

Table S3 Annotated loci within the 100 kb windows with the highest density of sex-specific SNPs.

Species	Number of sex-specific SNPs [#]	Position of the window	Annotated loci in window (GenBank or ENSEMBL accession number)
<i>Gambusia affinis</i>	708 ¹ (one window)	Chr1:4,822,500..4,922,500 ¹	<i>pgbd3-like</i> (XM_023326051.1) lncRNA (XR_002753294.1) <i>miR1-1</i> (ENSXMAG00000020423) miRNA (ENSXMAG00000026405)
	1058 ² (two windows)	NHOQ01000110.1:1,135,500..1,236,000 ²	ncRNA (XR_534006.2) <i>gata5-like</i> (XM_023341516.1) (located on platyfish Chr1:4,984,658-4,994,612)
<i>Gambusia holbrooki</i>	628 ¹ (three windows)	Chr16:10,229,000..10,330,500 ¹	<i>transmembrane emp24 domain-containing protein 1</i> (part) (XM_005801546.2) <i>dynamamin 2a</i> (ENSXMAG00000013164) lncRNA (XR_002754183.1) <i>queuine tRNA-ribosyltransferase catalytic subunit 1</i> (ENSXMAG00000025867) <i>potassium channel subfamily T member 2-like</i> (XM_023349273.1) <i>trim35-12</i> (ENSXMAG00000013120) lncRNA (XR_002754152.1) <i>GIPC PDZ domain containing family member 1</i> (XM_005801486.3) <i>dnaJ homolog subfamily B member 1-like</i> (XM_005801485.2) lncRNA (part) (XR_002754184.1)
	683 ² (two windows)	NHOQ01002357.1:1,311,000..1,412,500 ²	<i>dnaJ homolog subfamily B member 1-like</i> (part) (XM_008416085.2) <i>GIPC PDZ domain containing family member 1</i> (XM_007572292.2) ncRNA (XR_002754152.1) <i>E3 ubiquitin-protein ligase TRIM39-like</i> (XM_014469686.2) <i>potassium channel subfamily T member 2-like</i> (XM_023349273.1) <i>queuine tRNA-ribosyltransferase catalytic subunit 1</i> (XM_008416073.2) <i>dynamamin-2</i> (XM_005801492.2) <i>emp24 domain-containing protein 1</i> (part) (XM_005801546.2)

<i>Gambusia holbrooki</i> (continued)	565 ¹ (one window)	Chr16:11,798,000..11,898,000 ¹	<i>sox9-like</i> (XM_005801437.2)
	546 ² (one window)	NHOQ01002125.1:1000..101,000 ²	<i>sox9-like</i> (XM_005801437.2) ncRNA (XR_001776856.1) Rex3 retrotransposon, Rex2 retrotransposon, and Rex1 retrotransposon (AY298859.1)
	397 ¹ (one window)	Chr16:13,955,500..14,055,500 ¹	<i>target of myb1 like 2 membrane trafficking protein</i> (part) (ENSXMAG00000028211) <i>sterol regulatory element binding transcription factor 1</i> (XM_005808733.3) <i>retinoic acid induced 1</i> (part) (ENSXMAG00000014773)
	469 ² (one window on this scaffold)	NHOQ01002164.1:1,675,500..1,775,500 ²	<i>retinoic acid induced 1</i> (XM_014472413.2) <i>sterol regulatory element binding transcription factor 1</i> (XM_005808733.3) <i>target of myb1 like 2 membrane trafficking protein</i> (part) (XM_023349079.1)

Note: Annotations were derived from the platyfish chromosome assembly (GenBank accession number: GCF_002775205.1) or obtained by aligning the *G. affinis* candidate interval to the NCBI nr/nt databank using BLAST (only hits with E-value 0 are shown; if several hits to identical loci from different species were found, the best hit is given). One window: there is one window with such a number of SNPs. Two or three windows: there are two or three windows containing exactly this number of SNPs.

#Female-specific SNPs in *G. affinis* and male-specific SNPs in *G. holbrooki*

¹Compared to the platyfish genome assembly

²Compared to the *G. affinis* genome assembly