

Supplementary Table 1. Pairwise p-values between the four sets of linkage groups and the chromosomes of each species using Fisher's exact test. The p-values were corrected using the Benjamini-Hochberg procedure. Columns represent the merged LGs described in the Methods after Simakov et al. 2022.

Supplementary Table 2. Summary of the progressiveCactus alignment using halStats. Anc4 corresponds to the ancestor of SBEN and Clitellata (see also github repository for the full output, including a phylogenetic tree with the numbering of all nodes).

Supplementary Table 3. Transposable element insertions in each of the genomes assessed. TEs stands for the total number of insertions and Superfamilies to the number of TE superfamilies that have insertions in each genome. DNAcounts, LINEcounts, LTRcounts, RCcounts and SINEcounts refer to the number of insertions belonging to each of those orders while Unknowncounts refers to the number of insertions that were not classified into any of the orders. DNAcov, LINEcov, LTRcov, RCcov, SINEcov and Unknowncov stand for the % of bases covered by members of each order. Species codes are as indicated in the tab 'Species_codes'.

Supplementary Table 4. List of genes arising in the branch leading to Clitellata as a result of chromoanagenesis involved in response to abiotic stress. Corresponding HOG, composite/component nature, no. of genes in the same HOG, mechanism of origin (eg, fusion, fusion, de novo), putative function retrieved by BLAST and GO terms retrieved by FANTASIA (next tab) are indicated.

Supplementary Table 5. List of species, acronyms and data source for all datasets included in this study.

Supplementary Data 1. Substitution-rate-adjusted mixed paralog–ortholog Ks plot for *Eisenia andrei*, *Metaphire vulgaris*, *Hirudinaria manillensis* and *Enchytraeus crypticus*. The inferred putative WGD events are indicated with dashed lines labelled with letters (a, b, c, etc.) corresponding to the Ks-based WGD age estimates. The speciation events calculated using the rate-adjusted mode estimates of the ortholog Ks-distributions between each species and the other species are drawn as long-dashed lines labelled with numbers between 1 and 8 surrounded by coloured boxes ranging from one standard deviation below and above the mean mode estimate. Lines representing the same speciation event share number and colour.

Supplementary Data 2. Ks plot for the different retained gene duplicate pairs which originated on different nodes, indicative of whole genome duplication events. From left to right, from up to down, (1) the node including Clitellata and its closest common ancestor, (2). the node of Clitellata, (3) the node which includes leeches and earthworms, (4) the node which includes only earthworms (Crassiclitellata). Each species is represented with a different colour.

Supplementary Data 3. Reactome report for the functional pathway enrichment of genes belonging to Hierarchical Orthogroups (HOGs) lost in Clitellata present in *Sipunculus nudus*, showing the functional pathways of humans.

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Supplementary Data 5. Reactome report for the functional pathway enrichment of genes belonging to Hierarchical Orthogroups (HOGs) lost in Clitellata present in *Paraescarpia echinospica*, showing the functional pathways of humans.

Supplementary Data 6. Reactome report for the functional pathway enrichment of genes belonging to Hierarchical Orthogroups (HOGs) lost in Clitellata present in *Pecten maximus* (Mollusca), showing the functional pathways of humans.

Supplementary Data 7. CMC-Chapaev3 transposable elements in other earthworms (*Metaphire vulgaris*, MVUL, and *Carpetania matritensis*, CMAT), showing their overall overlap with centromeres (black lines).

Supplementary Data 8. CMC-Chapaev3 transposase phylogeny showing all terminals.

Supplementary Data 9. *Hox* gene cluster evolution in all species included in our dataset. Rectangular boxes represent *Hox* genes, while chromosomes or scaffolds (in the case of the draft genome of the enchytraeid) are symbolised as distinct horizontal lines. Tandem duplications are depicted by duplicated rectangles and are named with consecutive letters. Genes not classified as a *Hox* gene are represented by triangles, with the number of such genes depicted inside the triangle. Separations greater than 300kb between two non-consecutive *Hox* genes are represented by two vertical lines (||). Chromosomes are split in different lines when there are multiple copies of several genes of the *Hox* cluster in the same chromosome.

Supplementary Data 10. Gene Ontology (GO) enrichment of genes in HOGs lost in Clitellata. Up, treemap visualisation of all terms (BP, Biological Process; MF, Molecular Function, CC, Cellular Component). Down, bar plot of GOs enriched in functions related to cell cycle, chromatin remodelling, DNA repair and genome stability.

Supplementary Data 11. Report on the chromosome-level genome assemblies of *Norana najaformis* and *Carpetania matritensis*, newly generated for this study.

Supplementary Methods. Extended version of the methods.