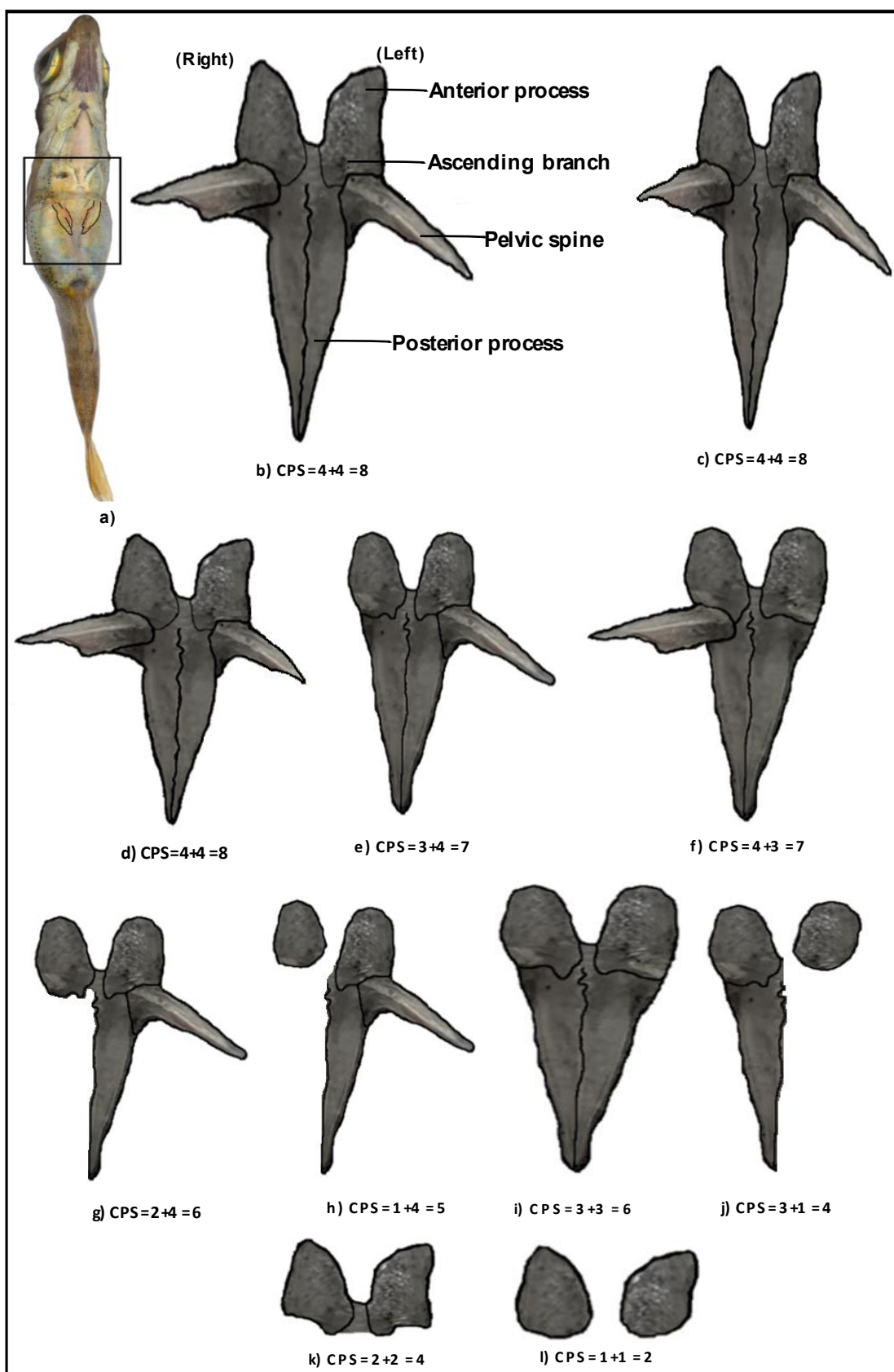


## Supplementary materials

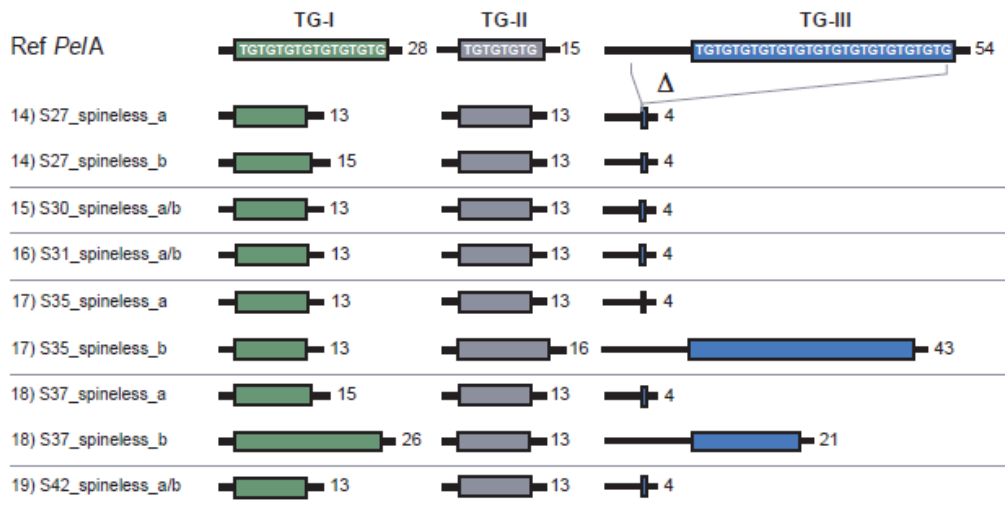
S1. (a) Ventral view of a three-spined stickleback and (b) the pelvis structure (also termed “pelvic girdle”) consisting of pairs of “anterior process”, “ascending branch”, “pelvic spine” and “posterior process” [18, 19]. The following varieties were found in Lake Storvatnet: (b) a symmetric specimen pelvis with complete pairs of pelvic spines, posterior processes, ascending branches, and anterior processes and a combined pelvic score (CPS) of 8; (c) a specimen with left-biased asymmetric pelvis and CPS 8; (d) right-biased asymmetric pelvis with CPS 8; (e) left-biased asymmetric pelvis with CPS 7; (f) right-biased asymmetric pelvis structure with CPS 7; (g) left-biased asymmetric pelvis with CPS 6; (h) left-biased asymmetric pelvis with CPS 5; (i) spineless pelvis structure with CPS 6; (j) spineless pelvis structure with CPS 4; (k) spineless pelvis structure with CPS 4; (l) spineless pelvis structure with CPS 2. Specimens with more than 0.2 mm difference in length between the right and left pelvic spine were categorized as “asymmetric spined” specimens (Fig. 2c – h) and varied from CPS 8 (Fig. 2c) to CPS 5 (Fig. 2h).



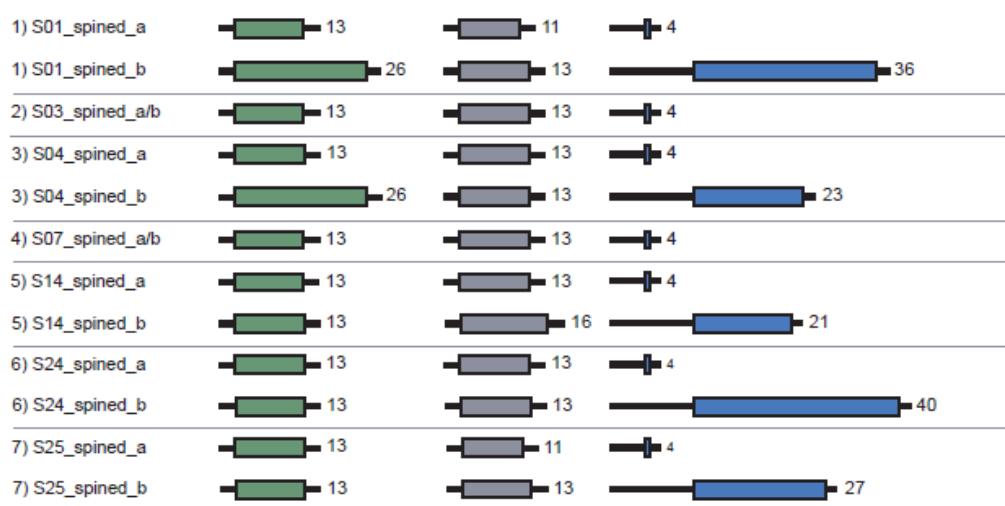
S2. Number of (TG)<sub>n</sub> at TG-repeat I (green), II (grey), and III (blue) from (a) six spineless, (b) seven symmetric spined, and (c) six asymmetric spined specimens from Lake Storvatnet.

Numbers to the right of TG-repeat I, II and III show the number of repeats. The reference sequence (GU130435) from Salmon River, British Columbia is shown in (a). Note that all the examined specimens from Lake Storvatnet (but not from Lake Gjerdhaugvatnet in Suppl. S3a or the marine site in Suppl. S3b) have an additional 58 bp deletion (which does not contain a TG-repeat) upstream of and flanking TG-repeat III. These deleted nucleotides are not shown here (but see Fig. 5, and Suppl. S6). Fragment analyses were carried out to identify the allelic variation of TG-repeats I-III. The two alleles in the diploid sticklebacks are indicated by a and b. Note that the alleles of TG-repeats I-III are unphased.

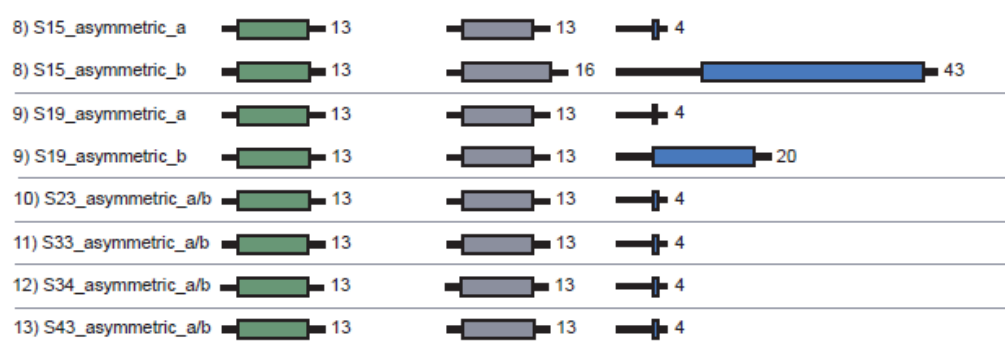
(a) *PeIA\_Lake Storvatn\_Spineless*



(b) *PeIA\_Lake Storvatn\_Spined*

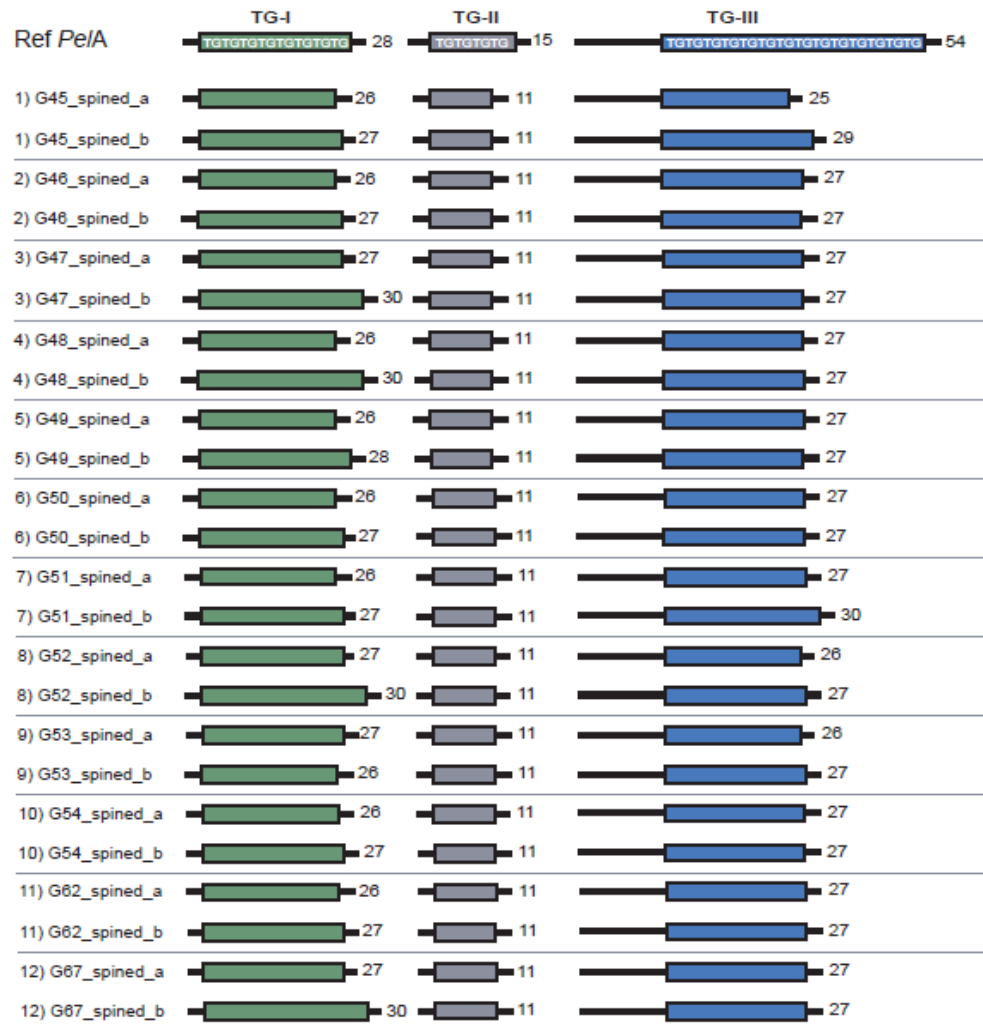


(c) *PeIA\_Lake Storvatn\_Asymmetric*

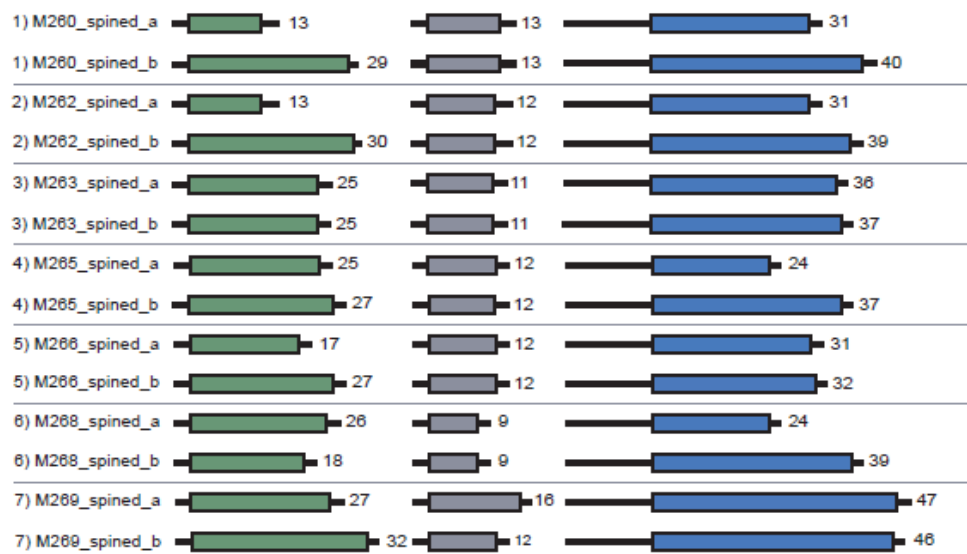


S3. Number of TG-repeats at TG-repeat I (green), II (grey), and III (blue) from (a) 12 specimens from Lake Gjerdhaugvatnet and (b) seven specimens from the marine site. All examined specimens from these two sites were spined (CPS is 8). Numbers to the right of TG-repeat I, II and III show the number of repeats. The reference sequence (GU130435.1) from Salmon River, British Columbia is shown in the upper line in (a). Fragment analyses were carried out to identify the allelic variation of TG-repeats I-III. The two alleles in the diploid sticklebacks are indicated by a and b. Note that the alleles of TG-repeats I-III are unphased.

(a) *PeIA\_Gjerdhaugvatn*



(b) *PeIA\_Marlne*



#### S4. Partial *Pitx1* reference sequence encompassing the *PelA* enhancer elements

Partial *Pitx1* reference sequence from a marine pelvic-complete stickleback from Salmon River, British Columbia, GenBank accession GU130435; 377 852 nt [128 457-131 624] with the *PelA* enhancer region extending from 128 457-131 382 (indicated by [ ]). Bold-Underlined-Red sequences indicate the forward and reverse primer sites for the PCR amplicon (2813 bp). TG-repeat I [(TG)<sub>25</sub>], TG-repeat II [(TG)<sub>15</sub>], and TG-repeat III [(TG)<sub>50</sub>] are underlined. Nucleotide sequences in bold within brackets, (1) and (2), (3) and (4), (5) and (6), indicate sites for the forward and reverse sequencing primers for TG-repeat I, TG-repeat II, and TG-repeat III, respectively.

[CACCGATGTTCCAACAGATGTTTGTCAACACCATCCGGCTGTGGTGGCCCTTGTGTGTTTTATTTGGT  
TGCTGCCATGGCGACGGGGGCCGGGGGGGATTCTCTCTTGCCGATGATGACGCTACGTCGGGGCCTC  
GCCGCTGCGTGAGAAATATGGGCATTAGGCGCTAAGCTGGGCGCCGTGCGGCGCTTTGAAGGGATTG  
GGGGGGGATTATCAAGGGGAGGGGGCGGTGCTAGTAACACACAAATGGCCTTTAATGAAGTGAGGGGA  
GGAGCCTAAAGCTGCAAATGGACCGGTTTGATTATTAGCACATATAAGATGTGTAGTTTGGGGTCTTT  
CTGCTGCTGGTCGACA**GCCCAAACTGACAAAGCA**CATTGCTCTTTTCATGGGGGGGTGCGGGGGGG  
GTTTCAGAGATGGAATGAAGCAGCTCTCTAAACACTGCTGATCTCAGCGGCTCCTCTGACATCACGTT  
GGAGCATCAAACCTCGTCCTCTCAGACTGAACCTG (1 : **AGGTCACAGTACAGTGCAG**) GTTCAAAG  
GTCACAAACCCCCCATCGACCCCCCCCCCTCCCTCGCCCGCGCCGTGACGTGTGCGCGCAGGTCAGAC  
GTGACGCGGCGCTCCATCACCGAGCCGCTTTGATGTGGGCCTAATATGGCTTGTAAGAAGGGGAACC  
CTGCACTTTTCAAGTGTTGCCTGACGCTGTCCAGACACAAGTGTGTGTGTGTGTGTGTGTGTGTGTGTG  
TGTGCGTG  
AACATTGAGAGGGTCTGGAGGAGCAGGTCTGGCTGGAGAATATGTCTCTGTCTATGGAGCACCACTCG  
TTC (2 : **CTGAAGGCATCTTCTCGTCCCA**) GCAGCAGTAACAACCTATGTGAGTTTTCCCTTTATTCAC  
CCAATAAAACCCATTTACTAAAAATGCTCAACTCGATTTCCATCACGTTGTTTATTAATGTTCTCTTC  
TGCACATGAAGGATCATTAAACAGTAATTCAGGTGCACAAACCTTTATTTTACCTTTTACCCCTCAA  
ATCTGCAGCGTTTTTCTGTGTTTACAGCCTGATGTGCAGCACACCTGGAGGATCCTTTCACTGTGCAA  
TCAGCTCTGAGATAAAAAGCAGTTACGACACACATAAACAGCCGAGATAAGCTGCTCTGCCCCACCC  
CCGAAATCCCCCCCC (3 : **GTCGAAGCAAAGAGGCGAGACATC**) ACGGCGTGAAGCGACTGTTATTCAT  
AAACATTCAGATATCAAACCAACAAGAACTGTTCAAATGTGTTCAAACAAACAGCGGGACGGGGGAG  
TG  
GGGGAACGGGGGGGATGGGGGGGGGGGTGAGGCGCGACCATCTAACTCTTCCACTGATT  
{ 5 : **GTTATGAAGG** (4 : **GCCGAGCGAC**) **CACCTTTAGAA** ) CTCTGCACAAGGTCCTAATGCATTCTGTTT  
GACCTCGCCGGAGTAAATCAAATACTGGCCTCAGATCTCCTGTGTGTGTGCGTGTGTGTGTGTGTGTGTG  
TG  
TG  
ATGAAGAAAGGAGGCTTATAAAACATTTGAGCTTCCA (6 : **CGGATTGTTGTGGTCAACGC**) CACACGTT  
GTCATAGCAACCAATAGTCATTTTGTACATCAGCATCATTTGTTCTGGAGACTGGGTACACAGCTAGC  
CGCTAACAGGTAGCATTAGCGGCTAACAGGTAGCATTAGCGGCTAACAAGTAGCGTTCAACTCTTTCT  
AGGCGAGACAGAACCAGAACCCGGCTGAGGAGCTGAGACGTGCGCGATGAAAGGCAGCAGGTTTATTG  
TCTCTATTTAAATAACCACCGTGTGATGAAAGAGACGTCTGATAGACCAGCATGTCCCCCCCCCCCC  
CCCCCCCCCCCCCATGTGTTCTTCATAATACAGAATCAGCATCTTCTGTATGTGTGTGTGTCTCCTGTTGA  
TCCGTCCTGAAAGACGTCTCCGTGCGAGTTGTCCCCCATGGGTACTGCGATAGATCTGAGGTCCACC  
TGCACCGGGGACACCGGGGACAATAATGAGAGAGAGAGACAGGCCGTCCCCAGAGGGAGGTCTGAGGG  
ACTAAGGTCCTCCAGCAGGAGGAAACCTCTCTGCTGACAAACACCACATCGTTTTGGTTGGAGGGGGG  
GGGTCAGCCAGCAGCTGCTGTGGTGGGAACGCCAAACCAAACAGAGGGAGGGGGGGCTCACCTCTGCCC  
AAACACACGCCCCGTCCTCCCGACCGCAGGAGACATATCAAAATAAAAAGGGTCAAATCTACAGAGGG  
ATTTAAGGCCTTATCGTTATCTGTCTCCACGGAGGACGGAGGCCTGCTCAGGACCAGCGGCTGTCTCT  
TGTCTCCACGGAGGACGGAGGCCTCCTCAGGACCAGCGGCTGTCTCGTCTCCACGGAGGACGGAGGC  
CTCCTCAGGACCAGCGGTTGTCTCTCCACGGAGGACGGAGGCCTCCTCGGGACCAGCGGCTGT  
CCTCGTCTCCACGGAGGACGGAGGCCTCCTCAGGACCAGCGGTTGTCTCTGTCTCCACGGAGGACGG  
AGGCCTCCTCAGGACCAGCGGCTGTCTCTGTCTCCACGGAGGACGGAGGCCTCTTGTGACTGGAGGA  
ATCAGAGAGGAAAGACGTCCGTTTTCTTCCCGTCTGAATCGTTTCTAAAAGCCTCCGTTGAGGATTTG  
CTCGTTATTCGTAAGCAGCTCGCTGTGAAAAC] ATTAATAACCGTCTTAATAACTGTTAGTTTGG

GTAAAGGACAGAAAAGTAGATGTGAAATGTTCTCATTTTCGTTTTTACATGTCATTTGTCTAATTGTC  
TGAAATATGTTATAGGTGCAAAATGCTGATAAAGAATGTTTGAGGTTTTAGTTTTGTGATTCCCTACA  
AACTGTTTTCTTTATTTATAATTAAAACCACGTGCTTCATTATAACAGAAATGTCTCATTTTGCTTTT  
GCTGCT

S5. DNA sequencing of *PeIb* enhancers from 9, 6 and 6 threespine sticklebacks from Lake Storvatnet, Lake Gjerdhaugvatnet and the marine site, respectively. All specimens were homozygous identical to the reference sequence for the 671 bp interrogated. Red sequences show primer sites used to produce an amplicon of 1009 bp. Black-bold-underlined sequences indicate the sites for sequencing primers.

*PeIb* enhancer region sequence from *Pitx1* reference GU130435; 377 852 [176 680-177 688].

CACGGATTACTGAGCAGCAATGGAAGGAGGACGCGTGGAGGCTCGCAGGGTTCCTGAAGCTC  
TTCTGCCCCCTGGAGGCCTCCAGAGGGTCTTCACCCCTCATCTCAAACCTCTCGGGGTCTGATC  
TCCTCGAGCCGATCGCTCGTCAGAGCGCTGAGACAGACAGACAGACAGACAGACAGACGGAG  
AAGGCTGCCAACATGTAGGGTTGGAACAACATGAGCTCATTCCCCACTAACATGAGGGAGGC  
CTGGGGGGGGCGAGGGGGCGAGGGGGGGGGCAGCGGTCTGCAAACCGGAGGAGACGTTTCTA  
CCAATTTGATTGTTTGTGAAAGAAATCATTCTCATCAAACCCCCAATTGCTGCCAACTGGG  
CGCCTGGCGTGTCTCAGGGTAATGCGGCCTGACGTGTGCGGGTAAGAGGAGAGGCCGCTCCA  
GCAGATGCCCCCCCCGACGCCGCCCCCCCCATCCCCCAACCACGACTTCAAACGCGGGGAAGTG  
TAAAGACAGACTAGTTTtagggcctaaaacaactaaagacgggattagaggaagtccgctaag  
TGGCCTTGAGATGCTGCAGCGAGTCCATGGCGGGACTCAGTGTCTCTGTTACCCCTGGGGGC  
GGGGGGGGGGGAGGGGGGGGTGATACTGTGATACTTGTGTTAAGTTTCCAGTGAGCTGGTAA  
AGTTAAACTACACACATCAAGTTGTTTGCATAAATTATTACACTAAAGAGGCAAAACCCAA  
AGGACGTCCTCAAGAAAACCCTTTCAACAATAAAAGAGGCTGCATTCTCTGTAGTGACCAGCA  
GGGGGCGACTCCTCCGACCCATAGACGTTTATGAGAAGATCCCTCTTCCTCTCTCGATTGAT  
ATATTGTTGGTTGTCAGTCCTCAACACACGCACGCACGCACGCACACACACACACACACC  
TGGTTGAACGTTTTGAAGTGAGCTCTTTGAAGATGGCGTCGCGCTCCTCCACCTCCACCCA  
TCCAGAGGTCTTGAGCT



## S6. DNA sequence alignments

Sequences upstream and downstream of TG-repeats I-III (1 – 3), and *PeIB* sequences (4a-b), aligned to the reference sequence GU130435. S, G and M represent the specimens from Lake Storvatnet, Lake Gjerdhaugvatnet and the marine site, respectively. ‘+’, ‘-’, and ‘\*’ indicate spined, spineless, and asymmetric spined specimens, respectively, from Lake Storvatnet. Ambiguous nucleotide positions are indicated by ‘N’. The number above each alignment represents the position of bases according to the reference. 4c shows the agarose gel bands of *PeIB* amplicons (approximately 1009 bp).

### 1. TG repeat I

#### Upstream to the TG-I repeat

	(129, 082)		(129, 153)
<b>Ref</b>	CACCGAGCCGCTTTGATGTGGGCCTAATATGGCTTGTAAGAAGGGGAACCCTGCACTTTCAAGTGTGCGC		
S03+	.....		
S07+	.....		
S14+	.....		
S24+	.....		
S15*	.....		
S19*	.....		
S23*	.....		
S33*	.....		
S43*	.....		
S30-	.....		
S31-	.....		
S35-	.....		

### 2. TG repeat II

#### Upstream of TG-II repeat

	(129, 805)		(129, 862)
<b>Ref</b>	TATCAAACCAACAAGAACTGTTCAAATGTGTTCAAACAAACAGCGGGACGGGGGAG (TG-II)		
S01+	.....		
S03+	.....		
S04+	.....		
S07+	.....		
S14+	.....		
S24+	.....		
S25+	.....		
S15*	.....		
S19*	.....		
S23*	.....		
S33*	.....		
S34*	.....		
S43*	.....		
S27-	.....		
S30-	.....		
S31-	.....		
S35-	.....		
S37-	.....		
S42-	.....		
G45	.....		
G46	.....		
G47	.....		
G48	.....		
G49	.....		
G50	.....		
G51	.....		
G52	.....		
G53	.....		
G54	.....		
G62	.....		
G67	.....		

M260	..... <b>T</b> .....
M262	.....
M263	.....
M265	..... <b>T</b> .....
M266	..... <b>T</b> .....
M268	..... <b>T</b> .....
M269	..... <b>T</b> .....

### 3. TG-III repeats

#### a. Deletion of unique 58-bp upstream of TG-III repeats found in Lake Storvatnet only

	(129, 974)	(130, 033)
<b>Ref:</b>	GGTCCTAATGCATTCTGTTTGACCTCGCCGGAGTAAATCAAATACTGGCCTCAGATCTCC ( <b>TG-III</b> )	
S04+:	GG-----	
S07+:	GG-----	
S23*:	GG-----	
S33*:	GG-----	
S34*:	GG-----	
S43*:	GG-----	
S27-:	GG-----	
S30-:	GG-----	
S42-:	GG-----	
G53:	.....	
G54:	.....	
M268:	.....	

#### b. Downstream of TG-III repeats

	(130, 134)	(130, 194)
<b>Ref</b>	TGTGTGTGACAGCACGCTAGACACAAGGAAGGAACGGAGGGAGAGAATTAGGAAAGTGGG (60)	
S01+	..... <b>A</b> .....	
S14+	..... <b>A</b> .....	
S43*	..... <b>A</b> .....	
S27-	..... <b>A</b> .....	
G53	..... <b>A</b> .....	
G54	..... <b>A</b> .....	
M269	..... <b>A</b> .....	

	(130, 195)	(130, 254)
<b>Ref</b>	ATAAATGAAGAAAGGAGGCTTATAAAACATTTGAGCTTCC-CGGATTGTTGTGGTCACGC (120)	
S01+	..... <b>A</b> .....	
S14+	..... <b>A</b> .....	
S43*	..... <b>A</b> .....	
S27-	..... <b>A</b> .....	
G53	..... <b>A</b> .....	
G54	..... <b>A</b> .....	
M269	..... <b>A</b> .....	

	(130, 255)	(130, 314)
<b>Ref</b>	CACACGTTGTCATAGCAACCAATAGTCATTTTGTACATCAGCATCATTTGTTCTGGAGAC (180)	
S01+	.....	
S14+	..... <b>G</b> .....	
S43*	.....	
S27-	.....	
G53	.....	
G54	.....	
M269	.....	

4. *PeIB*

a. Upstream of first poly-G tract

	(176,895)	(176,948)
<b>Ref</b>	CATGAGCTCATTCCCCACTAACATGAGGGAGGCCTGGGGGGGGCGAGGGGGCG	
S01+	.....	<b>N</b> .....
S03+	.....	.....
S04+	.....	.....
S23*	.....	.....
S33*	.....	.....
S34*	.....	.....
S30-	.....	<b>N</b> .....
S35-	.....	<b>N</b> .....
S37-	.....	.....
S42-	.....	.....
G45	.....	.....
G46	.....	<b>N</b> .....
G48	.....	.....
G49	.....	.....
G51	.....	.....
G67	.....	.....
M260	.....	<b>A</b> ..... <b>N</b> .....
M265	.....	<b>N</b> .....
M266	.....	.....
M268	.....	<b>N</b> .....
M269	.....	<b>N</b> .....

b. Downstream of last poly-G tract

	(177,313)	(177,378)
<b>Ref</b>	GGGGGGGGTGATACTGTGATACTTGTGTAAAGTTTCCAGTGAGCTGGTAAAGTTAAACTACACAC	
S03+	.....	<b>G</b> .....
S23*	.....	<b>G</b> .....
S37-	.....	<b>G</b> .....
G45	.....	<b>G</b> .....
M269	.....	<b>G</b> .....

c. Agarose gel of *PeIB* amplicons (1009 bp)

