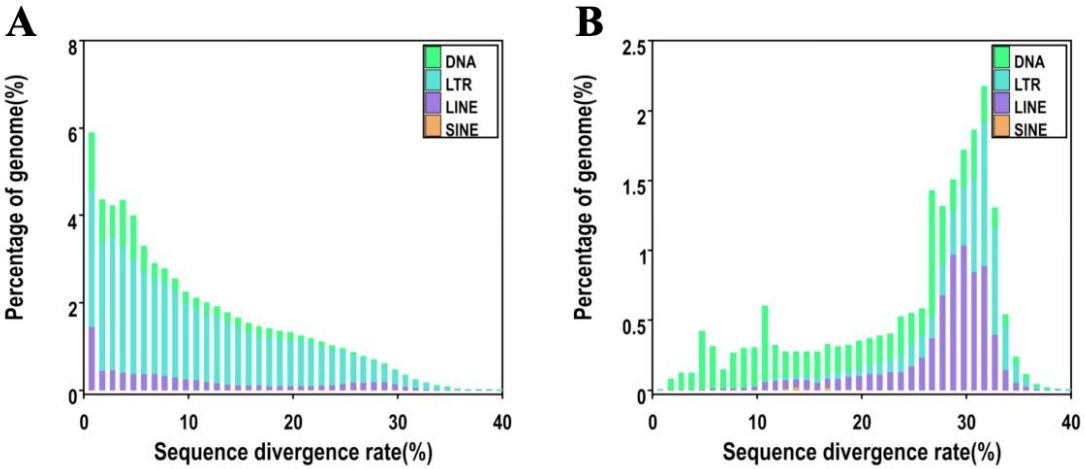
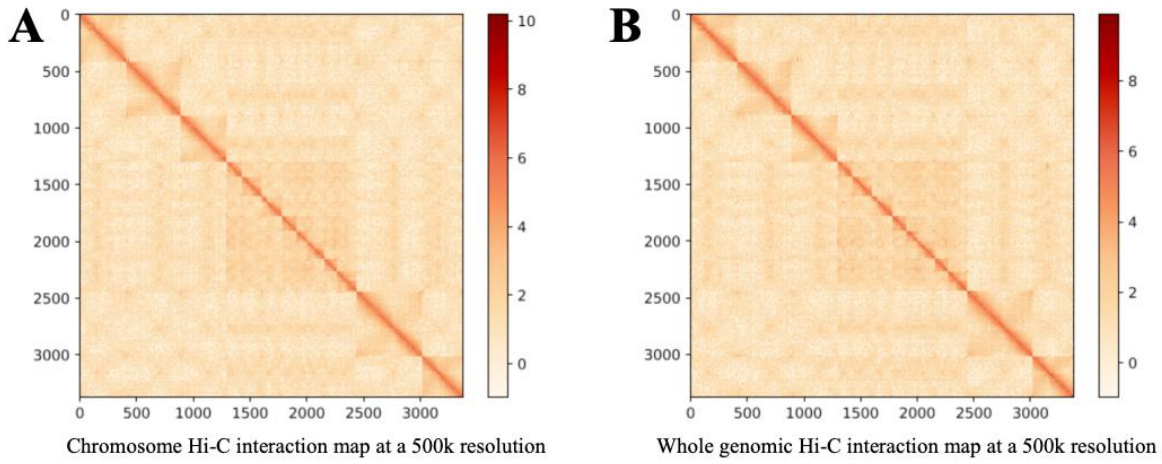
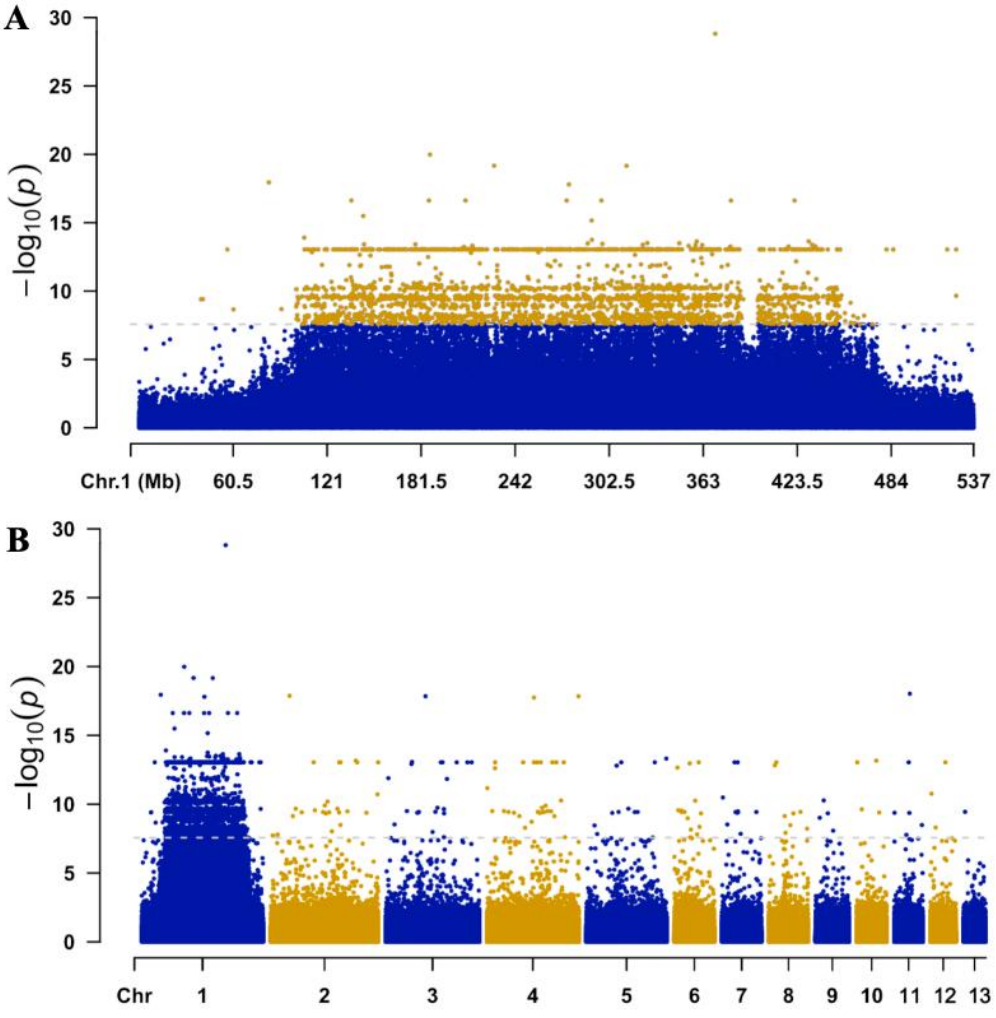
Supplemental Figures



Supplemental Figure S1. The divergence distribution of transposable elements (TEs).
A. Depicts the divergence distribution of transposable elements (TEs) annotated using RepeatMasker based on the Repbase library. B. Illustrates the divergence distribution of TEs predicted by the De novo method. The x-axis represents the divergence of TE sequences annotated in the genome, while the y-axis indicates the percentage of TE sequences at each divergence level in the genome. Different TEs are represented by different colors.

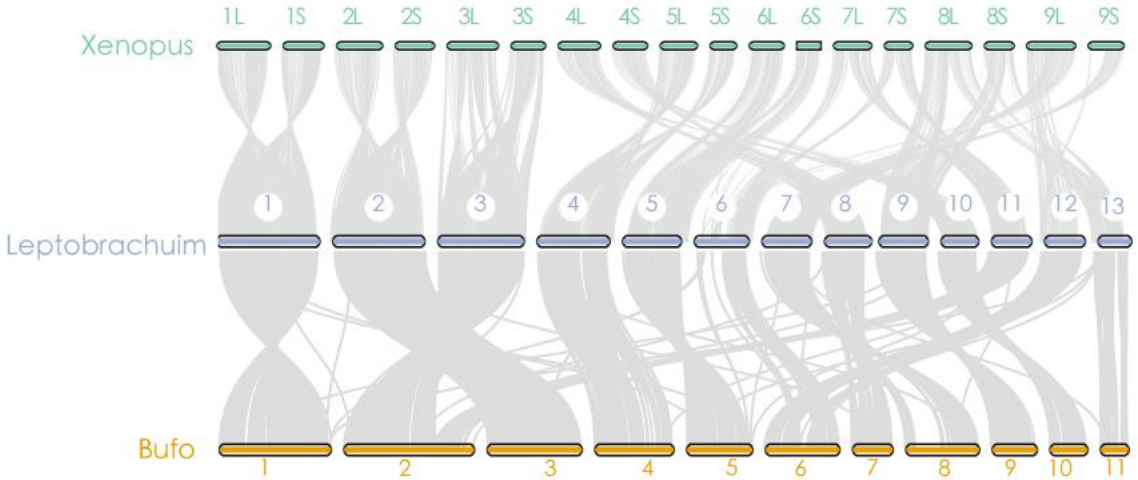


Supplemental Figure S2. The chromosome and whole-genome interaction map of *L.boringii*.
A. Chromosome Hi-C interaction map at a 500k resolution. B. Whole-genome Hi-C interaction map at a 500k resolution. In the spectra, colors ranging from light to dark indicate increasing interaction strength, with darker shades indicating stronger interactions. The x and y axes represent their respective N*bin positions on the genome. The first 13 squares in the figure represent the 13 chromosomes of *L. boringii*, while the sequences not clustered onto chromosomes are shown afterward.



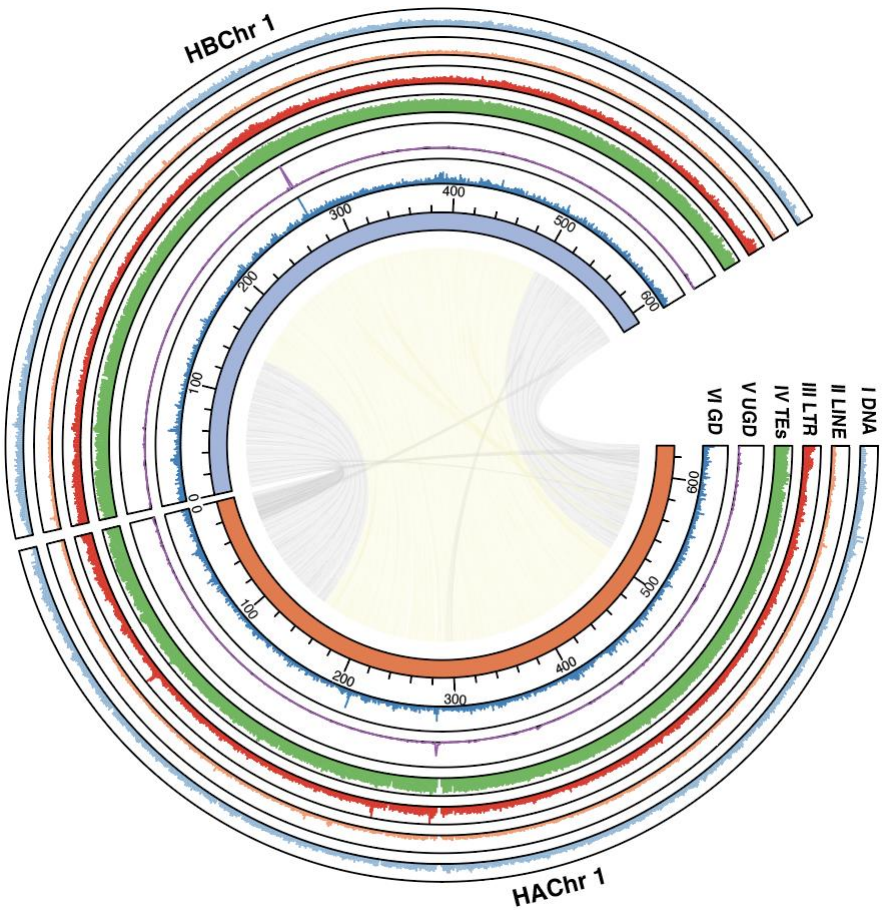
Supplemental Figure S3. The distribution of significant SNPs in the sex chromosome. (A) and whole genome (B) of *L. boringii*.

Grey dash line indicate threshold of GWAS (-log10 (0.05/No. of SNP)=7.568702), yellow regions above the threshold in (A) represent significant GWAS SNPs on chromosome.



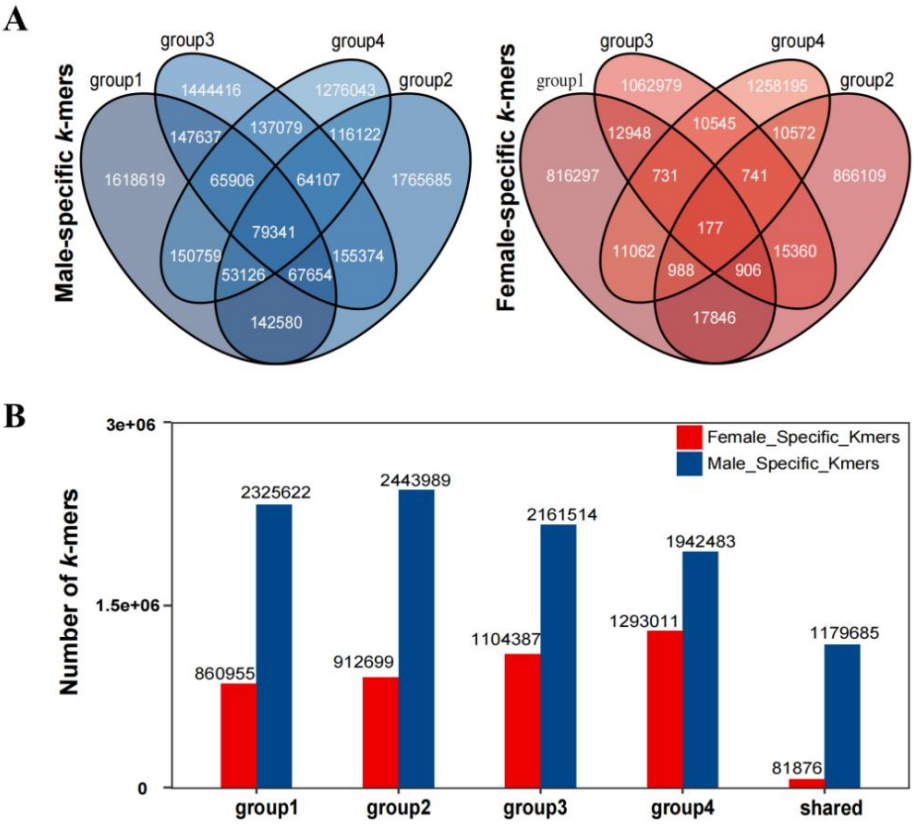
Supplemental Figure S4. Synteny alignment of three species (*L.boringii*, *Xenopus laevis* and *Bufo gargarizans*).

Result of synteny alignment shows the high degree of conservation of Chr1 in the *L.boringii*. The genome data of the other two species were downloaded from NCBI (PRJNA313213 and PRJNA628553).



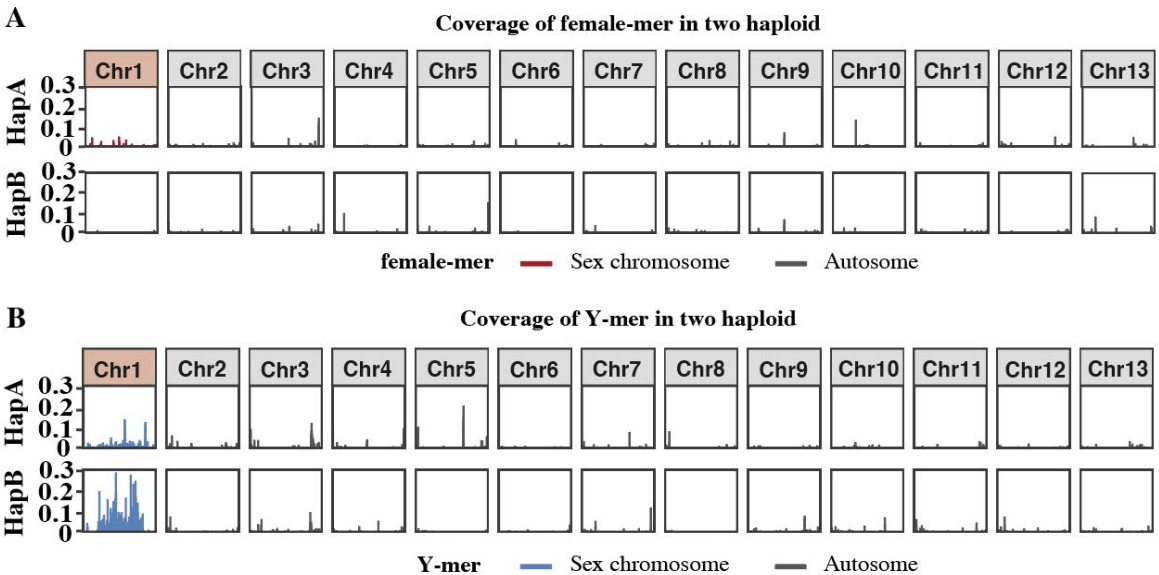
Supplemental Figure S5. Various genomic features were analyzed in 1 Mb sliding windows across HAChr 1 and HBChr 1.

From the outermost to the innermost rings: (I) distribution of DNA transposons; (II) distribution of LINE elements; (III) distribution of LTR retrotransposons; (IV) distribution of all transposable elements; (V) unassigned gene density (UGD); (VI) gene density (GD). The radial axis represents genomic positions (in Mb). Inner connecting lines depict syntenic regions and structural variations between HAChr 1 and HAChr 1, with yellow lines highlighting sex-linked region (SLR).



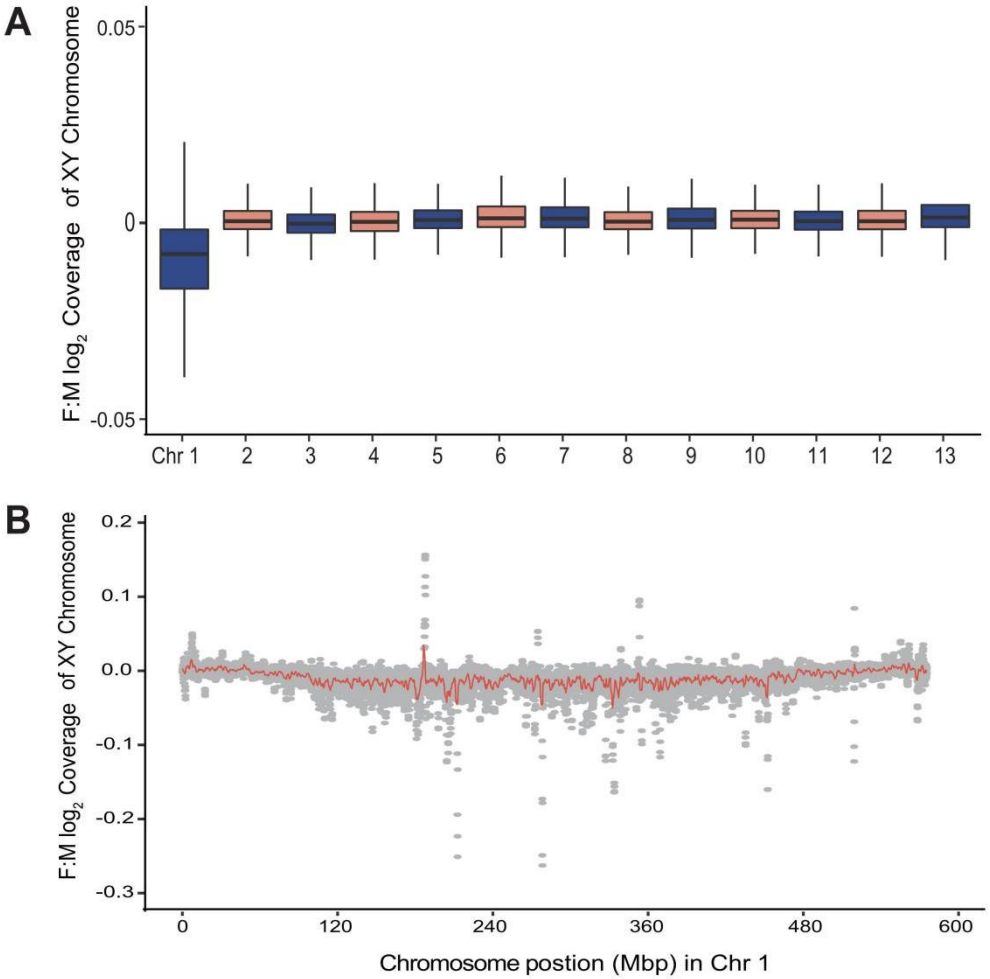
Supplemental Figure S6. Results of male-specific and female-specific kmers identified in four repeated grouping experiments.

A. Male-unique *k*-mers (Y-mer) count (blue) and female-unique *k*-mers (female-mer) counts (red) in 4 test group. The overlapping regions represent the k-mers shared by at least two test groups, representing a more accurate filtered *k*-mers data. B. In each group, the number of male kmers is significantly higher than that of female *k*-mers Shared *k*-mers represent specificity *k*-mers that are commonly identified in at least two grouping experiments.



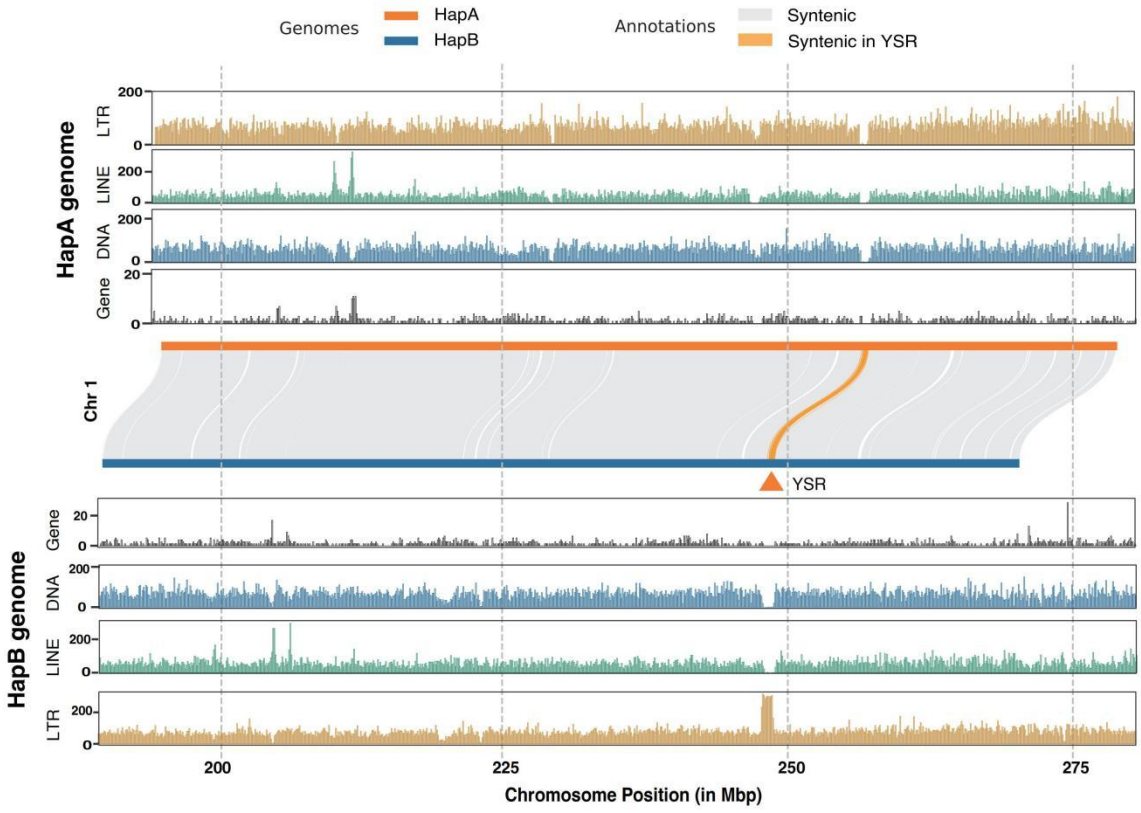
Supplemental Figure S7. Coverage analysis of sex-specific *k*-mers aligned to the HapA and HapB haplotypes.

A. Coverage of female-specific *k*-mers (female-mers) aligned to the HapA and HapB haplotypes (depicted in red). B. Coverage of male-specific k-mers (Y-mers) aligned to the HapA and HapB haplotypes (depicted in blue). Y-mers exhibit significant enrichment exclusively on the sex chromosome of HapB.



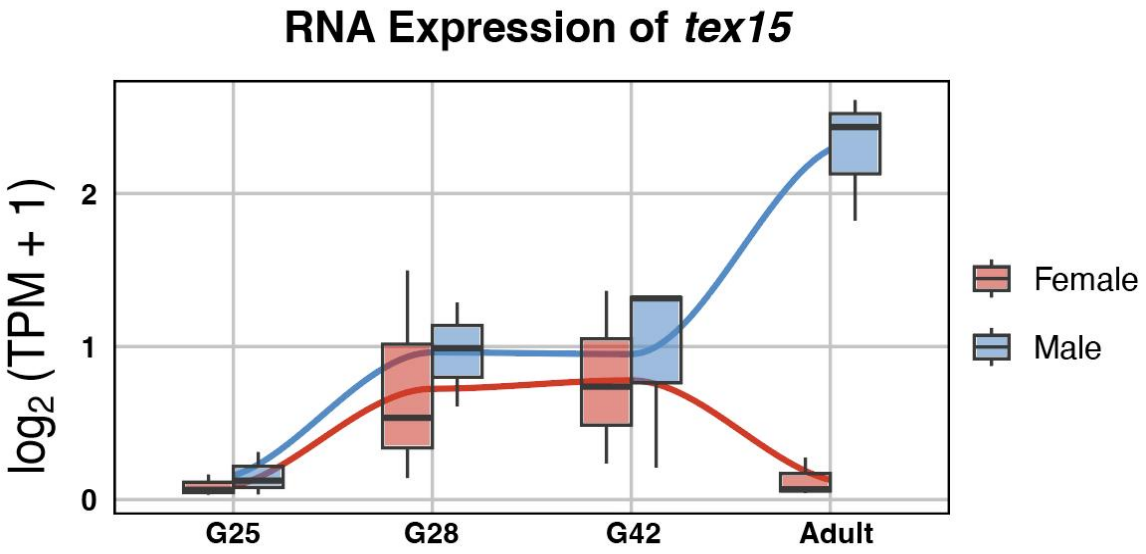
Supplemental Figure S8. A comparison of F:M $\log_2$ coverage of the XY Chromosomes across different chromosomes and positions of *L. boringii*.

A. Boxplots showing the F:M $\log_2$ coverage ratio across Chr 1 to Chr 13. B. Scatter plot with a smoothed trend line illustrating the F:M $\log_2$ coverage along Chr 1.



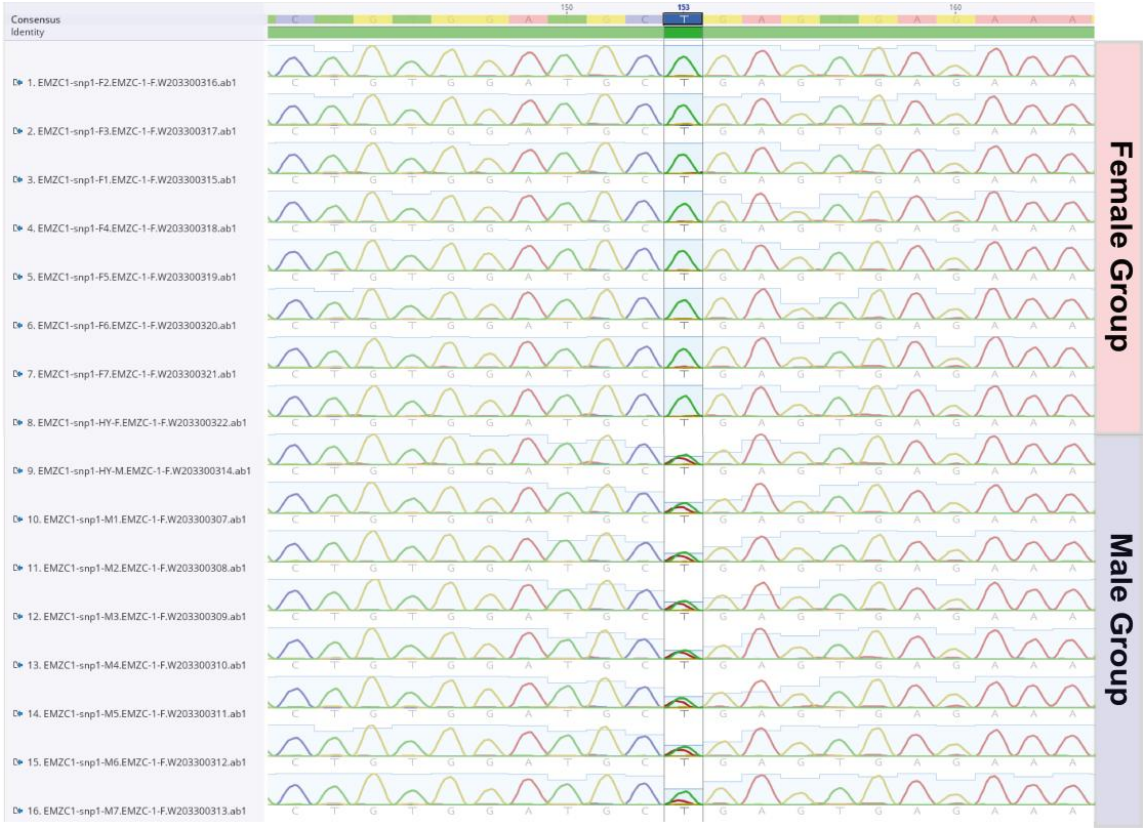
Supplemental Figure S9. Comparative visualization of the genomic features of HapA and HapB near the YSR.

Zooming in to show the distribution of transposable elements (LTR, LINE, DNA) and gene density and homologous regions near the YSR in two haploide genome, where the homologous collinear segment region between YSR (orange triangle) and HapA is highlighted with orange lines.



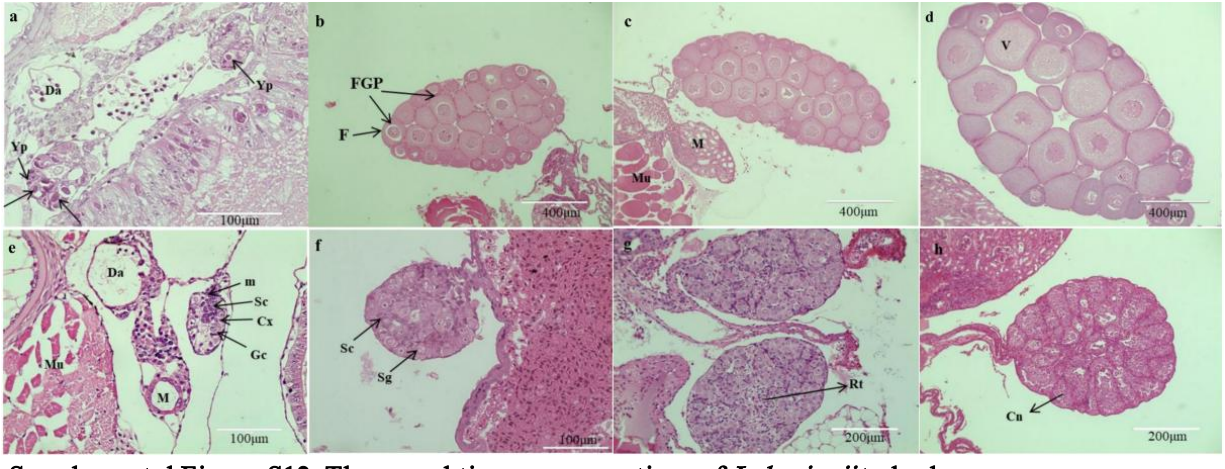
Supplemental Figure S10. RNA expression levels of the *tex15* gene across four developmental stages (G25, G28, G42, Adult) in both male and female individuals.

The expression is measured as $\log_2(\text{TPM} + 1)$. The boxplots represent the distribution of expression levels for each group at each stage, with males shown in blue and females in red.



Supplemental Figure S11. Validation of sex-specific SNPs (SNP1 locus, Chr 1:115680776) in *L. boringii* by Sanger sequencing.

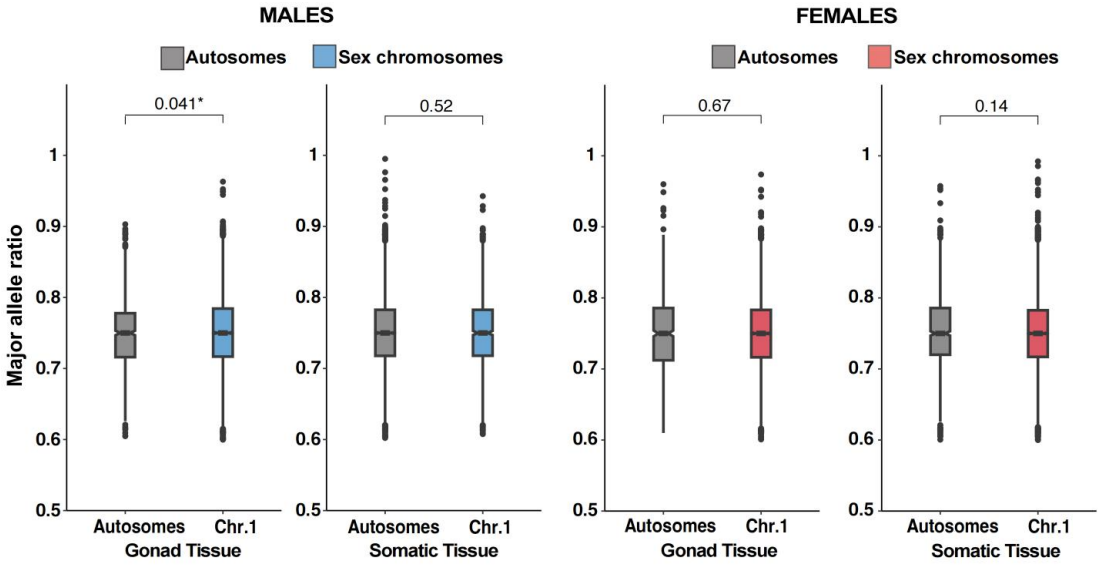
Sanger sequencing chromatograms illustrate nucleotide variation at SNP1 in male and female groups. A cohort of 48 individuals, evenly split between males and females, was used. The sample set included 7 individual females and 17 pooled females, as well as 7 individual males and 17 pooled males. The highlighted region (position 153, corresponding to genomic position Chr1:115680776) shows sex-specific polymorphism, with different nucleotide peaks: heterozygous (AT) in all male groups and homozygous (TT) in all female groups.



Supplemental Figure S12. The gonad tissue cross sections of *L. boringii* tadpoles.

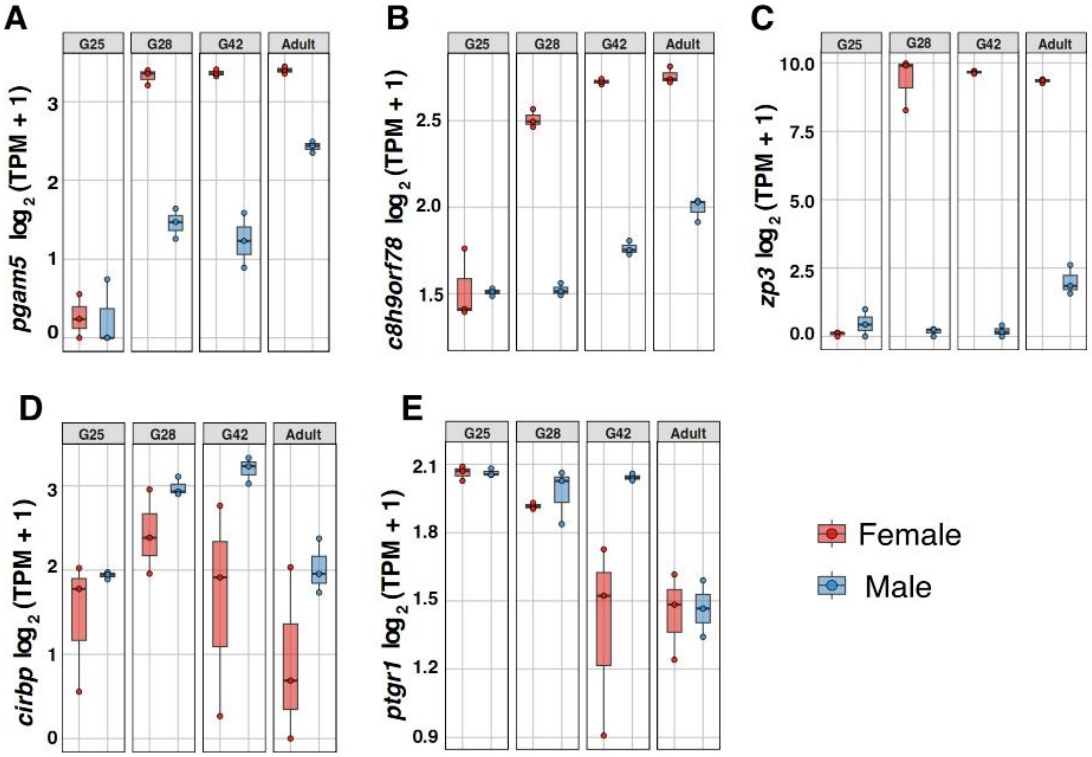
(a) The PGCs contain a few yolk platelet (arrow) and begin proliferate at Gosner stage 25; (b) The ovary at the end of Gosner stage 28; (c) The ovary at Gosner stage 42; (d) The ovary in Adult frog.; (e) The undifferentiated gonad at Gosner stage 26; (f) The testis at the end of Gosner stage 28 (g) The testis at Gosner stage 42 (h) The testis in Adult frog.

Cn: cell nests of secondary spermatocyte, Cx: cortex, Da: dorsal aorta, Dio: diplotene oocyte, F: follicle, FGP: first growth phase oocyte, Gc: germ cells, L: leptotene, m: medulla, M: mesonephros, Me: mesentery, Mu: muscle, O: ovary, Oc: ovarian cavity, V:vitellogenic oocy, Rt: rete testis, Sg: spermatogonia, St: seminiferous tubule, Sc: somatic cells, Sp: spine, T: testis, Tc: testis cord, Yp: yolk platelet.



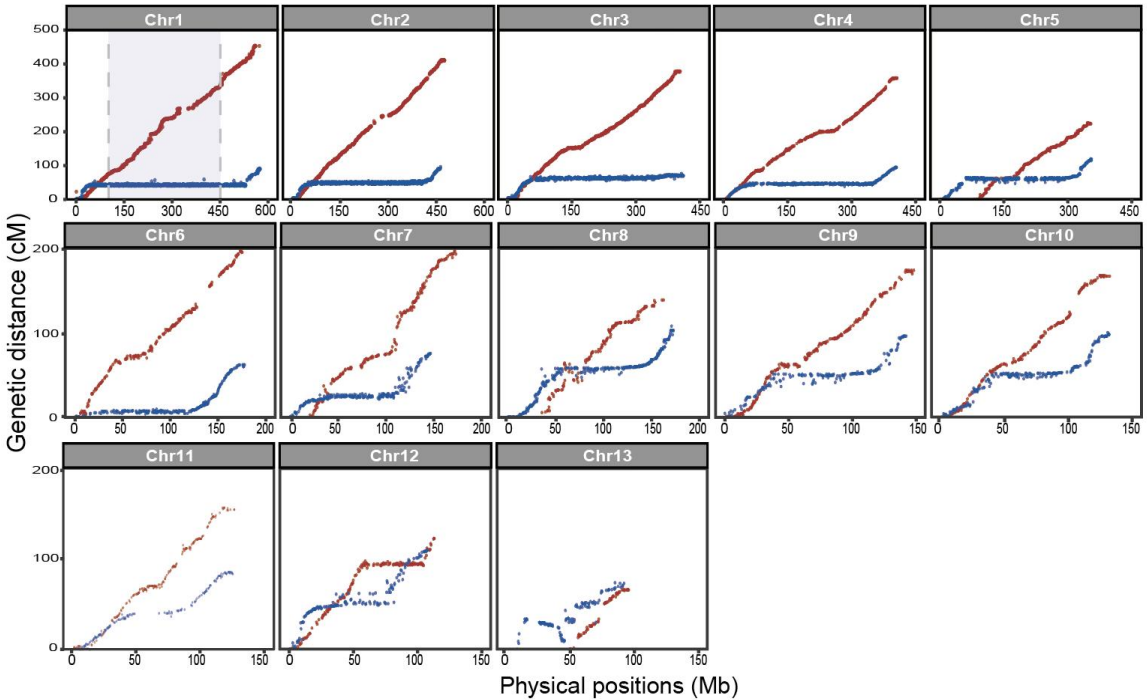
Supplemental Figure S13. Patterns of ASE of *L. boringii*.

Boxplots show differences of major allele ratio for autosomal genes (gray) and sex chromosome genes (blue in male and red in female) with an ASE pattern. *P* values are based on Wilcoxon rank sum tests. Significant differences in major allele ratio of genes between sex chromosomes and autosomes only in the male gonad.



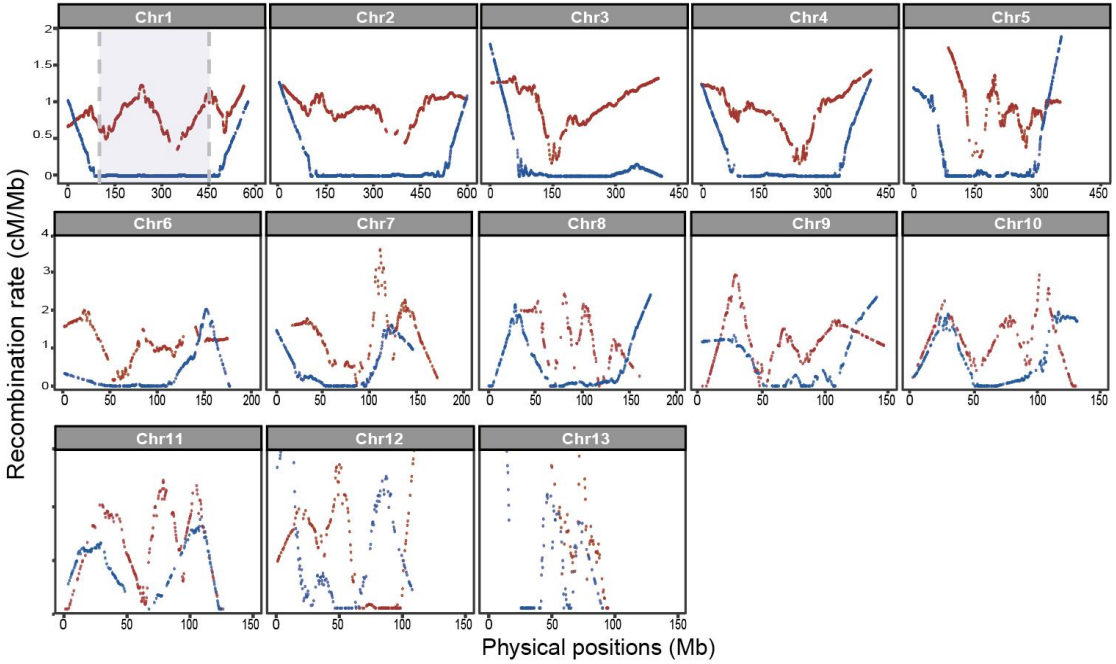
Supplemental Figure S14. The expression levels of five candidate genes (*pgam5, c8h9orf78, zp3, cribp,* and *ptgr1).*

This figure presents boxplots of the $\log_2$ (TPM + 1) expression values for each gene, separated by sex (females in red, males in blue) and developmental stage.



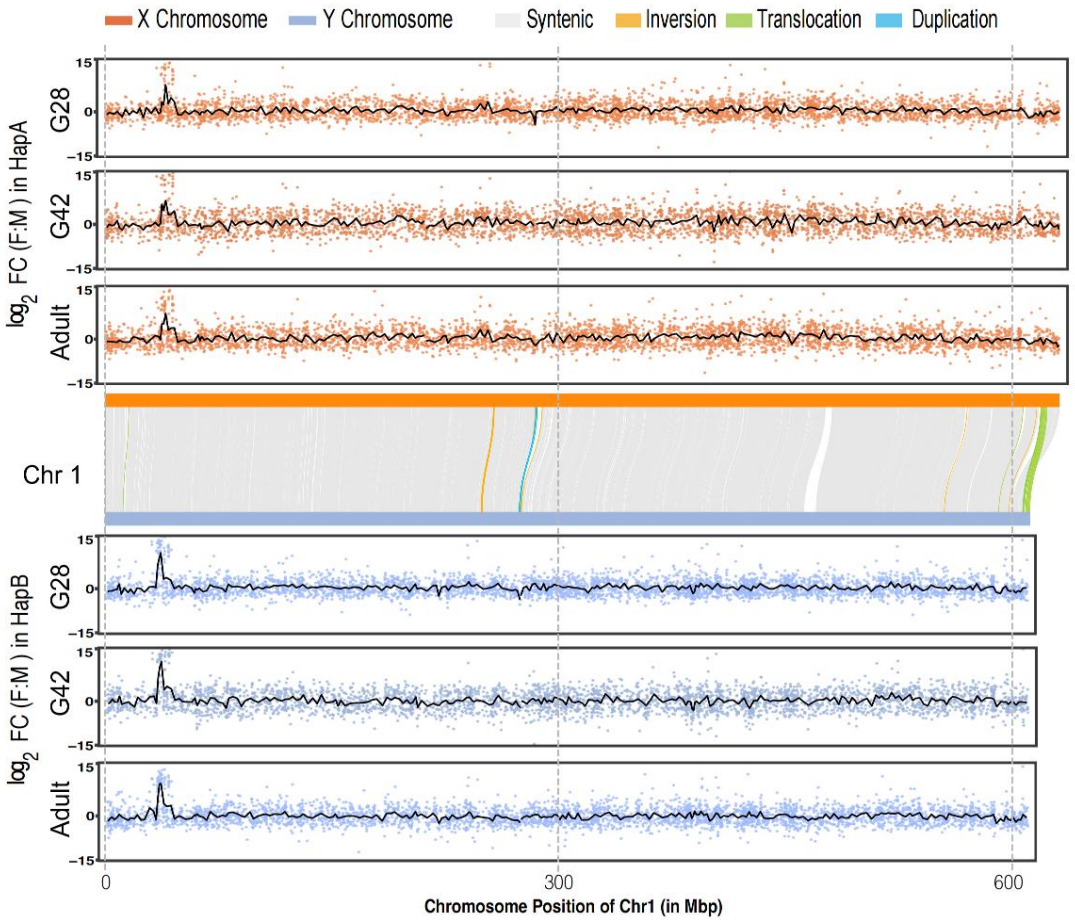
Supplemental Figure S15. Comparison of sex-specific genetic map length.

The x-axis represents the physical position (Mb) of markers, the y-axis represents the genetic positions of markers on the linkage map (measured in centimorgans, CM) between male (blue) and female (red) for each chromosome. Light purple region represents the position of SDR identified from GWAS result



Supplemental Figure S16. Comparison of sex-specific recombination maps.

The recombination rates for all chromosomes are compared between female (red) and male (blue). In male individuals, the central regions of most chromosomes exhibit a certain degree of recombination suppression. Light purple region represents the position of SDR identified from GWAS result



Supplemental Figure S17. Collinearity, structural and gene expression (log2 of the female-to-male ratio) comparison of HAChr 1 genome (orange) and HBChr 1 genome (blue). The gene expression (log_2 female:male FC) is shown along the X Chromosome (HAChr 1, upper panels) and Y Chromosome (HBChr 1, lower panels) across three developmental stages (G28, G42, and Adult). The black line represents the smoothed expression trend calculated for groups of 30 genes. The middle panel provides collinearity and structural annotations, including syntenic regions (gray), inversions (orange), translocations (green), and duplications (blue). Chromosomal positions are presented in megabase pairs (Mbp) along the x-axis, spanning from 0 to 600 Mbp.