-80	PE150	7.8	14.1
	Anj -12	4.7 Kb	45-80	PE150	6.1	11.0
	-	Total			214.3	387.5

Supplementary Table 2. Paired-end DNA libraries sequenced for feather star genome assembly. Sequencing depths were calculated for high-quality clean data based on genome size (553Mb for feather star). The basic statistics were performed on cleaned sequencing data.

	Contig		Sca	ffold
	Size (bp)	Number	Size (bp)	Number
N90	1,723	95,713	2,020	21,723
N80	3,903	60,286	24,482	2,201
N70	6,452	42,583	235,147	881
N60	8,984	30,763	434,435	582
N50	11,683	21,960	628,067	397
Max length (bp)	29	291126 4073095		73095
Total size (bp)	90164	5781	9738	80027
Total number (>100bp)	19	5320	1	13370
Total number (>2000bp)	8	6229		22255

Supplementary Table 3. Basic statistics of the assembled green sea urchin genome.

	Contig		Scat	ffold	
	Size (bp)	Number	Size (bp)	Number	
N90	1,478	48,708	1,805	9,910	
N80	4,505	26,019	67,678	989	
N70	8,618	17,091	231,513	528	
N60	12,948	11,747	419,248	343	
N50	18,075	8,045	623,489	228	
Max length (bp)	198	3,797	5,844,021		
Total size (bp)	565,8	29,874	589,638,548		
Total number (>100bp)	119,876		76,733		
Total number (>2000bp)	40,222		8,330		

Supplementary Table 4. Basic statistics of the assembled feather star genome.

	Co	Contig		ffold	
	Size (bp)	Number	Size (bp)	Number	
N90	1,651	355,751	19,599	38,746	
N80	2,768	253,412	32,996	28,075	
N70	3,873	186,181	45,744	20,984	
N60	5,082	136,373	58,599	15,651	
N50	6,474	97,791	72,780	11,418	
Max length (bp)	8	1,520	7	78,843	
Total size (bp)	2,210,14	0,678	2,764,315,159		
Total number (>100bp)	644,795 75,696		75,696		
Total number (>2000bp)	317,571		70,280		

Supplementary Table 5 Basic statistics of the brittle star genome obtained from Echinobase⁶⁵⁻⁶⁷.

Library	Description	Gene number	Coverage
	Complete BUSCOs (C)	270	89.1 %
	Complete and single-copy BUSCOs (S)	248	81.8 %
autromista adh0	Complete and duplicated BUSCOs (D)	22	7.3 %
eukaryota_odb9	Fragmented BUSCOs (F)	14	4.6 %
	Missing BUSCOs (M)	19	6.3 %
	Total BUSCO groups searched	303	-
	Complete BUSCOs (C)	882	90.2 %
	Complete and single-copy BUSCOs (S)	815	83.3 %
matazaa adh0	Complete and duplicated BUSCOs (D)	67	6.9 %
metazoa_odb9	Fragmented BUSCOs (F)	50	5.1 %
	Missing BUSCOs (M)	46	4.7 %
	Total BUSCO groups	978	-

Supplementary Table 6 Coverage rate of conserved genes in assembled green sea urchin genome by BUSCO.

Library	Description	Gene number	Coverage
Eukaryote-conserved genes (eukaryota_odb9)	Complete BUSCOs (C)	282	93.0 %
	Complete and single-copy BUSCOs (S)	274	90.4 %
	Complete and duplicated BUSCOs (D)	8	2.6 %
	Fragmented BUSCOs (F)	8	2.6 %
	Missing BUSCOs (M)	13	4.4 %
	Total BUSCO groups searched	303	-
	Complete BUSCOs (C)	916	93.6 %
Matazaa approximited	Complete and single-copy BUSCOs (S)	906	92.6 %
genes (metazoa_odb9)	Complete and duplicated BUSCOs (D)	10	1.0 %
	Fragmented BUSCOs (F)	23	2.4 %
	Missing BUSCOs (M)	39	4.0 %
	Total BUSCO groups searched	978	-

Supplementary Table 7 Coverage rate of conserved genes in assembled feather star genome by BUSCO

Library	Description	Gene number	Coverage
	Complete BUSCOs (C)	303	87.8 %
Eulermote concerned	Complete and single-copy BUSCOs (S)	175	57.8 %
Eukaryote-conserved	Complete and duplicated BUSCOs (D)	91	30.0 %
genes	Fragmented BUSCOs (F)	17	5.6 %
(eukaryota_odb9)	Missing BUSCOs (M)	20	6.6 %
	Total BUSCO groups searched	303	-
	Complete BUSCOs (C)	901	92.1 %
Matamaa assessmed	Complete and single-copy BUSCOs (S)	597	61.0 %
Metazoa-conserved genes	Complete and duplicated BUSCOs (D)	304	31.1 %
	Fragmented BUSCOs (F)	36	3.7 %
(metazoa_0009)	Missing BUSCOs (M)	41	4.2 %
	Total BUSCO groups searched	978	-

Supplementary Table 8 Coverage rate of conserved genes in brittle star genome by BUSCO.

Species	Total reads	Mapped reads	Reads map (%)
Feather star	838,964,826	782,414,226	93.26
Sea urchin	1,367,973,887	1,360,681,865	99.47

Supplementary Table 9 Statistics of the mapping ratio of RNAseq reads in two assembled genomes.

Statistical item	Length (bp)	Number
N90	426	
N80	711	
N70	1,097	
N60	1,568	
N50	2,091	
Average length	1,116	
Max length	31,011	
Total length	873,673,625	
Total number		782,334
Number>=1000bp		250,683

Supplementary Table 10 Statistics of feather star transcripts assembled by Trinity and clustered by TGICL.

Statistical item	Length (bp)	Number
N90	499	
N80	818	
N70	1,181	
N60	1,601	
N50	2,063	
Average length	1,217	
Max length	37,771	
Total length	239,193,964	
Total number		196,425
Number>=1000bp		74,038

Supplementary Table 11 Statistics of green sea urchin (*Lytechinus variegatus*) transcripts assembled by Trinity and clustered by TGICL.

Range of	Range of Total		Percent	>50% of sequence		>90% of sequence	
length	number	match number	natch (%) Nu		Percent(%)	Number	Percent (%)
All	453,163	447,015	98.64	430,467	94.99	320,783	70.79
>=500	227,528	225,406	99.07	217,710	95.68	162,479	71.41
>=1000	133,684	133,342	99.74	129,325	96.74	99,388	74.35

Supplementary Table 12 Assessment of the completeness of coding region using transcriptome data in the feather star genome.

Range of	Total	Total	Percent	>50% of sequence		>90% of	sequence
length (bp)	number	match number	(%)	Number	Percent (%)	Number	Percent (%)
All	187,965	187,078	99.53	179,153	95.31	120,108	63.90
>=500	94,870	94,551	99.66	90,635	95.54	55,675	58.69
>=1000	51,093	51,057	99.93	48,655	95.23	28,087	54.97

Supplementary Table 13 Assessment of the completeness of coding region using transcriptome data in the green sea urchin genome.

	Repbas	e TEs	TE pr	otiens	De ne	0V0	Combine	ed TEs
Туре	Length (bp)	% in genome						
DNA	4,329,259	0.73	2,776,494	0.47	60,982,279	10.34	65,802,851	11.16
LINE	5,273,685	0.89	20,453,430	3.47	63,607,826	10.79	86,507,741	14.67
SINE	816,335	0.14	0	0	18,668,683	3.16	18,957,279	3.22
LTR	5,897,757	1.00	7,628,210	1.30	77,710,356	13.18	88,461,182	15.00
Other	48	0.000008	0	0	14,785	0.0025	14,833	0.002
Unknown	0	0	0	0	96,699,545	16.40	96,699,545	16.40
Total	15,029,720	2.5	30,812,054	5.2	254,273,525	43.12	288,319,345	48.90

Supplementary Table 14 The statistics of transposable element in feather star genome.

Туре	Repeat Size	% of genome
Trf	27,484,869	4.66
Repeatmasker	15,029,720	2.55
Proteinmask	30,812,054	5.23
De novo	270,515,276	45.88
Total	313,512,480	53.12

Supplementary Table 15 The statistics of repeat sequences annotated by different methods in feather star genome

	Repbas	e TEs	TE pro	otiens	De no	0 V 0	Combine	ed TEs
Tyme	Length	% in	Length	% in	Length	% in	Length	% in
Type	(Bp)	genome	(Bp)	genome	(Bp)	genome	(Bp)	genome
DNA	15,726,621	1.61	2,700,815	0.28	104,346,168	10.71	109,578,386	11.25
LINE	10,611,419	1.09	24,815,386	2.55	26,910,567	2.76	55,762,446	5.72
SINE	2,822,575	0.29	0	0	19,535,922	2.01	20,452,191	2.10
LTR	5,661,477	0.58	5,259,001	0.54	5,808,334	0.60	16,180,259	1.66
Other	2,085	0.0002	0	0	0	0	2,085	0.0002
Unknown	0	0	0	0	253,607,055	26.04	253,607,055	26.04
Total	33.870.267	3.48	32,753,565	3.36	398.826.011	40.95	437.743.803	44.95

Supplementary Table 16 The statistics of transposable element in green sea urchin genome.

Туре	Repeat Size	% of genome
Trf	41429791	4.35
Repeatmasker	33870267	3.48
Proteinmask	32753565	3.36
De novo	403226206	41.40
Total	456240421	46.84

Supplementary Table 17 The statistics of repeat sequences annotated by different methods in green sea urchin genome.

Species	Feather star	Green sea urchin
Gene number	26,838	30,238
Average mRNA length (bp)	8,836.90	16,134.76
Total number of exon	162,961	198,447
Average length of exon (bp)	191.16	203.5
Average length of CDS (bp)	1,160.73	1,335.53
Average number of exon	6.07	6.56
Total length of intron (bp)	206,013,032	447,499,073

Supplementary Table 18 The statistics of annotated gene set in feather star.

Database	Number	Percent (%)
GO	17,034	63.46
InterPro	15,389	57.34
COG	5,577	20.78
KEGG	12,146	45.25
Nr	17,880	66.62
SwissProt	14,097	52.52
TrEMBL	17 374	64 73

Supplementary Table 19 The number of gene models in feather star genome that could be annotated by GO, InterPro, COG, KEGG, Nr (RefSeq non-redundant proteins database), SwissProt and TrEMBL databases.

Database	Number	Percent (%)
GO	18,042	59.66
InterPro	16,491	54.53
COG	6,210	20.53
KEGG	13,616	45.02
Nr	20,538	67.92
SwissProt	14,962	49.48

TrEMBL 20,041	66.27

Supplementary Table 20 The number of gene models in green sea urchin genome that could be a	annotated
by GO, InterPro, COG, KcGG, Nr, SwissProt and TrEMBL databases.	

Туре	Sub type	Copy (w)	Average length (bp)	Total length (bp)	% of
					genome
miRNA	-	330	92.60	30,559	0.0052
tRNA	-	785	73.09	57,375	0.0097
rRNA	-	365	97.33	35,524	0.0060
	-	150	146.74	22,011	0.0037
	CD-box	32	140.31	4,490	0.0008
SIKINA	HACA-box	3	154	462	0.00008
	splicing	115	148.34	17,059	0.0029

Supplementary Table 21 The statistic result of ncRNAs annotation in feather star genome.

Type	Sub type	Conv(w)	Average length	Total length	% of
турс	Sub type	Cob?(")	(bp)	(bp)	genome
miRNA	-	595	97.84	58,213	0.0060
tRNA	-	1,131	74.45	84,206	0.0086
rRNA	-	12	334.50	4,014	0.0004
	-	187	125.89	23,542	0.0024
snRNA	Cc-box	64	101.41	6,490	0.0007
	splicing	123	138.63	17,052	0.0018

Supplementary Table 22 The statistic result of ncRNAs annotation in the green sea urchin genome.

Species	Species name	Gene count	Gene family count	Average # of genes per family
Green sea urchin	Lytechinus variegatus	30,238	12,877	1.65
Purple sea urchin	Strongylocentrotus purpuratus	27,741	13,798	1.65
Sea cucumber (<i>Apj</i>)	Apostichopus japonicus	21,771	10,209	1.48
Brittle star	Ophiothrix spiculata	22,904	7,252	2.58
Sea star	Acanthaster planci	24,323	11,522	1.63
Feather star (Anj)	Anneissia japonica	26,762	11,071	1.78
Acorn worm	Saccoglossus kowalevskii	20,935	11,101	1.56
amphioxus	Branchiostoma belcheri	25,135	11,167	1.84
Ciona	Ciona intestinalis	13,531	8,280	1.34
Lamprey	Petromyzon marinus	10,415	6,845	1.26
zebrafish	Danio rerio	25,832	13,419	1.74
Medaka	O. lattipes	19,699	11,934	1.44
Western clawed frog (frog)	Xenopus laevis	45,099	16,428	2.18
chicken	Gallus gallus	18,346	12,172	1.38
Chinese softcshell turtle	Pelodiscus sinensis	18,189	12,432	1.35
Mouse	Mus musculus	22,808	13,389	1.55
Fruit fly	Drosophila melanogaster	13,918	6,979	1.49

Supplementary Table 23 Basic statistics of genes in each species

Species name	Assembly/Gene models made by	Version information
Anolis carolinensis	Ensembl	AnoCar2.0
Branchiostoma belcheri	LanceletDB	v18h27.r3_ref
Ciona intestinalis	Ensembl	GCA_000224145.1
Danio rerio	Ensembl	GRCz10
Gallus gallus	Ensembl	Gallus_gallus-5.0
Strongylocentrotus purpuratus	NCBI	GCF_000002235.4
Saccoglossus kowalevskii	NCBI	GCF_000003605.2
Homo sapiens	Ensembl	GRCh38
Mus musculus	Ensembl	GRCm38
Petromyzon marinus	Ensembl	Pmarinus_7.0
Xenopus tropicalis	Ensembl	JGI_4.2
Drosophila melanogaster	Ensembl	BDGP6
Alligator mississippiensis	NCBI	GCF_000281125.3
Ophiothrix spiculata	Echinobase ⁶⁵⁻⁶⁷	PRJNA182997
Apostichopus japonicus	GigaDB / NCBI	Jo J et al. ⁶⁸ and Zhang X et al. ³³
Acanthaster planci	marinegenomics.oist.jp	cotsv1.0
Xenopus laevis	Xenbase	Xenla9.1_v1.8.3.2

Supplementary Table 24 Gene set and genomes used in this project.

Species	Relative branch length
A. japonica (Anj)	0.676
A. planci	0.457
O. spiculata	0.720
L. variegatus	0.198
S. purpuratus	0.099
A. japonicus (Apj)	0.726
S. kowalevskii	0.603
P. marinus	0.596
G. gallus	0.131
P. sinensis	0.142
M. musculus	0.206
X. laevis	0.245
D. rerrio	0.208
O. lattipes	0.272
C. inttestinalis	1.291
B. floridae	0.619
D. melanogaster	0.849

Supplementary Table 25 Branch length estimated from orthologous protein sequences using RAxML

Outgroup	Ingroup 1	Ingroup 2	bA	bB	delta	Z score	СР
Fruit fly	Purple sea urchin	Feather star	0.357167	0.357832	0.000664	0.248722	18.96%
Fruit fly	Sea cucumber	Feather star	0.405544	0.338478	0.067065	20.519726	99.96%
Fruit fly	Sea star (A. planci)	Feather star	0.325828	0.358536	0.032708	12.516316	99.96%
Fruit fly	Ciona	Feather star	0.638331	0.493120	0.145211	44.751784	99.96%
Fruit fly	Frog	Feather star	0.430825	0.497370	0.066545	23.667571	99.96%
Fruit fly	Mouse	Feather star	0.426553	0.503514	0.076962	27.491514	99.96%
Fruit fly	Amphioxus	Feather star	0.356322	0.434225	0.077903	29.249280	99.96%
Fruit fly	Acorn worm	Feather star	0.348458	0.401235	0.052777	19.409783	99.96%
Fruit fly	Turtle	Feather star	0.438257	0.484672	0.046415	16.226124	99.96%
Fruit fly	Medaka	Feather star	0.441844	0.489073	0.047229	16.736153	99.96%
Fruit fly	Zebrafish	Feather star	0.436039	0.495892	0.059853	21.198209	99.96%
Fruit fly	Chicken	Feather star	0.426225	0.491857	0.065632	23.285712	99.96%
Fruit fly	Brittle star	Feather star	0.438306	0.322925	0.115381	36.881544	99.96%
Fruit fly	Lamprey	Feather star	0.468829	0.447759	0.021071	6.902717	99.96%
Fruit fly	Green sea urchin	Feather star	0.434961	0.357281	0.077681	26.119120	99.96%

Supplementary Table 26 The result of evolutionary rate by LINTRE.

The outgroup is Drosophila, and feather star as the reference. Z-statistic was used to test whether the distances between ingroups (bA, bB) to outgroup is significantly different to 0 or not. Delta is the absolute difference between bA and bB (delta = | bA - bB |). Z-statistics(Z) is delta/standard error (Z = delta/s.e.). CP (confident probability) is equal to 1-P-value (CP= 1 - pvalue).

Outgroup	Ingroup A	Ingroup B	Identical	Ingroup A specific	Ingroup B specific	Chi-score	P-value
Fruit fly	Purple sea urchin	Feather star	249278	50403	50482	0.06	0.80358
Fruit fly	Sea cucumber	Feather star	163070	38339	32852	422.91	< 0.000001
Fruit fly	Sea star (A. planci)	Feather star	252455	46664	50569	156.83	< 0.000001
Fruit fly	Ciona	Feather star	228412	77874	61072	2031.78	< 0.000001
Fruit fly	Frog	Feather star	256298	60342	68865	562.21	< 0.000001
Fruit fly	Mouse	Feather star	258718	60113	70056	759.50	< 0.000001
Fruit fly	Amphioxus	Feather star	242897	50022	59741	860.57	< 0.000001
Fruit fly	Acorn worm	Feather star	239633	48178	54403	377.76	< 0.000001
Fruit fly	Turtle	Feather star	244650	59439	65172	263.76	< 0.000001
Fruit fly	Medaka	Feather star	247908	60186	66140	280.62	< 0.000001
Fruit fly	Zebrafish	Feather star	253733	60393	68000	450.70	< 0.000001
Fruit fly	Chicken	Feather star	251967	59428	67747	544.18	< 0.000001
Fruit fly	Brittle star	Feather star	193593	49011	38062	1376.78	< 0.000001
Fruit fly	Lamprey	Feather star	201026	53043	50818	47.67	< 0.000001
Fruit fly	Green sea urchin	Feather star	231579	57268	48742	685.72	< 0.000001

Supplementary Table 27 The result of evolutionary rate by RRT (Tajima's <u>Relative Rate Test</u>)

	Anj	Ар	Os	Lv	Sp	Apj	Sk	Pm	Gg	Ps	Mm	Xl	Dr	Ol	Ci	Bf	Dm
Anj	0.00	1.31	1.57	1.47	1.37	1.59	1.43	2.00	1.90	1.91	1.92	1.89	1.91	1.97	2.43	1.64	2.65
Ар	1.31	0.00	1.18	1.21	1.11	1.33	1.39	1.96	1.85	1.87	1.87	1.84	1.86	1.92	2.39	1.59	2.61
Os	1.57	1.18	0.00	1.47	1.37	1.59	1.65	2.22	2.12	2.13	2.13	2.11	2.12	2.19	2.65	1.85	2.87
Lv	1.47	1.21	1.47	0.00	0.30	1.33	1.56	2.12	2.02	2.03	2.04	2.01	2.03	2.09	2.55	1.76	2.77
Sp	1.37	1.11	1.37	0.30	0.00	1.24	1.46	2.02	1.92	1.93	1.94	1.91	1.93	1.99	2.45	1.66	2.68
Apj	1.59	1.33	1.59	1.33	1.24	0.00	1.67	2.24	2.14	2.15	2.16	2.13	2.15	2.21	2.67	1.88	2.89
Sk	1.43	1.39	1.65	1.56	1.46	1.67	0.00	1.77	1.67	1.68	1.69	1.66	1.68	1.74	2.20	1.41	2.42
Pm	2.00	1.96	2.22	2.12	2.02	2.24	1.77	0.00	1.09	1.10	1.11	1.08	1.10	1.16	2.15	1.60	2.74
Gg	1.90	1.85	2.12	2.02	1.92	2.14	1.67	1.09	0.00	0.27	0.40	0.50	0.69	0.75	2.05	1.50	2.64
Ps	1.91	1.87	2.13	2.03	1.93	2.15	1.68	1.10	0.27	0.00	0.41	0.51	0.70	0.76	2.06	1.51	2.65
Mm	1.92	1.87	2.13	2.04	1.94	2.16	1.69	1.11	0.40	0.41	0.00	0.52	0.70	0.77	2.07	1.52	2.66
Xl	1.89	1.84	2.11	2.01	1.91	2.13	1.66	1.08	0.50	0.51	0.52	0.00	0.68	0.74	2.04	1.49	2.63
Dr	1.91	1.86	2.12	2.03	1.93	2.15	1.68	1.10	0.69	0.70	0.70	0.68	0.00	0.48	2.06	1.51	2.65
Ol	1.97	1.92	2.19	2.09	1.99	2.21	1.74	1.16	0.75	0.76	0.77	0.74	0.48	0.00	2.12	1.57	2.71
Ci	2.43	2.39	2.65	2.55	2.45	2.67	2.20	2.15	2.05	2.06	2.07	2.04	2.06	2.12	0.00	2.03	3.17
Bf	1.64	1.59	1.85	1.76	1.66	1.88	1.41	1.60	1.50	1.51	1.52	1.49	1.51	1.57	2.03	0.00	2.38
Dm	2.65	2.61	2.87	2.77	2.68	2.89	2.42	2.74	2.64	2.65	2.66	2.63	2.65	2.71	3.17	2.38	0.00

Supplementary Table 28 The result of evolutionary rate by APE.

Gene ID	KO ID	Name	Definition
scaffold288031 len342695 cov171.5	K09511	DNAJB5	DnaJ homolog subfamily B member 5
scaffold287712 len2945570 cov188.9	K10397	KIF6 9	kinesin family member 6/9
scaffold287714 len2871179 cov188.42	K03130	TAF5	transcription initiation factor TFIID subunit 5
scaffold2707 len876726 cov182.13	K12571	PAN2	PAB-dependent poly(A)-specific ribonuclease subunit 2
scaffold287743 len2803612 cov181.129	K01958	PC, pyc	pyruvate carboxylase
scaffold32248 len650992 cov182.16	K12812	UAP56, BAT1, SUB2	ATP-dependent RNA helicase UAP56/SUB2
scaffold287717 len1600092 cov198.49	K12197	CHMP1, VPS46, DID2	charged multivesicular body protein 1
scaffold522 len1101056 cov227.41	K10134	EI24	etoposide-induced 2.4 mRNA
scaffold287938 len408866 cov212.4	K08469	GPR158	G protein-coupled receptor 158
scaffold287861 len940595 cov192.24	K00924	E2.7.1	kinase
scaffold7731 len1735946 cov199.45	K02209	MCM5, CDC46	DNA replication licensing factor MCM5
scaffold1483 len710385 cov172.5	K08857	NEK1 4 5	NIMA (never in mitosis gene a)-related kinase 1/4/5
scaffold288275 len480233 cov166.13	K04601	CELSR2	cadherin EGF LAG seven-pass G-type receptor 2
scaffold2030 len652032 cov178.10	K03251	EIF3D	translation initiation factor 3 subunit D
scaffold287715 len1027211 cov185.10	K04560	STX1A	syntaxin 1A
scaffold1489_len2284818_cov182.8	K10364	CAPZA	capping protein (actin filament) muscle Z-line alpha
scaffold287949_len505691_cov196.9	K10733	GINS2_PSF2	GINS complex subunit 2
scaffold11779_len1430905_cov172_34	K12035	TRIM71	tripartite motif-containing protein 71
scaffold284860_len1800451_cov186_35	K08332	VAC8	vacuolar protein 8
scaffold685_len2926416_cov193_50	K12380	RASA3	Ras GTPase-activating protein 3
scaffold288014_len389662_cov180_3	K12606	RCD1 CNOT9 CAF40	CCR4-NOT transcription complex subunit 9
scaffold287861_len940595_cov192_15	K02901	RP-L27e RPL27	large subunit ribosomal protein L 27e
scaffold287835_len3118868_cov185.83	K09871	AOP12	aquanorin-12
scaffold287706_len398110_cov188_11	K07739	FLP3 KAT9	elongator complex protein 3
scaffold287847_len925385_cov155.22	K11885	DDI1	DNA damage_inducible protein 1
scaffold4054 len1135799 cov19515	K12796	ERBB2IP ERBIN	erbb2-interacting protein
scaffold287704_len1198815_cov201_33	K10570	FRCC8 CKN1 CSA	DNA excision repair protein FRCC-8
scaffold287754_len4335492_cov202_56	K12829	SF3B2 SAP145 CUS1	splicing factor 3B subunit 2
scaffold287868_len458714_cov195_5	K12392	AP1B1	AP-1 complex subunit beta-1
scaffold211_len1577895_cov191_47	K10151	CCND2	G1/S-specific cyclin-D2
scaffold287710 len1884721 cov185 57	K12831	SF3BA SAPAQ	splicing factor 3B subunit A
scaffold288015_len651517_cov101.8	K04460	PPP5C	serine/threenine_protein phosphatase 5
scaffold288191_len216021_cov166_10	K02885	RP_I 10e RPI 10	large subunit ribosomal protein L 19e
scaffold2040_len624021_cov100.10	K02885	KCNIK13	notassium channel subfamily K member 12
scaffold1504_lon1068242_cov182.20	K04922 K10380	ANK	ankurin
scalfold1374_lcll1008242_c0v185.27	K10380	TUDD	ankynn tybylin dolto
scaffold275 len1210475 cov179.5	K10390	KLC	kinesin light chain
$s_{a110102/3} = 10112194/3 = 0001/8.3$ $s_{aaffold} = 202612 = 0001/8.3$	K10407	DDVAD	5' AMD activated protein kinase, regulatory beta subunit
scalfold287726 lon1801122 ocv214.91	K0/199	TINAD DID5V	1 phosphatidulinosital 4 phosphate 5 kinase
scallolu20//50_lell1091122_c0V214.81	KUU009	TITUN DDD1D0 NIDD1	nuclear inhibitor of protoin phosphates 1
scaffold276 lon1572418 cov104.22	K13210	PP S50 DDS5	small subunit ribosomal protein S5a
scalibius /0_lell15/2416_c0v194.32	NU2909	LIDV	homoodomain interacting protein kinaco
scanolu2.34 len10/2027 cov1/8.46	KU8820	TIIT N	nomeodomam interacting protein kinase

scaffold306325_len3074_cov500_single.1	K13125	NOSIP	nitric oxide synthase-interacting protein				
scaffold737_len1565949_cov176.8	K08291	GRK4 5 6	G protein-coupled receptor kinase				
scaffold287755_len519135_cov135.3	K11338	RUVBL2, RVB2, INO80J	RuvB-like protein 2				
scaffold211_len1577895_cov191.25	K02865	RP-L10Ae, RPL10A	large subunit ribosomal protein L10Ae				
scaffold3782_len1007564_cov172.50	K09402	FOXJ1	forkhead box protein J1				
scaffold3934_len446420_cov169.6	K14561	IMP4	U3 small nucleolar ribonucleoprotein protein				
scaffold284860_len1800451_cov186.50	K00599	METTL6	methyltransferase-like protein 6				
scaffold2756_len2360902_cov194.74	K00006	GPD1	glycerol-3-phosphate dehydrogenase (NAD+)				
scaffold352_len1594369_cov220.6	K10165	NOD2, CARD15	nucleotide-binding oligomerization domain-containing protein 2				
scaffold287949_len505691_cov196.2	K05726	BCAR1, CAS	breast cancer anti-estrogen resistance 1				
scaffold1826_len1998817_cov189.4	K10085	EDEM2	ER degradation enhancer, mannosidase alpha-like 2				
scaffold288039_len1115542_cov189.4	K12815	DHX38, PRP16	pre-mRNA-splicing factor ATP-dependent RNA helicase				
scaffold288221_len503873_cov180.18	K05305	FUK	fucokinase				
scaffold7370_len818893_cov191.42	K04638	IFT57, HIPPI, ESRRBL1	intraflagellar transport protein 57				
scaffold287579_len849171_cov184.46	K10406	KIFC2_3	kinesin family member C2/C3				
scaffold287711_len853857_cov187.4	K05862	VDAC1	voltage-dependent anion channel protein 1				
scaffold287990_len610157_cov183.15	K14163	EPRS	bifunctional glutamyl/prolyl-tRNA synthetase				
scaffold287834_len5846547_cov211.150	K14021	BAK, BAK1	Bcl-2 homologous antagonist/killer				
scaffold522_len1101056_cov227.7	K11797	PHIP, WDR11	PH-interacting protein				
scaffold352_len1594369_cov220.71	K09498	CCT6	T-complex protein 1 subunit zeta				
scaffold287540_len12866_cov218.1	K14717	SLC39A11, ZIP11	solute carrier family 39 (zinc transporter), member 11				
scaffold483_len1788099_cov177.12	K01238	SUN	SUN family beta-glucosidase				
scaffold287769_len469884_cov163.11	K07561	DPH1, dph2	2-(3-amino-3-carboxypropyl)histidine synthase				
scaffold585_len4108843_cov191.75	K10631	TOPORS	E3 ubiquitin-protein ligase				
scaffold287790_len634930_cov199.5	K02908	RP-L30e, RPL30	large subunit ribosomal protein L30e				
scaffold1696_len571195_cov180.20	K14403	CPSF3, YSH1	cleavage and polyadenylation specificity factor subunit 3				
upplementary Table 20 KECC annotations of positively selected genes during achinederm evolution							

Supplementary Table 29 KEGG annotations of positively selected genes during echinoderm evolution.

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