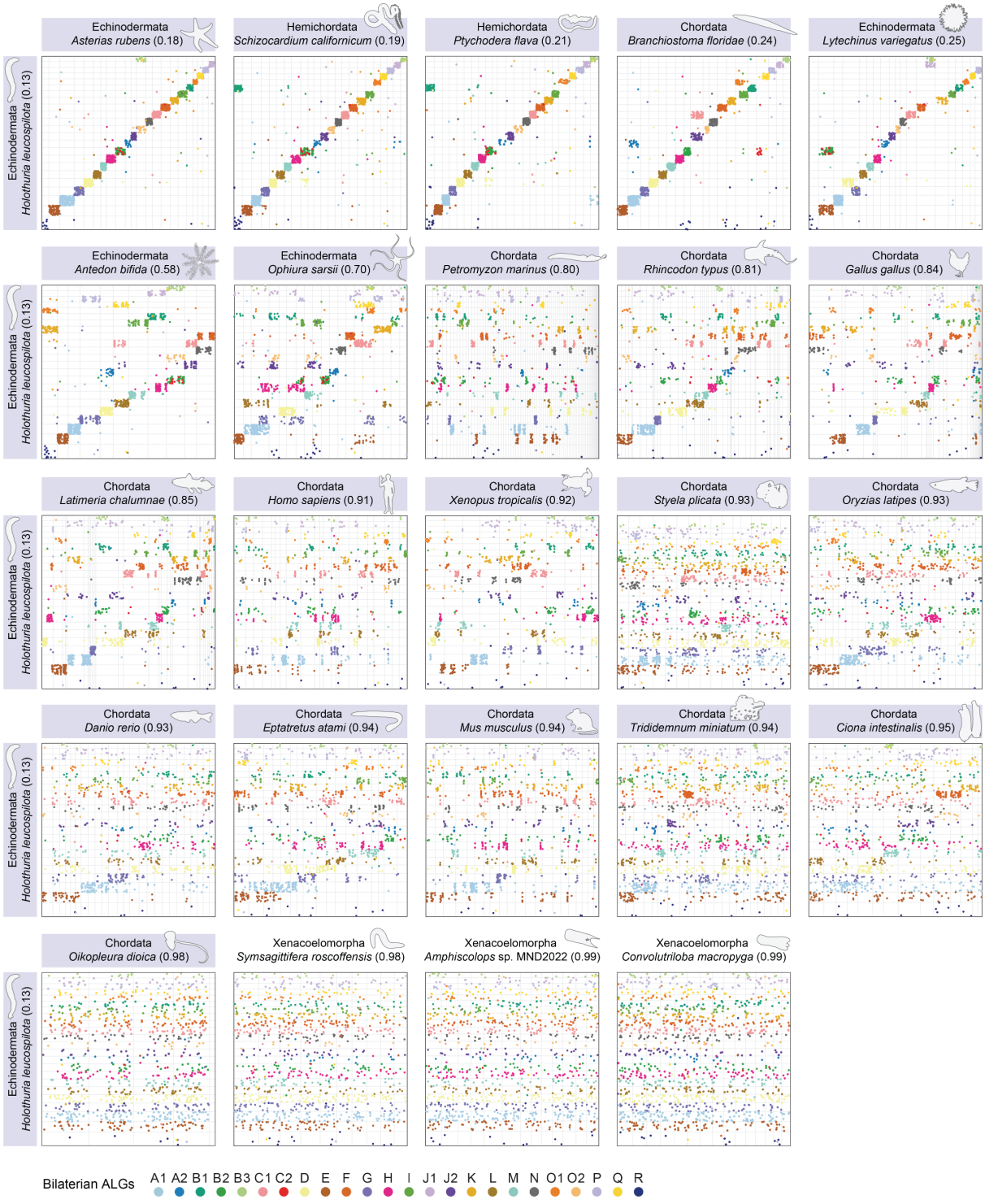

Fig. S1 Genome structure in deuterostomes and acoels. Each box represents a pairwise comparison of two genomes. Dots represent the position of orthologous genes in each genome, colour-coded by their bilaterian ancestral linkage group (ALG). Chromosomes are separated by grey lines. In each case, the most conserved species in the dataset (sea cucumber *Holothuria leucospilota*, rearrangement index [RI] = 0.13) is on the Y-axis. *H. leucospilota* has 23 chromosomes; 22 of which represent single bilaterian ALGs and one of which is a fusion-with-mixing event between ALGs B2 and C2. The number in parentheses is the RI for each species.

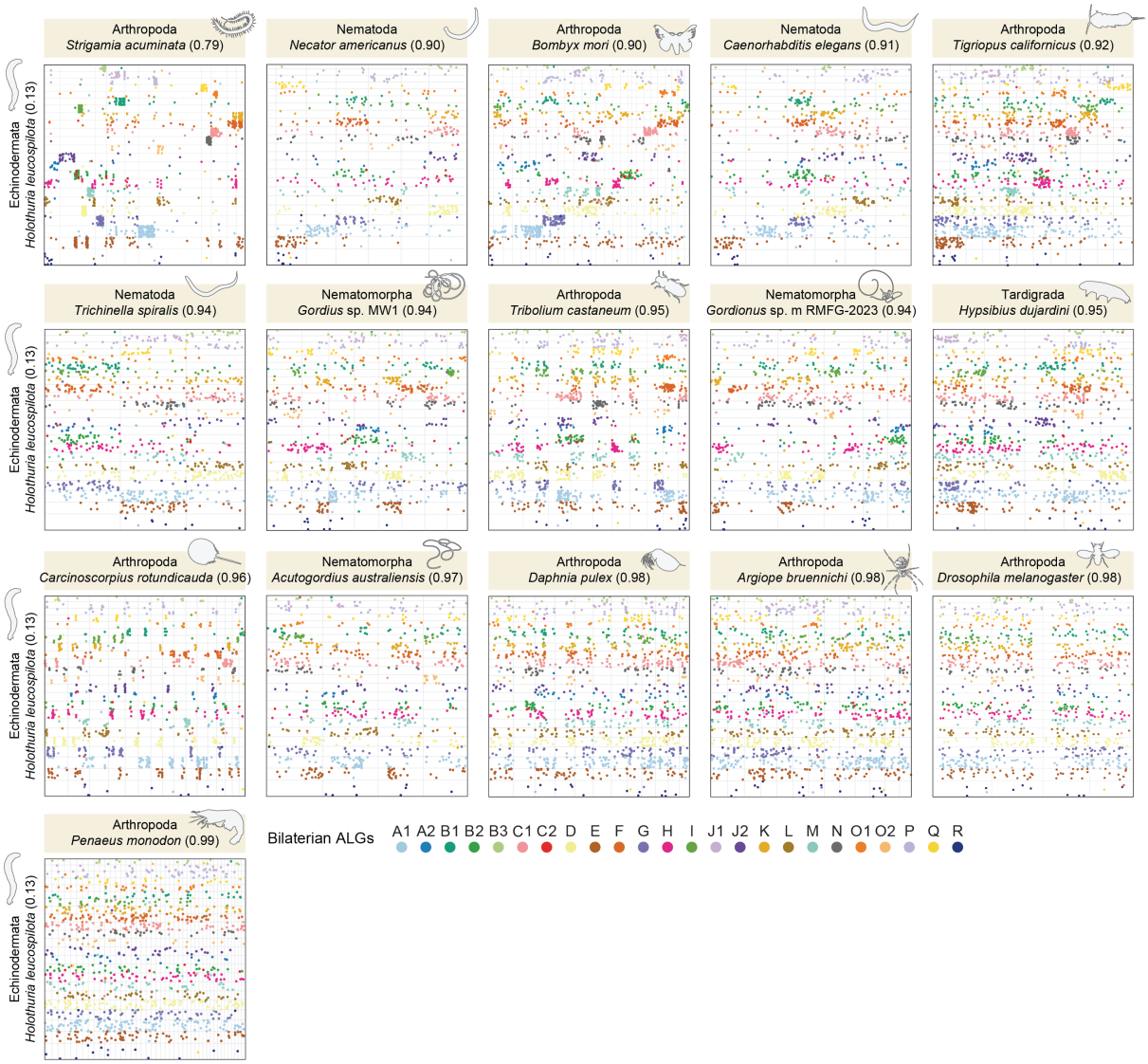

Fig. S2 Genome structure in ecdysozoans. Each box represents a pairwise comparison of two genomes. Dots represent the position of orthologous genes in each genome, colour-coded by their bilaterian ALG. Chromosomes are separated by grey lines. In each case, the most conserved species in the dataset (sea cucumber *Holothuria leucospilota*, rearrangement index [RI] = 0.13) is on the Y-axis. *H. leucospilota* has 23 chromosomes; 22 of which represent single bilaterian ALGs and one of which is a fusion-with-mixing event between ALGs B2 and C2. The number in parentheses is the RI for each species.

Fig. S3 Genome structure in spiralians. Each box represents a pairwise comparison of two genomes. Dots represent the position of orthologous genes in each genome, colour-coded by their bilaterian ALG. Chromosomes are separated by grey lines. In each case, the most conserved species in the dataset (sea cucumber *Holothuria leucospilota*, rearrangement index [RI] = 0.13) is on the Y-axis. *H. leucospilota* has 23 chromosomes; 22 of which represent single bilaterian ALGs and one of which is a fusion-with-mixing event between ALGs B2 and C2. The number in parentheses is the RI for each species.

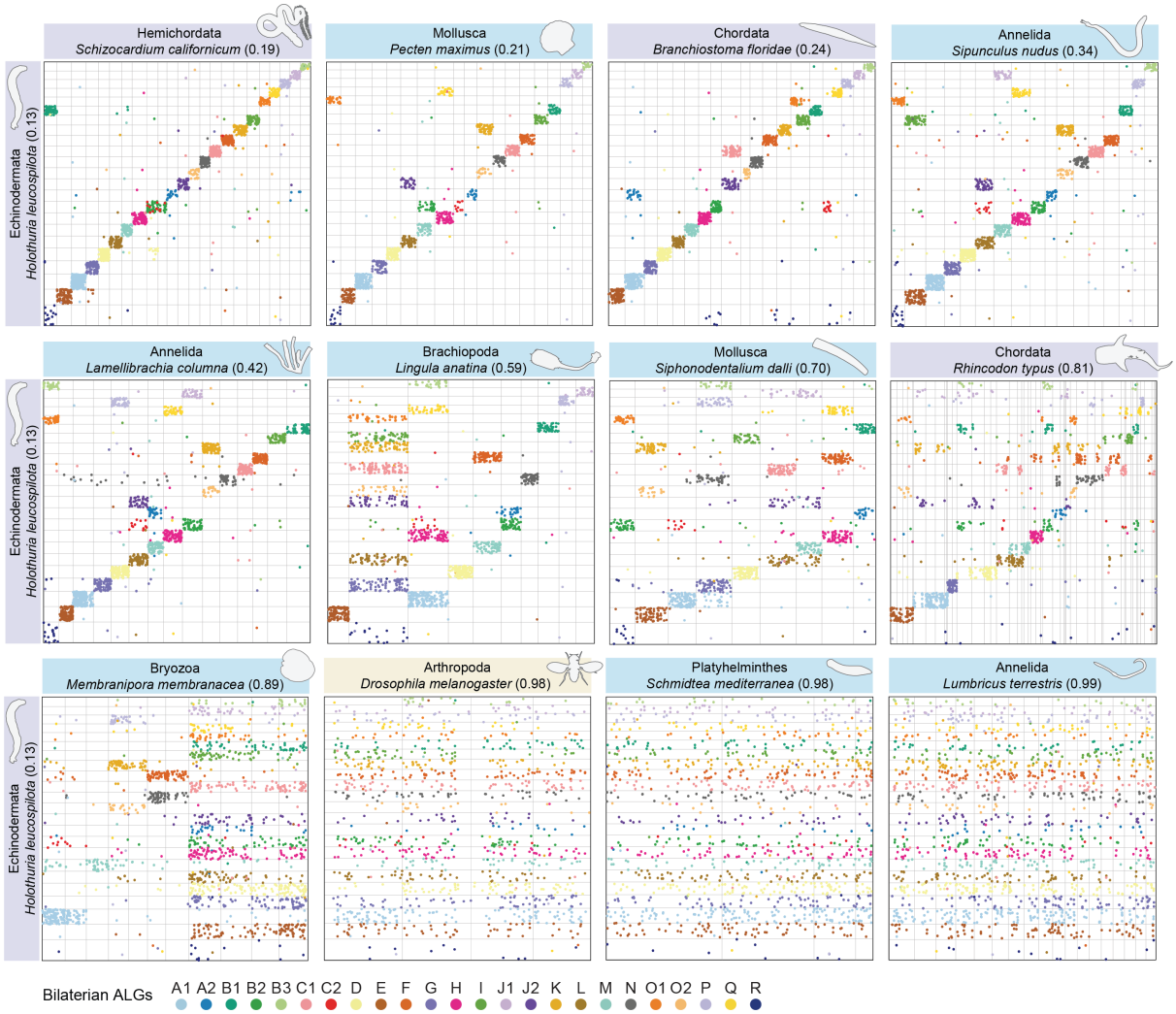

Fig. S4 Validation of the rearrangement index (RI). Synteny plots for species with varied levels of genome rearrangements reported in the literature. The RI for each of these species (parentheses) is consistent with the levels of ALG rearrangement its genome, demonstrating that the index is a valid measure of rearrangement level:

1. The hemichordate *Schizocardium californicum* (RI = 0.19): 4 ALGs involved in fusion events; 2 fission events.
2. The scallop *Pecten maximus* (RI = 0.21): 10 ALGs involved in fusion events, no fission events.
3. The amphioxus *Branchiostoma floridae* (RI = 0.24): 8 ALGs involved in fusion events, 1 ALG lost (R).
4. The annelid *Sipunculus nudus* (RI = 0.34): 13 ALGs involved in fusion events, no fission events.
5. The tube worm *Lamellibrachia columna* (RI = 0.42): 16 ALGs involved in fusion events, no fission events.
6. The brachiopod *Lingula anatina* (RI = 0.59): 18 ALGs involved in fusion events, no fission events.
7. The tusk shell *Siphonodentalium dalli* (RI = 0.70): 24 ALGs involved in fusion events, 3 fission events.
8. The whale shark *Rhincodon typus* (RI = 0.81): Extensive ALG fissions resulting in 50 chromosomes.

9. The bryozoan *Membranipora membranacea* (RI = 0.89): 23 ALGs involved in fusion events, 23 ALGs involved in fission events.
10. The fruit fly *Drosophila melanogaster* (RI = 0.98): complete loss of ALGs.
11. The planarian *Schmidtea mediterranea* (RI = 0.98): complete loss of ALGs.
12. The earthworm *Lumbricus terrestris* (RI = 0.99): complete loss of ALGs.

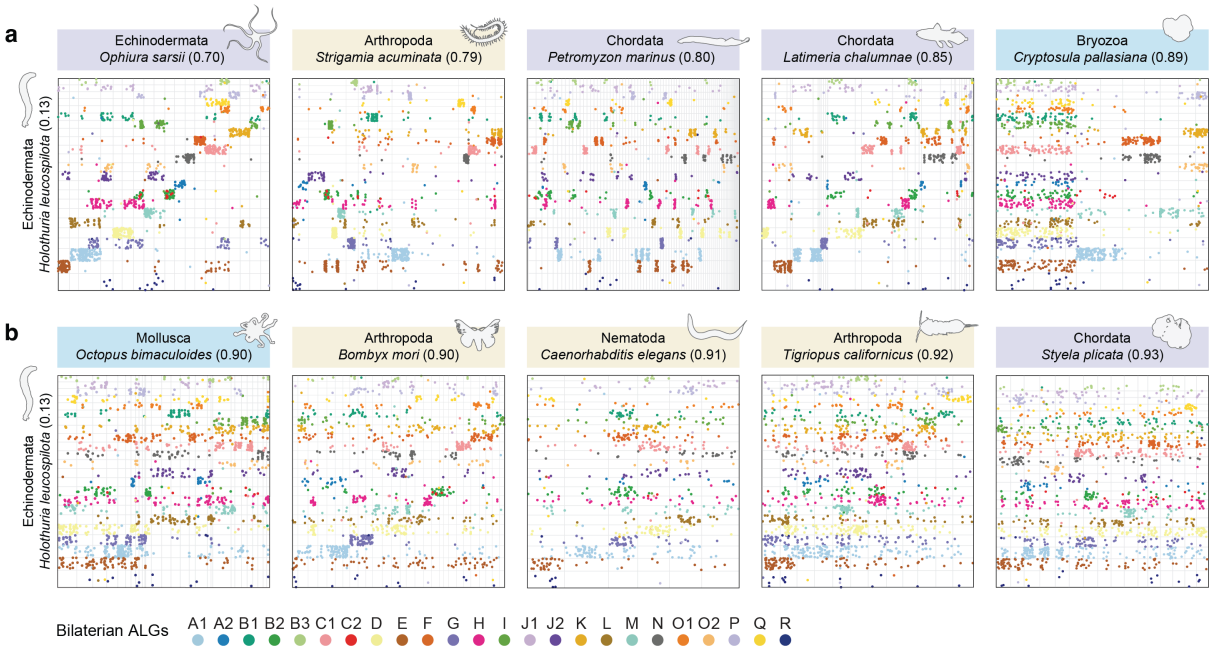

Fig. S5 An RI of 0.9 represents a highly rearranged genome. Over 50% of species in our dataset have an RI > 0.9. To understand what level of rearrangement this corresponds to, we produced synteny dot plots for species with RI just under 0.9 (**a**) and just over 0.9 (**b**). Genomes in both groups represent extensive departures from the bilaterian ancestral state of 24 ALGs. In **a**, most ALGs are still detectable, but many are combined by fusions or split by fissions. In **b**, few ALGs are detectable, and those that are have invariably been subjected to both fusion and fission.

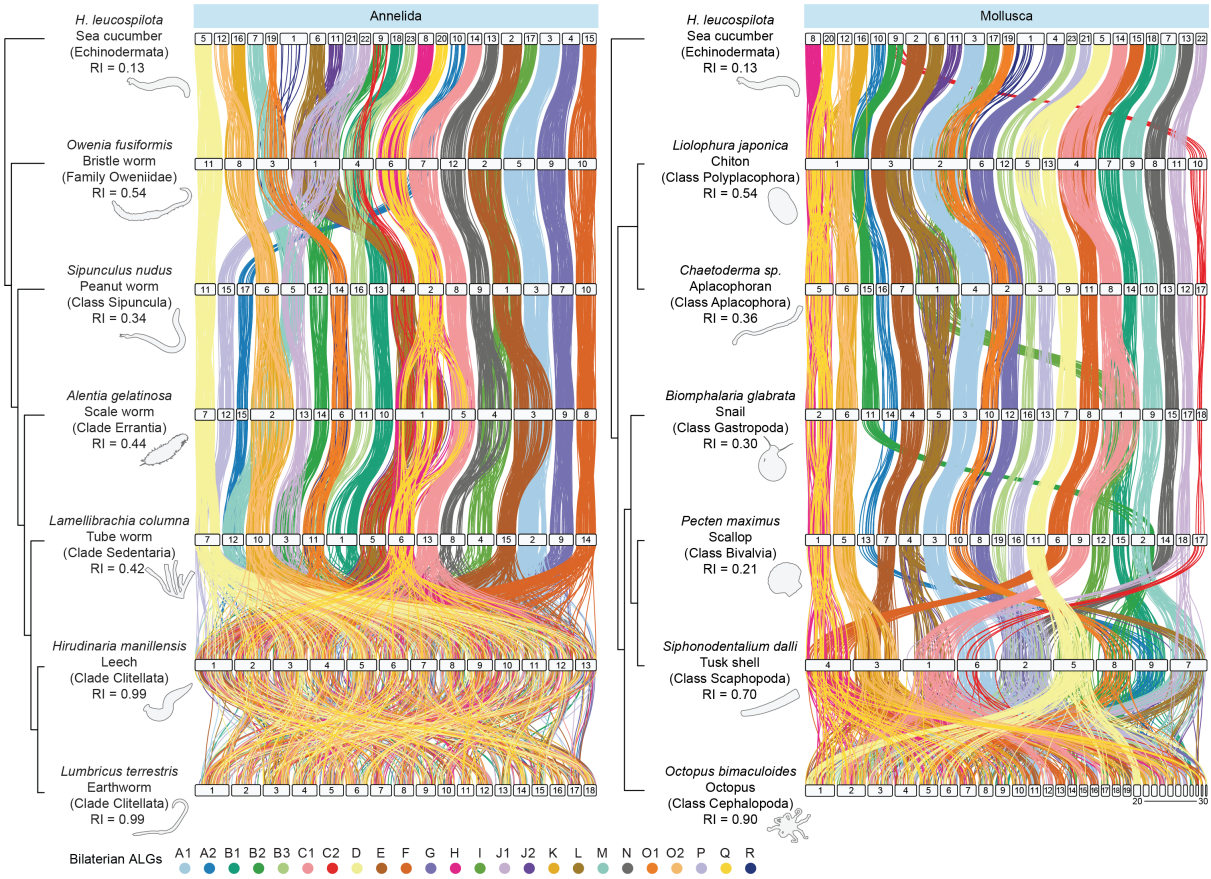

Fig. S6 Loss of bilaterian ALGs can occur in specific lineages within phyla. Idiogram plots for annelids and molluscs. White bars represent chromosomes. Vertical lines connect the genomic positions of orthologous genes, colour-coded by the bilaterian ALG to which the genes belong. In both annelids and molluscs, most lineages retain a relatively conserved genome structure characterised by large-scale maintenance of ALGs with few lineage-specific chromosome fusion events. Within annelids, the genomes of clitellates (leeches and earthworms) are completely scrambled. Within molluscs, the genome of *Octopus bimaculoides* is highly scrambled.

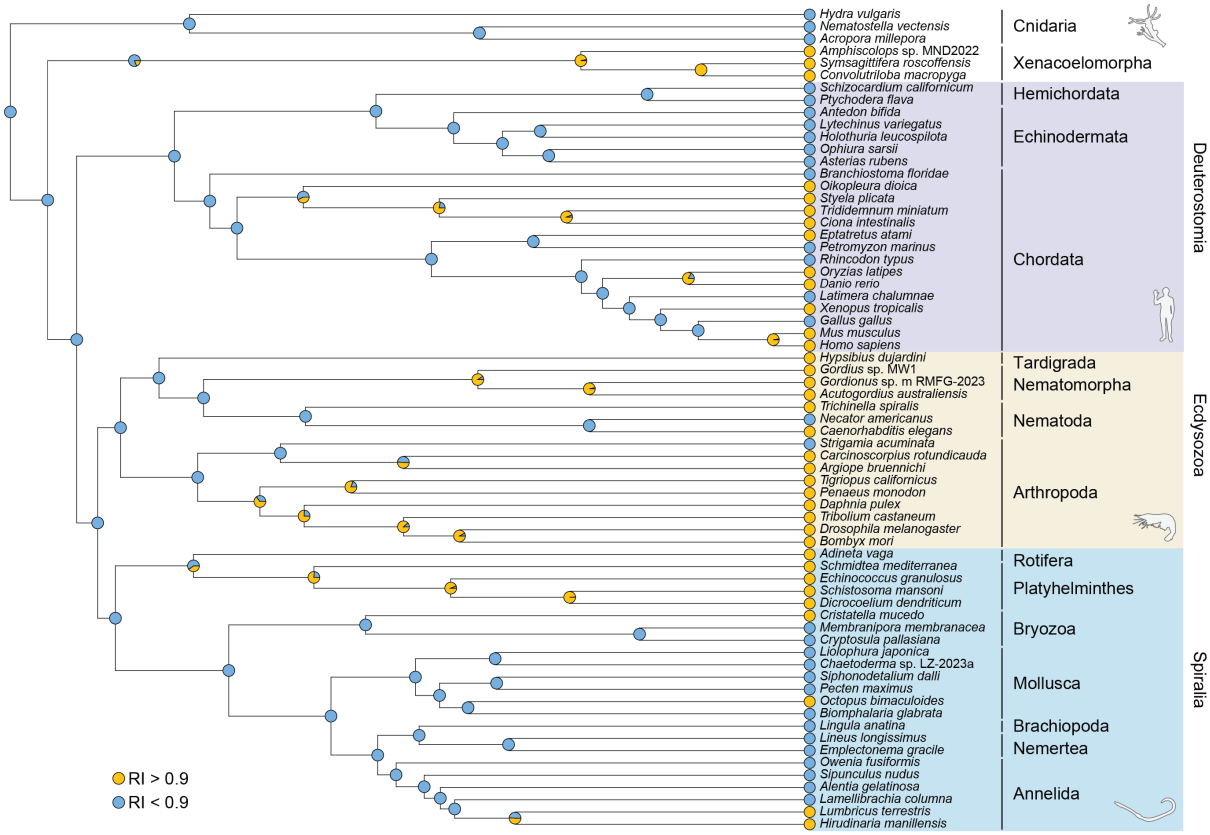

Fig. S7 Highly rearranged genomes (RI > 0.9) have evolved many times independently. Ancestral state reconstruction using RI > 0.9 (yellow) or RI > 0.9 (blue) as a discrete character. The analysis used a unidirectional character state transition model where RI can only increase, given the assumption that once lost, ALGs cannot be reconstituted. Maximum likelihood phylogeny built from a supermatrix of 148 concatenated single-copy protein sequences with species occupancy of at least 75%. The Huelsenbeck et al. (2003) Markov-chain-Monte-Carlo-based stochastic character mapping approach was used for ancestral state reconstruction.

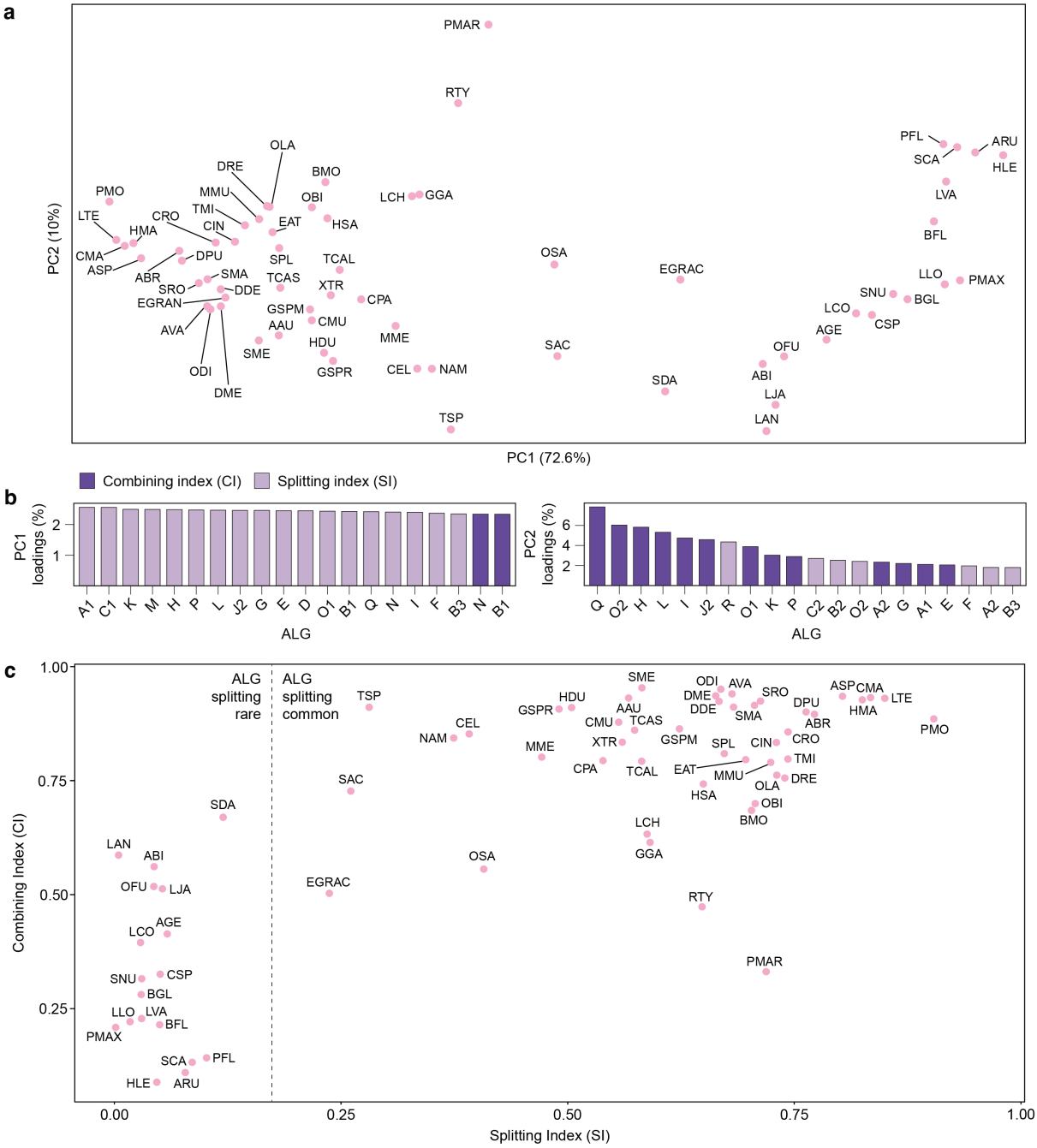

Fig. S8 The main driver of RI variation is ALG splitting. **a** The ALG combining index measures the extent to which genes from different bilaterian ALGs are combined together on the same chromosome. The ALG splitting index measures the extent to which genes from a single ALG are split apart over different chromosomes. Higher index scores indicate more rearranged genomes. The principal component analysis (PCA) shows the contribution of combining and splitting indices of each of the 24 individual ALGs to variation in the dataset. PC1 explains 72.6% of the variation. **b** Loadings for PC1 and PC2. The top 18 loadings for PC1, which explains 72.6% of the variation in the dataset, are all splitting indices. This suggests that the main difference between species' genome structures is the extent to which genes from a single ALG can be split apart by processes such as chromosome fission or translocation. **c** Combining index versus splitting index in bilaterian genomes. There are two

clusters of species along the ALG splitting axis, a group where ALG splitting is minimal and one where it is relatively frequent. Species abbreviations are reported in Table S1.

Table S2 Proportion of ‘jumping genes’—genes located on a chromosome not containing their reconstructed ALG—in a subset of eight species from six phyla

Phylum	**Species**	**RI**	**Jumping genes (%)**
Echinodermata	*Asterias rubens*	0.18338923	4.550946436
Chordata	*Branchiostoma floridae*	0.23666861	13.24422843
Mollusca	*Biomphalaria glabrata*	0.30479256	6.024626209
Echinodermata	*Holothuria leucospilota*	0.13237787	5.682715075
Brachiopoda	*Lingula anatina*	0.58843233	1.534828808
Nemertea	*Lineus longissimus*	0.23553018	2.912239276
Mollusca	*Pecten maximus*	0.21032758	3.202846975
Annelida	*Sipunculus nudus*	0.34094679	3.743104807