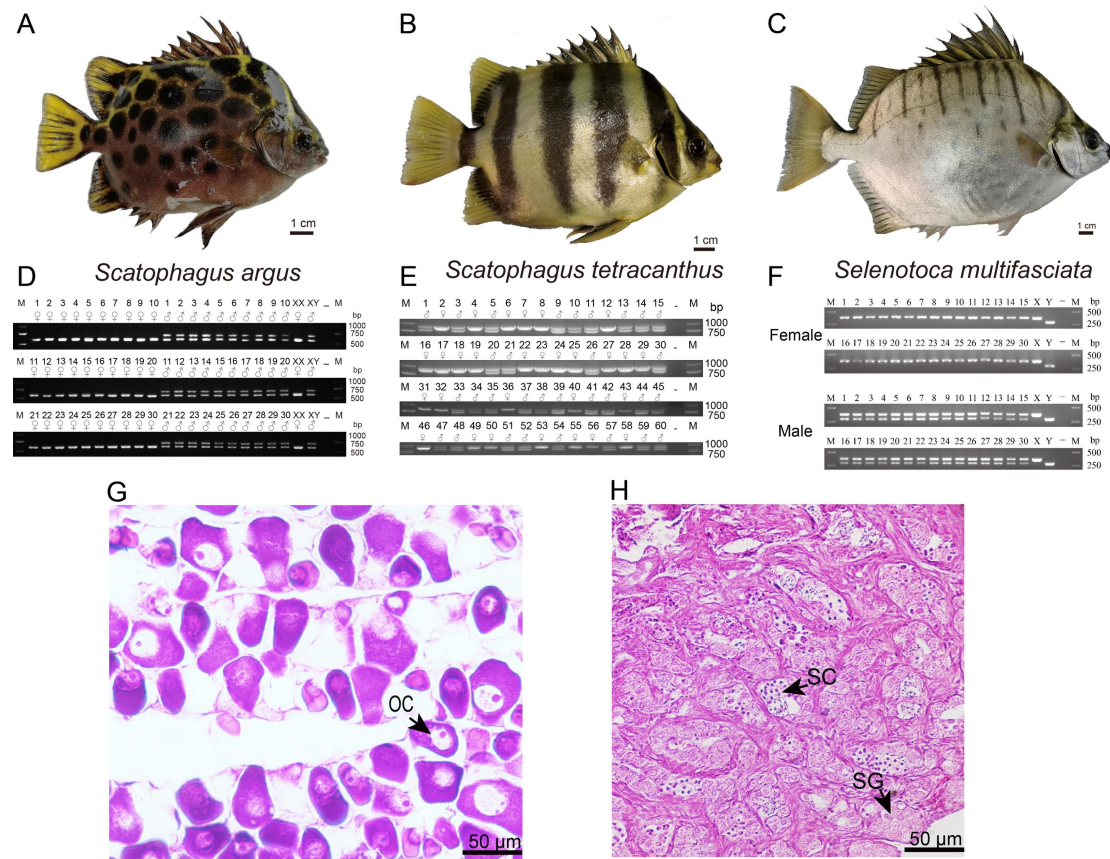
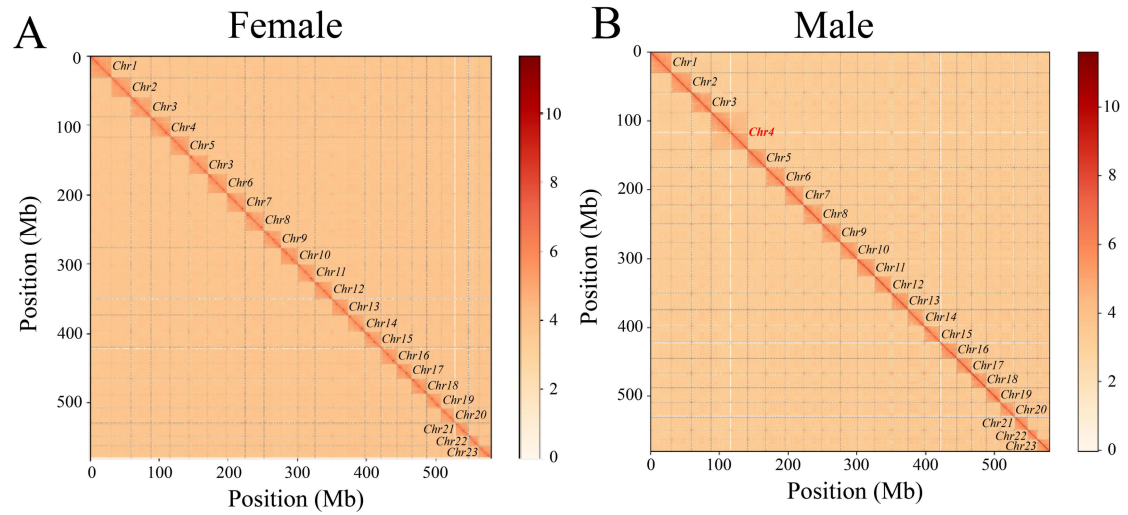


Supplementary Figures

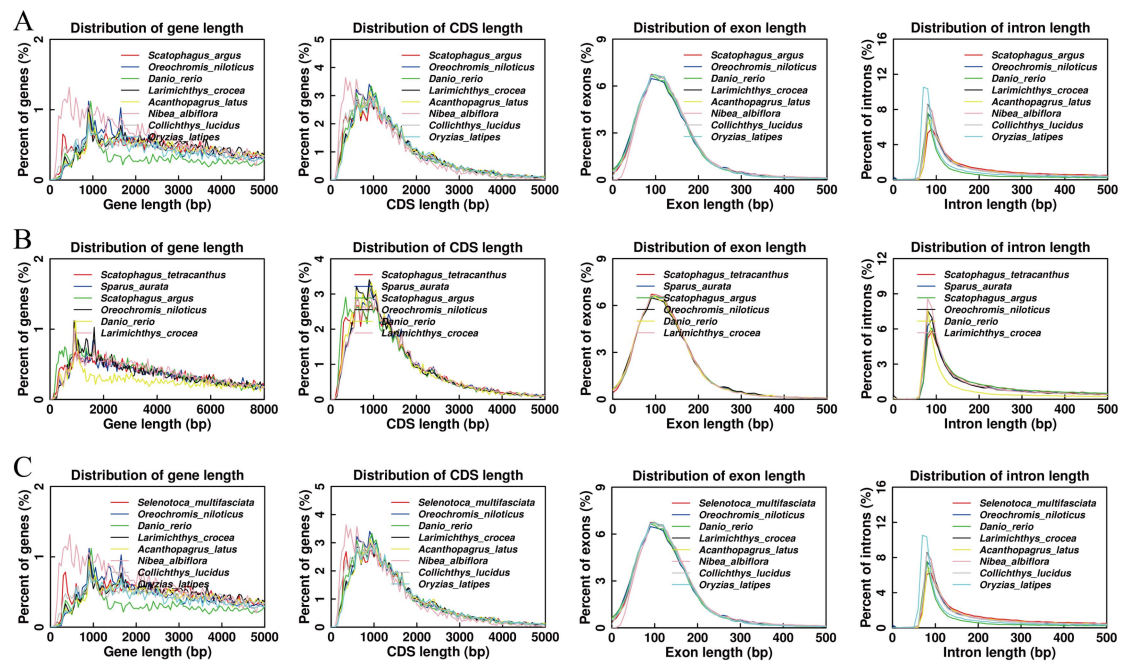


Supplementary Figure 1. Photographs and sex identification of experimental specimens.

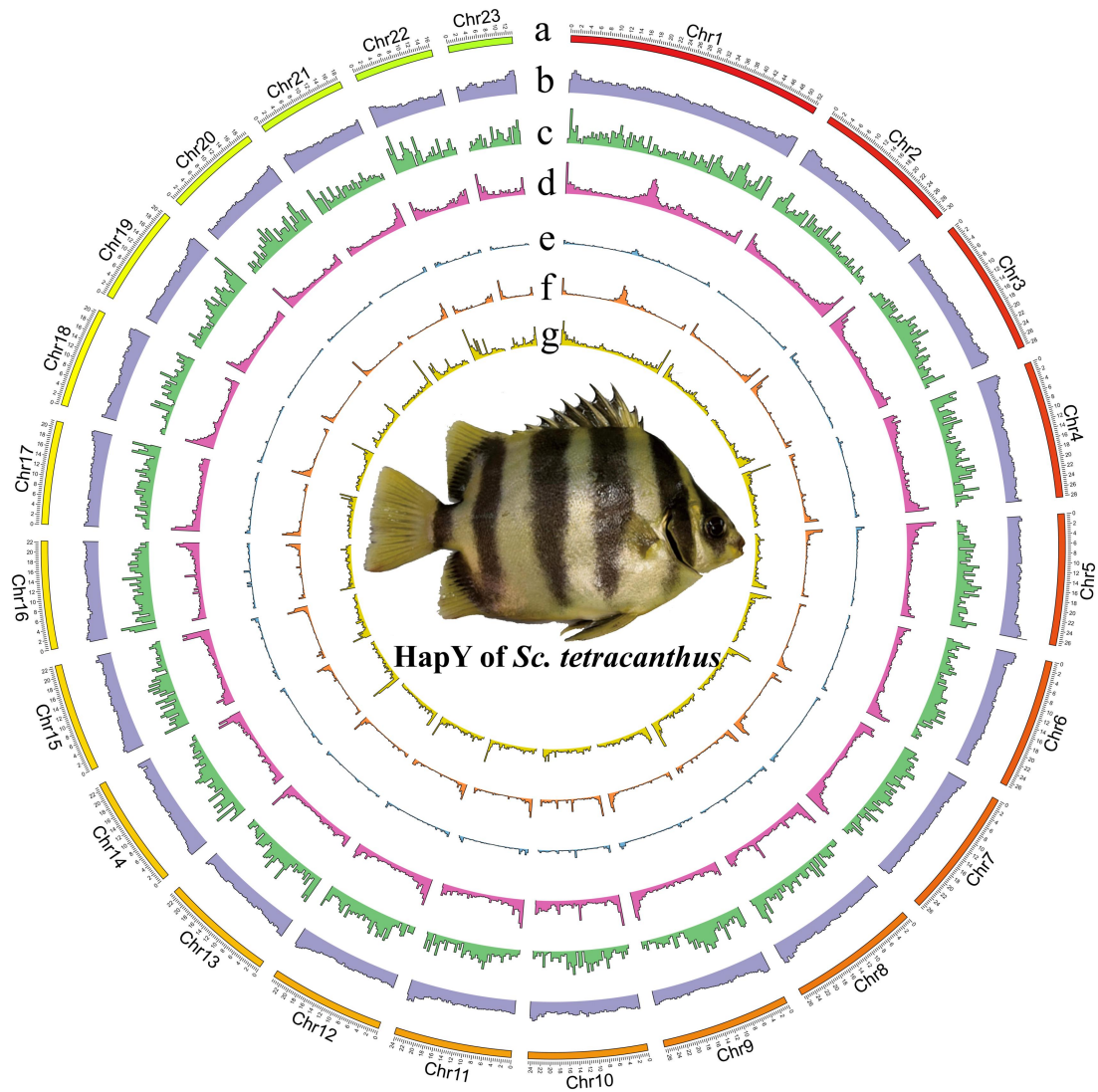
(A) *Scatophagus argus*. (B) *Scatophagus tetracanthus*. (C) *Selenotoca multifasciata*. (D), (E) and (F), genetic sexing of *Sc. argus*, *Sc. tetracanthus* and *Se. multifasciata*, respectively. (G) and (H), histological sections of ovary and testis of *Sc. tetracanthus*.



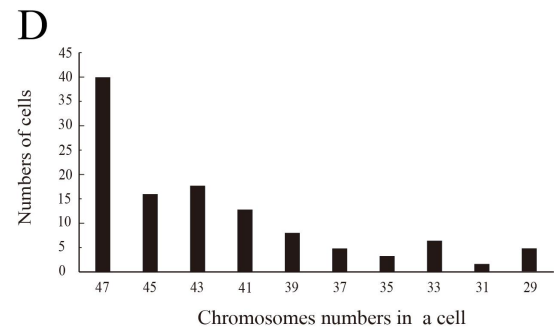
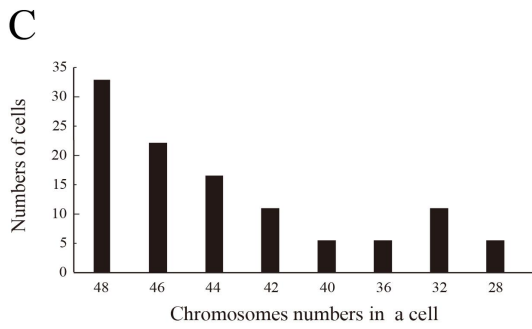
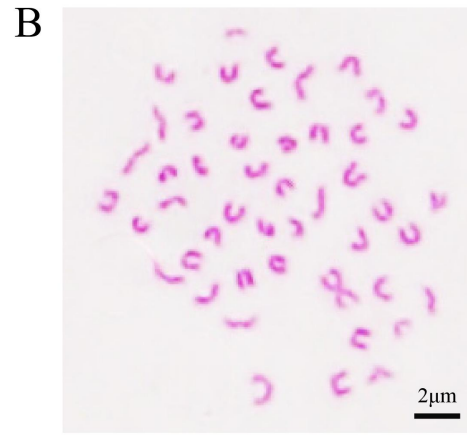
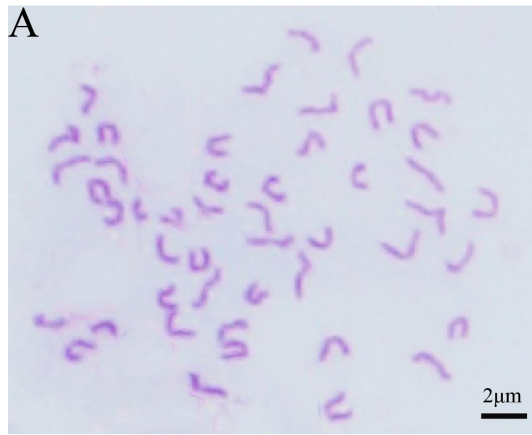
Supplementary Figure 2. Hi-C interactions among chromosomes in *Sc. tetracanthus* genome. (A) Hi-C interactions among 24 chromosomes in female genomes. (B) Hi-C interactions among 23 chromosomes in male genomes. Strong interactions are indicated in dark red and weak interactions are indicated in yellow.



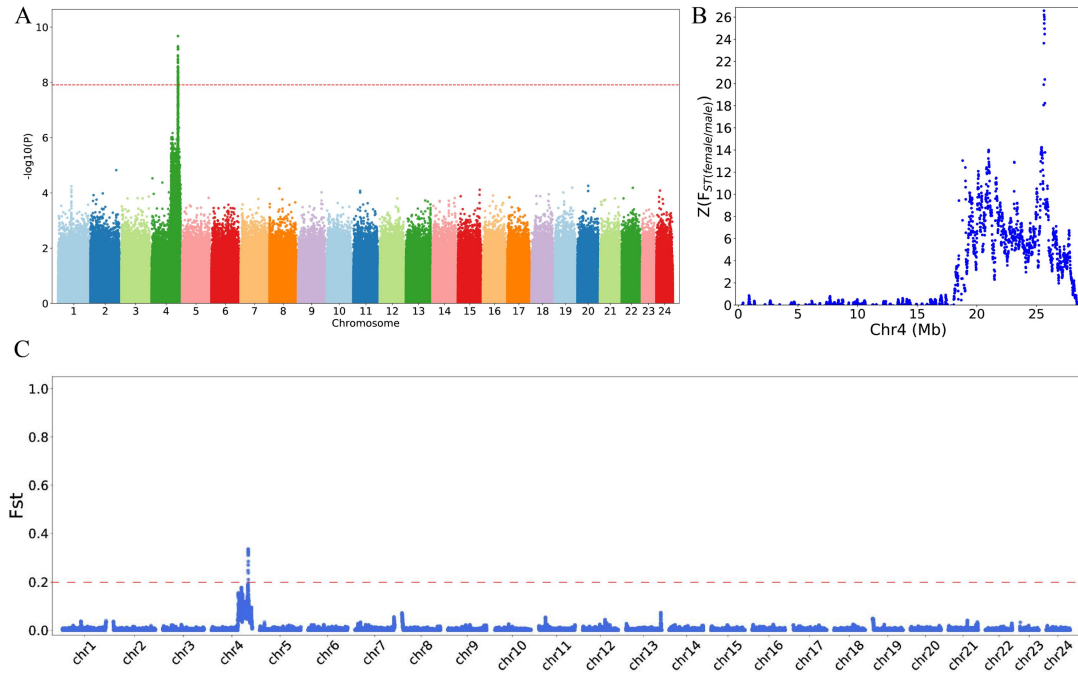
Supplementary Figure 3. Length distribution comparison on total gene, CDS, exon, and intron of annotated gene models of three Scatophagidae species with other closely related teleost fish species. Length distribution of total gene, CDS, exon, and intron were compared of *Sc. argus* (A), *Sc. tetracanthus* (B) and *Se. multifasciata* (C), respectively.



Supplementary Figure 4. Features of male *Sc. tetracanthus* genome (HapY). (a) Chromosome length; (b) GC content; (c) Gene density; (d) Repeat sequence; (e) Long terminal repeated (LTR); (f) Long interspersed nuclear elements (LINE); (g) Simple sequence repeat (SSR).

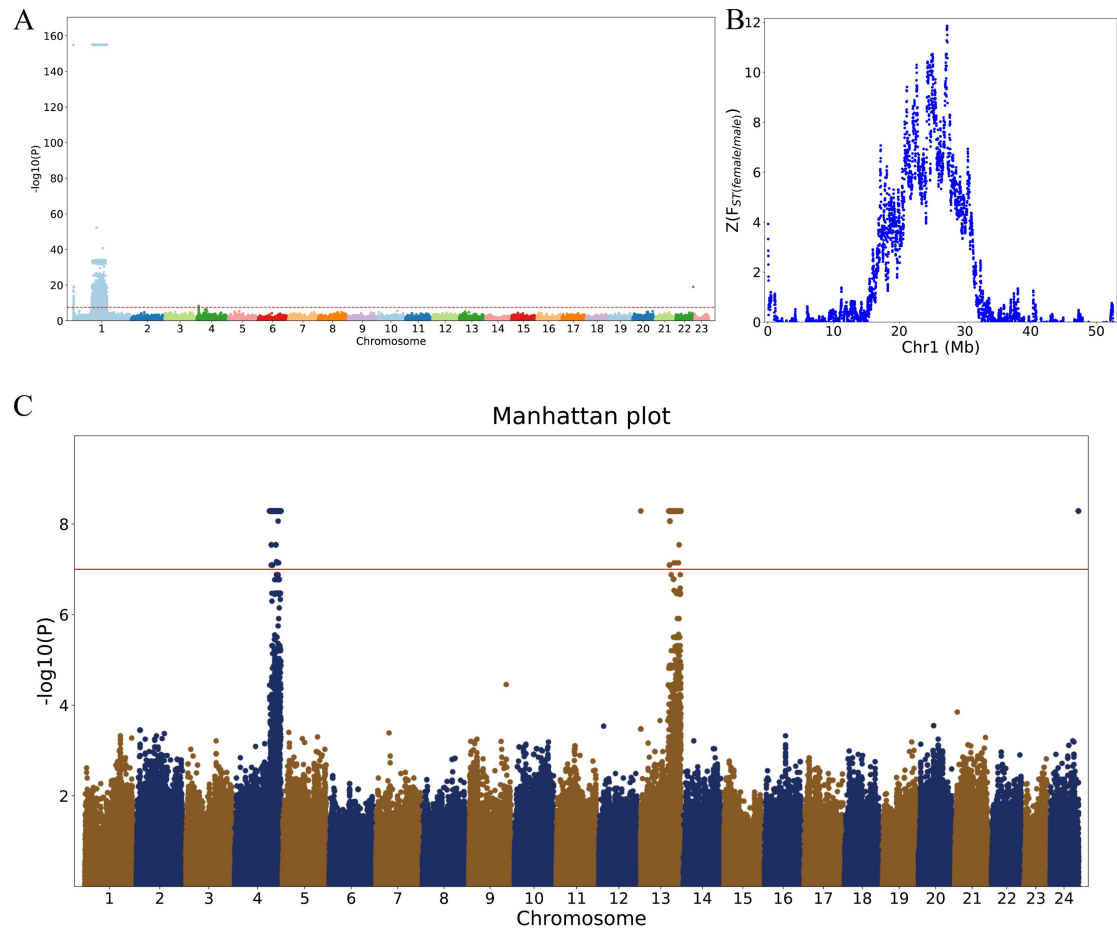


Supplementary Figure 5. Karyotypes of *Sc. tetracanthus*. (A) Karyotypes result statistics of female *Sc. tetracanthus*. (B) Karyotypes result statistics of male *Sc. tetracanthus*. (C) Single cell chromosome number statistics in female *Sc. tetracanthus*. (D) Single cell chromosome number statistics in male *Sc. tetracanthus*.

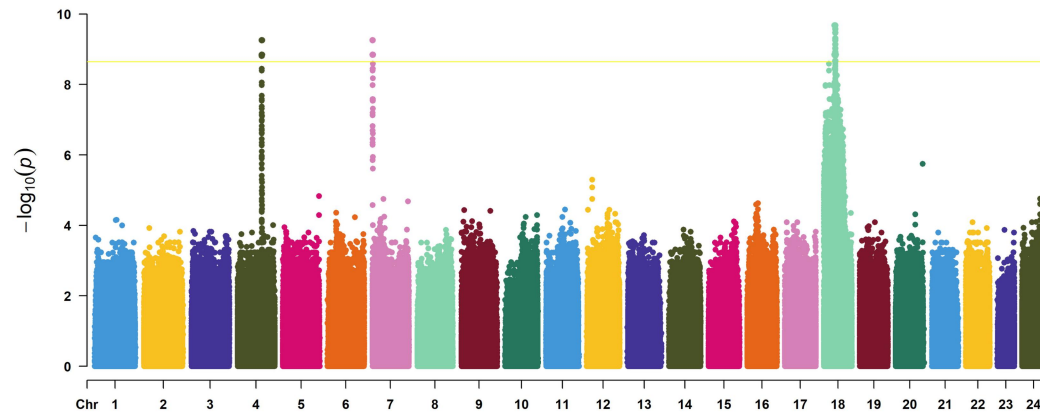


Supplementary Figure 6. Manhattan plot of *Sc. argus* for sex trait association analysis. (A)

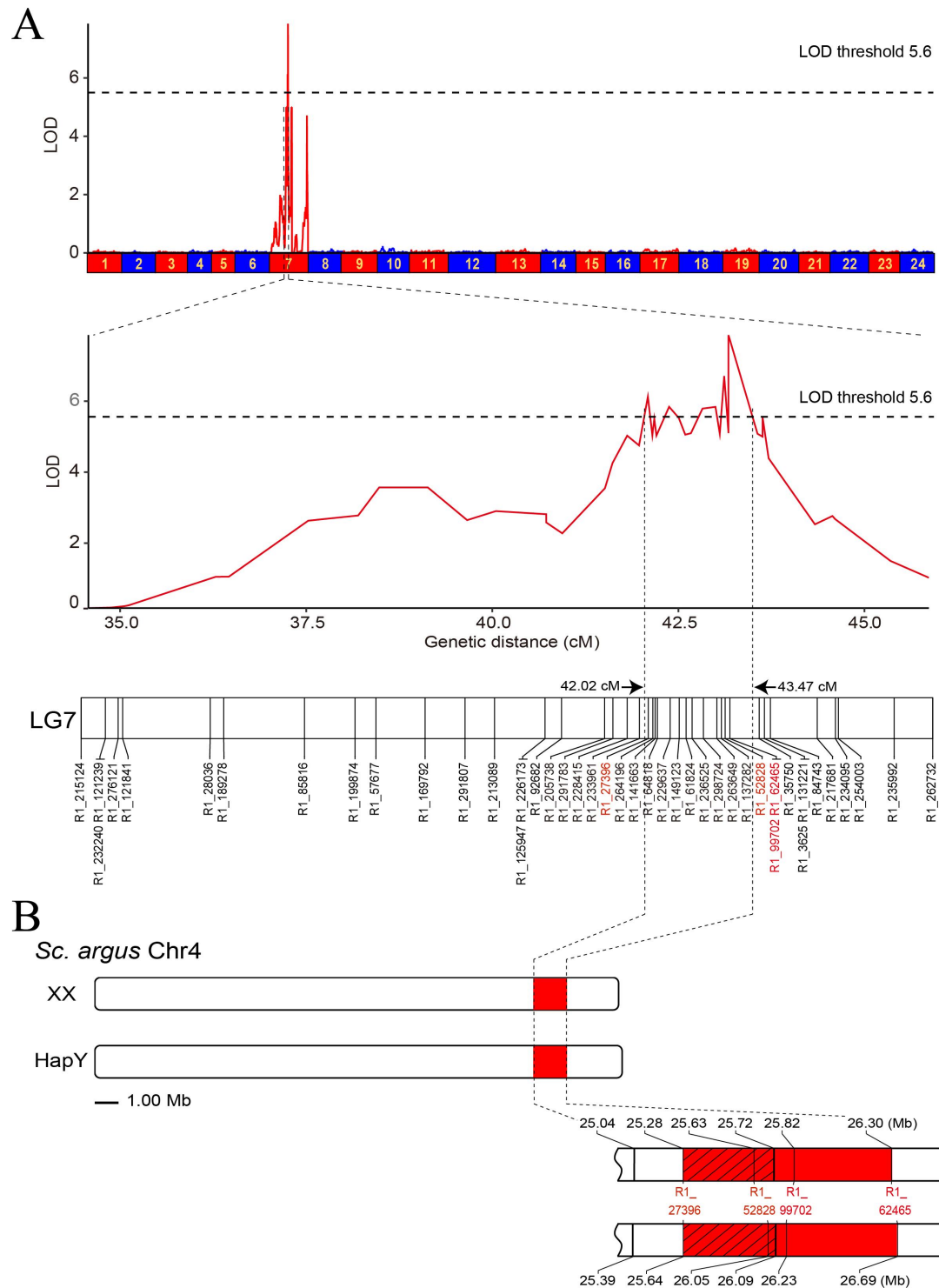
Genome-wide association study for a sex determining locus based on 30 females and 30 males, where the genome-wide significant cutoff value after Bonferroni corrections is shown (red horizontal line), using the male genome (hapY) as a reference genome. **(B)** Z-transformed F_{ST} scan for sex determining locus on Chr4. **(C)** F_{ST} scan for sex determining locus on Chr4, using the female genome as a reference genome.



Supplementary Figure 7. Manhattan plot of *Sc. tetracanthus* for sex trait association analysis. (A) Genome-wide association study for a sex determining locus based on 30 females and 30 males, where the genome-wide significant cutoff value after Bonferroni corrections is shown (red horizontal line), using the male genome (hapY) as a reference genome. (B) Z-transformed F_{ST} scan for sex determining locus on Chr1. (C) Genome-wide association study for a sex determining locus based on 30 females and 30 males, where the genome-wide significant cutoff value after Bonferroni corrections is shown (red horizontal line), using the female genome as a reference genome.

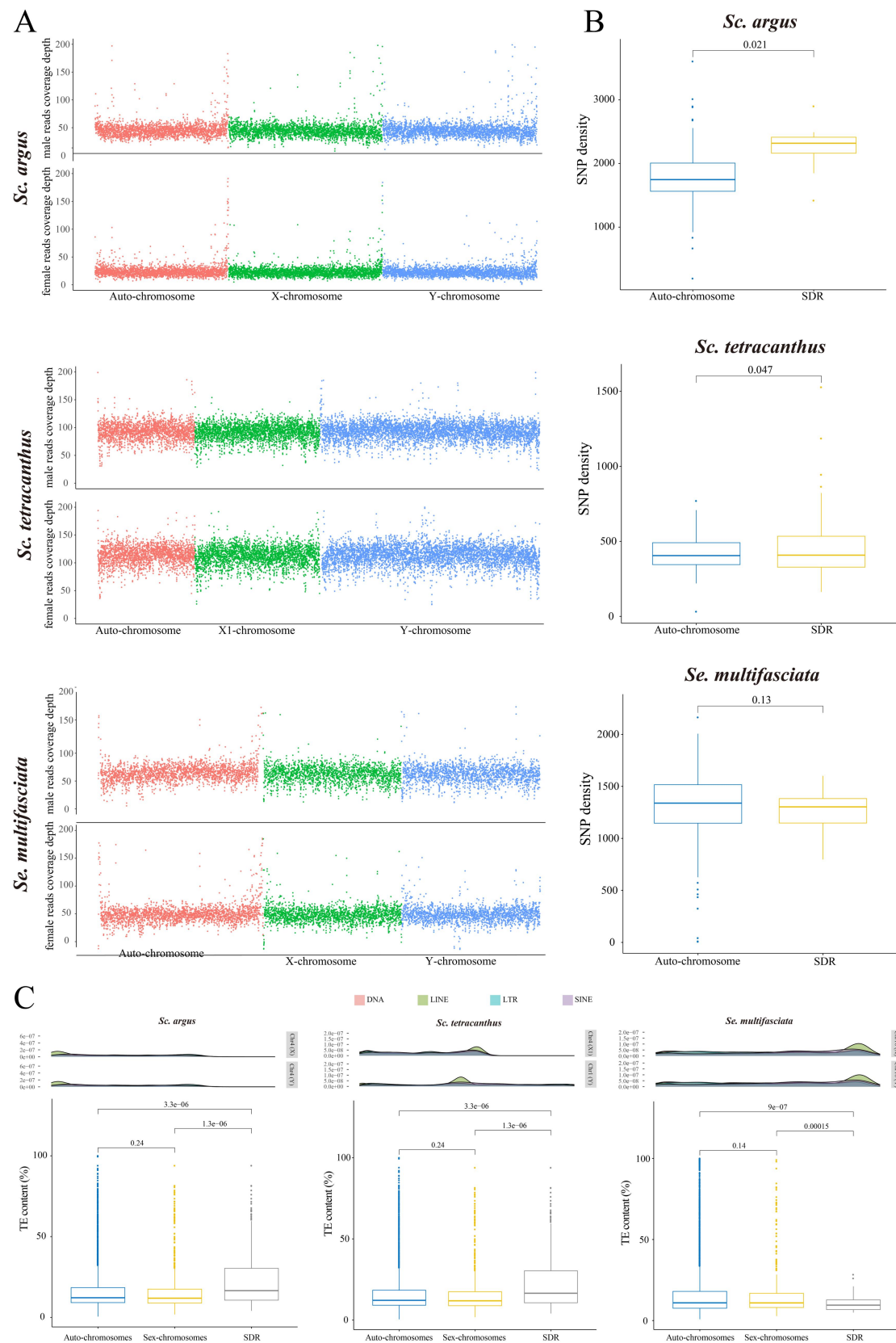


Supplementary Figure 8. Manhattan plot of female *Se. multifasciata* for sex trait association analysis. The genome-wide significant cutoff value after Bonferroni corrections is shown (yellow horizontal line), using the female genome as a reference genome.



Supplementary Figure 9. Quantitative trait locus (QTL) mapping of sex trait based on the high-resolution genetic map of *Sc. argus*. (A) The location of sex-related QTLs on LG7 and their respective SNP markers. The X-axis represents the genetic length of linkage groups, and the Y-axis represents the corresponding LOD values. (B) Identification of sex-specific

genomic region on Chr4 of the female (XX) and male (HapY) genomes using sex-linked SNPs within the highly significant QTLs. Shaded area indicates X and Y chromosome SDR overlap with corresponding QTL region.



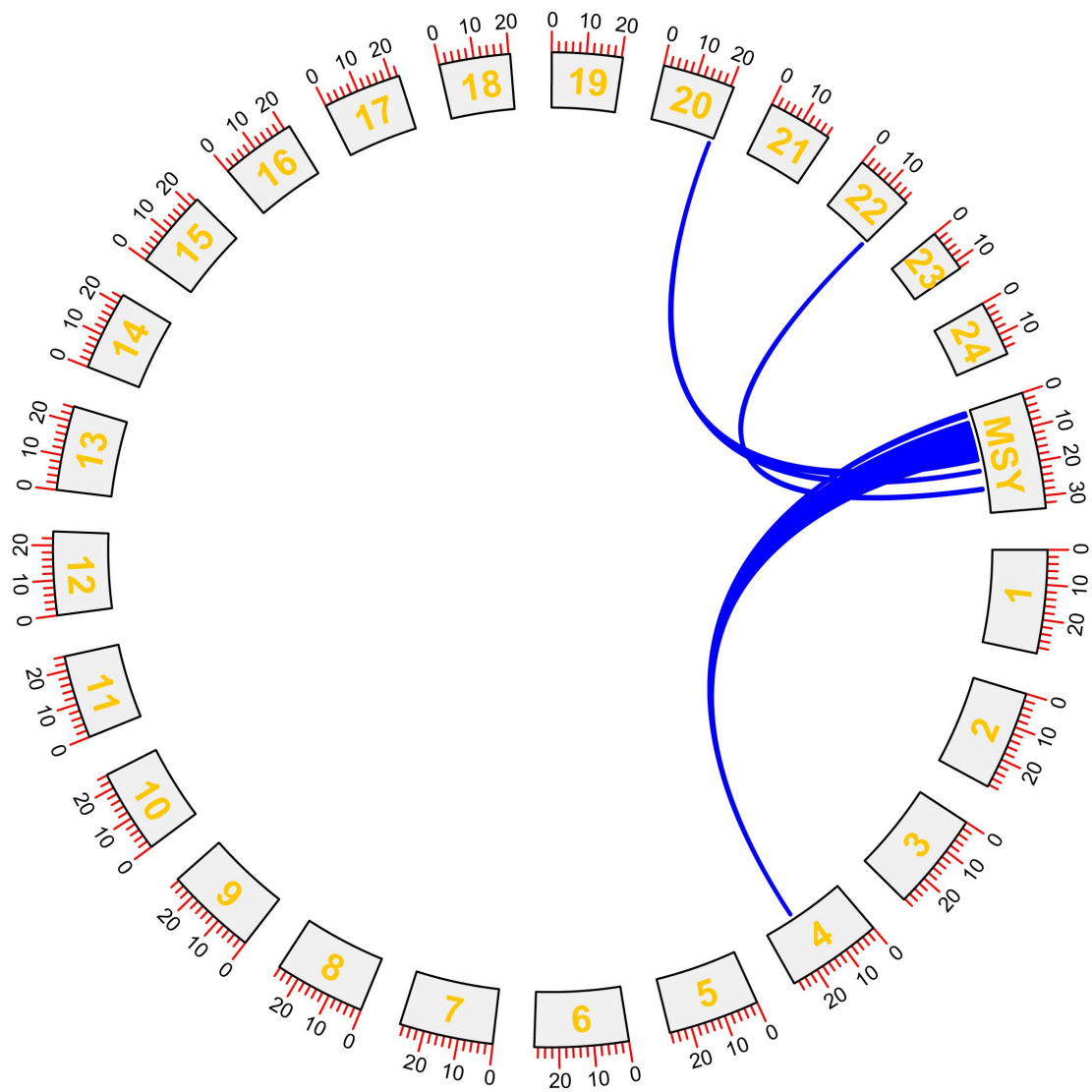
Supplementary Figure 10. Analysis of sex chromosome in Scatophagidae species. (A)

HiFi sequencing coverage from a male and a female over the X and Y (using chromosome 12

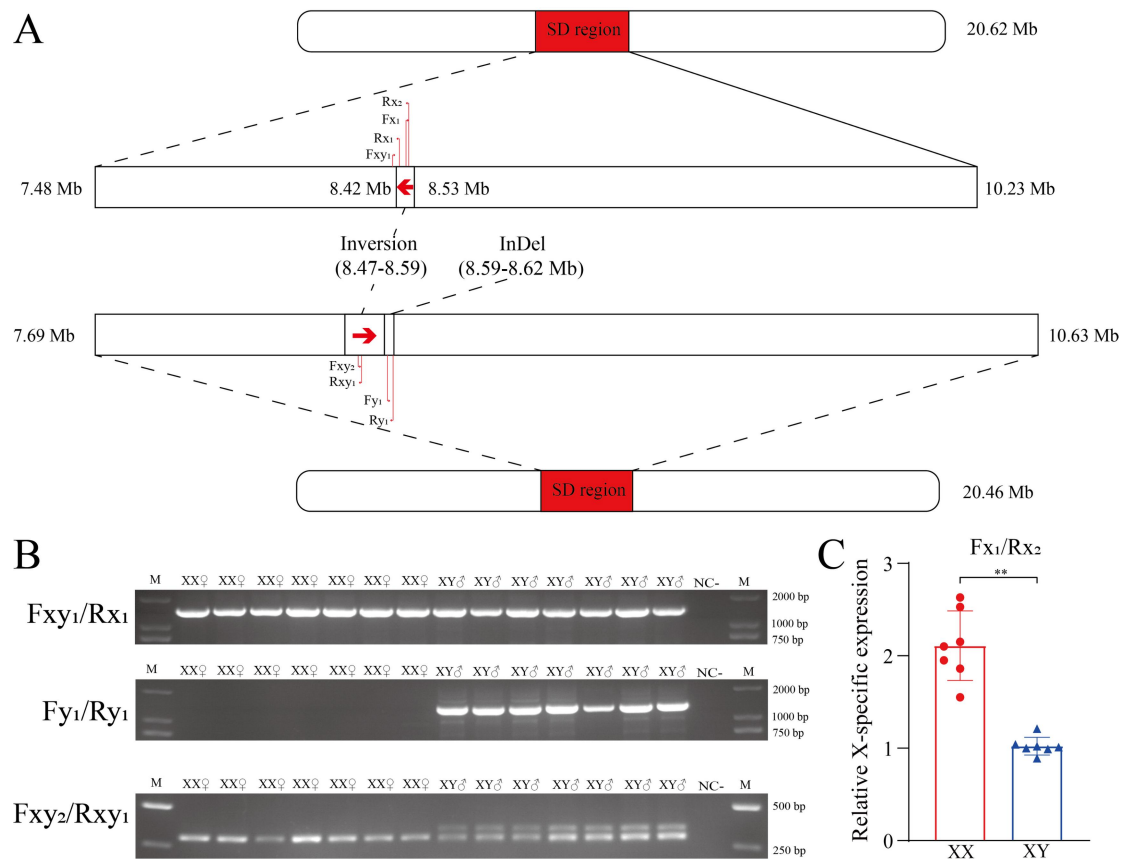
as auto-chromosomes). **(B)** The SNP density of sex-linked region on sex chromosome.

Sequencing coverage was calculated per 10-kb window (dot). **(C)** Contents of TE of sex-linked region on sex chromosome.

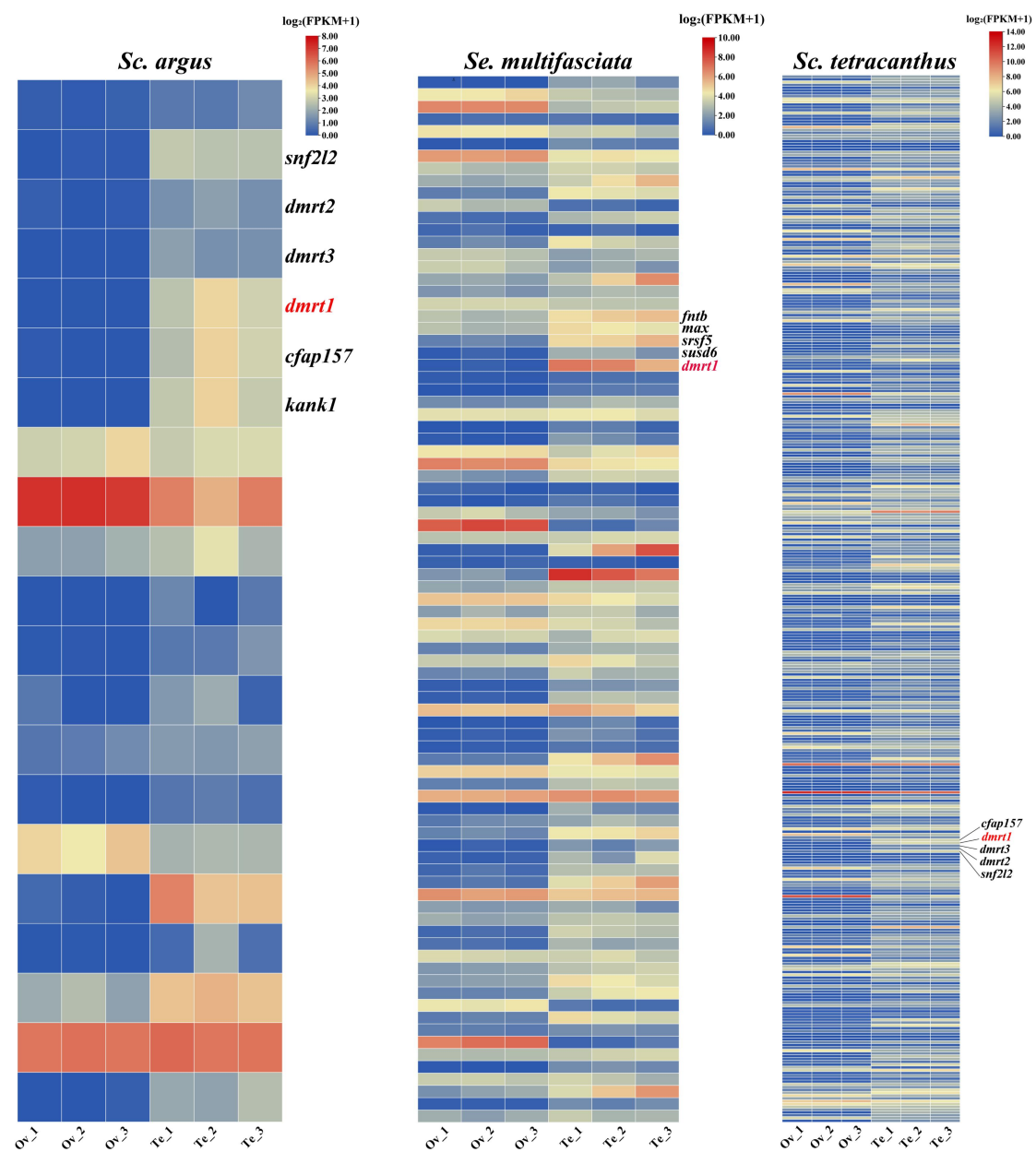
multifasciata.



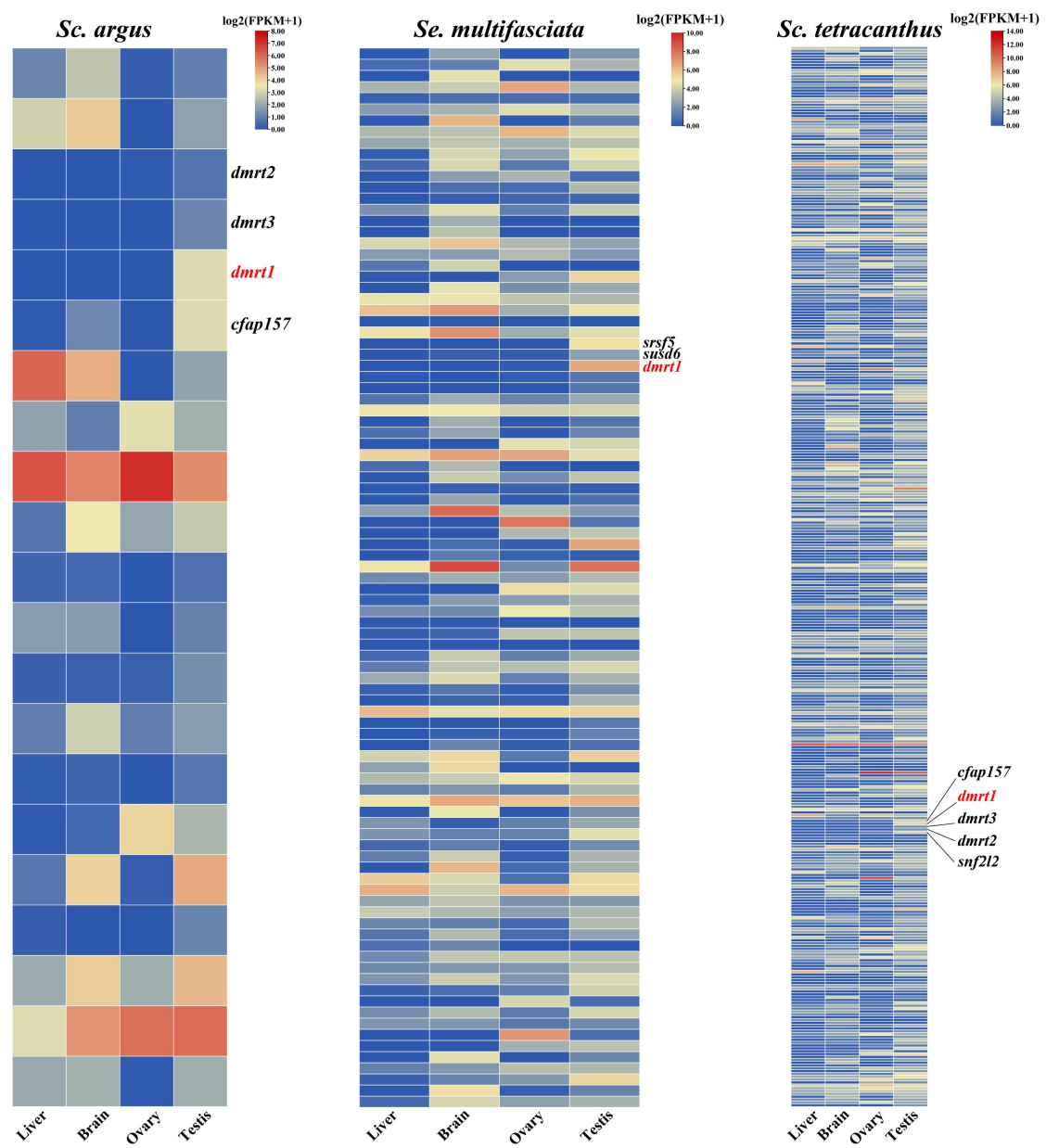
Supplementary Figure 12. Circos plots showing the syntenic relationships between the male-specific region and other parts of the genome in *Se. multifasciata*. The male-specific regions (MSY) shown in the lower part of plot are scaled in kilobases.



Supplementary Figure 13. Analysis and validation of structural variation (SV) between X and Y chromosomes in sex determining region of *Se. multifasciata*. (A) Schematic diagram showed two SVs including an inversion and an InDel between X and Y chromosomes. (B) Validation of the inversion by PCR in 7 XX and 7 XY individuals. (C) Validation of the X specific sequence by qPCR in 7 XX and 7 XY individuals. **: Significant at the 0.01 level.



Supplementary Figure 14. Expression patterns of sex determining region genes in gonads.



Supplementary Figure 15. Expression patterns of sex determining region genes in different tissues.

Sc. argus

*dmrt1*Δ*X*: CAGACACTGTGGTAAGGAGGTACACTGATATTTAATTGGAAGAACAATATCAATTTTCAGTTTCAGTCAAAACAAACCAAAATATATATATATATTTGTTGT -1141
*dmrt1*Y: CAGACACTGTGGTAAGGAGGTACACTGATATTTAATTGGAAGAACAATATCAATTTTCAGTTTCAGTCAAAACAAACCAAAATATATATATATATTTGTTGT -1027

*dmrt1*Δ*X*: TGTGTGTGTGCCACCAAAATATTTTCAGGAATTCCTTCAGCAGCTACACACACAGGTTTTCATGTTTTTCAGTTAATGAAAGGATCCAGTTAAAC -1046
*dmrt1*Y: TGTGTGTGTGTGTTGCCAAATATTTTCAGGAATTCCTTCAGCAGCTACACACACAGGTTTTCATGTTTTTCAGTTAATGAAAGGATCCAGTTAAAC -932

*dmrt1*Δ*X*: ATTTGTAAATGCGAGGTATGTAATACCCCTTTGAGATAAAGTACCTGCTCACCATACCTGACTCAGACTCAGGTATTTCTCAAAAGTAGATACA -951
*dmrt1*Y: ATTTGTAAATGCGAGGTATGTAATACCCCTTTGAGATAAAGTACCTGCTCACCATACCTGA-----CTCAGGTATTTCTCAAAAGTAGATACA -843

*dmrt1*Δ*X*: TTGCTGTGTGCAGATGCCAGGATCGATCTCAAATCTTGAGTTAAAGCGGACTTCTATTTTAACTAAATATGTTTGAAGGTTATACAGCAGATTC -856
*dmrt1*Y: TTGCTGTGTGCAGATGCCAGGATCGATCTCAAATCTTGAGTTAAAGCGGACTTCTATTTTAACTAAATATGTTTGAAGGTTATACAGCAGATTC -748

*dmrt1*Δ*X*: AGAATTCAAATCTGTTGTACAGTTTGGTTATACCTTCTCTTCAGCTACTACTCCTGAAAGCTCTTTCAAAAGATAGCAACTCTCTATG -761
*dmrt1*Y: AGAATTCAAATCAAGGTTATACCTTT-----CTTTCAGCTAGGTTACTACTCCTGAATTTGAAGCTTTTTCAAAAGATAGCAATCTCTCTATG -660

*dmrt1*Δ*X*: TTTCATGATTCTAATAACACCCCTGGCCTAGAGGTTCCGAGAACGGGTTCTGATCGGAGACTCACCGGTTCAATTTCCCCACTTCGATGGCCAGGAA -666
*dmrt1*Y: ACCATAGA-CAGATTGTAATCTT--TTTATTTATTTCTCAA-CA-TTATGACTTTTGAGAT-TGGGATTAAT-----AATGC AAAATCAATCA -577

*dmrt1*Δ*X*: AAATTTGGCTGTGCTGCACTGATTAATGAGAAATATGCCCCCAATTACCCGGCTGATCTGGCCTTGAGCAAGGCACHTAACCCGCCAATATCTCT -571
*dmrt1*Y: GCATACAAAT---TATTAATC--TAAATATGATTTTCCCGGGGGGGCGGCTTAATGAGCTATGAGGACAG-----TAAACC---ATATGA- -498

*dmrt1*Δ*X*: CCTTCGCCCTCATGCTGCCACTTCTCTCTGTGTCTTTCAGTCGATCTAAATTTCCCGGGTGTTCATGCTGTGCTTCAACTTAGGATGGCTC -476
*dmrt1*Y: ---AGAACTCAA-----ATTACTGCTAT-----TTTCACCAAGCTGCAATCT-----GAAATGC--ATTATGCAAGAAATAATTG -433

*dmrt1*Δ*X*: AAATCCAGAAAGCAATTCAGTTGTGTATGTA AAAATATATATATCTGTC AATTAAGCTGATTTCTGTTCTTTTACTTCCACTTTATTTATTTG -381
*dmrt1*Y: TAATACACT-GATATGTATAATTTTCA-CTATTAAGCTAATTTT--TCTGCTGATA-----TTACTTTCTGCTTAGGCCAAATTA AAAAATCC -348

*dmrt1*Δ*X*: CTAACTTTTGCTCACTTTGCAGAAATCCGATTAATAATCCCAATCAATCAACATACAATTAATATATATTTATTTATTTAGTTTAAATCGGTA -286
*dmrt1*Y: --AATATTTG--AATTTG--AAATTTG-----TAGAGCTGAGCTACTTTGAGTCGTAGA--ACTAT-ACAAGCAG-AGGTTC-CAATG--GAACA -272

*dmrt1*Δ*X*: ATCTTTATTTATTTATTTATTTATCATATAATATATATATTCATTTTACCAGGTCCAAAGTGAAAGTGATAAATCCAGAAATTAATTTGATAACAGT -182
*dmrt1*Y: ATATGC-TTTTGGCTCAT-CTATTTTATCAAAAACTGTAAACCCAATTGGGTGCAGAGAGCTCATGCCAC-TACTGTGCCAACTCTCTCCCGG -180

*dmrt1*Δ*X*: GATATGTATTTATTTCTCAGTACTAAGTAGTA--AATATATTTTCTGCTATCTTATTTTCCAGTTAGGCCAAATTA AAAAATCCGAAAGTTTGAAT -98
*dmrt1*Y: CTTCACCGCTGTTTCTCTCCACAAGTCCCGGAGGAGCGCGCGGAAGGACCGTGACCTTATAGCTT--CCAGCTTGGC--CCAGCA---AAGT -93

*dmrt1*Δ*X*: TTGAAAGCTTTAGAGCTGAGCTACTTCACTGCTGTTGACTATACAGCACAGCCGAGAAACCGACATAGTTTCTTAAGTGACTTCTATTTCCAGTCC -3
*dmrt1*Y: CTTTAAACACCCGAGGCT-GCTGAGACAAATCCGACATAT-GTATCTTAAGTGACTG-TTCTTGTCTGCTTTTATAGTC-CT-TTATTTCCAGTAC -3

TSS →

*dmrt1*Δ*X*: CCATGACACAAGGACACCGAGAGCAAGCAGGTGCCGGAGTCCACCGGAACCTCTC CCCCATCCACGGCCAGAGAACTCCAGGATCTTCAAGTGC 93
*dmrt1*Y: CCATGACACAAGGACACCGAGAGCAAGCAGGTGCCGGAGTCCACCGGAACCTCTC CCCCATCCAAACAACAGAAACCTCCAGGATGCCCAAGTGC 93

*dmrt1*Δ*X*: TCCCTTCTGTAGGAACACCGGCTGTATAATTCCTGTGAGGGGCACAAAAGCTTCTGCAACTGGAGGACTGCCAGTGTCCCAAAATGTAAGTAC 188
*dmrt1*Y: TCCCTTCTGTAGGAACACCGGCTGTATAATTCCTGTGAGGGGCACAAAAGCTTCTGCAACTGGAGGACTGCCAGTGTCCCAAAATGTAAGTAC 188

Sc. tetracanthus

*dmrt1*ΔX: CCACCTTGGCTGACTGACTGACCAACATTTGAGGTAAATTCAGTTTCAGCTCAGTTAAGCCTTGAGAAATTAAT AAGAGAGTAATTCACCAAGTAACA -1356
*dmrt1*LY: CCACCTTGGCTGACTGACTGACCAACAATTTGAGGTAAATTCAGTTTCAGCTCAGTTAAGCCTTGAGAAATTAAT AAGAGAGTAATTCACCAAGTAACA -1026

*dmrt1*ΔX: GCTCCACTGTCATAAAAAAGAACTACAGAAAAATGTAATTTAATAGTATGCAACTCAGATATTTGAGCATAACTACAAGCCCAGTAACACCCG --- -1264
*dmrt1*LY: GCTCCACTGTCATAAAAAAGAACTACAGAAAAATGTAATTTAATAGTATGCAACTCAGATATTTGAGCATAACTACAAGCCCAGTAACACCCGCTG -932

*dmrt1*ΔX: -----GACACAGACACTGCGGTAAAGGAGTACACTGATATTTAATTTGGAAGAAGTAATCATTTTCAGTTTCAGTCAAAACAAG --- -1187
*dmrt1*LY: GCTATTGTAAATTTATAGCCCAACAGACACTGCGGTAAAGGAGTACACTGATATTTAATTTGGAAGAAGTAATCATTTTCAGTTTCAGTCAAAACAAG -837

*dmrt1*ΔX: CAAATTAATTAATTTGTT-----GTTATTTGTTGTTGCCAAATTTTTCAGGAATGCTTCTCAGCAGCTACACAGCCAGGTTTTCATGT -1104
*dmrt1*LY: CAAATTAATTAATTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGCCAAATTTTTCAGGAATGCTTCTCAGCAGCTACACAGCCAGGTTTTCATGT -742

*dmrt1*ΔX: TTTTCAGTTAAATGAAAGGATGCAGTTAAACATTTGTAATTTGGTAGGTATGTAATAGCCCTTGAGATAACCTGACCTTGTTTACCATACCTTCACTCA -1009
*dmrt1*LY: TTTTCAGTTAAATGAAAGGATGCAGTTAAACATTTGTAATTTGGTAGGTATGTAATAGCCCTTGAGATAACCTGACCTTGTTTACCATACCTTCACTCA -647

*dmrt1*ΔX: GACTCCGGTATTTTCAAAAGTAGATACATTTGCTGTGTGCAGATGCCAGGATGGATCTCAAATCTTGAGTTAAAGGGACTTTCTCTTTTAACTAA -914
*dmrt1*LY: GACTCAGGTAATTTTCAAAAGTAGATACATTTGCTGTGTGCAGATGCCAGGATGGATCTCAAATCTTGAGTTAAAGGGACTTTCTCTTTTAACTAA -552

*dmrt1*ΔX: ATATGTTTGCAGGGTATACAGCAGATTGAGAAATTCAAATCTGTTCTACAGTTTGGTTTATACCTTCTCTTCAGCTACTACTCCTGAAACTGAAG -819
*dmrt1*LY: ATATGTTTGCAGGGTATACAGCAGATTGAGAAATTCAAATCTGTTCTACAGTTTGGTTTATACCTTCTCTTCAGCTACTACTCCTGAAACTGAAG -494

*dmrt1*ΔX: CGAGATTACAGATTACCAACTCTCTATCTTCATGATTGCAATACGAGCCCTGCCCTACAGCTTTGGAGAAGTGGCTTGTGATTTGGGGGTCAGTC -724
*dmrt1*LY: -GAGGTTCAA--ATTACC--TGC--ATTTCAC-----CAGCTCAGAGTGAAAGT-----GAT----- -447

*dmrt1*ΔX: GTTTCATTTCCCCACCCGGACCGCCAGCAAAATTTGGCTGTGCTGGAGTGATTAATGCAAAAAATGCTCCCCACCCCCATTAGCTGGCTCATC -629
*dmrt1*LY: --TAAAT-----G--CAGAAATAAT--GTA--ATACAGTGAAT-----ATC -415

*dmrt1*ΔX: TTCTCTTGAGCAAGGCACTTAACCCCCCAATATGCTCCCCGGGGCCCTTGATGCAGCCCACTGGCTCTGCTCTTTTCAGTGCATGTAATTTGCTG -534
*dmrt1*LY: TTA-----AAAT-----CTCAGATTTCAAGTAAATTTTGTCTG--TATATTACG-- -373


*dmrt1*ΔX: GGTCTTGCAATGTTGTTGTTTCACTAAGGACCGCTCAAAATGCAGAAAGCAAAATCACTGCTCTATGTAATAAATATATATATATCTGCAATTAAGCTG -439
*dmrt1*LY: --TT-----GTACTTA-----CGCCAAATTAATAATCGAA--TATTTGAATTTCAAA--CTG--TAGAGCTG -316

*dmrt1*ΔX: ATTCTCTTCTCTTTTACCTGACTTAATTAATTTGCTAACCTTTTGTGACTTTGCAGAAATTCGAATTAATAATGCCAATCAATCAACTAGAAATT -344
*dmrt1*LY: A--G-----GTACTTCACTTATAG--A-----ACTATACAGCAGAGCTT-----CAGATGCACATAGCC--TT -261

*dmrt1*ΔX: CTAAATAATTAATAAGCAATTAATTAATTTGTTATTTATATATTTAGTATTAATTCGTAATCTTATTATATTTTATTTATTTATTTATTTATTA -249
*dmrt1*LY: TTGCTTATTTCT--ATTATTAATAAAAGTGTGAACCGAATTTGGGTGACGCACTTCTGTC--AGTCTTTGCCAAG--TTCTCTTC -181

*dmrt1*ΔX: TCCATTTTTCACCGAGCTGCAAGTGGAAGTATGAATTCGCAAAATTAATTTCAATACAGTATATCTATTTATTTCTCAGTACTAAGTAGTAAGTATA -155
*dmrt1*LY: CCGGCTTCCAGCTCTTTTCTCTCCCAAGTTCCTGCGCAGCGGGGG--GAAGCAGCTGACCTT-TATAGCTTCAC--G-- -106

*dmrt1*ΔX: TTTTCTGCTATGTTATTTTGTGCTTTAGCCGAAATTAATAAATCGAATGTTTGAATTTTGAAAACTGTAGAGCTGAGCTAGCTTGAAGCTCTTTCAAC -60
*dmrt1*LY: -----CTGGAC--GACCAAGTC--TTTAACCGCCGAGC--CTGCTGAGCGCAACTTC--GACATAGTAT -47



*dmrt1*ΔX: TATACAGCAGAGGGACAAACCGACATAGTATCTTAAGTCACTGCTATTTCCGATAGCAGTGAACAAGGCCAGCAAGTTCGAAGCAGGTGCCCGAG -36
*dmrt1*LY: CTAA--AGTG-----AGCTTCTGCTCTTTTAGTCTTT--TATTTCCGATAGCAGTGAACAAGGCCAGCGAGCAAGCAGGTGCCCGAG -36

*dmrt1*ΔX: TCCACCGGAAGCTCTCTCCCATCCACGGGCCAAGACAGCAATGATGCCCAAGTGTCCCGCTGTAGGAACCCAGGCTGTATATCTCTCTGTGA -131
*dmrt1*LY: TCCACCGGAAGCTCTCTCCCATCCCAAGGCCAAGACCTCCAGATGCCCAAGTGTCCCGCTGTAGGAACCCAGGCTGTATATCTCTCTGTGA -131

C

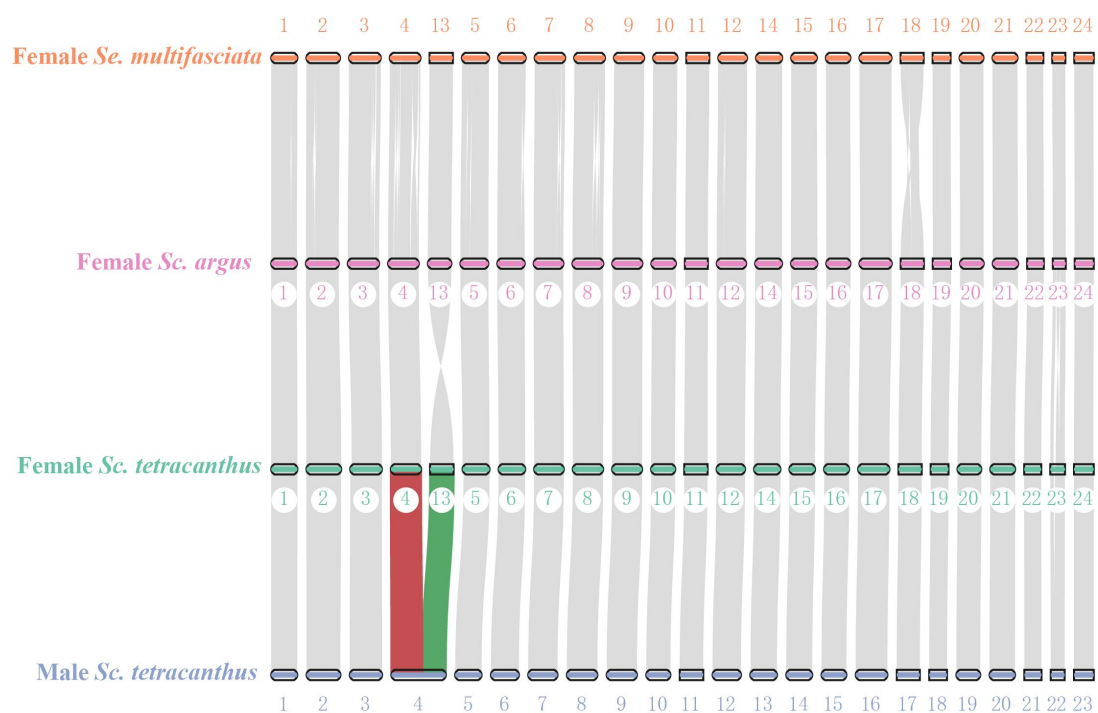
Se. multifasciata

<i>dmrt1a</i> :	TCCACT- TCGGTCACTGACTGACCAGCATTGACGTTAATTAGTTTCAGCT- CAGTCAAGCCTTGAGAATTAAATAGAGAATAATTCACCAAGTA	-1039
<i>dmrt1Y</i> :	TCAGGGGTGAAAAAGTCGGCAGGAAGCATCACTGCAGACCCCTCAAGCCCTGCACACAAACTGTTGATCTGCTCTCTC- TGGTAGGGGTTACA	-1054
<i>dmrt1a</i> :	ACACTTCCACTCTCATAAAAAAGAACTACAGAAATGCATTTTAGTAGGATGT- TAT- TCAGATAT- TACAGCATAACTACAAACCCAGAGC	-947
<i>dmrt1Y</i> :	GAGCTCTGTATGCCAAACTACCAAGGTAC- AAAAATAGCTTTTTCGCCAGACGTCTGTATTAATTATATCTAGCTTCTTCTCTCTCTCATTA	-960
<i>dmrt1a</i> :	CAATATTCCACTAACACTCTCTGGCTATTCTAAATTTATAGCCCAACAGATATTGTTCTAGGAAATACACTGATATT- CAATTGGAGGAATA- -	-855
<i>dmrt1Y</i> :	TATTATTATTATTATTATTATTATTATTATTA- TTTATT	-866
<i>dmrt1a</i> :	ATCATTGAGTTGAGTCAAAACAACTAAATTATTATTATTATTATTATTATTATTATTCGCAAAATATTGTCAGGAATGCATCTCAGCAGCTACAAACAG	-760
<i>dmrt1Y</i> :	ATTATTATTCTTATTGTTATTGTTATT	-771
<i>dmrt1a</i> :	AGGTCCTCTGTGTTTTTCAGTAAATGAAAGGATCCAGTTAAACATTTGCAAAATCGTAGCTCTCTGATAAATAGTCTTGAGATAACCAATCTGACTTGGC	-665
<i>dmrt1Y</i> :	AGGTCCTCTGTGTTTTTCAGTAAATGAAAGGATCCAGTTAAACATTTGCTTAATGGTAGATATGTAAATAGTGGTTGAGATAACCAATATGTA- TTGCT	-677
<i>dmrt1a</i> :	CACCTTATCTGACTCAGACTCAGGATTTCTCAAAAGTAGATCCATTGGTGTG- - - - CATAAGCAGGGTGTCACTTTGCAGATT- - - - - ATTA	-580
<i>dmrt1Y</i> :	CACCTTACCTGACTCAGACTCAGGATTTCTCAAAAGTAGATAATTGGTGTGTGTGCATATGCTAGCATGTCACTTTGCAGATTGGGATTATTG	-582
<i>dmrt1a</i> :	ATGCTAAATCAATCAACATACAAA- TTTATAATGTAATATTACAGATTTCTTTAGAGGCAAAATATAGAGCTATGGAGAAGATAAAGCATCTGAAG	-486
<i>dmrt1Y</i> :	ATGCTAAATCAACCAACATACAAAATCTTAATGTAATATTACAGATTTCTTTTCGGCAAAATTATCGACCC- - - - - ATAAAGCATATGAAG	-496
<i>dmrt1a</i> :	CAGTTCAAAATTAGCTCCATTTTACCAGCTGCAACATTAAGCTGCTTAATGCAGCAAAATTTGTATACATGTATATACATTATTCTCAGTACTC	-391
<i>dmrt1Y</i> :	AAGTTCAAAATTAGCTCCATTTTACCAGCTGCAACATTAAGCTGCTTAATGCAGCAAAATTTGTATACATGTATATACATTCTTTTCAGTACTA	-401
<i>dmrt1a</i> :	AGCTATATTTTGGTCTATATTACTTTTGTACTCAGGCCAAATTAATAATTTGAATCCATCGTTGTTCTAA- - ACTCTAGAGTTGCTCTTTAAGT	-298
<i>dmrt1Y</i> :	CGCCATATTTTGCAGCTATATTACTTTTGTACTCAGGCCAAATTAATAATTTGAATCCATCGCTAGTAAAGTTACTGAGATTTCCTCTTTAAGT	-306
<i>dmrt1a</i> :	CGTA- - - - - AGCAGAGGTTACATGAACAATGGGCTTTTAGTCATTCTATTTTACTAAAAATGTGTGAATGAAATCGGTGCACAGAGACT	-213
<i>dmrt1Y</i> :	CATGAAGTACACAAGCAGACATTCACATGAACAATGGGCTTTTAGTCATTCTATTTTATTAATAATGTGTGAATGCAATTCAGTGCACAGAGACA	-211
<i>dmrt1a</i> :	CATGCCACTG- - - - - CTGTGCCAACTCTCCCCCGCTTCCAGCTGTCTCCCTCCCAAGTCCAGCAGCAGGGCGGGGAAAGACGGTGTAC	-126
<i>dmrt1Y</i> :	CATGCCACTGATAAACTCTGTGCCAACTCTCCCCCGCTTCCAGCTGTCTCCCTCCCAAGTCCAGCAGCAGGGCGGGGAAAGACGGTGTAC	-116
<i>dmrt1a</i> :	CTTATAGCTTCCAGCTGGCACCAAGTCTTTAACCAGTCCAGCCAGCTGGGACAACTCCGACATAGTAACATAAGTCTCACTGTTGTGTGT	-31
<i>dmrt1Y</i> :	CTTGAAGCTTCCAGCTGGCCCCAGAAAAGTCTTTAACCAGCC- A- - - - - GACAACTCCGACATAGTAACATAAGTCTCACTGTTGTGTGT	-31
<i>dmrt1a</i> :	CGTCTTTTGGTGTCTTATTTCCAGTAGCCATGAGCAAGGACAAGCAGCAGCAAGCAGCTGCCGGAAATCCACCGGAACTCTGTCTCCCATCCAAAGG	65
<i>dmrt1Y</i> :	TGTCTTTTGGTGTCTTATTTCCAGTAGCCATGAGCAAG- - - - - CAGATGCCGGAGTCCACTGGTACTCTGTGCC- - - - - AAAGG	44
<i>dmrt1a</i> :	CCAGAAACCTTCCAGGATGCCCAAGTCTCTCGCTGTAGGAACACGGCTATGTATCTCCTCTGAAGGGACACAAACGCTTCTGCAACTGGAGGG	160
<i>dmrt1Y</i> :	ACAGAAACCTTCCAGGATGCCCAAGTCTCTCGCTGTAGGAATCAGGCTATGTATCTCCTCTGAAGGGACACAAACGCTTCTGCAACTGGAGGG	139

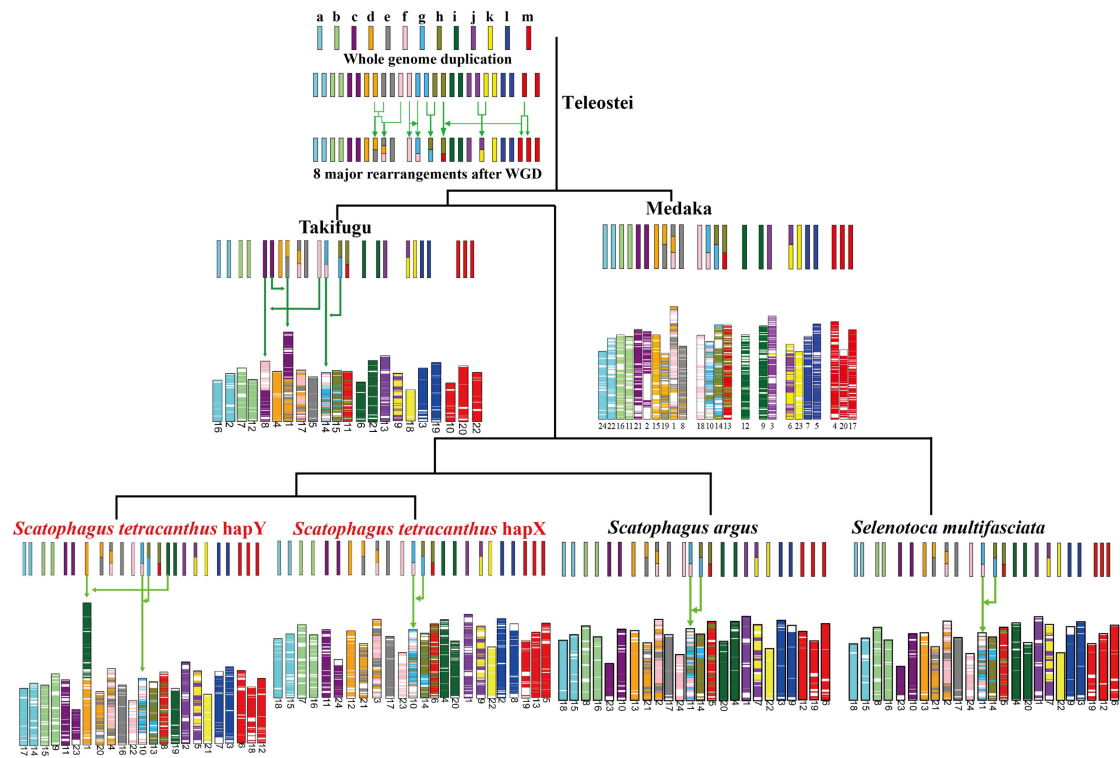
Supplementary Figure 16. Comparative analysis of promoter sequences (about -1k bp) of

dmrt1a/dmrt1ΔX and *dmrt1Y*. The start codon of each gene is positioned at 0 kb. TSS,

transcriptional start site.



Supplementary Figure 17. Chromosome conserved synteny in Scatophagidae species.



Supplementary Figure 18. Reconstruction of the ancestral chromosomes for the Scatophagidae species. Different colored bars represent 13 ancestral chromosomes. Gene sequences from the same ancestral chromosomes were presented in the same color. Green arrows mark some translocation, fusion, and fission events.



Supplementary Figure 19. Geographical distribution of the three Scatophagidae species in this study.