

Supplemental Material for

Fusion, fission, and scrambling of the bilaterian genome in Bryozoa

Thomas D. Lewin, Isabel Jiah-Yih Liao, Mu-En Chen, John D. D. Bishop, Peter W. H. Holland and Yi-Jyun Luo.

Corresponding author: Yi-Jyun Luo

Email: yjluo@gate.sinica.edu.tw

This PDF file includes:

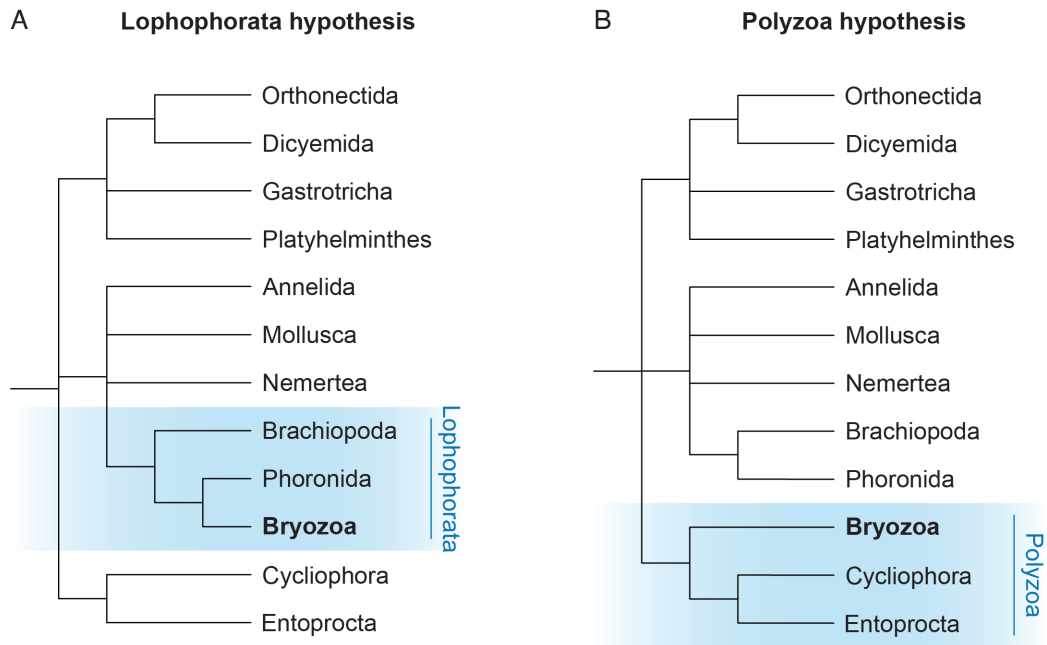
- Supplemental Figs. S1 to S13
- Supplemental Tables S1 to S14
- Supplemental References

Other supporting materials for this manuscript include the following:

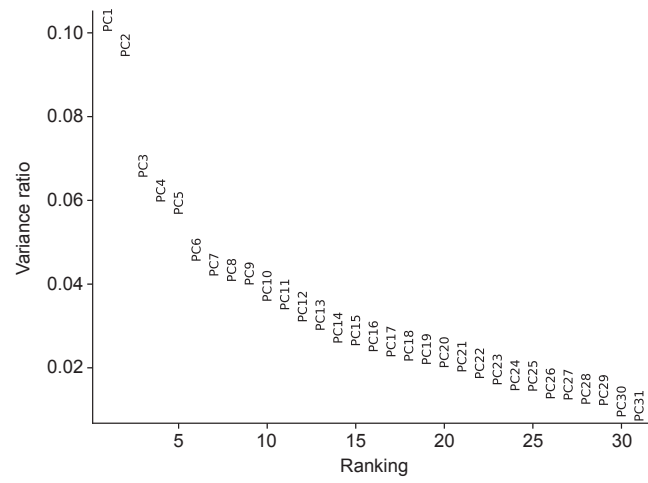
- Supplemental Data S1 to S2

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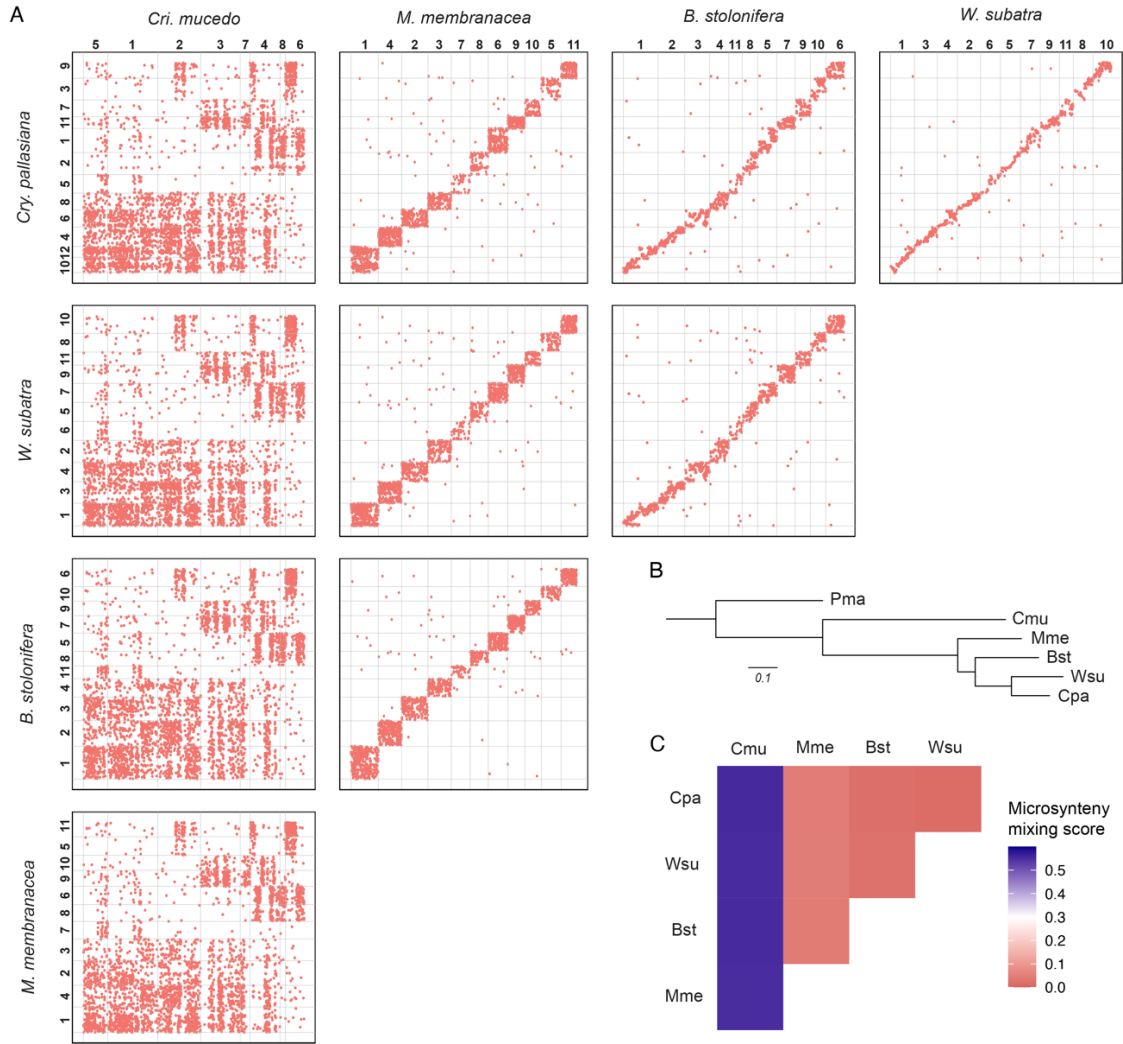
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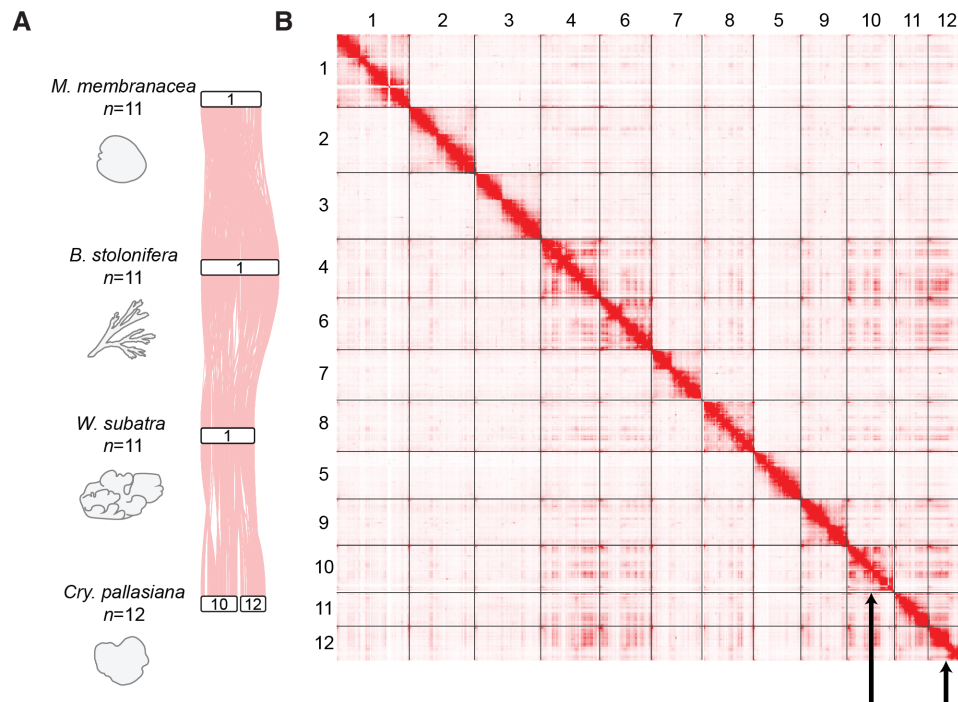
Supplemental Fig. S1. Lophophorata and Polyzoa hypotheses for the phylogenetic position of Bryozoa. (A) Phylogeny of spiralian phyla under Lophophorata hypothesis. (B) Phylogeny of spiralian phyla under Polyzoa hypothesis. Consensus phylogenies are from Liao et al. (2023).



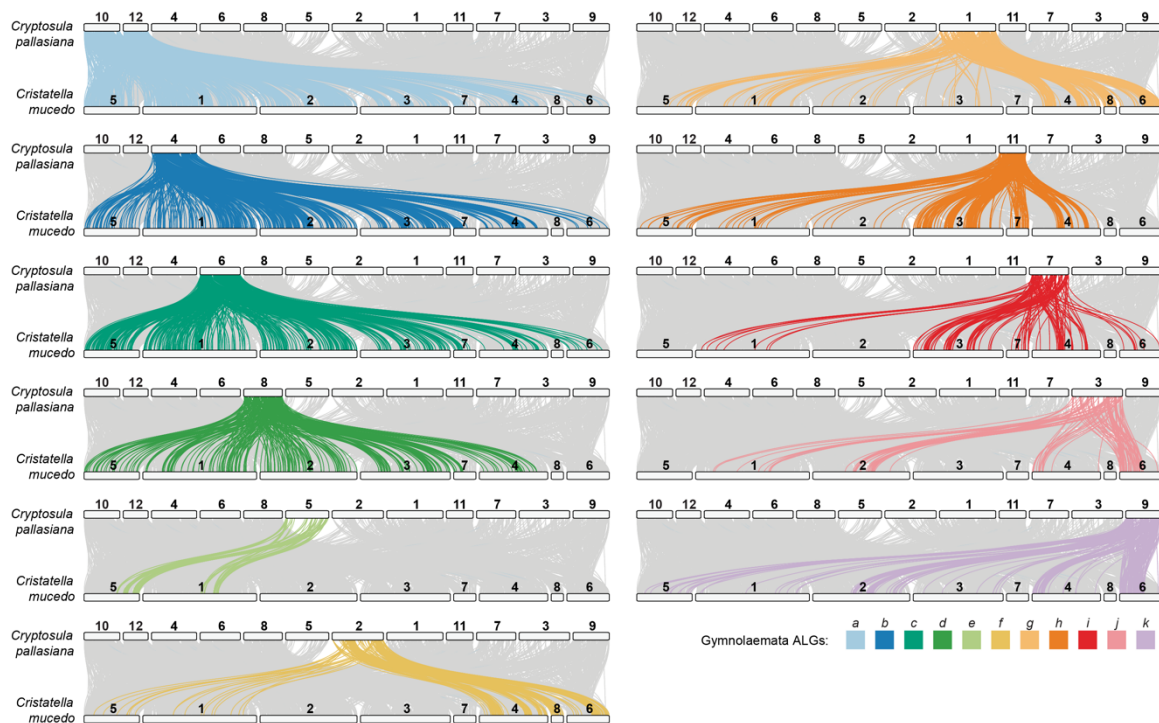
Supplemental Fig. S2. Variance ratios for the top 30 principal components for gene family analysis. Main figure for principal component analysis is Main Text Fig.1E.



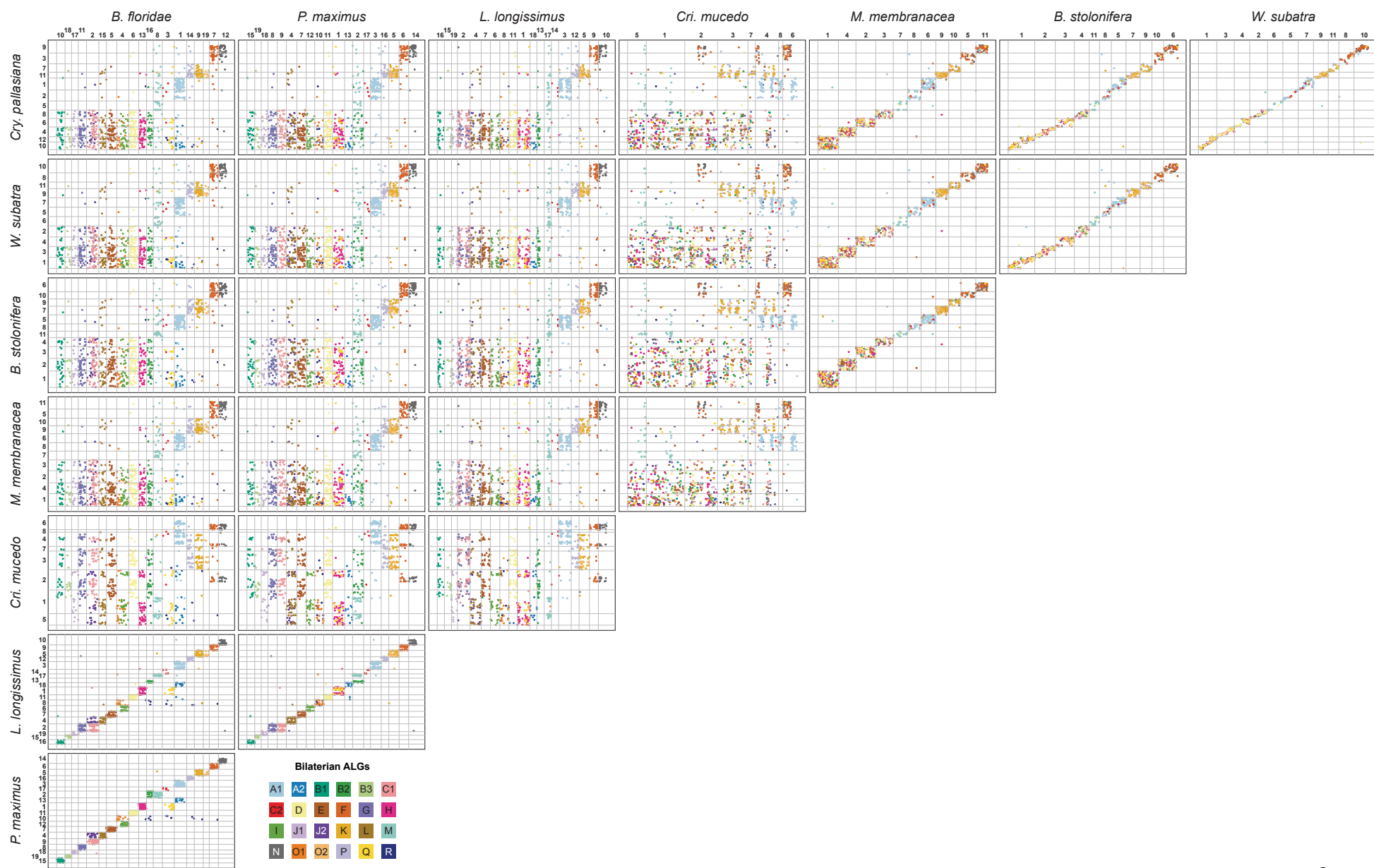
Supplemental Fig. S3. Chromosome evolution within bryozoans revealed by Oxford dot plots. (A) Oxford dot plots show pairwise comparisons of the position of orthologs in two genomes. Red points represent orthologous genes, axes represent entire genome length. Pale gray bars mark chromosome boundaries. Bold numbers show chromosome number. A straight line from bottom left to top right would represent complete conservation of macrosynteny and gene order (*Watersipora subatra* vs. *Cryptosula pallasiana* is the closest). The widening of the line into square blocks of genes represents the shuffling of orthologous genes within orthologous chromosomes (e.g. *Membranipora membranacea* vs. *Cry. pallasiana*) but not interchromosomal rearrangements, so macrosynteny is conserved. Blocks covering one chromosome in one species but multiple chromosomes in another species represent fusion or fission events (e.g. *Cristatella mucedo* vs. *Cry. pallasiana*). *Cry. pallasiana*, *W. subatra*, *Bugulina stolonifera* and *M. membranacea* have highly conserved genome structure with minimal interchromosomal rearrangement. The *Cri. mucedo* genome is highly rearranged in comparison. (B) Phylogeny of bryozoan study species reproduced from Main Text Figure 1C. (C) Quantification of microsynteny (shuffling of genes within chromosomes) between species with our ‘microsynteny mixing score’. More closely related species have a lower microsynteny mixing score, indicating lower rates of shuffling and more highly conserved gene order. Scores are listed as Table S7. Abbreviations: *Bst*, *B. stolonifera*; *Cmu*, *Cri. mucedo*; *Cpa*, *Cry. pallasiana*; *Mme*, *M. membranacea*; *Pma*, *P. maximus*; *Wsu*, *W. subatra*.



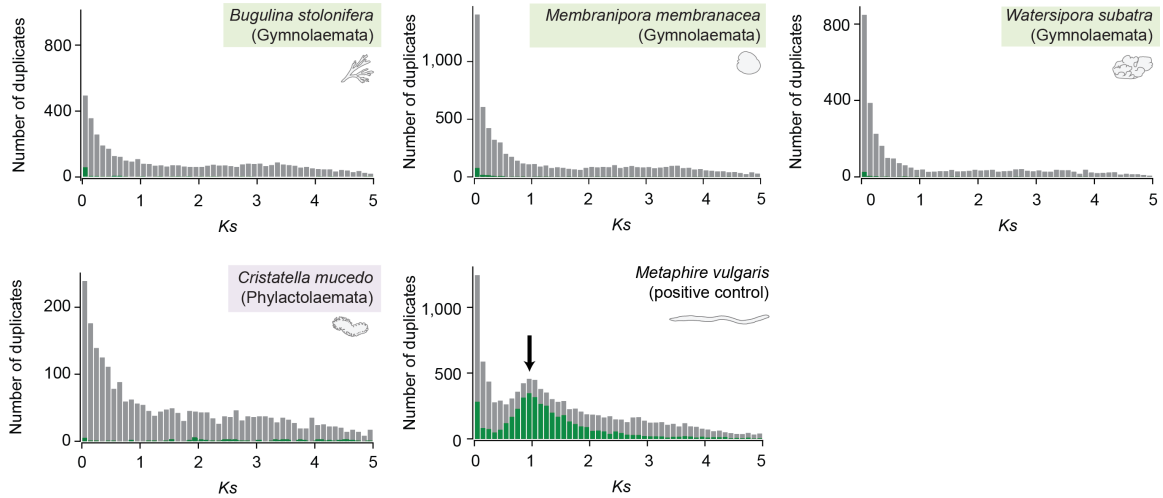
Supplemental Fig. S4. Macrosynten analysis together with Hi-C contact map for *Cry. pallasiana* reveals chromosome fission. (A) Syntenic analysis across bryozoan genomes shows lineage-specific chromosome fission in *Cry. pallasiana*. (B) *Cry. pallasiana* chromosomes 10 and 12 (arrows) are clearly separated on the contact map, demonstrating that these chromosomes are the products of a genuine chromosome fission and not a mis-assembly. Darker colors indicate a higher frequency of contacts.



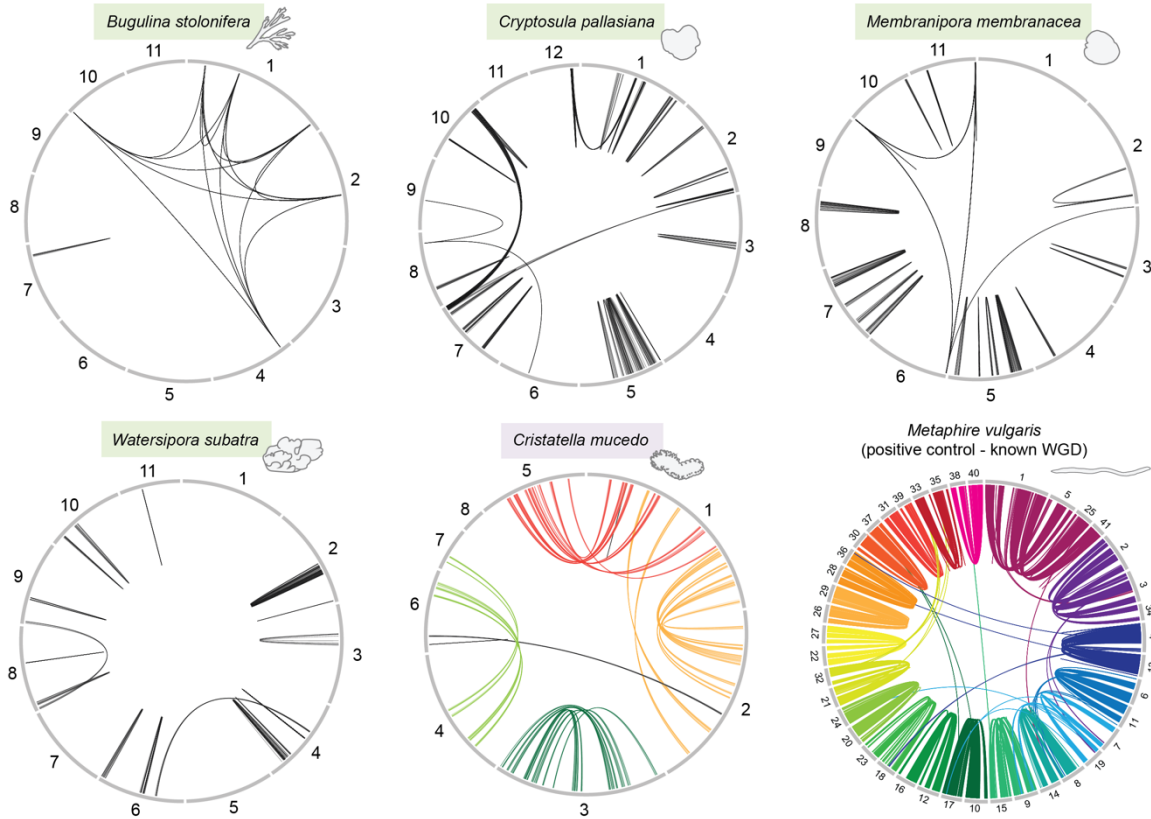
Supplemental Fig. S5. Relationships between *Cry. pallasiana* and *Cri. mucedo* chromosomes. Chromosome-scale gene linkage between *Cry. pallasiana* (representative of Gymnolaemata) and *Cri. mucedo* (Phylactolaemata) represented by ribbon plots. Horizontal bars represent chromosomes. Vertical lines connect the genomic position of orthologous genes in each genome. Each plot highlights genes from one Gymnolaemata ancestral linkage group (ALG). In Gymnolaemata species, 11 ALGs are highly conserved, though the chromosome containing ALG a underwent a fission event to form Chr 10 and Chr 12 in *Cry. pallasiana*. These ALGs are not conserved in the Phylactolaemata species *Cri. mucedo*, and are distributed across the genome.



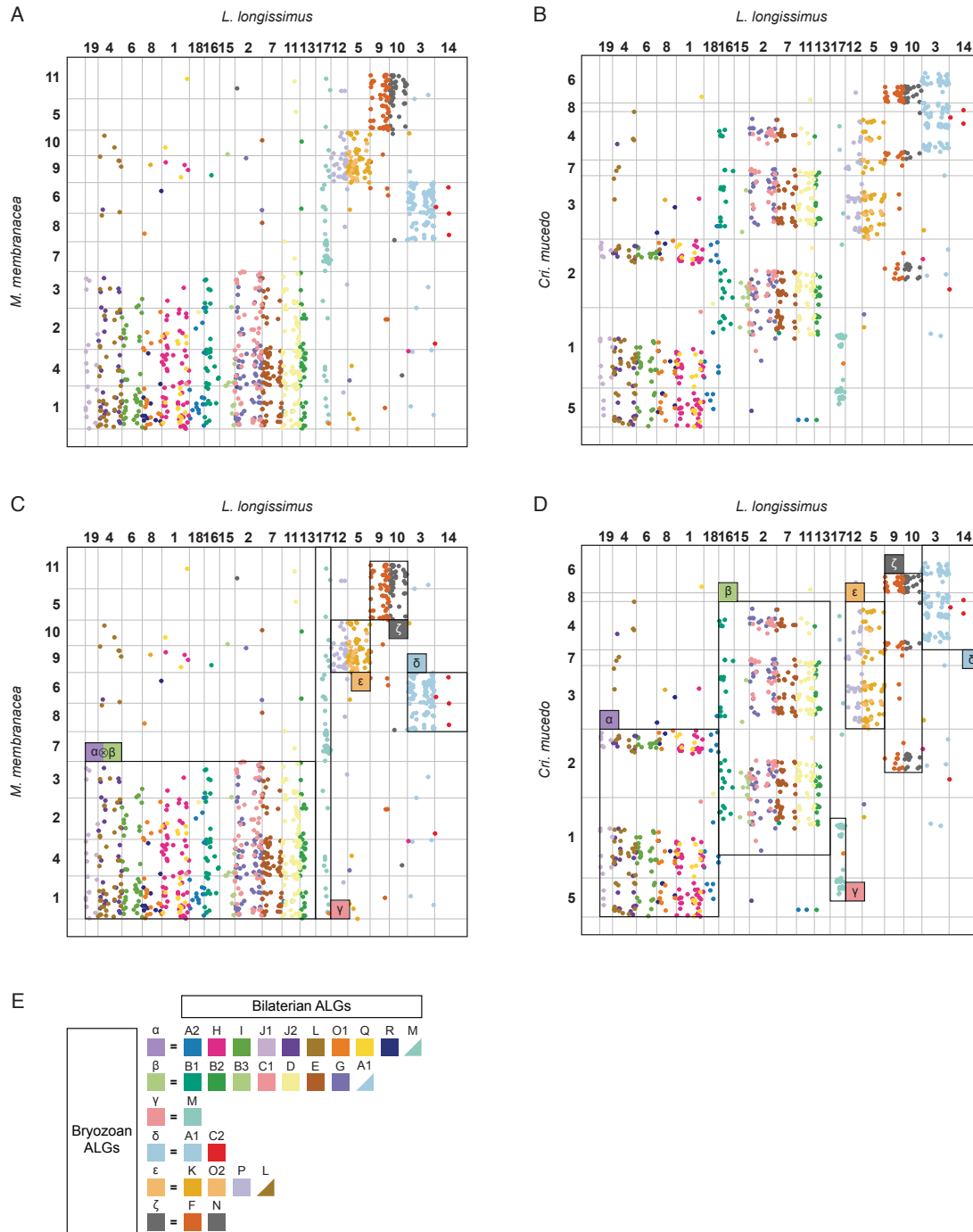
Supplemental Fig. S6. Chromosome relationships between bryozoans, spiralian and other bilaterians revealed by Oxford dot plots. Oxford dot plots show chromosome-scale gene linkage for species used in this work. Each point represents a gene. Genes are colored by their bilaterian ALG. Gray lines mark chromosome boundaries. Macrosynteny is highly conserved between *B. floridae*, *P. maximus* and *L. longissimus*, reflecting the bilaterian ancestral state. Macrosynteny is also highly conserved within Gymnolaemata bryozoans, but this state represents a substantial rearrangement from the bilaterian ancestral state.



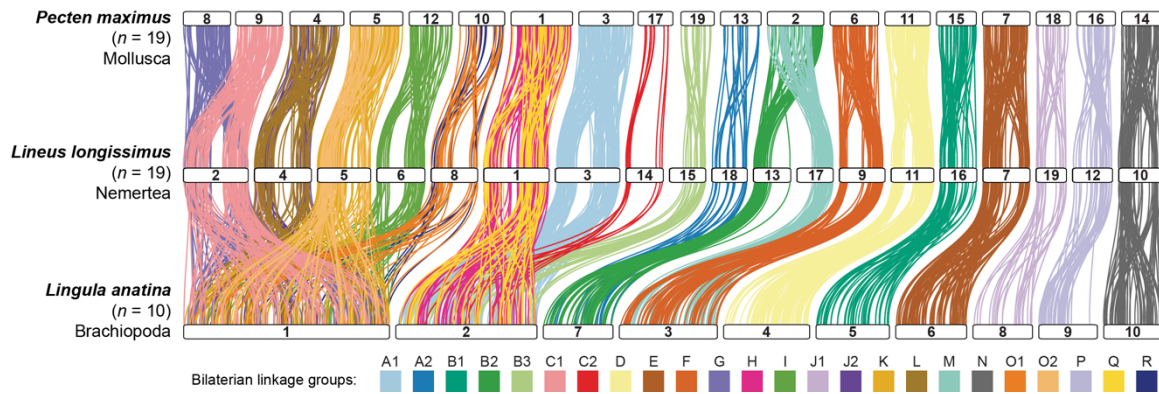
Supplemental Fig. S7. Assessment of whole genome duplication (WGD) in bryozoan genomes using synonymous substitution rate (K_s). Gray bars show histograms for all duplicated genes, while green bars show only anchor duplicates: these are genes found in duplicated collinear blocks of genes in the genome. Genomes with no WGD are expected to show exponential decay of duplicate numbers as K_s increases. *M. vulgaris* is a positive control with a known WGD (Jin et al. 2020): the plot for this species is interrupted by a large normally distributed peak at $K_s = 1$, indicating recent WGD (arrow). The plots of the bryozoans have no similar large peak at a low K_s , suggesting no recent WGD. The possible presence of a very shallow, very broad peak at a higher K_s in gymnolaemate bryozoans means that an ancient WGD in this lineage cannot conclusively be ruled out. The phylactolaemate bryozoan *Cri. mucedo* has several very small peaks of anchor gene duplicates (green), possibly representing segmental duplication events.



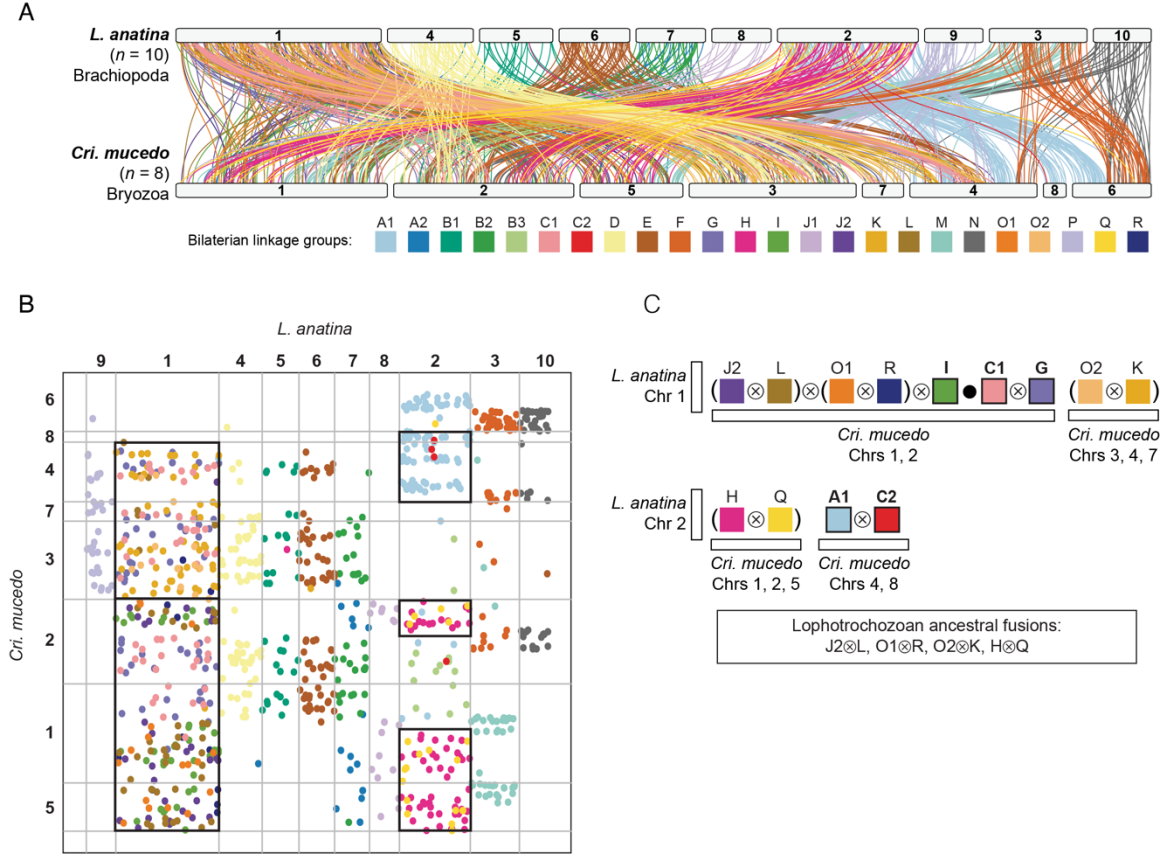
Supplemental Fig. S8. Duplicated collinear gene blocks in bryozoan genomes. Duplicated collinear blocks indicate that chromosomes likely emerged through WGD or large segmental duplications. *M. vulgaris* is a positive control with a known WGD: the plot for this species shows clear pairs of chromosomes with conserved collinear gene blocks; these emerged from WGD. The plots for gymnolaemate bryozoans show very few such blocks and no pairwise relationships of chromosomes. This does not support the presence of WGD in gymnolaemates. The plot for the phylactolaemate *Cri. mucedo* has several sections of homology (chromosome 1 with 2; 1 with 5; 4 with 6 and 7), suggesting possible segmental duplication or WGD events in this lineage.



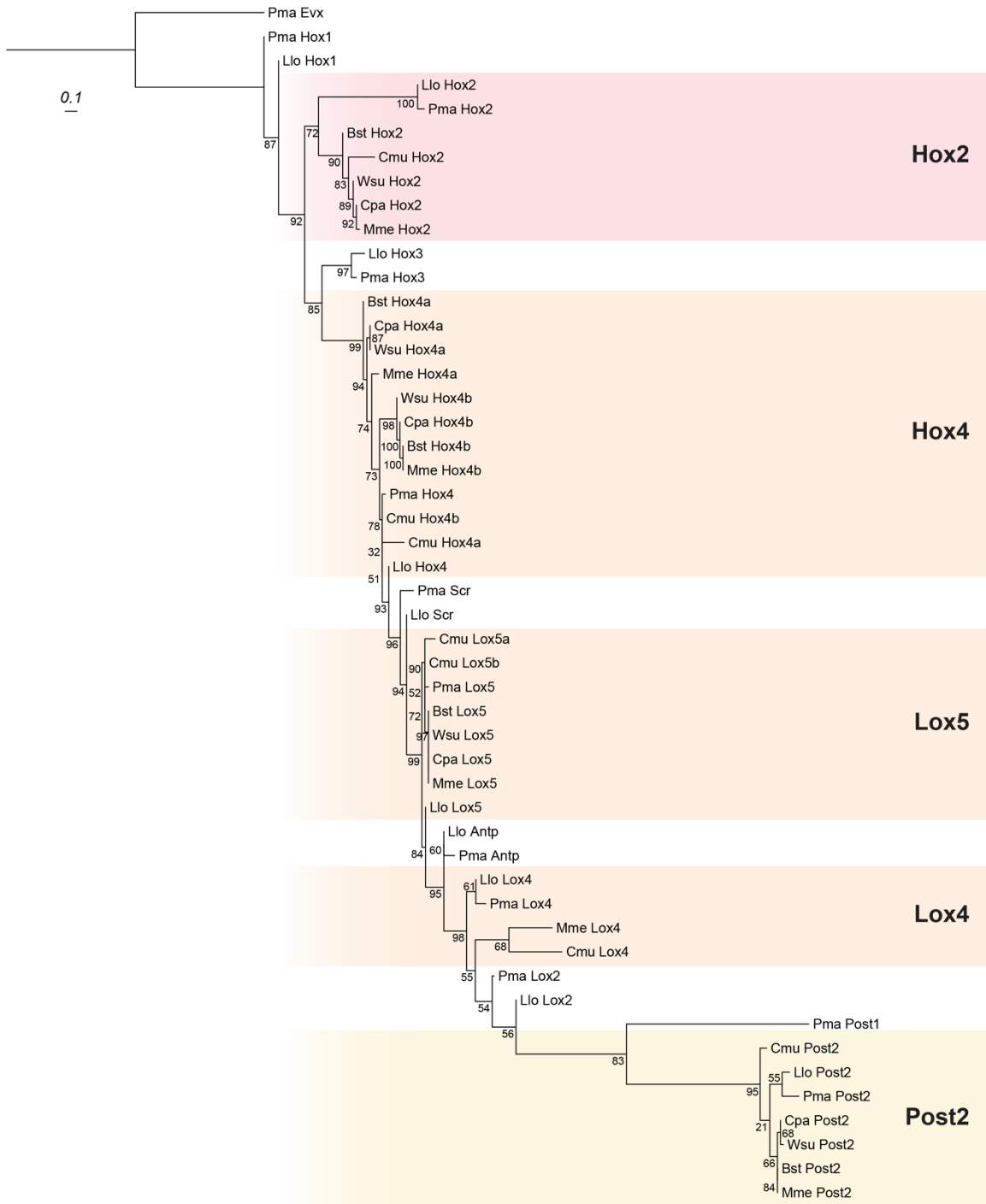
Supplemental Fig. S9. Oxford dot plots reveal bryozoan ALGs. Oxford dot plots show chromosome-scale gene linkage of *L. longissimus*, which closely resembles the bilaterian ancestral state with (A) *M. membranacea* and (B) *Cri. mucedo*. Each point represents a gene. Genes are colored by their bilaterian ALG. Gray lines mark chromosome boundaries. Six bryozoan ALGs can be identified by sets of genes grouped together. In parts (C) and (D), the six bryozoan ALGs are highlighted with black boxes. (E) Composition of bryozoan ALGs by bilaterian ALGs reproduced from Main Text Figure 4B. Triangles represent the presence of a small subset of genes from that ALG.



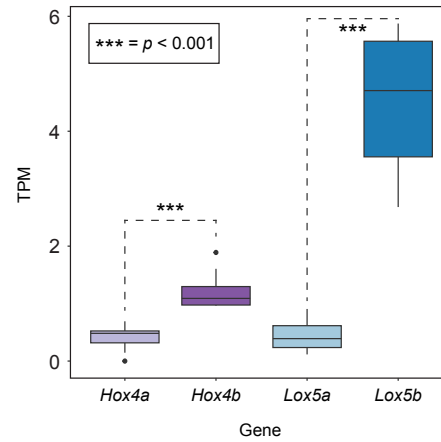
Supplemental Fig. S10. Chromosome evolution in *L. anatina*. Chromosome relationships between *P. maximus*, *L. longissimus* and *L. anatina* revealed by ribbon plots. Horizontal white bars represent chromosomes. Vertical colored lines connect orthologous genes on chromosomes. Four *L. anatina* chromosomes (Chr 1, Chr 2, Chr 3, and Chr 7) are the products of fusion-with-mixing events. Chr 1 contains nine bilaterian ALGs (C1⊗G⊗I⊗J2⊗K⊗L⊗O1⊗O2⊗R); Chr 2 contains five ALGs (A1⊗B3⊗C2⊗H⊗Q); Chr 3 and Chr 7 contain two ALGs each (F⊗M and A2⊗B2, respectively). No fission events can be detected in the *L. anatina* assembly.



Supplemental Fig. S11. Shared fusion events between *L. anatina* and *Cri. mucedo*. (A) Chromosome-scale gene linkage between the brachiopod *L. anatina* and the bryozoan *Cri. mucedo*. Horizontal bars represent chromosomes. Vertical lines connect the genomic position of orthologous genes in each genome. Lines are colored by bilaterian ALGs. (B) Oxford dot plot revealing chromosome-scale gene linkage between *L. anatina* and *Cri. mucedo*. Black boxes highlight shared fusion events. (C) Representation of the nine ALG fusion events shared between *L. anatina* and *Cri. mucedo*. Colored squares represent ALGs; white rectangles represent chromosomes; ⊗ marks ALG fusion-with-mixing events; • marks ALG fusion events without mixing. The ALG fusions shared between *L. anatina* and *Cri. mucedo* are identical to those shared between *L. anatina* and *M. membranacea* (Main text Fig. 5), except in *Cri. mucedo* the C1⊗G group has fused but not mixed (•) with the (J2⊗L)⊗(O1⊗R)⊗I group. To summarise, ALGs J2, L, O1, R, C1, G, I, O2, and K are co-located on Chr 1 in *L. anatina*. ALGs J2, L, O1, R, C1, G, I (Chrs 1, 2) and O2, K (Chrs 3, 4, 7) are also co-located in *Cri. mucedo*. Therefore, (J2⊗L)⊗(O1⊗R)⊗I•C1⊗G and (O2⊗K) represent seven fusion events shared by both species. ALGs H, Q, A1 and C2 are co-located on Chr 2 in *L. anatina*. H, Q (Chrs 1, 2, 5) and A1, C2 (Chrs 4, 8) are also co-located in *Cri. mucedo*. Therefore, (H⊗Q) and A1⊗C2 represent two further fusion events shared by both species, for a total of nine. We note that several spiralian phyla share four of these events ('lophotrochozoan ancestral fusions'), represented by ⊗ within parentheses, but their timing is uncertain. If these events are present in all spirilians, for instance, they may not be useful phylogenetic markers for the position of bryozoans. This leaves five confirmed derived fusion events shared between bryozoans and brachiopods but not present in molluscs and annelids. These events suggest a close evolutionary relationship between bryozoans and brachiopods, and are marked by ⊗ and • occurring outside of parentheses.



Supplemental Fig. S12. Tree of bryozoan Hox sequences. Tree of bryozoan Hox protein sequences used in this analysis. Tree is made using the maximum likelihood method (LG+G4 model) with 1000 bootstrap replicates in IQ-TREE (Minh et al. 2020). *Pecten maximus* Evx is the outgroup. Abbreviations: Bst, *B. stolonifera*; Cmu, *Cri. mucedo*; Cpa, *Cry. pallasiana*; Llo, *L. longissimus*; Mme, *M. membranacea*; Pma, *P. maximus*; Wsu, *W. subatra*.



Supplemental Fig. S13. Expression of *Hox4a* versus *Hox4b* and *Lox5a* versus *Lox5b* in *Cri. mucedo* adults. In both cases, there is a significant difference ($p < 0.001$) between the expression level of the gene duplicates. Abbreviations: TPM, transcripts per million.

Supplemental Table S1. Assembly statistics for bryozoan genomes.

Assembly	<i>Cri. mucedo</i>	<i>M. membranacea</i>	<i>B. stolonifera</i>	<i>W. subatra</i>	<i>Cry. pallasiana</i>
Reference	Hoencamp et al. 2021	Bishop et al. 2023a	Wood et al. 2023	Bishop et al. 2024	Bishop et al. 2023b
Assembly level	Chromosome	Chromosome	Chromosome	Chromosome	Chromosome
Sequencing technology	PacBio, Illumina, Hi-C	PacBio, 10X Genomics Chromium, Arima Hi-C	PacBio, 10X Genomics Chromium, Arima2 Hi-C	PacBio, 10X Genomics Chromium, Arima Hi-C	PacBio, 10X Genomics Chromium, Arima2 Hi-C
Genome size (Mb)	556	339	235	784	606
Chromosome number	8	11	11	11	12
Sequencing coverage	?	66X	52X	39X	35X
Number of scaffolds	753	21	30	240	847
Scaffold N50 (Mb)	97	30	20.3	66.5	46.3
Contig N50 (Mb)	0.4	7	12.6	4.5	1.8
GC content (%)	47.0	38.0	37.0	35.5	37.2
Repeats (%)	47.5	35.5	32.2	53.2	57.0
Number of genes	29904	25909	20502	25115	30048
Number of transcripts	32678	31934	25449	21344	35283
Mean gene length (bp)	10823	6430	6766	14392	7863
Mean number of introns per gene	6.0	8.6	9.1	7.9	6.9
Mean intron size (bp)	1704	683	658	1847	1005
Genome BUSCO metazoa odb10 (n = 954)	C:88.6% [S:87.4%, D:1.2%] F:6.2% M:5.2%	C:82.7% [S:81.0%, D:1.7%] F:8.0% M:9.3%	C:84.5% [S:83.8%, D:0.7%] F:6.5% M:9.0%	C:83.4% [S:81.7%, D:1.7%] F:8.6% M:8.0%	C:83.6% [S:82.4%, D:1.2%] F:8.0% M:8.4%
Transcriptome BUSCO metazoa odb10 (n = 954)	C:86.7% [S:85.3%, D:1.4%] F:6.8% M:6.5%	C:94.9% [S:91.9%, D:3.0%] F:2.6% M:2.5%	C:96.6% [S:95.4%, D:1.2%] F:1.8% M:1.6%	C:86.2% [S:84.8%, D:1.4%] F:6.7% M:7.1%	C:94.7% [S:93.1%, D:1.6%] F:2.2% M:3.1%
Proteome BUSCO metazoa odb10 (n = 954)	C:86.6% [S:85.3%, D:1.3%] F:6.3% M:7.1%	C:95.4% [S:92.0%, D:3.4%] F:2.3% M:2.3%	C:97.0% [S:95.8%, D:1.2%] F:1.5% M:1.5%	C:87.8% [S:86.3%, D:1.5%] F:5.5% M:6.7%	C:95.3% [S:93.7%, D:1.6%] F:1.7% M:3.0%

Supplemental Table S2. RNA-sequencing datasets used for gene prediction.

Species	SRA accession
<i>Cri. mucedo</i>	SRR10620224
<i>Cri. mucedo</i>	SRR10620225
<i>Cri. mucedo</i>	SRR10620226
<i>Cri. mucedo</i>	SRR10620227
<i>Cri. mucedo</i>	SRR10620228
<i>Cri. mucedo</i>	SRR10620229
<i>Cri. mucedo</i>	SRR10620230
<i>Cri. mucedo</i>	SRR10620231
<i>W. subatra</i>	ERR11641139
<i>M. membranacea</i>	ERR6464929
<i>M. membranacea</i>	SRR2131259
<i>Cry. pallasiana</i>	ERR9866430
<i>B. stolonifera</i>	ERR10123683
<i>B. stolonifera</i>	SRR11096622
<i>B. stolonifera</i>	SRR11096623
<i>B. stolonifera</i>	SRR11096624
<i>B. stolonifera</i>	SRR11096625
<i>B. stolonifera</i>	SRR11096626
<i>B. stolonifera</i>	SRR11096627
<i>B. stolonifera</i>	SRR11096628
<i>B. stolonifera</i>	SRR11096629
<i>B. stolonifera</i>	SRR11096631
<i>B. stolonifera</i>	SRR11096632
<i>B. stolonifera</i>	SRR11096632
<i>B. stolonifera</i>	SRR11096633
<i>B. stolonifera</i>	SRR11096634
<i>B. stolonifera</i>	SRR11096635
<i>B. stolonifera</i>	SRR11096636
<i>B. stolonifera</i>	SRR11096637
<i>B. stolonifera</i>	SRR11096638
<i>B. stolonifera</i>	SRR11096639
<i>B. stolonifera</i>	SRR11096640
<i>B. stolonifera</i>	SRR9667735

Supplemental Table S3. Repeat content of bryozoan genomes. Numbers correspond to the percentage of the genome composed by each type of element.

	<i>B. stolonifera</i> (% of genome)	<i>Cri. mucedo</i> (% of genome)	<i>Cry. pallasiana</i> (%) of genome)	<i>M. membranacea</i> (% of genome)	<i>W. subatra</i> (% of genome)
Bases masked	32.15	47.52	57.01	35.5	53.21
Retroelements	9.31	25.19	20.91	8.99	9.43
SINEs:	0.28	0.02	0.08	0.36	0.14
Penelope	0.69	0.01	0	0	0.01
LINEs	4.01	23.96	3.64	5.01	4.18
CRE/SLACS	0	0	0	0.02	0
L2/CR1/Rex	2.99	23.22	2.51	2.91	1.63
R1/LOA/Jockey	0	0.07	0.02	0.13	0.02
R2/R4/NeSL	0.05	0.25	0.23	0.89	1.1
RTE/Bov-B	0.25	0.01	0.46	0.42	0.36
L1/CIN4	0	0.1	0.15	0.26	0.52
LTR	5.01	1.2	17.19	3.62	5.1
BEL/Pao	0.45	0	0.25	0.39	0.44
Ty1/Copia	0.24	0.11	0.22	0.34	0.37
Gypsy/DIRS1	2.9	1.01	12.77	1.76	4.03
Retroviral	0.15	0.08	0.13	0.22	0.23
DNA transposons	4.3	7.03	9.03	3.04	11.37
hobo-Activator	1.09	1.25	1.87	0.36	1.53
Tc1-IS630-Pogo	0.2	1.64	0.13	0.29	5.21
En-Spm	0	0	0	0	0
MULE-MuDR	0.09	0.26	0.21	0.28	0.19
PiggyBac	0.02	0	0.03	0.07	0.32
Tourist/Harbinger	0.11	0.02	0.17	0.21	0.07
Other (Mirage, P-element, Transib)	0.06	0.02	0.04	0.26	0.3
Rolling Circles	0.03	0.1	0.24	0.1	1.24
Unclassified	15.9	12.09	25.33	22.5	29.8
Satellites	0.17	0.09	0.03	0.03	0.17
Simple Repeats	1.68	2.68	1.4	0.76	1.09
Low complexity repeats	0.07	0.33	0.06	0.05	0.09

Supplemental Table S4. Genomes used for principal component analysis (PCA). Bryozoan species are in bold.

Taxon	Species	Species ID	Assembly source	Assembly accession	Annotation source
Porifera	<i>Amphimedon queenslandica</i>	Aqu	NCBI	GCF_000090795.2	NCBI RefSeq
Echinodermata	<i>Asterias rubens</i>	Aru	NCBI	GCF_902459465.1	NCBI RefSeq
Rotifera	<i>Adineta vaga</i>	Ava	NCBI	GCA_021613535.1	NCBI GenBank
Chordata	<i>Branchiostoma lanceolatum</i>	Bla	NCBI	GCA_927797965.1	NCBI GenBank
Ctenophora	<i>Bolinopsis microptera</i>	Bmi	NCBI	GCA_026151205.1	This work
Bryozoa	<i>Bugulina stolonifera</i>	Bst	NCBI	GCA_935421135.1	This work
Nematoda	<i>Caenorhabditis elegans</i>	Cel	NCBI	GCA_000002985.3	NCBI RefSeq
Chordata	<i>Ciona intestinalis</i>	Cin	NCBI	GCF_000224145.3	NCBI RefSeq
Bryozoa	<i>Cristatella mucedo</i>	Cmu	GEO	GSE169088	This work
Bryozoa	<i>Cryptosula pallasiana</i>	Cpa	NCBI	GCA_945261195.1	This work
Annelida	<i>Capitella teleta</i>	Cte	NCBI	GCA_000328365.1	NCBI GenBank
Arthropoda	<i>Drosophila melanogaster</i>	Dme	NCBI	GCF_000001215.4	NCBI RefSeq
Arthropoda	<i>Daphnia pulex</i>	Dpu	NCBI	GCF_021134715.1	NCBI RefSeq
Chordata	<i>Danio rerio</i>	Dre	NCBI	GCF_000002035.6	NCBI RefSeq
Cnidaria	<i>Galaxea fascicularis</i>	Gfa	NCBI	GCA_948470475.1	This work
Xenacoelomorpha	<i>Hofstenia miamia</i>	Hmi	Ensembl Metazoa	GCA_004352715.1	Ensembl
Annelida	<i>Helobdella robusta</i>	Hro	NCBI	GCF_000326865.2	NCBI RefSeq
Chordata	<i>Homo sapiens</i>	Hsa	NCBI	GCF_000001405.40	NCBI RefSeq
Cnidaria	<i>Hydra vulgaris</i>	Hvu	NCBI	GCF_022113875.1	NCBI RefSeq
Brachiopoda	<i>Lingula anatina</i>	Lan	Unpublished	Unpublished	This work
Nemertea	<i>Lineus longissimus</i>	Llo	NCBI	GCA_910592395.2	This work
Echinodermata	<i>Lytechinus variegatus</i>	Lva	NCBI	GCF_018143015.1	NCBI RefSeq
Bryozoa	<i>Membranipora membranacea</i>	Mme	NCBI	GCA_914767715.1	This work
Nemertea	<i>Notospermus geniculatus</i>	Nge	OIST	nge_genome_v2.0.fa.gz	OIST
Cnidaria	<i>Nematostella vectensis</i>	Nve	NCBI	GCF_932526225.1	NCBI RefSeq
Mollusca	<i>Octopus bimaculoides</i>	Obi	NCBI	GCF_001194135.2	NCBI RefSeq
Annelida	<i>Owenia fusiformis</i>	Ofu	NCBI	GCA_903813345.2	NCBI RefSeq
Phoronida	<i>Phoronis australis</i>	Pau	OIST	pau_genome_v2.0.fa.gz	OIST

Mollusca	<i>Pecten maximus</i>	Pma	NCBI	GCF_902652985.1	NCBI RefSeq
Mollusca	<i>Patella vulgata</i>	Pvu	NCBI	GCF_932274485.2	NCBI RefSeq
Hemichordata	<i>Saccoglossus kowalevskii</i>	Sko	NCBI	GCF_000003605.2	NCBI RefSeq
Platyhelminthes	<i>Schistosoma mansoni</i>	Sma	NCBI	GCF_000237925.1	NCBI RefSeq
Platyhelminthes	<i>Schmidtea mediterranea</i>	Sme	SmedGD	SmedSxl_genome_v4.0	SmedGD
Xenacoelomorpha	<i>Symsagittifera roscoffensis</i>	Sro	http://gb.molgen.mpg.de/	N/A	http://gb.molgen.mpg.de/
Placozoa	<i>Trichoplax adhaerens</i>	Tad	NCBI	GCF_000150275.1	NCBI RefSeq
Arthropoda	<i>Tribolium castaneum</i>	Tca	NCBI	GCF_000002335.3	NCBI RefSeq
Bryozoa	<i>Watersipora subatra</i>	Wsu	NCBI	GCA_963576615.1	This work
Chordata	<i>Xenopus tropicalis</i>	Xtr	NCBI	GCF_000004195.4	NCBI RefSeq

Supplemental Table S5. OrthoFinder output for bryozoan species.

Number of species	5
Number of genes	127,707
Number of genes in orthogroups	97,475
Number of unassigned genes	30,232
Percentage of genes in orthogroups	76.3
Percentage of unassigned genes	23.7
Number of orthogroups	16,984
Number of species-specific orthogroups	4621
Number of genes in species-specific orthogroups	22,441
Percentage of genes in species-specific orthogroups	17.6
Mean orthogroup size	5.7
Median orthogroup size	5
G50 (assigned genes)	6
G50 (all genes)	5
O50 (assigned genes)	4,544
O50 (all genes)	7,483
Number of orthogroups with all species present	6,765
Number of single-copy orthogroups	4,242

Supplemental Table S6. Pairwise microsynteny mixing scores for entire bryozoan genomes.

Species 1	Species 2	Mixing score
<i>Cri. mucedo</i>	<i>M. membranacea</i>	0.551
<i>Cri. mucedo</i>	<i>B. stolonifera</i>	0.553
<i>Cri. mucedo</i>	<i>W. subatra</i>	0.552
<i>Cri. mucedo</i>	<i>Cry. pallasiana</i>	0.554
<i>M. membranacea</i>	<i>Cry. pallasiana</i>	0.046
<i>M. membranacea</i>	<i>W. subatra</i>	0.046
<i>M. membranacea</i>	<i>B. stolonifera</i>	0.043
<i>B. stolonifera</i>	<i>Cry. pallasiana</i>	0.019
<i>B. stolonifera</i>	<i>W. subatra</i>	0.024
<i>W. subatra</i>	<i>Cry. pallasiana</i>	0.013

Supplemental Table S7. Pairwise microsynteny mixing scores for individual gymnolaemate bryozoan chromosomes.

Species 1	Species 2	Species 1 chromosome	Species 2 chromosome	Order on dot plots	Mixing Rate	Estimated divergence time (MYA)
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	10	1	1	0.281	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	12	1	2	0.8	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	4	2	3	0.111	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	6	3	4	0.634	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	8	4	5	0.363	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	5	11	6	0.407	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	2	8	7	0.349	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	1	5	8	0.343	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	11	7	9	0.514	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	7	9	10	0.732	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	3	10	11	0.612	128
<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	9	6	12	0.638	128
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	10	1	1	0.902	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	12	1	2	0.667	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	4	4	3	0.952	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	6	2	4	0.914	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	8	3	5	0.906	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	5	7	6	0.715	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	2	8	7	0.817	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	1	6	8	0.968	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	11	9	9	0.968	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	7	10	10	0.856	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	3	5	11	0.604	256
<i>Cry. pallasiana</i>	<i>M. membranacea</i>	9	11	12	0.926	256
<i>Cry. pallasiana</i>	<i>W. subatra</i>	10	1	1	0.134	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	12	1	2	0.689	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	4	3	3	0.099	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	6	4	4	0.528	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	8	2	5	0.081	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	5	6	6	0.062	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	2	5	7	0.02	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	1	7	8	0.458	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	11	9	9	0.499	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	7	11	10	0.42	64
<i>Cry. pallasiana</i>	<i>W. subatra</i>	3	8	11	0.279	64

<i>Cry. pallasiana</i>	<i>W. subatra</i>	9	10	12	0.252	64
<i>M. membranacea</i>	<i>B. stolonifera</i>	1	1	1	0.9515372	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	4	2	2	0.943304013	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	2	3	3	0.853222734	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	3	4	4	0.96521043	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	7	11	5	0.700711183	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	8	8	6	0.946323022	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	6	5	7	0.794550167	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	9	7	8	0.82755028	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	10	9	9	0.934399245	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	5	10	10	0.964606918	256
<i>M. membranacea</i>	<i>B. stolonifera</i>	11	6	11	0.999654756	256
<i>M. membranacea</i>	<i>W. subatra</i>	1	1	1	0.9515372	256
<i>M. membranacea</i>	<i>W. subatra</i>	4	3	2	0.943304013	256
<i>M. membranacea</i>	<i>W. subatra</i>	2	4	3	0.853222734	256
<i>M. membranacea</i>	<i>W. subatra</i>	3	2	4	0.96521043	256
<i>M. membranacea</i>	<i>W. subatra</i>	7	6	5	0.700711183	256
<i>M. membranacea</i>	<i>W. subatra</i>	8	5	6	0.946323022	256
<i>M. membranacea</i>	<i>W. subatra</i>	6	7	7	0.794550167	256
<i>M. membranacea</i>	<i>W. subatra</i>	9	9	8	0.82755028	256
<i>M. membranacea</i>	<i>W. subatra</i>	10	11	9	0.934399245	256
<i>M. membranacea</i>	<i>W. subatra</i>	5	8	10	0.964606918	256
<i>M. membranacea</i>	<i>W. subatra</i>	11	10	11	0.999654756	256
<i>B. stolonifera</i>	<i>W. subatra</i>	1	1	1	0.161199763	128
<i>B. stolonifera</i>	<i>W. subatra</i>	2	3	2	0.211119054	128
<i>B. stolonifera</i>	<i>W. subatra</i>	3	4	3	0.930367893	128
<i>B. stolonifera</i>	<i>W. subatra</i>	4	2	4	0.472108731	128
<i>B. stolonifera</i>	<i>W. subatra</i>	11	6	5	0.621155978	128
<i>B. stolonifera</i>	<i>W. subatra</i>	8	5	6	0.522689494	128
<i>B. stolonifera</i>	<i>W. subatra</i>	5	7	7	0.961633944	128
<i>B. stolonifera</i>	<i>W. subatra</i>	7	9	8	0.384844331	128
<i>B. stolonifera</i>	<i>W. subatra</i>	9	11	9	0.933948334	128
<i>B. stolonifera</i>	<i>W. subatra</i>	10	8	10	0.680422252	128
<i>B. stolonifera</i>	<i>W. subatra</i>	6	10	11	0.409792086	128

Supplemental Table S8. ALG composition of bilaterian genomes.

Phylum	Species	Chr.	Bilaterian ALGs
Chordata	<i>B. floridae</i>	1	A1, A2
		2	C1, J2
		3	C2, Q
		4	I, O1
		5	E
		6	D
		7	F
		8	M
		9	K
		10	B1
		11	G
		12	N
		13	H
		14	P
		15	L
		16	B2
		17	J1
		18	B3
		19	O2
Mollusca	<i>P. maximus</i>	1	H, Q
		2	B2, M
		3	A1
		4	J2, L
		5	K, O2
		6	F
		7	E
		8	G
		9	C1
		10	O1, R
		11	D
		12	I
		13	A2
		14	N
		15	B1
		16	P
		17	C2

		18	J1
		19	B3
Nemertea	<i>L. longissimus</i>	1	H, Q
		2	C1, G
		3	A1
		4	J2, L
		5	K, O2
		6	I
		7	E
		8	O1, R
		9	F
		10	N
		11	D
		12	P
		13	B2
		14	C2
		15	B3
		16	B1
		17	M
		18	A2
		19	J1
Brachiopoda	<i>L. anatina</i>	1	C1, G, I, J2, K, L, O1, O2, R
		2	A1, B3, C2, H, Q
		3	F, M
		4	D
		5	B1
		6	E
		7	A2, B2
		8	J1
		9	P
		10	N

Supplemental Table S9. ALG composition of bryozoan genomes.

Species	Chr.	Bilaterian ALGs	Bryozoan ALGs	Gymnolaemata ALGs
<i>Cri. mucedo</i>	1	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, M	α, β, γ	<i>a, b, c, d, e, f, g, h, i, j, k</i>
	2	A1, A2, B1, B2, B3, C1, D, E, F, G, H, I, J1, J2, L, N, O1, Q, R, (M)	α, β, ζ	<i>a, b, c, d, g, h, j, k</i>
	3	A1, B1, B2, B3, C1, D, E, G, K, L, O2, P	β, ϵ	<i>a, b, c, d, g, h, i, j, k</i>
	4	A1, B1, B2, B3, C1, C2, D, E, F, G, K, L, N, O2, P	$\beta, \delta, \epsilon, \zeta$	<i>a, b, c, d, f, g, h, i, j, k</i>
	5	A2, H, I, J1, J2, L, M O1, Q, R	α, γ	<i>a, b, c, d, e, f, g, h, i, k</i>
	6	A1, F, N	δ, ζ	<i>a, b, c, f, g, j, k</i>
	7	A1, B1, B2, B3, C1, D, E, G, K, L, O2, P	β, ϵ	<i>a, b, c, d, h, i</i>
	8	A1, C2	δ	<i>f, g</i>
<i>M. membranacea</i>	1	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>a</i>
	2	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>c</i>
	3	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>d</i>
	4	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>b</i>
	5	F, N, (M)	$\zeta, (\gamma)$	<i>j</i>
	6	A1, C2, (M)	$\delta, (\gamma)$	<i>g</i>
	7	M	γ	<i>e</i>
	8	A1, C2, (M)	$\delta, (\gamma)$	<i>f</i>
	9	K, L, O2, P, (M)	$\epsilon, (\gamma)$	<i>h</i>
	10	K, L, O2, P, (M)	$\epsilon, (\gamma)$	<i>i</i>
	11	F, N, (M)	$\zeta, (\gamma)$	<i>k</i>
<i>B. stolonifera</i>	1	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>a</i>
	2	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>b</i>
	3	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>c</i>
	4	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>d</i>
	5	A1, C2, (M)	$\delta, (\gamma)$	<i>g</i>
	6	F, N, (M)	$\zeta, (\gamma)$	<i>k</i>
	7	K, L, O2, P, (M)	$\epsilon, (\gamma)$	<i>h</i>
	8	A1, C2, (M)	$\delta, (\gamma)$	<i>f</i>
	9	K, L, O2, P, (M)	$\epsilon, (\gamma)$	<i>i</i>
	10	F, N, (M)	$\zeta, (\gamma)$	<i>j</i>
	11	M	γ	<i>e</i>
<i>W. subatra</i>	1	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>a</i>
	2	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	<i>d</i>

	3	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	b
	4	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	c
	5	A1, C2, (M)	$\delta, (\gamma)$	f
	6	M	γ	e
	7	A1, C2, (M)	$\delta, (\gamma)$	g
	8	F, N, (M)	$\zeta, (\gamma)$	j
	9	K, L, O2, P, (M)	$\varepsilon, (\gamma)$	h
	10	K, L, O2, P, (M)	$\varepsilon, (\gamma)$	k
	11	F, N, (M)	$\zeta, (\gamma)$	i
<i>Cry. pallasiana</i>	1	A1, C2, (M)	$\delta, (\gamma)$	g
	2	A1, C2, (M)	$\delta, (\gamma)$	f
	3	F, N, (M)	$\zeta, (\gamma)$	j
	4	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	b
	5	M	γ	e
	6	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	c
	7	K, L, O2, P, (M)	$\varepsilon, (\gamma)$	i
	8	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	d
	9	F, N, (M)	$\zeta, (\gamma)$	k
	10	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	a
	11	K, L, O2, P, (M)	$\varepsilon, (\gamma)$	h
	12	A1, A2, B1, B2, B3, C1, D, E, G, H, I, J1, J2, L, O1, Q, R, (M)	$\alpha, \beta, (\gamma)$	a

Supplemental Table S10. Origins of genes in bryozoan genomes with the annelid *Metaphire vulgaris* for comparison.

	<i>Metaphire vulgaris</i> (known WGD)	<i>M. membranacea</i>	<i>Cry. pallasiana</i>	<i>B. stolonifera</i>	<i>W. subatra</i>	<i>Cri. mucedo</i>
Singleton	43.0	39.8	36.1	44.7	40.1	68.6
Dispersed duplicate	25.0	33.4	34.0	32.8	34.9	17.7
Proximal duplicate	3.9	12.2	13.6	7.8	11.0	6.6
Tandem duplicate	4.6	13.1	14.6	14.3	10.4	5.0
WGD/segmental duplicate	23.5	1.5	1.7	0.4	3.6	2.1

Supplemental Table S11. Composition of bryozoan ALGs.

Bryozoan ALG	Bilaterian ALGs
α	A2, H, I, J1, J2, L, O1, Q, R, M
β	B1, B2, B3, C1, D, E, G, A1
γ	M
δ	A1, C2
ε	K, O2, P, L
ζ	F, N

Supplemental Table S12. Summary statistics for the *Lingula anatina* genome assembly.

Description	<i>L. anatina</i> statistic
Genome size (Mb)	329
Genome size (chromosome-level scaffolds only, Mb)	322
Sequencing coverage	405-fold
Number of scaffolds	16
Number of scaffolds > 10 Mb	10
Scaffold N50 (Mb)	30.1
Contig N50 (kb)	22,282
GC content (%)	36.5
Repeats (%)	31.7
Genome BUSCO metazoa odb10 (n = 954)	C:97.5%,S:96.6%,D:0.9%,F:1.8%,M:0.7%

Supplemental Table S13. Hi-C scaffolding statistics for the *L. anatina* genome assembly.

Superscaffold	Number of contigs	Length of contigs	Length of superscaffold
chr1	6	71,918,473	71,920,973
chr2	2	49,366,815	49,367,315
chr3	1	34,252,355	34,252,355
chr4	4	30,084,556	30,086,056
chr5	6	25,661,712	25,664,212
chr6	2	24,769,930	24,770,430
chr7	3	24,364,059	24,365,059
chr8	3	20,822,434	20,823,434
chr9	4	20,476,484	20,477,984
chr10	3	20,310,182	20,311,182
TOTAL	34	322,027,000	322,039,000

Supplemental Table S14. Expression levels of bryozoan Hox genes in RNA-seq datasets. TPM = transcripts per [kilobase] million.

Species	Stage	SRA accession	Transcript	Gene	TPM
<i>Cri. mucedo</i>	Adult	SRR10620224	g8621.t1	<i>Hox4a</i>	0.692
<i>Cri. mucedo</i>	Adult	SRR10620224	g5048.t1	<i>Hox4b</i>	0.966
<i>Cri. mucedo</i>	Adult	SRR10620224	g8615.t1	<i>Lox5a</i>	0.611
<i>Cri. mucedo</i>	Adult	SRR10620224	g5021.t1	<i>Lox5b</i>	5.712
<i>Cri. mucedo</i>	Adult	SRR10620225	g8621.t1	<i>Hox4a</i>	0.000
<i>Cri. mucedo</i>	Adult	SRR10620225	g5048.t1	<i>Hox4b</i>	0.964
<i>Cri. mucedo</i>	Adult	SRR10620225	g8615.t1	<i>Lox5a</i>	0.445
<i>Cri. mucedo</i>	Adult	SRR10620225	g5021.t1	<i>Lox5b</i>	3.563
<i>Cri. mucedo</i>	Adult	SRR10620226	g8621.t1	<i>Hox4a</i>	0.487
<i>Cri. mucedo</i>	Adult	SRR10620226	g5048.t1	<i>Hox4b</i>	1.606
<i>Cri. mucedo</i>	Adult	SRR10620226	g8615.t1	<i>Lox5a</i>	0.233
<i>Cri. mucedo</i>	Adult	SRR10620226	g5021.t1	<i>Lox5b</i>	5.139
<i>Cri. mucedo</i>	Adult	SRR10620227	g8621.t1	<i>Hox4a</i>	0.374
<i>Cri. mucedo</i>	Adult	SRR10620227	g5048.t1	<i>Hox4b</i>	1.890
<i>Cri. mucedo</i>	Adult	SRR10620227	g8615.t1	<i>Lox5a</i>	0.630
<i>Cri. mucedo</i>	Adult	SRR10620227	g5021.t1	<i>Lox5b</i>	5.518
<i>Cri. mucedo</i>	Adult	SRR10620228	g8621.t1	<i>Hox4a</i>	0.146
<i>Cri. mucedo</i>	Adult	SRR10620228	g5048.t1	<i>Hox4b</i>	1.194
<i>Cri. mucedo</i>	Adult	SRR10620228	g8615.t1	<i>Lox5a</i>	0.907
<i>Cri. mucedo</i>	Adult	SRR10620228	g5021.t1	<i>Lox5b</i>	5.874
<i>Cri. mucedo</i>	Adult	SRR10620229	g8621.t1	<i>Hox4a</i>	0.478
<i>Cri. mucedo</i>	Adult	SRR10620229	g5048.t1	<i>Hox4b</i>	1.076
<i>Cri. mucedo</i>	Adult	SRR10620229	g8615.t1	<i>Lox5a</i>	0.336
<i>Cri. mucedo</i>	Adult	SRR10620229	g5021.t1	<i>Lox5b</i>	4.276
<i>Cri. mucedo</i>	Adult	SRR10620230	g8621.t1	<i>Hox4a</i>	0.602
<i>Cri. mucedo</i>	Adult	SRR10620230	g5048.t1	<i>Hox4b</i>	0.978
<i>Cri. mucedo</i>	Adult	SRR10620230	g8615.t1	<i>Lox5a</i>	0.116
<i>Cri. mucedo</i>	Adult	SRR10620230	g5021.t1	<i>Lox5b</i>	3.529
<i>Cri. mucedo</i>	Adult	SRR10620231	g8621.t1	<i>Hox4a</i>	0.498
<i>Cri. mucedo</i>	Adult	SRR10620231	g5048.t1	<i>Hox4b</i>	1.106
<i>Cri. mucedo</i>	Adult	SRR10620231	g8615.t1	<i>Lox5a</i>	0.236
<i>Cri. mucedo</i>	Adult	SRR10620231	g5021.t1	<i>Lox5b</i>	2.681

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