

Supplemental Fig. 1: PCR validation of male-specific amplification of sex-linked markers in *Tachyglossus aculeatus*. The marker *AMH* was validated twice in two locations, North America (US) and Australia (AUS), using seven males/females and three males/females, respectively. PCRs were amplified for 32 cycles with annealing temperatures of 54°C. The marker 16S was used to confirm proper amplification of all samples without any sex-specific pattern of amplification in the US Lab. The marker *CRSPX* was used to confirm proper amplification of all samples in the AUS lab.

