### **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

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Lef-1     KDM3A     JAZF1     IGHMBP2     ID4     HSD17B3     HOXA2     HMG81     HMG81     HMG81     HKS1     GL12     GL13     GL12     GL14     GL15     GL16     GL17     GATA5     GATA5     GATA5     GATA4     GATA5     GATA5     GATA5     GATA5     GATA6     GATA7     FST     FST     FOXP1     FOXP1     FOX12     FGR2     EGR1     DMC1     CYP	Leif-3       JAZF1       IGHMBP2       ID4       HSD17B3       HOKA2       HMG81       HMG81       HEY1       GLI2       GLI2       GLI2       GLI4       GATA5       GATA6       GATA7       GATA6       GATA7       GATA6       GATA7       GATA6       GATA7       GATA6       GATA7       GATA6       GATA7       GATA7       GATA6       GATA7       GATA7       GATA8       GATA9       GATA4       GATA1       GATA2       FST       FST       FST       FOK12       FOK12       FOK12       FOK12       FOK12       FOK12       FOK12       FOK14       DDX4       CDH       GL11A <td>LGR5 -</td> <td></td> <td></td> <td></td> <td></td>	LGR5 -				
KDM3A   JAZF1     JAZF1   JAZF1     IGHMBP2   JA     HA   JA     HSD17B3   JA     HOXA2   JA     HMGA1   JA     HMGA1   JA     HKS1   JA     GL12   GE     GL13   GE     GL14   GE     GL15   GE     GATA5   GATA5     GATA4   GATA3     GATA3   GATA4     GATA2   FOXP1     FOXP1   FOXP2     FOXP1   FOXP1     FOX12   FGR2     FGR2   GE     EMX2   GE     EGR1   GE     DMC1   DHH     DMC1   DHH     DDX4   CYP17A1     CYP17A1   CYP17A1     CYP17A1   GE     CVP17A1   GE     GDM1   GE     BMP2   GE     BMP2   GE     BMP2   GE	KDMsa     JAZF1     ID4     ID4     HSD17B3     HOKA2     HMG81     HMG81     HMG81     HMG81     GL12     GL13     GL14     GL15     GL16     GFR41     GATA5     GATA6     GATA7     FST     FSK     FOXP1     FOXP2     FOXP1     FOXP1     FOXP2     FOXP1     FOX12     FGR2     ETV5     EMX2     EGR1     DMC1     DHH     DA4     ODX4     CYP19A1     CYP19A1     CYP17A1     CYP17A1<	Lef-1				
JACF1     JAKP1     IGHMBP2     ID4     HSD17B3     HOXA2     HMGA1     HMGA1     HEY1     GL13     GL14     GSATA5     GATA5     GATA6     GATA7     GATA6     GATA7     GATA6     GATA7     GATA6     GATA7     GATA6     GATA7     GATA7     GATA6     GATA7     GATA7     GATA7     GATA7     GATA7     GATA7     GATA7     GATA7     GATA7     HEV1     HEV1     HEV1     HEV1     HEV1     HEV1     HEV1	JAZE1     JAKE1     IGHMBP2     ID4     HSD17B3     HOXA2     HMGA1     HKS17B3     HMGA1     HKS17B3     GL12     GL13     GL14     GL15     GL16     GL17     GATA5	KDM3A -				
IGHMBP2   ID4     ID4   ID4     HSD17B3   ID4     HMKA1   ID4     HMKA1   ID4     HMGA1   ID4     HMGA1   ID4     HEY1   ID4     GL12   ID4     GL13   ID4     GL14   ID4     GATA5   ID4     GATA6   ID4     GATA7   ID4     GATA4   ID4     GATA5   ID4     GATA6   ID4     GATA7   ID4     GATA8   ID4     GATA9   ID4     GATA4   ID4     GATA5   ID4     GATA6   ID4     GATA7   ID4     GATA8   ID4     GATA9   ID4     GATA9   ID4     GATA9   ID4     GATA9   ID4     FST   ID4     FOXP2   ID4     FOXP2   ID4     FOX12   ID4     FGR1   ID4     DMC1	IGHMBP2   ID4     ID4   ID4     HSD17B3   ID4     HOX42   ID4     HM681   ID4     HM681   ID4     HKS1   ID4     GL12   ID4     GL13   ID4     GL14   ID4     GENF   ID4     GATA5   ID4     GATA5   ID4     GATA2   ID4     FST   FST     FOXP1   ID4     FOXP2   ID4     FOXP1   ID4     FOX12   ID4     FGFR2   ID5     EMX2   ID4     EGR1   ID4     DMC1   ID4     DMR11   ID4     DMC1   ID5     DMC1   ID4     ID4   ID4     ID71A1   ID5     CVP19A1   ID4     CVP17A1   ID5     CVP17A1   ID7     CVP17A1   ID7     CVP17A1   ID7     CVP17A1   ID7     CVP17A1<	IAZE1 -				
IDA   IDA     HSD17B3   IDA     HOXA2   IDA     HMGB1   IDA     HMGA1   IDA     HMGA1   IDA     IDA   IDA     GL13   IDA     GL14   IDA     GL15   IDA     GL16   IDA     GL17   IDA     GDNF   IDA     GATA1   IDA     GATA2   IDA     FST   IDA     FOXP2   IDA     FOXP1   IDA     FOXP1   IDA     IDA   IDA <	No. minut     Historic       HISDT7B3     HOXA2       HMGB1     HMGB1       HMGB1     HMGB1       HMGB1     HMGB1       GLI3     GLI2       GLI4     GLI4       GEV     GATA5       GATA5     GATA5       GATA6     GATA5       GATA7     GATA5       GATA6     GATA5       GATA7     GATA5       GATA5     GATA5       GATA6     GATA5       GATA7     GATA5       GATA5     GATA5       FOXP1     FOXP1       FOXP2     FOXP1       FOXP1     FOXP1       DMR1     DMR1       DMR1     DMA1       DDX4     CYP19A1       CYP17A1     CON1       GDM1 </td <td>IGHMBP2</td> <td></td> <td></td> <td></td> <td></td>	IGHMBP2				
HSD17B3   HMXA2     HMMGA1   HMGA1     HMGA1   HMGA1     HEV1   GLI3     GLI3   GLI4     GLI4   GLI5     GLI5   GLI6     GLI6   GLI6     GATA5   GATA5     GATA4   GATA4     GATA5   GATA4     GATA4   GATA4     GATA5   GATA4     GATA4   GATA5     GATA5   GATA4     GATA5   GATA4     GATA4   GATA5     GATA5   GATA4     GATA5   GATA4     GATA5   GATA5     GATA6   GATA5     GATA7   GATA5     GATA6   GATA6     GATA7   GATA7     GATA6   GATA6     GATA7   GATA7     GATA7   GATA7     GATA6   GATA7     GATA7   GATA7     GATA7   GATA7     GATA7   GATA7     GATA8   GATA7     GATA9   GATA7     GATA7 <td>HSD 7/83   HOXA2     HMGB1   HMGA1     HMGA1   HMGA1     HEY1   GLI     GLI3   GLI     GLI4   GLI5     GLI5   GLI6     GLI6   GLI6     GLI7   GLI7     GLI8   GLI7     GLI9   GLI1     GERA1   GATA5     GATA5   GATA4     GATA4   GATA5     GATA5   GATA4     GATA2   FST     FST   FST     FOXP2   FOX01     FOX12   FGR2     EGR1   DMC1     DMRT1   DMC1     DMRT1   DMC1     DMC1   DMC1     DMC1   CDN4     CYP17A1   CYP17A1     CYP17A1   CPCN1     BMP2   BMP2     BCD1   BMP2     BCL11A   A     APAMTS19   Aparapulchella   C marmoratus</td> <td>ID4 -</td> <td></td> <td></td> <td></td> <td></td>	HSD 7/83   HOXA2     HMGB1   HMGA1     HMGA1   HMGA1     HEY1   GLI     GLI3   GLI     GLI4   GLI5     GLI5   GLI6     GLI6   GLI6     GLI7   GLI7     GLI8   GLI7     GLI9   GLI1     GERA1   GATA5     GATA5   GATA4     GATA4   GATA5     GATA5   GATA4     GATA2   FST     FST   FST     FOXP2   FOX01     FOX12   FGR2     EGR1   DMC1     DMRT1   DMC1     DMRT1   DMC1     DMC1   DMC1     DMC1   CDN4     CYP17A1   CYP17A1     CYP17A1   CPCN1     BMP2   BMP2     BCD1   BMP2     BCL11A   A     APAMTS19   Aparapulchella   C marmoratus	ID4 -				
HOXA2     HMGB1     HMGA1     HEY1     GL13     GL14     GL15     GL16     GERA1     GATA5     GATA5     GATA4     GATA5     GATA5     GATA4     GATA5     GATA5     GATA6     GATA7     GATA7     GATA8     GATA9     GATA4     GATA5     GATA6     GATA7     GATA6     GATA7     GATA6     GATA7	HOXA2 HMGB1 HMGB1 HMGB1 HMGB1 HMGB1 HMGB1 HMGB1 HMGB1 GL12 GL12 GL12 GL12 GL13 GATA5	HSD17B3				•
HMGB1   HIMGA1     HHKV1   GLI     GLI3   GLI     GLI4   GLI5     GLI5   GLI6     GENF   GLI7     GATA4   GATA5     GATA5   GATA4     GATA2   GATA2     FST   FSHR     FOXP2   FOXP2     FOXP1   FOXP2     FOXP2   GATA5     GATA2   GATA2     FOXP2   GATA2     FOXP1   GATA2     FOXP2   GATA2     FOXP1   GATA2     FOXP2   GATA2     FOXP2   GATA2     FOXP1   GATA2     FOXP2   GATA2     FOXP1   GATA2     FOXP2   GATA2     FOXP1   GATA2     FOXP2   GATA2     FOXP1   GATA2     FOXP1   GATA2     GATA2   GATA2     GATA2   GATA2     GATA3   GATA3     GATA4   GATA4     GATA4   GATA4     GATA4	HMGB1   HMGA1     HFEY1   GL13     GL13   GL14     GL14   GL15     GL15   GL16     GDNF   GATA1     GATA5   GATA2     GATA4   GATA2     FST   FSHR     FOXP2   FOXP1     FOXP1   FOX01     FOX01   FOX01     FOX01   FOX01     FOX12   GATA2     EGR1   GATA2     EGR1   GATA2     DMRT1   GATA2     DDX4   CYP19A1     CYP17A1   GATA2     GATA2   GATA2     BMP2   GATA2     BMP2   GATA2     GATA3   GATA2     GATA2   GATA2     GATA2   GATA2     GATA2   GATA2     FST   GATA2     FOXP2   GATA2     GATA2   GATA2     GATA2   GATA2     GATA2   GATA2     GATA2   GATA2     GATA2   GATA2     GATA2	HOXA2				•
HMGA1   HEY1     GL13   GL13     GL14   GL14     GFRA1   GL15     GDNF   GL16     GATA5   GATA5     GATA4   GATA4     GATA5   GATA4     GATA2   FST     FST   FST     FSNR   FSNR     FOXP2   FOXP1     FOX12   FGR2     FGR2   GATA4     GATA4   GATA4     GATA5   GATA4     GATA4   GATA5     GATA5   GATA4     GATA4   GATA5     GATA5   GATA6     GATA6   GATA6     GATA7   GATA7     GATA5   GATA6     GATA6   GATA6     GATA7   GATA6     FOXP2   GATA6     FOX1   FOX1     FOX2   FGR2     FGR1   GATA6     DMC1   DDMC1     DMA1   DDMC1     DMA1   GATA6     CYP19A1   GATA6     CYP19A1   GAT	HMGA1     HEY1     GL13     GL14     GERA1     GATA5     GATA4     GATA5     GATA4     GATA5     GATA6     GATA7     GATA7     GATA8     GATA9     FST     FST     FOXP2     FOXP1     FOXP2     FOX1     FOX12     FGR1     DMRT1     DMRT1     DMRT1     DMA1     CYP19A1     CYP19A1     CYP17A1     CDH1     BMP2     BCL11A     ADAMTS19	HMGB1	•		•	•
HEY1   GL12     GL12   GL12     GL14   GL15     GFRA1   GL16     GATA5   GATA5     GATA5   GATA5     GATA5   GATA5     GATA4   GATA5     GATA5   GATA5     GATA6   GATA5     GATA5   GATA5     GATA5   GATA5     GATA5   GATA5     GATA5   GATA5     GATA5   GATA5     FST   FST     FOXP2   GATA5     FOXP1   FOXP1     FOX01   FO     FOX12   GATA5     EGR1   GATA5     DMR11   GATA5     DDX4   GATA5     CYP17A1   GATA5     CVP17A1	HEY1   GL13     GL12   GL14     GL17   GATA1     GATA5   GATA5     GATA5   GATA5     GATA4   GATA5     GATA5   GATA5     GATA4   GATA5     GATA5   GATA5     GATA4   GATA5     GATA5   GATA5     GATA6   GATA6     GATA7   GATA7     FST   FST     FON01   FON01     FOX01   FON01     FOX12   FON1     DMRT1   DMRT1     DMRT1   DMC1     DH   GATA7     CYP17A1   GATA7     CYP17A1   GATA7     CYP17A1   GATA7     CYP17A1   GATA7     CYP17A1   GATA7     CYP17A	HMGA1				•
GLI3	GLI3   GLI4     GLI1   GLI4     GLI3   GLI4     GLI4   GLI4     GLI5   GLI4     GDNF   GLI4     GATA1   GLI5     GATA2   GLI5     FST   GLI5     FOXP2   FOXP1     FOXP1   FOXP1     FOX01   FOX01     FOX01   FOX01     FOXP2   GLI5     FOXP1   GLI5     FOX01   FOX01     FOX01   GLI5     FOX01   GLI5     FOX01   GLI5     FOX01   GLI5     FOX01   GLI5     FOX12   GLI5     FOX14   GLI5     GLI5   GLI5	HEY1	•	•	•	•
GL12   GL1     GFRA1   GFRA1     GDNF   GATA5     GATA5   GATA5     GATA4   GATA5     GATA5   GATA4     GATA2   GATA5     GATA4   GATA5     GATA5   GATA6     GATA6   GATA6     GATA72   GATA6     GATA2   GATA6     GATA5   GATA6     GATA6   GATA6     GATA7   GATA6     GATA6   GATA6     GATA7   GATA6     GATA6   GATA6     GATA6   GATA6     GATA7   GATA6     GATA6   GATA6     GATA7   GATA6     FST   F     FOXP1   F     FOX12   F     FGR1   GATA6     DMC1   D     DMC1   D     DMA1   GATA6     CYP19A1   GATA7     CYP19A1   GATA7     CVP11A1   GATA7     CDA1   GATA7     BMP2   GATA7<	GL12     GL1       GERA1     GFRA1       GDNF     GATA5       GATA5     GATA5       FST     FST       FOXP1     FOXP1       DMR1     DMC1       DMA1     GATA5       CVP17A1     CVP17A1       CVP11A1     GATA5       CDK1     GATA5       BMP2     GATA5       GATA5     GATA5       GATA5	GLI3		•	•	•
GLI1   GATA     GATA5   GATA5     GATA5   GATA5     GATA2   GATA2     FST   FST     FOXP2   FOXP2     FOXP1   FOX01     FOX01   FOX01     FOX12   GATA2     EGR1   GATA2     DMRT1   GATA2     DMC1   GATA2     DDX4   GATA2     CYP13A1   GATA2     CVP17A1   GATA2     GATA2   GATA2     GATA2   GATA2     FOXP2   GATA2     FOXP2   GATA2     FOXP2   GATA3     GATA4   GATA2     FOX01   GATA2     FOX12   GATA2     FOX12   GATA2     FOX12   GATA3     GATA3   GATA3     GATA4   GATA3     GATA5   GATA3     GATA5   GATA3     GATA5   GATA3     GATA5   GATA3     GATA5   GATA3     GATA4   GATA4     GATA5 </td <td>GLI1     GRA1       GFRA1     GATA5       GATA5     GATA5       GATA4     GATA5       GATA5     GATA6       GATA2     GATA2       FST     FST       FOXP2     FOXP1       FOX01     FOX01       FOX12     GATA2       FGFR2     GATA5       EATA     GATA2       FGFR2     GATA5       EATA5     GATA2       FOXP2     GATA5       FOXP2     GATA5       FOX01     FOX01       FOX12     GATA5       FGFR2     GATA5       EATA5     GATA5       EATA5     GATA5       DMRT1     GATA5       DMR1     GATA5       DAT     GATA5       CYP17A1     GATA5       CYP17A1     GATA5       CYP17A1     GATA5       GATA5     GATA5       GATA5     GATA5       GATA5     GATA5       GATA5     GATA5       GATA5</td> <td>GLI2</td> <td>•</td> <td>•</td> <td>•</td> <td>•</td>	GLI1     GRA1       GFRA1     GATA5       GATA5     GATA5       GATA4     GATA5       GATA5     GATA6       GATA2     GATA2       FST     FST       FOXP2     FOXP1       FOX01     FOX01       FOX12     GATA2       FGFR2     GATA5       EATA     GATA2       FGFR2     GATA5       EATA5     GATA2       FOXP2     GATA5       FOXP2     GATA5       FOX01     FOX01       FOX12     GATA5       FGFR2     GATA5       EATA5     GATA5       EATA5     GATA5       DMRT1     GATA5       DMR1     GATA5       DAT     GATA5       CYP17A1     GATA5       CYP17A1     GATA5       CYP17A1     GATA5       GATA5     GATA5       GATA5     GATA5       GATA5     GATA5       GATA5     GATA5       GATA5	GLI2	•	•	•	•
GFRA1   GATA5     GATA5   GATA4     GATA4   GATA2     GATA2   FST     FST   FST     FOXP2   FOXP2     FOX1   FOX2     FOX2   FST     FOX1   FOX2     FGR4   GATA2     FOX1   FOX2     FOX1   FOX2     FGR4   GATA4     GATA2   FOX2     FOX1   FOX2     FOX2   FOX2     FOX1   FOX2     FOX2   FOX2     FOX1   FOX2     FOX2   FOX2     FOX2   FOX2     FOX2   FOX2     FOX2   FOX2     FOX2   FOX2     FOX2   FOX2     FOX4   FOX2     FOX4   FOX4     FOX4   FOX4     C	GFRA1   GATA5     GATA5   GATA4     GATA4   GATA3     GATA2   FST     FST   FST     FOXP2   FOXP1     FOX12   FGT     FGR1   FOXP2     FOX12   FGT     FGR1   FOXP3     DMRT1   DMC1     DMC1   DMC1     DHH   FOXP4     CYP19A1   CYP19A1     CYP17A1   CON1     BMP2   BCD11     ADAMTS19   Aparapulchella   C marmoratus   V acanthurus	GLI1				•
GDNF   GATADI     GATA5   GATA5     GATA4   GATA3     GATA3   GATA3     GATA4   GATA3     GATA2   GATA4     FST   F     FST   F     FOXP1   F     FOX01   F     FOX01   F     FQR2   F     FQR2   F     FQR1   F     FGFR2   F     EGR1   F     DMRT1   F     DHH   F     DDX4   F     CYP11A1   C     CDX4   F     CDN4   F     BMP2   BCL11A     AR   ADAMTS19	GDNF       GATA5       FST       FON01       FON01       FON01       FON01       FON01       DMR1       DMR1       DMR1       DMR1       DMA1       CYP17A1       CYP17A1       CVP11A1       CDK1       BUP2       BUP2       AR       ADAMTS19	GFRA1	•	•	•	•
GATAD1   GATA2     GATA2   GATA2     FST   FST     FOXP2   FOXP2     FOXP1   FOX01     FOX01   FOX01     FOX12   FOX01     FOX12   FOX01     FOX11   FOX01     FOX12   FOX01     FOX11   FOX01     FOX12   FOX01     FOX13   FOX01     FOX14   FOX01     FOX15   FOX01     FOX14   FOX01     FOX15   FOX01     FOX14   FOX01     FOX15   FOX01     FOX14   FOX01     FOX15   FOX01     FOX17   FOX01     FOX17   FOX01     FOX17   FOX01     DMRT1   FOX01     DMC1   FOX01     DX4   FOX01     CYP19A1   FOX01     CVP17A1   FOX02     CVP17A1   FOX02     FOX18   FOX04     FOX19   FOX12     FOX10   FOX12     FO	GATAD1 GATA5 GATA4 GATA3 GATA2 FST FSHR FOXP2 FOXP1 FOX01 FOX01 FOX12 FGFR2 ETV5 EMX2 EGR1 DHH DEAF1 DHH DEAF1 CYP17A1	GDNF		•		
GATA5   GATA4     GATA4   GATA3     GATA2   GATA2     GATA2   GATA3     GATA4   GATA5     FST   GATA6     FOXP2   FOXP2     FOXP1   FOXP2     FOXP2   FOXP2     FOXP1   FOXP2     FOXP2   FOXP2     FOXP2   FOXP2     FOXP2   FOXP2     FOX12   FOX12     FGFR2   FOX12     EMX2   FOX12     DMRT1   DMC1     DMR1   DDX4     DDX4   CYP19A1     CYP19A1   CYP17A1     CVP17A1   GUN     CDH1   GUN     BMP2   FOX14     AR   ADAMTS19	GATA5   GATA3     GATA3   GATA3     GATA3   GATA4     GATA4   GATA5     FST   FST     FSHR   FST     FOXP1   FOXP1     FOX12   FGFR2     FGFR2   FGFR2     EMX2   EMX2     EAR1   DMRT1     DMC1   DH     DDX4   CYP17A1     CYP17A1   CYP17A1     CDH1   EMP2     BCL11A   AR     AR   ADAMTS19	GATAD1	•	•	•	•
GATA4   GATA2   FST   FST   FOXP2   FOXP1   FOX01   FOX01   FOX2   ERT   ERT   DMRT1   DMRT1   DMC1   DHH   DEAF1   DDX4   CYP19A1   CYP17A1   CVP11A1   CDH1   BMP2   BMP2   BMP2   AR	GATA4     GATA2     FST     FSHR     FOXP2     FOXP1     FOX01     FOX01     FOX01     FOX12     FGFR2     EGR1     DMRT1     DMC1     DHH     DEAF1     DAX4     CYP17A1     CYP17A1     CVP11A1     CDH1     BMP2     BCL11A     AR     ADAMTS19	GATA5			•	•
GATA3	GATA3	GATA4	•		•	•
GATA2   FST     FST   FST     FOXP2   FOXP1     FOX01   FOX01     FOX12   FOX12     FGFR2   FGFR2     EGR1   DMRT1     DMC1   DMC1     DHH   FOX4     CYP17A1   CYP17A1     CYP17A1   CVP17A1     CDL1   FOX12     BMP2   BMP2     BMP2   FOX12     AR   ADAMTS19	GATA2     FST     FSHR     FOXP1     FOXP1     FOX01     FOX12     FGR7     EGR1     DMRT1     DMRT1     DMRT1     DMR1     DK1     DK2     EGR1     DK1     DK2     GVP17A1     CVP17A1     CVP17A1     CVP17A1     CVP17A1     CDKN1B     CDH1     BM2     BCL11A     AR     ADAMTS19	GATA3				•
FST   FST     FOXP2   FOXP2     FOXP1   FOXP1     FOX01   FOXP2     FOX2   FOXP2     FGFR2   FOXP2     ETV5   FOXP2     EGR1   FOXP2     DMRT1   FOXP2     DMC1   FOXP2     DHH   FOXP2     CYP19A1   FOXP2     CYP17A1   FOXP2     CODH1   FOXP2     BMP2   FOXP2     BMP2   FOXP2     AR   FOXP2	FST   FST     FOXP2   FOXP2     FOXP1   FOXP1     FOXP1   FOXP2     FOXP2   FOXP1     FOX01   FOXP2     FOXP1   FOXP1     FOXP2   FOXP1     FOXP1   FOXP1     FOXP2   FOXP1     FOXP1   FOXP1     DMRT1   DMRT1     DMRT1   DMR1     DHH   DHH     DEAF1   DDX4     CYP17A1   CYP17A1     CYP17A1   CYP17A1     CDH1   BMP2     BMP2   BCL11A     AR   APAMTS19	GATA2				•
FSHR	FSHR   FOXP2     FOXP1   FOXP1     FOX01   FOX01     FOX12   FGFR2     EGR1   EMX2     EGR1   DMRT1     DMRT1   DMC1     DHH   DEAF1     DDX4   CYP19A1     CYP17A1   CYP17A1     CDKN1B   CDH1     BMP2   BCL11A     AR   AParapulchella   C. marmoratus   V acanthurus	FST -		•		•
FOXP1   FOX01     FOX01   FOX02     FOX12   FOX02     FGFR2   FOX02     EGR1   FOX02     DMRT1   DMC1     DMC1   FOX02     DDX4   FOX02     CYP19A1   FOX02     CYP17A1   FOX02     COD1   FOX02     BMP2   FOX02     BMP2   FOX02     AR   FOX02	FOXP2     FOXP1     FOX01     FOX12     FOX12     FOX12     FGFR2     EGR1     DMRT1     DMRT1     DMRT1     DMRT1     DMRT1     DMRT1     DMRT1     DMRT1     DMR1     DH     DEAF1     DDX4     CYP17A1     CYP17A1     CYP17A1     CVP17A1     CVP11A1     BMP2     BCL11A     AR     ADAMTS19	FSHR			•	•
FOXP1   FOX01     FOX12   FOX12     FGR2   FOX12     ETV5   FOX12     EMX2   FOX12     DMRT1   FOX12     DMRT1   FOX12     DMC1   FOX12     DMC1   FOX12     DMC1   FOX12     DMC1   FOX12     DMC1   FOX12     DMC1   FOX12     DX4   FOX12     CYP19A1   FOX14     CYP17A1   FOX14     COPU1   FOX14     BMP2   FOX14     BMP2   FOX14     AR   FOX14     ADAMTS19   FOX15	FOXF1     FOX01       FOX01     FOX01       FQX12     FGR2       ETV5     EGR1       DMRT1     DMC1       DHH     DDX4       CYP19A1     CYP19A1       CYP17A1     CYP17A1       CVP11A1     CDKN1B       DDAH     BMP2       BCL11A     AR       ADAMTS19     E. macquarii	FOXP2			•	•
FOXU1   FGFR2     FGFR2   FGFR2     ETV5   FGFR2     EMX2   FGFR2     EQR1   FGFR2     DMRT1   FGFR2     DMRT1   FGFR2     DMRT1   FGFR2     DMRT1   FGFR2     DV1   FGFR2     CYP17A1   FGFR2     CYP17A1   FGFR2     CDH1   FGFR2     BMP2   FGFR2     BCL11A   FGFR2     AR   ADAMTS19	FOXU1     FOXU2       FGFR2     FGFR2       ETV5     EMX2       EGR1     DMRT1       DMRT1     DMRT1       DMC1     DH       DEAF1     DDX4       CYP19A1     CYP17A1       CVP17A1     CVP17A1       CDH1     BMP2       BCL11A     AR       APAMTS19     E. macquarii	FOXP1				•
FOGL2     FGFR2     ETV5     EMX2     EGR1     DMRT1     DMC1     DHH     DEAF1     DDX4     CYP19A1     CYP17A1     CVP11A1     CDL1     BMP2     BMP2     BMP2     AR	FORE2     FGFR2     ETV5     EMX2     EGR1     DMRT1     DMC1     DHH     DEAF1     DX4     CYP19A1     CYP17A1     CYP11A1     CVP17A1     CDH1     BMP2     BCL11A     AR     ADAMTS19	FOX01			1	
FIGTR2   EV5     EVX2   EGR1     DMRT1   DMRT1     DMC1   DMRT1     DHH   DDX4     CYP19A1   CYP17A1     CYP17A1   CYP17A1     BMP2   BUP2     BKP2   ECL11A     AR   ADAMTS19	FGFR2     EGFR3       ETV5     EMX2       EGR1     DMRT1       DMC1     DMRT1       DEX4     CYP19A1       CYP17A1     CYP17A1       CDKN1B     CDK1       CDK11     BMP2       BCL11A     AR       ADAMTS19     E. macquarii	FOXL2		800		
E IV 3 EMX2 EGR1 DMRT1 DMRT1 DMC1 DHH DEAF1 DDX4 CYP17A1 CYP17	E W2 EGR1 DMRT1 DMRT1 DMC1 DHH DEAF1 DDX4 CYP17A1 CYP17A1 CYP17A1 CVP17A1 CVP17A1 CDKN1B CDH1 BMP2 BCL11A AR ADAMTS19 E. macguarii A. parapulchella C. marmoratus V acanthurus	FGFR2				
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LGN1     DMRT1       DMC1     DH       DHH     DEAF1       DDX4     CYP19A1       CYP17A1     CYP17A1       CDKN1B     CDH1       BMP2     BCL11A       AR     ADAMTS19	EURN DMRT1 DMC1 DHH DEAF1 DDX4 CYP19A1 CYP19A1 CYP17A1 CDKN1B CDH1 BMP2 BCL11A AR ADAMTS19 E. macquarii A. parapulchella C. marmoratus V acanthurus	ENIX2				
DMC1       DH1       DEAF1       DDX4       CYP19A1       CYP17A1       CYP17A1       CDKN1B       CDH1       BMP2       BCL11A       AR	DHAT DHA DEAF1 DDX4 CYP17A1 CYP17A1 CVP17A1 CDKN1B CDH1 BMP2 BCL11A AR ADAMTS19 E. macguarii A. parapulchella C. marmoratus V acanthurus	DMPT1				
DHH       DEAF1       DDX4       CYP19A1       CYP17A1       CYP17A1       CVP17A1       BMP2       BMP2       BCL11A       AR	DHH DEAF1 DDX4 CYP19A1 CYP17A1 CYP17A1 CDKN1B CDH1 BMP2 BCL11A AR ADAMTS19	DMC1				
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CYP11A1 CDKN1B CDH1 BMP2 BCL11A AR ADAMTS19	CYP11A1 CDKN1B CDH1 BMP2 BCL11A AR ADAMTS19 • E. macquarii • A.parapulchella • C. marmoratus • V acanthurus	CYP17A1				
CDKN1B CDH1 BMP2 BCL11A AR ADAMTS19	CDKN1B CDH1 BMP2 BCL11A AR ADAMTS19 E. macquarii A. parapulchella C. marmoratus V acanthurus	CYP11A1				
CDH1 BMP2 BCL11A AR ADAMTS19	CDH1 BMP2 BCL11A AR ADAMTS19 E. macquarii A. parapulchella C. marmoratus V acanthurus	CDKN1B				
BMP2 BCL11A AR ADAMTS19	BMP2 BCL11A AR ADAMTS19 • E. macquarii • A.parapulchella • C. marmoratus • V acanthurus	CDH1 -				
BCL11A AR ADAMTS19	BCL11A AR ADAMTS19 • E. macquarii • A.parapulchella • C. marmoratus • V acanthurus	BMP2				
AR ADAMTS19	AR ADAMTS19 • E. macquarii • A.parapulchella • C. marmoratus • V acanthurus	BCI 11A				
ADAMTS19 •	ADAMTS19	AR -				
e manuel de la companya de la company	E. macquarii A.parapulchella C. marmoratus V acanthurus	ADAMTS19		X N		
	• E. macquarii • A.parapulchella • C. marmoratus • V. acanthurus				10	31

# 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 log2 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 log2 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.