

A

```

gipc1Y    ATGCTCTGCGGTCTGGGAAGAAGGAAGAAGGCGTCCCCCTTGGTGGAGAATGAGGAAGCG 60
gipc1X    ATGCTCTGCGGTCTGGGAAGAAGGAAGAAGGCGTCCCCCTTGGTGGAGAATGAGGAAGCG 60
*****

gipc1Y    GAGCCCATCCGCGGTGGTCTCAACAATGCCGGGTATGGAGGGCTTGGATGGAGGAGTCGCT 120
gipc1X    GAGCCCATCCGCGGTGGTCTCAACAATGCCGGGTATGGAGGGCTTGGATGGAGGAGTCGCT 120
*****

gipc1Y    GGGGAGGCTGCAGGGTCTGACGGTCTTCCTCCCCACCAAACAACATGAGACCCCGGCTG 180
gipc1X    GGGGAGGCTGCAGGGTCTGACGGTCTTCCTCCCCACCAAACAACATGAGACCCCGGCTG 180
*****

gipc1Y    ATTTTCCACACCCAACCTGGCTCACGGCAGCCCCACAGCCGCATTTAGGGCTTTAGCAAT 240
gipc1X    ATTTTCCACACCCAACCTGGCTCACGGCAGCCCCACAGCCGCATTTAGGGCTTTAGCAAT 240
*****

gipc1Y    GTGCGAGAGCTCTATGCCAAAATTGGAGAGGCCTTTGGCATCCCAGCATCTGAGGTTATG 300
gipc1X    GTGCGAGAGCTCTATGCCAAAATTGGAGAGGCCTTTGGCATCCCAGCATCTGAGGTTATG 300
*****

gipc1Y    TTTTGCACGCTGAACACTCACAAGGTGGACATGGATAAACTTTTAGGGGGACAGATTGGG 360
gipc1X    TTTTGCACGCTGAACACTCACAAGGTGGACATGGATAAACTTTTAGGGGGACAGATTGGG 360
*****

gipc1Y    CTGGAGGACTTCATTTTGGCCACATTAAAGGCCAGAAGAAGGAAATAGAAATCTTCAAA 420
gipc1X    CTGGAGGACTTCATTTTGGCCACATTAAAGGCCAGAAGAAGGAAATAGAAATCTTCAAA 420
*****

gipc1Y    GGAGAAGATGCTTTAGGACTGACAATCACCGACAACGGAGCAGGTTATGCTTTCATCAAG 480
gipc1X    GGAGAAGATGCTTTAGGACTGACAATCACCGACAACGGAGCAGGTTATGCTTTCATCAAG 480
*****

gipc1Y    AGGATCCGGGAGGGGAGCATCATCCACCAAAATCCAGGTCATTAACGTTGGTGACATGATC 540
gipc1X    AGGATCCGGGAGGGGAGCATCATCCACCAAAATCCAGGTCATTAACGTTGGTGACATGATC 540
*****

gipc1Y    GAGTCCATTAAACGGCCATCGCCTCATTGGCTGTGCGACTACGAAAGTTGCCAAAATGCTG 600
gipc1X    GAGTCCATTAAACGGCCATCGCCTCATTGGCTGTGCGACTACGAAAGTTGCCAAAATGCTG 600
*****

gipc1Y    AAGGAGCTTTCTAAGGGGAAAATGTTACCATCAAGCTTGTGGAGCCCCCTCAAAGCTTTT 660
gipc1X    AAGGAGCTTTCTAAGGGGAAAATGTTACCATCAAGCTTGTGGAGCCCCCTCAAAGCTTTT 660
*****

gipc1Y    GATATGATTGGTCAGAGGTCGTGAGGCTCCAGGTCGGGCTCAGCGGTCCAGCTCGGGACG 720
gipc1X    GATATGATTGGTCAGAGGTCGTGAGGCTCCAGGTCGGGCTCAGCGGTCCAGCTCGGGACG 720
*****

gipc1Y    GGCAGAGGGACCCCTTCGTCTGCGCTCTAAAGGTCGCGCCACCGTGGAAGAGCTGCCTTCT 780
gipc1X    GGCAGAGGGACCCCTTCGTCTGCGCTCTAAAGGTCGCGCCACCGTGGAAGAGCTGCCTTCT 780
*****

gipc1Y    GCATTTGAGGAAAAAGCGATTGAGAAGGTGGATGATTTGCTAGAGAGCTACATGGGCATC 840
gipc1X    GCATTTGAGGAAAAAGCGATTGAGAAGGTGGATGATTTGCTAGAGAGCTACATGGGCATC 840
*****

gipc1Y    AGAGACAGCGAACTGGCCGCCACTATGGTGGAGCTGGGCAAAGACAAGAAGAACCCAGAC 900
gipc1X    AGAGACAGCGAACTGGCCGCCACTATGGTGGAGCTGGGCAAAGACAAGAAGAACCCAGAC 900
*****

gipc1Y    GAGTTTGCTGAAGCTTTGGATGAGACTCTGGGAGACTTCGCCCTCCCCGACGAGTTTGTT 960
gipc1X    GAGTTTGCTGAAGCTTTGGATGAGACTCTGGGAGACTTCGCCCTCCCCGACGAGTTTGTT 960
*****

gipc1Y    TTCGACGCTCTGGGGCGCCATCGGTGACGCGAAGGTTGGACGTGTGTAA 1008
gipc1X    TTCGACGCTCTGGGGCGCCATCGGTGACGCGAAGGTTGGACGTGTGTAA 1008
*****

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B

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gipc1Y    MPLLGLGRRKKASPLVENEEAEPIRGGLNMPGMEGLDGGVAGEAAGSDGLPPPPNNMRPRL 60
gipc1X    MPLLGLGRRKKASPLVENEEAEPIRGGLNLPGMEGLDGGVAGEAAGSDGLPPPPNNMRPRL 60
          *****:*****

gipc1Y    IFHTQLAHGSPTGRIEGFSNVRELYAKIGEAFGIPASEVMFCTLNTHKVDMDKLLGGQIG 120
gipc1X    IFHTQLAHGSPTGRIEGFSNVRELYAKIGEAFGIPASEVMFCTLNTHKVDMDKLLGGQIG 120
          *****

gipc1Y    LEDFIFAHIKGQKKEIEIFKGEDALGLTITDNGAGYAFIKRIREGSIIHQIQVINVGDMI 180
gipc1X    LEDFIFAHIKGQKKEIEIFKGEDALGLTITDNGAGYAFIKRIREGSIIHQIQVINVGDMI 180
          *****

gipc1Y    ESINGHRLIGCRHYEVAKMLKELSKGKMFTIKLVEPLKAFDMIGQRSSGSRSGSAVQLGT 240
gipc1X    ESINGHRLIGCRHYEVAKMLKELPKGKMFTIKLVEPLKAFDMIGQRSSGSRSGSGVQLGT 240
          *****

gipc1Y    GRGTLRLRSKGLATVDELPSAFEEKAIEKVDDLLESYMGIRDSELAATMVDELGDKKKNPD 300
gipc1X    GRGTLRLRSKGPATVEELPSAFEEKAIEKVDDLLESYMGIRDSELAATMVDELGDKKKNPD 300
          *****

gipc1Y    EFAEALDETLGDFAFPDEFVFDVWGAIGDAKVGRV 335
gipc1X    EFAEALDETLGDFAFPDEFVFDVWGAIGDAKVGRV 335
          *****

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C

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sox9bX    ATGAATCTCCTCGACCCCTTACCTGAAGATGACAGAAGAACAGGAGAAATGTCATTTCAGAC 60
sox9bY    ATGAATCTCCTCGACCCCTTACCTGAAGATGACAGAAGAACAGGAGAAATGTCATTTCAGAC 60
          *****

sox9bX    GCTCCGAGCCCCAGCATGTCTGAAGACTCAGCGGGCTCGCCGTGCCCGTCCGGCTCCGGT 120
sox9bY    GCTCCGAGCCCCAGCATGTCTGAAGACTCAGCGGGCTCGCCGTGCCCGTCCGGCTCCGGT 120
          *****

sox9bX    TCGGACACCGAGAACACCCGGCCGTCCGACAACACCTCCTCAGGGGACCGGACTACAAG 180
sox9bY    TCGGACACCGAGAACACCCGGCCGTCCGACAACACCTCCTCAGGGGACCGGACTACAAG 180
          *****

sox9bX    AAGGAAGGCGAGGAAGAGAAGTTCCCGGTGTGCATCAGAGATGCGGTGTCCAGGTGCTG 240
sox9bY    AAGGAAGGCGAGGAAGAGAAGTTCCCGGTGTGCATCAGAGATGCGGTGTCCAGGTGCTG 240
          *****

sox9bX    AAGGGCTATGACTGGACGCTCGTACCCATGCCGGTGCGCGTCAACGGCTCCAGCAAGAGC 300
sox9bY    AAGGGCTATGACTGGACGCTCGTACCCATGCCGGTGCGCGTCAACGGCTCCAGCAAGAGC 300
          *****

sox9bX    AAACCACACGTTAAGAGACCCATGAACGCCTTCATGGTTTGGGCTCAGGCGGCGCGGAGG 360
sox9bY    AAACCACACGTTAAGAGACCCATGAACGCCTTCATGGTTTGGGCTCAGGCGGCGCGGAGG 360
          *****

sox9bX    AAGCTGGCCGATCAGTATCCGCATCTGCACAACGCAGAACTCAGCAAAACCCTGGGAAAA 420
sox9bY    AAGTTGGCCGATCAGTATCCGCATCTGCACAACGCAGAACTCAGCAAAACCCTGGGAAAA 420
          *** *****

sox9bX    CTTTGGAGGCTTCTCAATGAGGTGGAGAAGCGCCGTTTGTGGAGGAAGCTGAACGCCTG 480
sox9bY    CTTTGGAGGCTTCTCAATGAGGTGGAGAAGCGCCGTTTGTGGAGGAAGCTGAACGCCTG 480
          *****

sox9bX    AGAGTGCAGCACAGAAGGATCACCCGACTACAAATATCAACCGAGGCGGAGGAAATCT 540
sox9bY    AGAGTGCAGCACAGAAGGATCACCCGACTATAAATATCAACCGAGGCGGAGGAAATCT 540
          *****

sox9bX    GTTAAGAATGGGCAAAATGAATCCGAGGATGGCGAACAGACGCACATCTCTCCGAACGCT 600
sox9bY    GTTAAGAATGGGCAAAATGAATCCGAGGATGGCGAACAGACGCACATCTCTCCGAACGCT 600
          *****

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sox9bX	ATATTCAAGGCGCTGCAGCAAGCAGACTCTCCTGCGTCCAGCATGGGCGAGGTGCACTCT	660
sox9bY	ATATTCAAGGCGCTGCAGCAAGCAGACTCTCCTGCGTCCAGCATGGGCGAGGTGCACTCT	660

sox9bX	CCGGGAGAACATTTCGGTTCAGTCACAGGGACCACCAACACCCCGACAACCCCAAAGACA	720
sox9bY	CCGGGAGAACATTTCGGTTCAGTCACAGGGACCACCAACACCCCGACAACCCCAAAGACA	720

sox9bX	GATCTTTCTCCAGCAAAGCTGACCTGAAGCGTGAGGGCCGCCCCATTCAAGAGGGCAGC	780
sox9bY	GATCTTTCTCCAGCAAAGCTGACCTGAAGCGTGAGGGCCGCCCCATTCAAGAGGGCAGC	780

sox9bX	AGTCGGCAGCTCAACATAGACTTTGGAGCTGTGGACATTGGTGAGCTTAGCAGCGAAGTC	840
sox9bY	AGTCGGCAGCTCAACATAGACTTTGGAGCTGTGGACATTGGTGAGCTTAGCTGCGAAGTC	840

sox9bX	ATCTCCAACATCGGGAGCTTTGATGTTGATGAGTTCGACCAGTACCTGCCACCTCACAGC	900
sox9bY	ATCTCCAACATCGGGAGCTTTGATGTTGATGAGTTCGACCAGTACCTGCCACCTCACAGC	900

sox9bX	CACGCTGGGGTTGCTGGTCCAGGCCAGGCTGGCTACACAGGCAGCTACGGTATTAGCACC	960
sox9bY	CACGCTGGGGTTGCTGGTCCAGGCCAGGCTGGCTACACAGGCAGCTACGGTATTAGCACC	960

sox9bX	TCCTCAGTCAGTCAGGGAGCCGGTGTGGGAGCTCATGCTTGGATGTCCAAGCAGCAGCAG	1020
sox9bY	TCCTCAGTCAGTCAGGGAGCCAGTGTGGGAGCTCATGCTTGGATGTCCAAGCAGCAGCAG	1020

sox9bX	CAGCAGCATACTCTGACCAACCTTGGTGGAGCAGAGAGCAAGGTCAACAGGGCCAGCAG	1080
sox9bY	CAGCAGCATACTCTGACCAACCTTGGTGGAGCAGAGAGCAAGGTCAACAGGGCCAGCAG	1080

sox9bX	AGAACCACCCAGATCAAGACGGAGCAGCTCAGCCCAGCCACTACAGTGACCAACAGGGA	1140
sox9bY	AGAACCACCCAGATCAAGACGGAGCAGCTCAGCCCAGCCACTACAGTGACCAACAGGGA	1140

sox9bX	TCCCCGCAGCACATCACCTATGGCTCATTCAACCTACAGCACTACAGCCCCTCTTCTTAT	1200
sox9bY	TCCCCGCAGCACATCACCTATGGCTCATTCAACTTACAGCACTACAGCCCCTCTTCTTAT	1200

sox9bX	CCGTCCATCACAAGAGCACAATATGACTATTGAGAACCAGAGCAGTGCCAACTCTTAC	1260
sox9bY	CCGTCCATCACAAGAGCACAATATGACTATTGAGAACCAGAGCAGTGCCAACTCTTAC	1260

sox9bX	TACAGCCACGCAGCTGGCCAAGGTTCCAGCCTGTACTCCACCTTCAGCTACATGAGCCCC	1320
sox9bY	TACAGCCACGCAGCTGGCCAAGGTTCCAGCCTGTACTCCACCTTCAGCTACATGAGCCCC	1320

sox9bX	AGCCAGAGGCCGATGTACACCCCAATAGCCGACAGCACTGGGGTGCCCTCTGTGCCGCAG	1380
sox9bY	AGCCAGAGGCCGATGTACACCCCAATAGCCGACAGCACTGGGGTGCCCTCTGTGCCGCAG	1380

sox9bX	ACCCACAGTCCACAGCACTGGGAGCAGCAGCCGATTTACACACAACCTGTCCAGGCCCTGA	1440
sox9bY	ACCCACAGTCCACAGTACTGGGAGCAGCAGCCGATTTACACACAACCTGTCCAGGCCCTGA	1440

D

sox9bX	MNLLDPYLKMTTEEQEKCHSDAPSPMSSEDSAGSPCPSGSGSDTENTRPSDNHLLRGPDYK	60
sox9bY	MNLLDPYLKMTTEEQEKCHSDAPSPMSSEDSAGSPCPSGSGSDTENTRPSDNYLLRGPDYK	60

sox9bX	KEGEEEFKFPVCIRDAVSQVLKGYDWTLPMPVRVNGSSKSKPHVKRPMNAFMVWAQAARR	120
sox9bY	KEGEEEFKFPVCIRDAVSQVLKGYDWTLPMPVRVNGSSKSKPHVKRPMNAFMVWAQAARR	120

sox9bX	KLADQYPHLHNAELSKTLGKLWRLNNEVEKRPFVEEAERLRVQHKKDHPDYKYQPRRRKS	180
sox9bY	KLADQYPHLHNAELSKTLGKLWRLNNEVEKRPFVEEAERLRVQHKKDHPDYKYQPRRRKS	180

sox9bX	VKNGQNESEDGEQTHISPNAIFKALQQADSPASSMGEVHSPGEHS	GQSQGPPTPPTPKT	240
sox9bY	VKNGQNESEDGEQTHISPNAIFKALQQADSPASSMGEVHSPGEHS	GQSQGPPTPPTPKT	240

sox9bX	DLSSSKADLKREGRPIQEGSSRQLNIDFGAVDIGELS	SEVISNIGSFDVDEFDQYLPPHS	300
sox9bY	DLSSSKADLKREGRPIQEGSSRQLNIDFGAVDIGELS	SEVISNIGSFDVDEFDQYLPPHS	300

sox9bX	HAGVAGPAQAGYTGSYGISTSSVSQGA	VGAAHAWMSKQQQQHTLT	360
sox9bY	HAGVAGPAQAGYTGSYGISTSSVSQGA	VGAAHAWMSKQQQQHTLT	360

sox9bX	RTTQIKTEQLSPSHYSQQGSPQHI	TYGSFNLQHYSPSSYPSITRAQYDYSEHQSSANSY	420
sox9bY	RTTQIKTEQLSPSHYSQQGSPQHI	TYGSFNLQHYSPSSYPSITRAQYDYSEHQSSANSY	420

sox9bX	YSHAAGQGSSLYSTFSYMSPSRPMYTP	ADSTGVPSVPQTHSPQ	479
sox9bY	YSHAAGQGSSLYSTFSYMSPSRPMYTP	ADSTGVPSVPQTHSPQ	479

Figure S4 Alignments of male *G. holbrooki* *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 X and Y chromosome. The sequences were aligned with Clustal Omega. (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.