

A

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sox9bY ATGAATCTCCTCGACCTTACCTGAAGATGACAGAAGAACAGGAGAAATGTCATTTCAGAC 60

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sox9bY RTTQIKTEQLSPSHYSDQQGSPQHI TYGSFNLQH YSPSSYPSITRAQYDYSEHQSSANSY 420
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Figure S4 Alignments of male *G. holbrooki* and *G. affinis* *gipc1* and *sox9b* alleles.

Alignment of (A) *gipc1* ORF and (B) predicted protein sequences and (C) *sox9b* ORF and (D) putative protein sequences from the *G. holbrooki* X and Y chromosome and *G. affinis*. The *G. affinis* sequences were derived from the *G. affinis* genome (HOFFBERG *et al.* 2018). The sequences were aligned with Clustal Omega. Based on the distribution of SNPs, the respective alleles from the X and from *G. affinis* appear to correspond to the ancestral state of *Gambusia*. (B) Orange, GH1 domain; light blue, PDZ domain; red, GH2 domain. (D) Green, DIM domain; dark blue, HMG domain; brown, K2 domain; violet, TA domain. Accession numbers of *G. affinis* sequences: *sox9b* ORF, NHOQ01002125.1:61568-61995,62336-62583,62831-63594; *sox9b* protein sequence, PWA19450.1; *gipc1* ORF, NHOQ01002357.1: 1316372-1316665,1316740-1316925,1317062-1317242,1318142-1318254,1318339-1318420, 1318497-1318640,1318740-1318819,1319648-1319671 (shortened as probably not correctly assembled (does not end with stop codon)); *gipc1* protein sequence, PWA17858.1 (shortened in accordance with ORF sequence).

Reference

Hoffberg, S. L., N. J. Troendle, T. C. Glenn, O. Mahmud, S. Louha *et al.*, 2018 A high-quality reference genome for the invasive mosquitofish *Gambusia affinis* using a Chicago Library. G3 (Bethesda) 8: 1855-1861.