Additional File 11. Sex determination by PCR.

DNA was extracted with the DNA Purification System Kit (High pure PCR template preparation kit (Roche). The sex chromosome genes *zinc finger protein X-linked* (*ZFX*) and *zinc finger protein Y-linked* (*ZFY*) located on the X and Y chromosomes respectively, were amplified by PCR as previously described by Weiss and Johnston (1999).

Briefly, the PCR mix contain the forward primer ZF_F and two reverse primers specific of the X (ZFX_R) and Y (ZFY_R) chromosomes. Thus, one amplicon of 488 base pairs (bp) (X chromosome specific) is amplified in XX females and two amplicons of 488 and 340 bp (Y chromosome specific) in XY males.

Primer	**Forward sequence (5´-3´)**
ZF_F	ATTGTTCTAAGTCGCCATATTCTCT
ZFX_R	GAACACACTACTGAGCAAAATGTATA
ZFY_R	CATCTTTACAAGCTTGTAGACACACT

The PCR reaction was performed with 2.5µl of 10x Reaction Buffer (Biotools) with a final concentration of 1x, 0.3µM of each of the three primers, 1mM of dNTPs, 2mM of $MgCl^{2+}$, 0.6 U of DNA Polimerase (Biotools) and 50ng of DNA in a final volume of 25ul.

The thermal cycling profile was initiated with 95 ◦C enzyme activation incubation for 3 minutes. Next 35 cycles of 94 ◦C for 15 seconds (s), 60.1 ◦C for 30 s and 72 ◦C for 30 s. After cycling a final elongation was run 7 minutes at 72 ◦C. Sex discrimination was performed by electrophoresis in agarose gels at 2.5%.