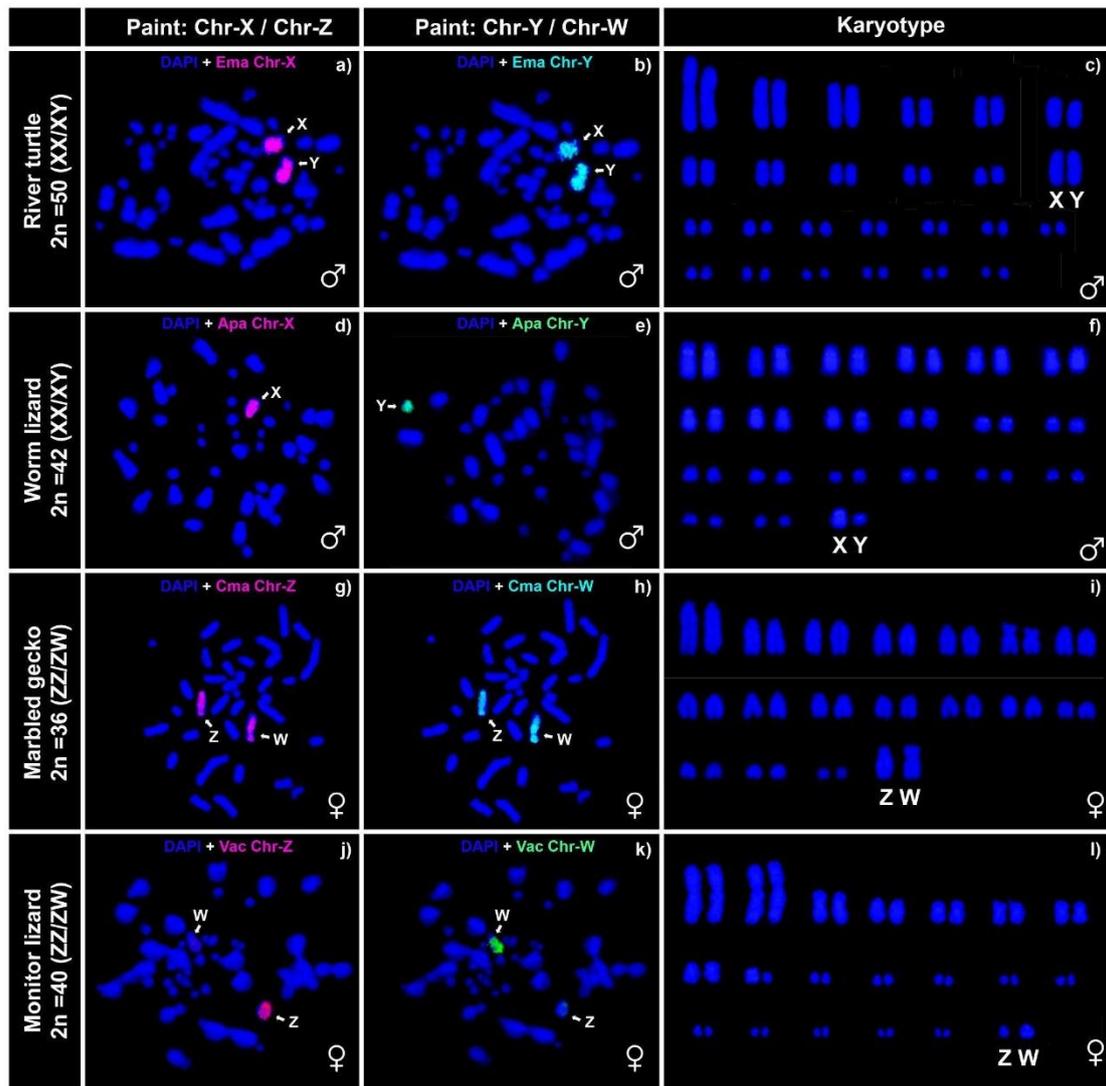
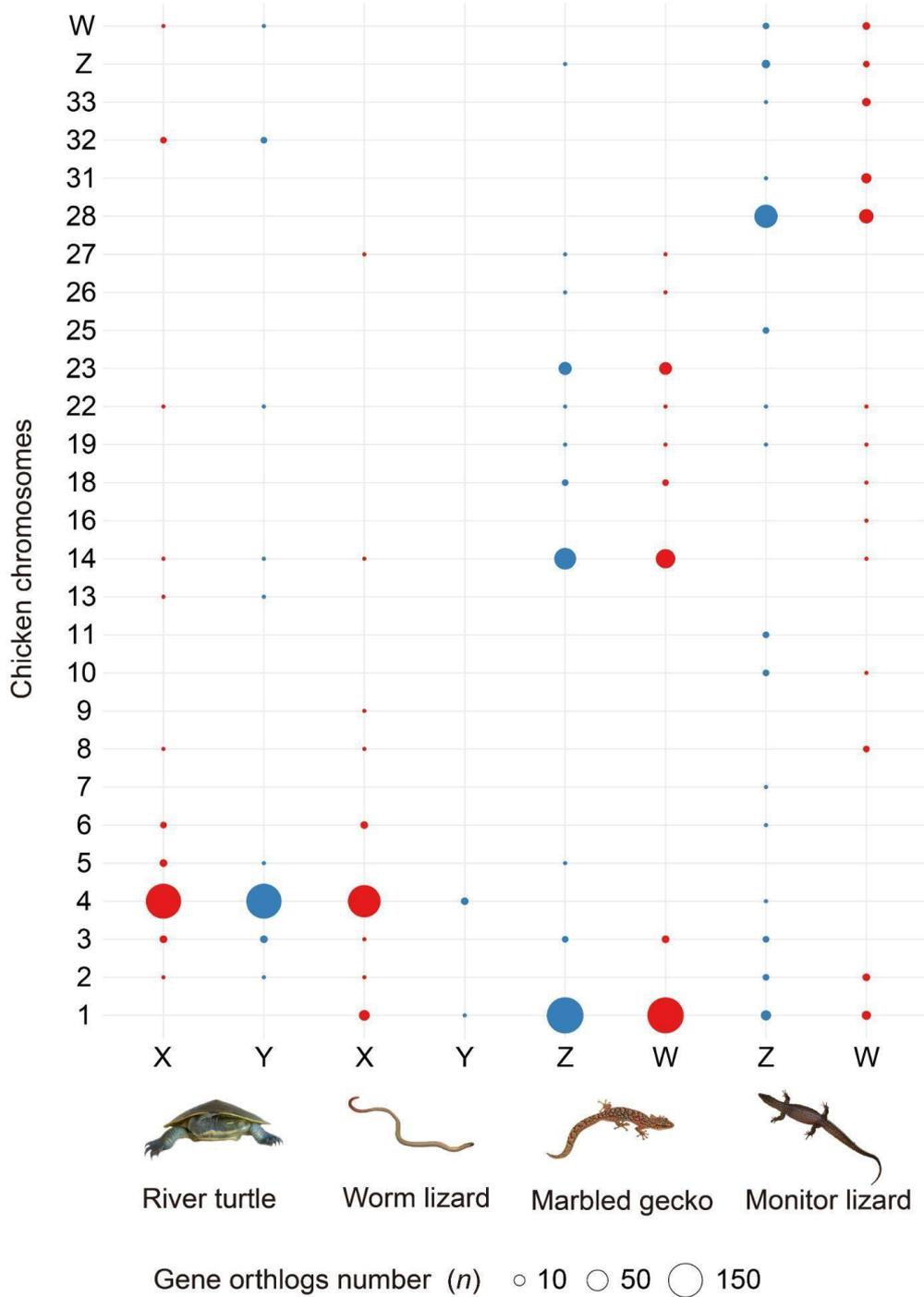


## Supplementary Materials

### Supplementary Figures



Supplementary Figure S1 Fluorescence in situ hybridization (FISH) images of the four reptile species showing validation of microdissected chromosome probes.



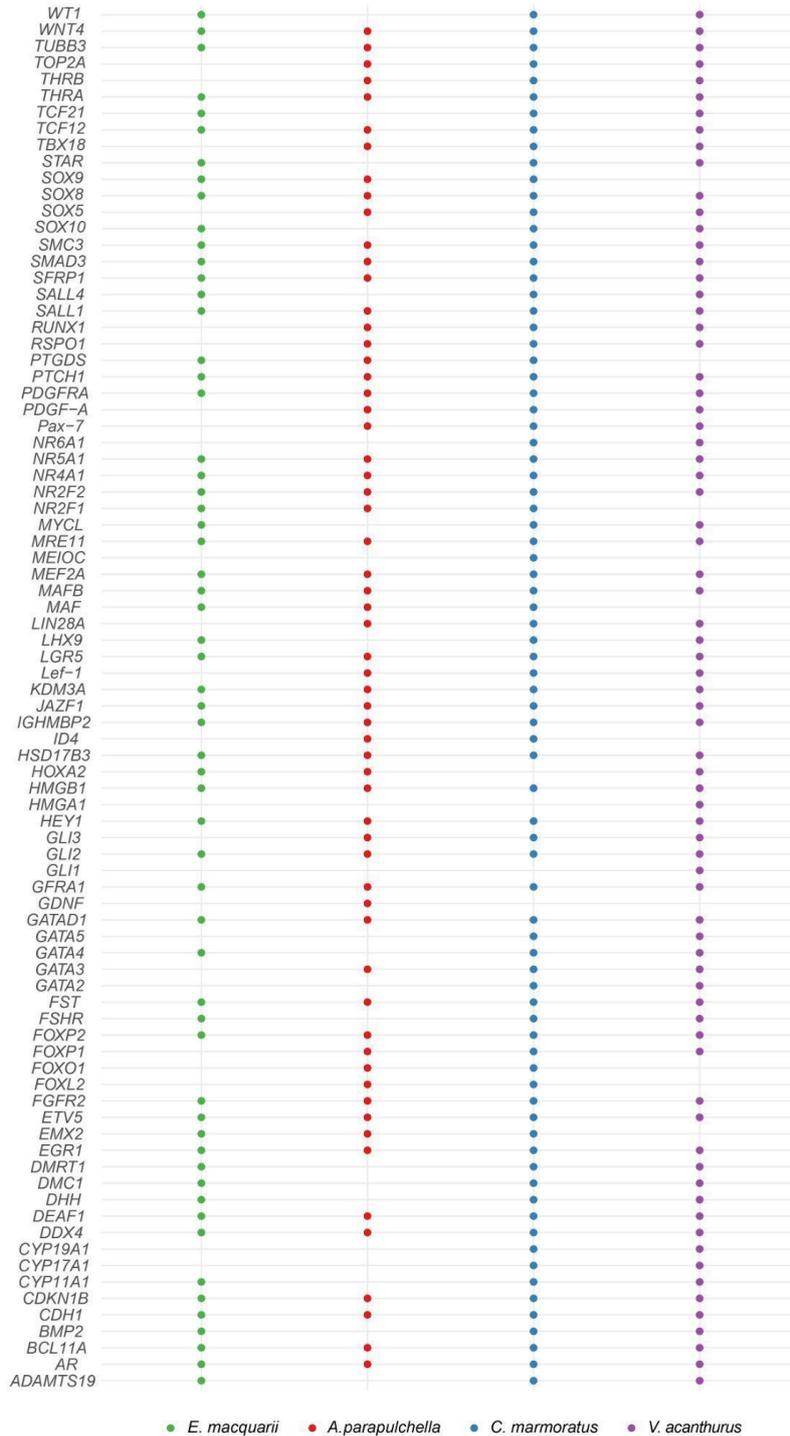
**Supplementary Figure S2 Bubbles showing orthologs of sex chromosomes of the four reptile species.**

The figure shows the origins of the sex chromosomes(X-axis) of four reptile species against the chicken genome(Y-axis). Dot sizes refers to the number of orthologs, and the color indicates the sex chromosomes of the four reptiles, which is red by X and W, and blue by Y and Z.



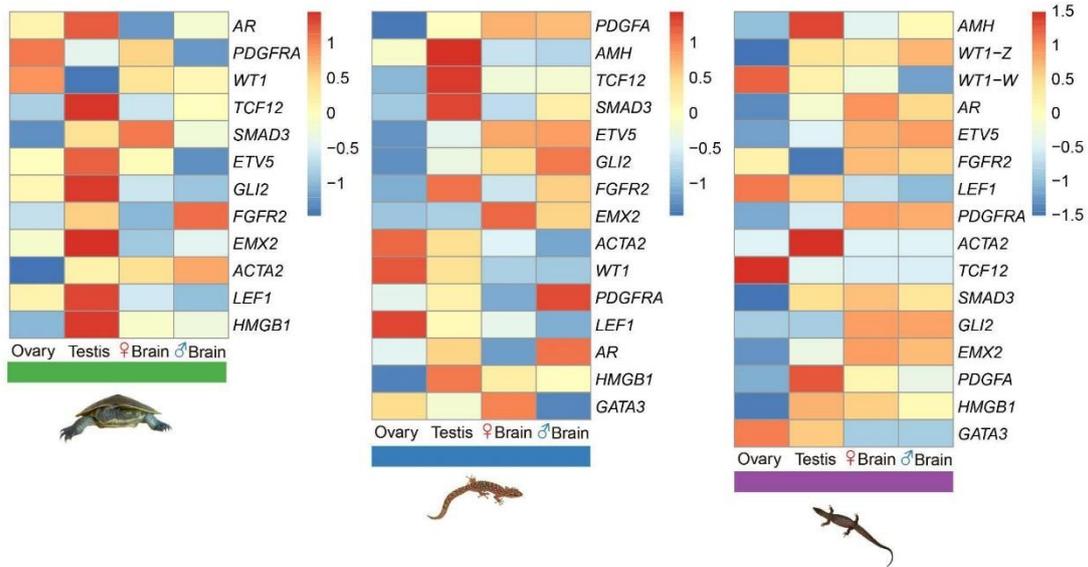
**Supplementary Figure S3 Location of orthologs of sex-determining genes on the homologous chicken chromosomes**

We labelled the ortholog of known sex-determining genes in the chicken (Gga6a) genome. And different colors refer to genes of different species, with red to Worm lizard, green to River turtle, blue to Marbled gecko and purple to Monitor lizard. Lines in orange refers to the orthologs of sex-determining genes that have been studied. SD-genes: Candidate Sex determining genes



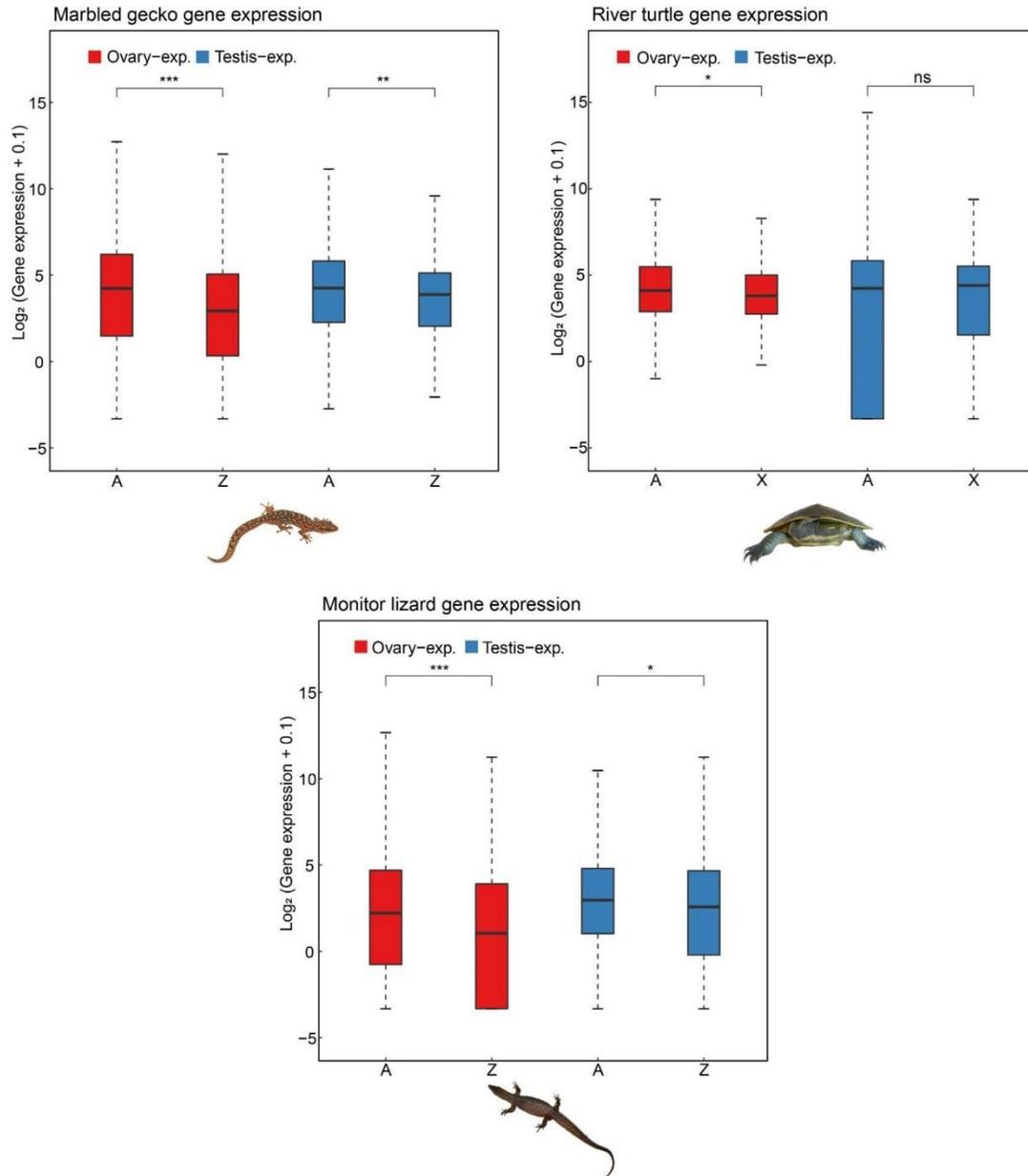
**Supplementary Figure S4 Orthologs of sex-determining genes ag. Gga6a in 4 species.**

The figure shows the presence (shown as a dot in the figure, otherwise no dot) of assembled orthologs of known sex-determining genes in the four species. And different colors refer to different species, which are red to Worm lizard, green to River turtle, blue to Marbled gecko and purple to Monitor lizard.



**Supplementary Figure S5 Comparisons of sex-determining genes' expression patterns in different tissues. (Except for APA with only sexed somatic tissue transcriptomes.).**

The figure shows the log<sub>2</sub> values of expressions (TPM) of assembled orthologs of known sex-determining genes in the three species. And different colors refer to different species, which are green to River turtle, blue to Marbled gecko and purple to Monitor lizard.



**Supplementary Figure S6 Gene expression of River turtle, Marbled gecko and Monitor lizard.**

Each box shows the log<sub>2</sub> values of absolute gene expressions (TPM). A: autosomal genes; Z: Z-linked genes; X: X-linked genes. For XY species, to see masculinization, autosomal genes will generally have a higher testis expression. For ZW species, it is the opposite, and for ovary, it is all opposite.