

**Table S2 Annotated loci within the 100 kb windows with the highest density of sex-specific SNPs.**

| Species                   | Number of sex-specific SNPs <sup>#</sup> | Position of the window                           | Annotated loci in window (GenBank or ENSEMBL accession number)  |
|---------------------------|--|--|---|
| <i>Gambusia affinis</i>   | 727 <sup>1</sup><br>(one window)         | Chr1:4,822,500..4,922,500 <sup>1</sup>           | <i>pgbd3-like</i> (XM_023326051.1)<br>lncRNA (XR_002753294.1)<br><i>miR1-1</i> (ENSXMAG00000020423)<br>miRNA (ENSXMAG00000026405)   |
|                           | 1002 <sup>2</sup><br>(two windows)       | NHOQ01000110.1:1,135,500..1,236,000 <sup>2</sup> | ncRNA (XR_534006.2)<br><i>gata5-like</i> (XM_023341516.1)   |
| <i>Gambusia holbrooki</i> | 641 <sup>1</sup><br>(three windows)      | Chr16:10,229,000..10,330,500 <sup>1</sup>        | <i>transmembrane emp24 domain-containing protein 1</i> (part) (XM_005801546.2)<br><i>dynamin 2a</i> (ENSXMAG00000013164)<br>lncRNA (XR_002754183.1)<br><i>queuine tRNA-ribosyltransferase catalytic subunit 1</i> (ENSXMAG00000025867)<br><i>potassium channel subfamily T member 2-like</i> (XM_023349273.1)<br><i>trim35-12</i> (ENSXMAG00000013120)<br>lncRNA (XR_002754152.1)<br><i>GIPC PDZ domain containing family member 1</i> (XM_005801486.3)<br><i>dnaJ homolog subfamily B member 1-like</i> (XM_005801485.2)<br>lncRNA (part) (XR_002754184.1) |
|                           | 758 <sup>2</sup><br>(two windows)        | NHOQ01002357.1:1,311,000..1,412,500 <sup>2</sup> | <i>dnaJ homolog subfamily B member 1-like</i> (part) (XM_008416085.2)<br><i>GIPC PDZ domain containing family member 1</i> (XM_007572292.2)<br>ncRNA (XR_002754152.1)<br><i>E3 ubiquitin-protein ligase TRIM39-like</i> (XM_014469686.2)<br><i>potassium channel subfamily T member 2-like</i> (XM_023349273.1)<br><i>queuine tRNA-ribosyltransferase catalytic subunit 1</i> (XM_008416073.2)<br><i>dynamin-2</i> (XM_005801492.2)<br><i>emp24 domain-containing protein 1</i> (part) (XM_005801546.2)   |

|  |  |  |   |
|--|--|--|---|
| <i>Gambusia holbrooki</i><br>(continued) | 505 <sup>1</sup><br>(one window)                     | Chr16:11,798,000..11,898,000 <sup>1</sup>        | <i>sox9-like</i> (XM_005801437.2)   |
|  | 556 <sup>2</sup><br>(one window)                     | NHOQ01002125.1:1000..101,000 <sup>2</sup>        | <i>sox9-like</i> (XM_005801437.2)<br>ncRNA (XR_001776856.1)<br>Rex3 retrotransposon, Rex2 retrotransposon, and Rex1 retrotransposon (AY298859.1)  |
|  | 368 <sup>1</sup><br>(one window)                     | Chr16:13,955,500..14,055,500 <sup>1</sup>        | <i>target of myb1 like 2 membrane trafficking protein</i> (part) (ENSXMAG00000028211)<br><i>sterol regulatory element binding transcription factor 1</i> (XM_005808733.3)<br><i>retinoic acid induced 1</i> (part) (ENSXMAG00000014773) |
|  | 405 <sup>2</sup><br>(one window on<br>this scaffold) | NHOQ01002164.1:1,675,500..1,775,500 <sup>2</sup> | <i>retinoic acid induced 1</i> (XM_014472413.2)<br><i>sterol regulatory element binding transcription factor 1</i> (XM_005808733.3)<br><i>target of myb1 like 2 membrane trafficking protein</i> (part) (XM_023349079.1)                |

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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).

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

<sup>1</sup>Compared to the platyfish genome assembly

<sup>2</sup>Compared to the *G. affinis* genome assembly