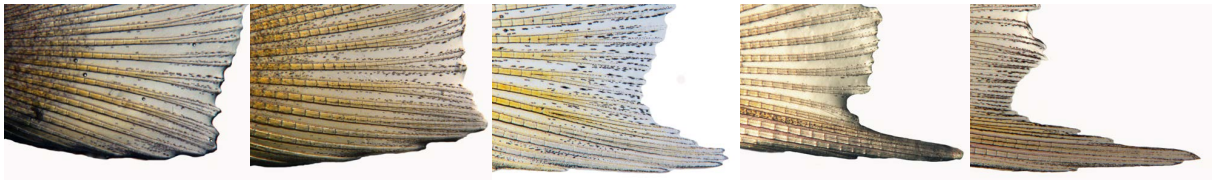
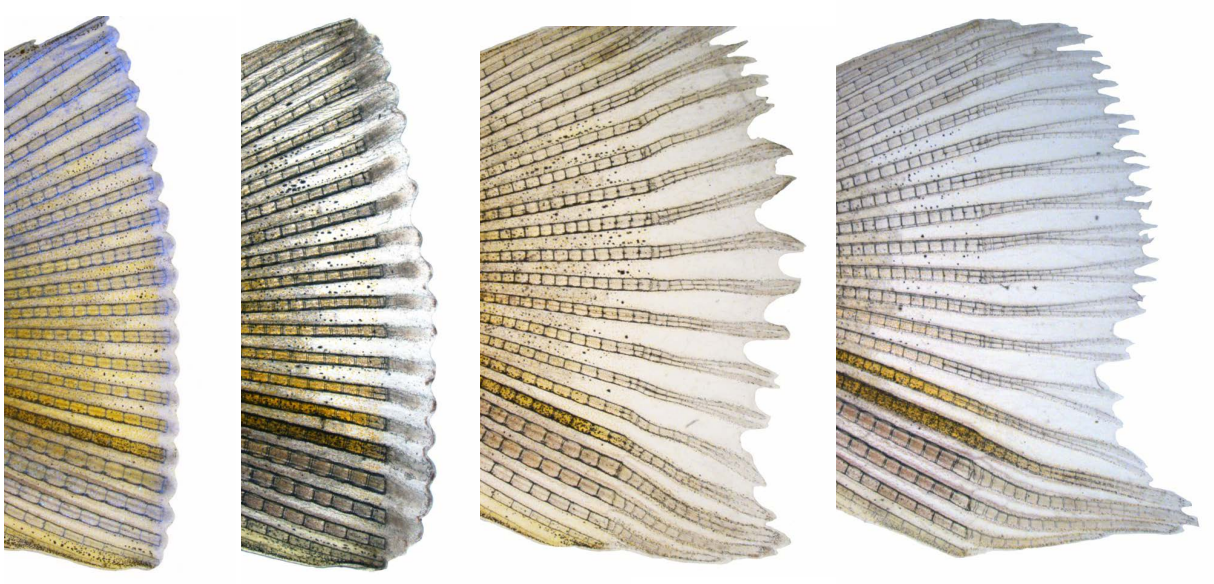
A



Stage 1 Stage 2 Stage 3 Stage 4 Stage 5

B



C

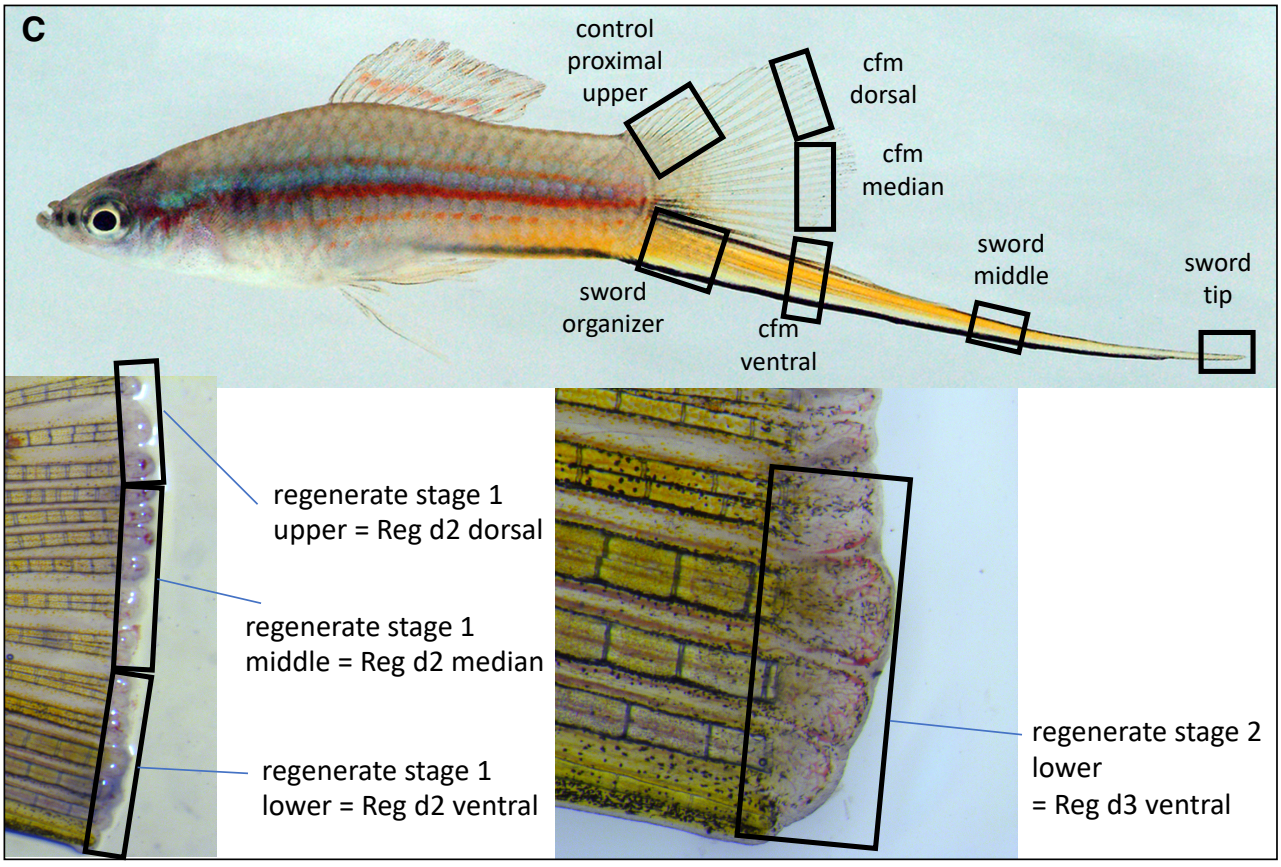
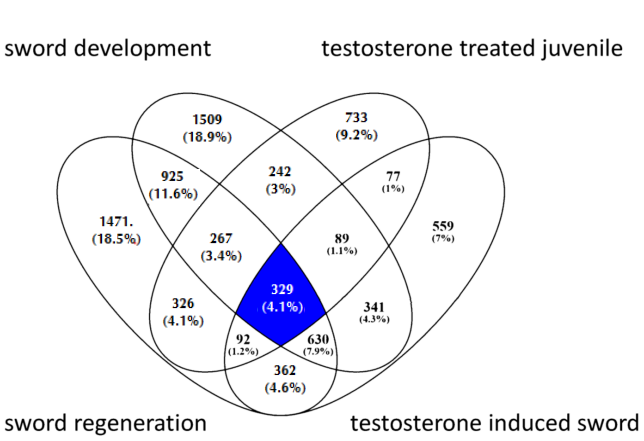
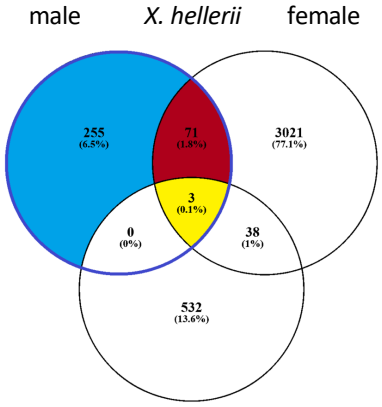


Figure S1. Stages of normal sword development, regeneration and sampled compartments, related to Figure 2-4, 6, and STAR Methods section "Experimental animals". (A) Stages of normal sword development in *Xiphohorus he*llerii males during puberty. Stage 1 is reached, depending on the genotype at the *P*-locus, between 2.5 and 5 months of age, stage 2 is 3-5 days after onset of puberty, stage 3 8-12 days, stage 4 14-18 days and stage 5 20-24 days on average. **(B)** Stages of sword regeneration in *Xiphophorus hellerii* males. Stage 1: day 0, stage 2: 2-4 days after amputation (daa), stage 3: 5-7 daa, stage 4: 8-10 daa. **(C)** Compartments used for sampling (indicated by black boxes). Top, regions of the tail fin taken for amputation. Cfm. Caudal fin margin. Bottom left, regenerate blastema at 2 dpa and regions taken for RNA extractions. Bottom right, regenerate blastema at 3dpa, the ventral part (boxed) starts to grow more than median and dorsal.

A



B



X. maculatus, male

C

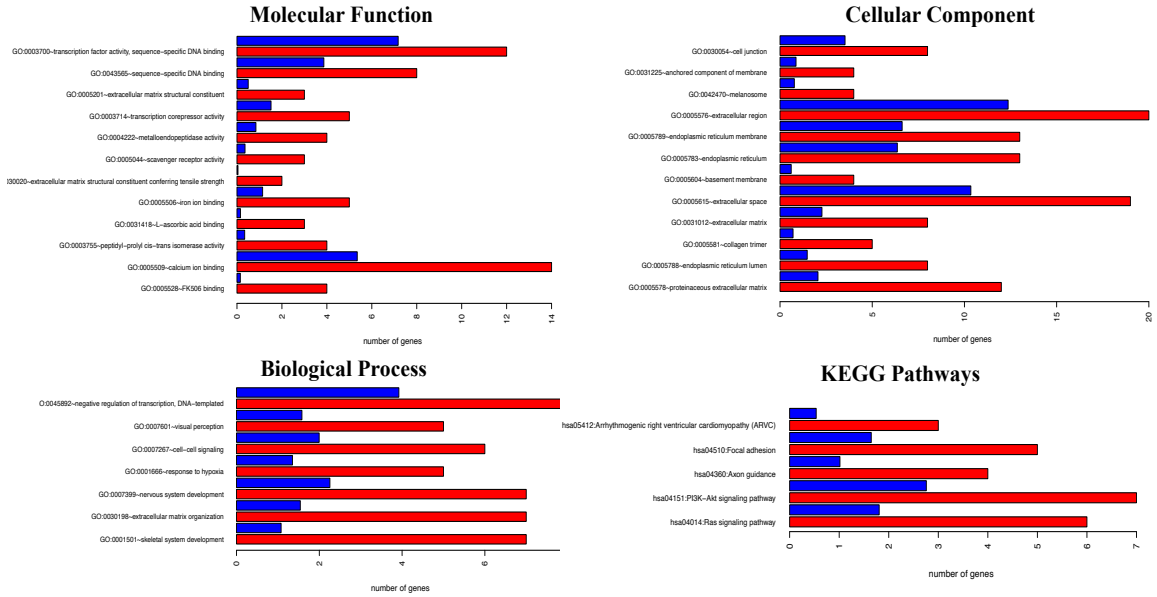


Figure S2. Differentially expressed genes in caudal fin transcriptomes, related to Figures 2-4, 6 and STAR Methods section "Differential gene expression analysis". (**A**) Venn diagram for numbers of differentially expressed genes with log2FC>=1 between upper and lower caudal fin margin during natural sword development (stage 1-5), sword regeneration (days 0-10), testosterone induced sword in females and testosterone treated juvenile *Xiphophorus hellerii*. 329 DEGs are common to all datasets (blue field, see table S1). (**B**) Venn diagram for numbers of differentially expressed genes with log2FC>=1 from the sword transcriptome (blue field in (A)), female *X. hellerii* and male *X. maculatus* caudal fin regeneration. From the sword transcriptome of *X.hellerii* 74 DEGs overlap with female (red and yellow fields, see table S1) and 3 with *X. maculatus* male (yellow field, see table S1). **(C)** Barplots of enriched gene ontology (GO) terms in the sword transcriptome for Molecular Function, Cellular Component, Biological Process, and KEGG pathways for genes related to sword development. Blue bars indicate the number of expected genes, red indicate the number of observed genes. Significance of GO-term enrichment is decreasing from bottom to top.

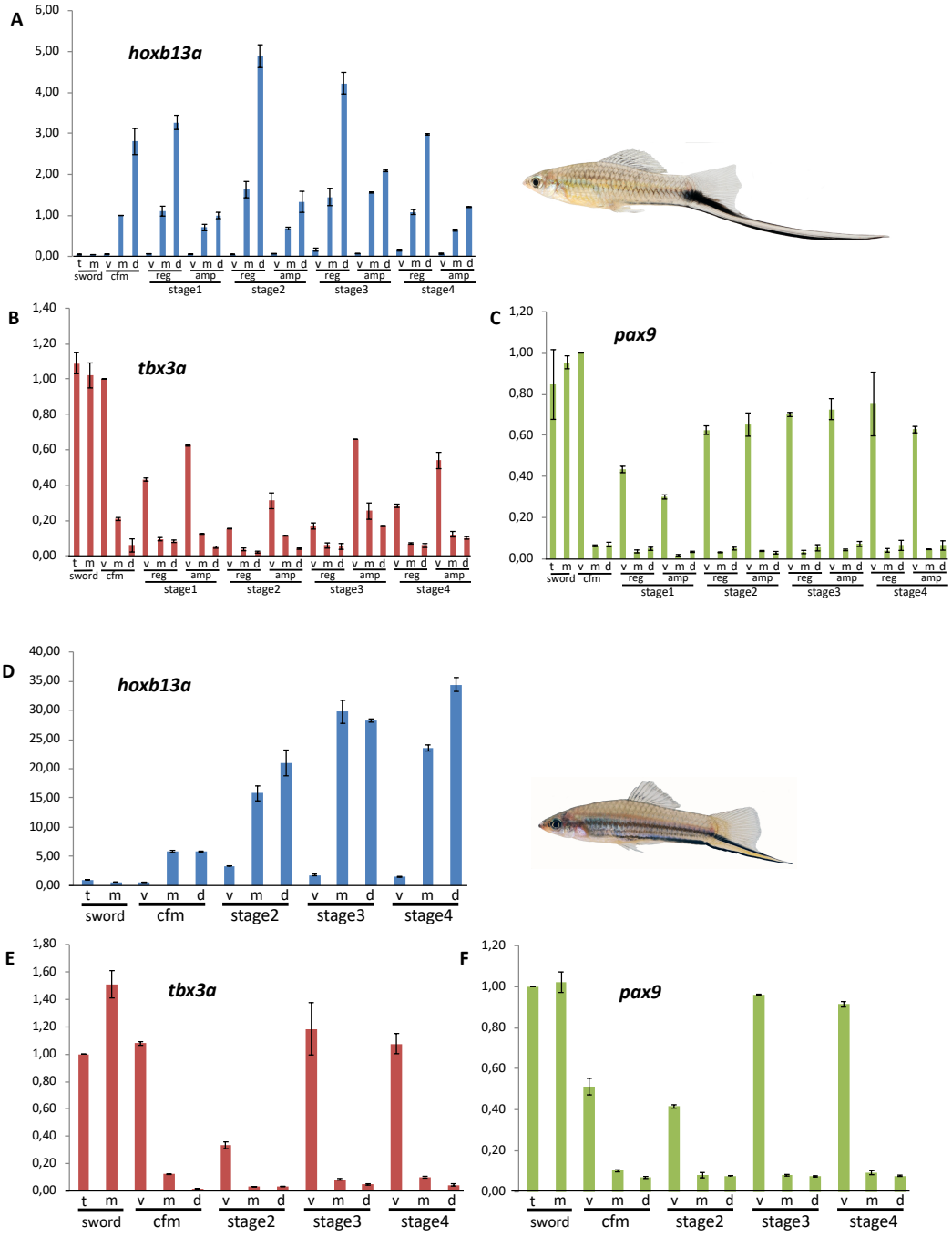


Figure S3. Spatial expression pattern of transcription factor genes in the caudal fin and sword of *Xiphophorus montezumae* (A-C) and *Xiphophorus monticolus* (D-F) males, related to Figure 2 and STAR Methods section "qPCR expression analysis". Expression of transcription factor genes *hoxb13a* (**A. D**)*, tbx3a* **(B, E**) and *pax9* (**C, F**) in the caudal fin margin of the tail fin (cfm), the median sector (m) and tip (t) of the sword and during sword regeneration (v, ventral, m, median, d, dorsal compartment) in the regenerating tissue (reg) and the compartment proximal to the regenerate (amp). Vertical axis indicates fold change of expression normalized to cfm, v (B, C) or cfm, m (A) or to sword, t (D-F). Data are represented as mean ± SEM.

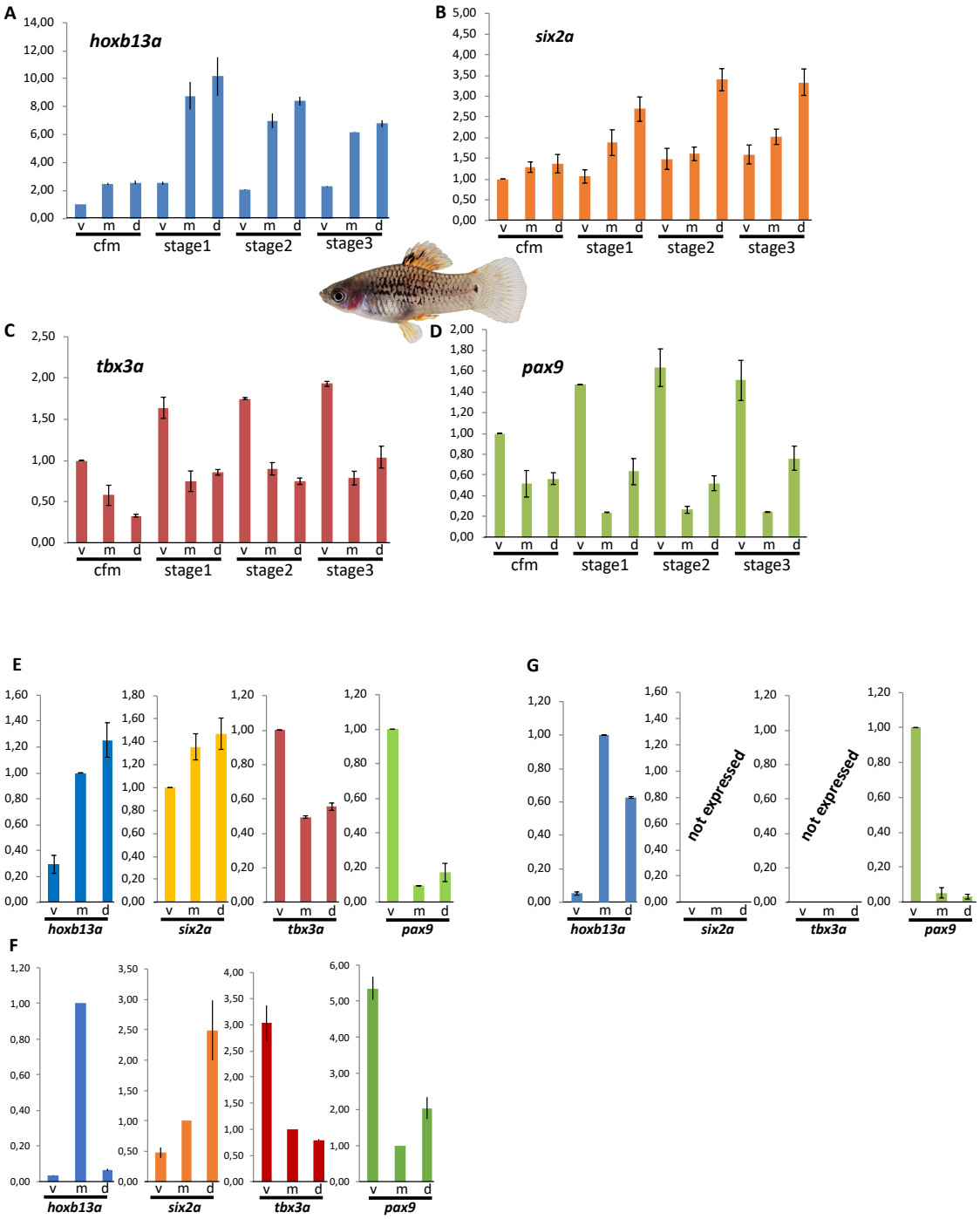


Figure S4. Comparison of transcription factor expression patterns in males of different species, related to Figure 2, 3 and STAR Methods section "qPCR expression analysis". (A-D) Spatial expression pattern of transcription factor genes in the caudal fin of *Xiphophorus maculatus* males. Expression of transcription factor genes *hoxb13a* (A), *six2a* (B), *tbx3a* (C) and *pax9* (D) in the caudal fin margin of the tail fin (cfm) and during tail fin regeneration (v, ventral, m, median, d, dorsal compartment). Vertical axis indicates fold change of expression normalized to cfm, v. Expression of *hoxb13a*, *six2a*, *tbx3a* and *pax9* in the caudal fin margin of the tail fin (cfm) of (**E**) adult pygmy swordtails, *Xiphophorus pygmaeus*, (**F**) *Priapella lacandonae* and (**G**) medaka, *Oryzias latipes*. v, ventral, m, median, d, dorsal compartment. Vertical axis indicates fold change of expression normalized to cfm, m, except for medaka *pax9* and *X. pygmaeus six2a*, *tbx3a* and *pax9*, cfm, v. Data are represented as mean ± SEM.

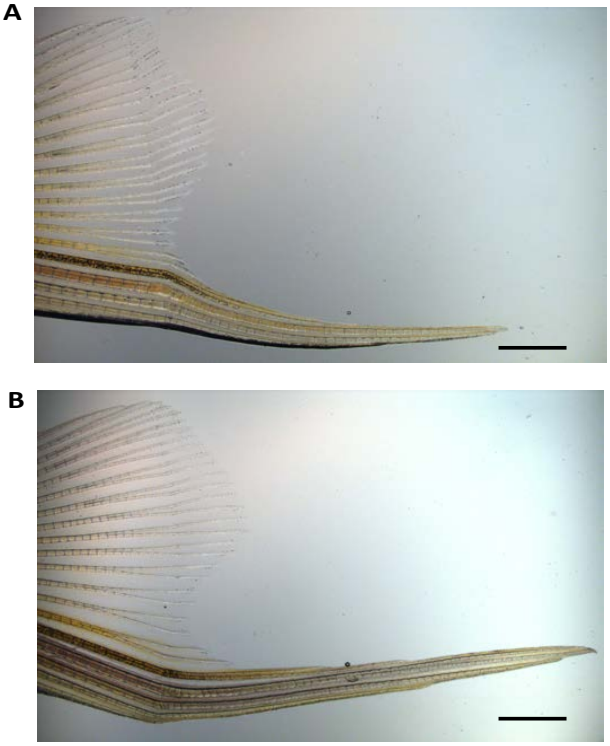


Figure S5: Regeneration of the sword in the presence of potassium channel modulator, related to Figure 7 and STAR Methods section "Experimental Animals". (**A**) Sword regeneration after 50 days of treatment with 1µM 4-aminopyridine. 38 +/- 2 bony elements were added to sword fin ray V8. No reduction of length was observed for non-sword fin rays. (B) Control, 51+/-3 bony elements were added to V8. Bar represents 2 mm.

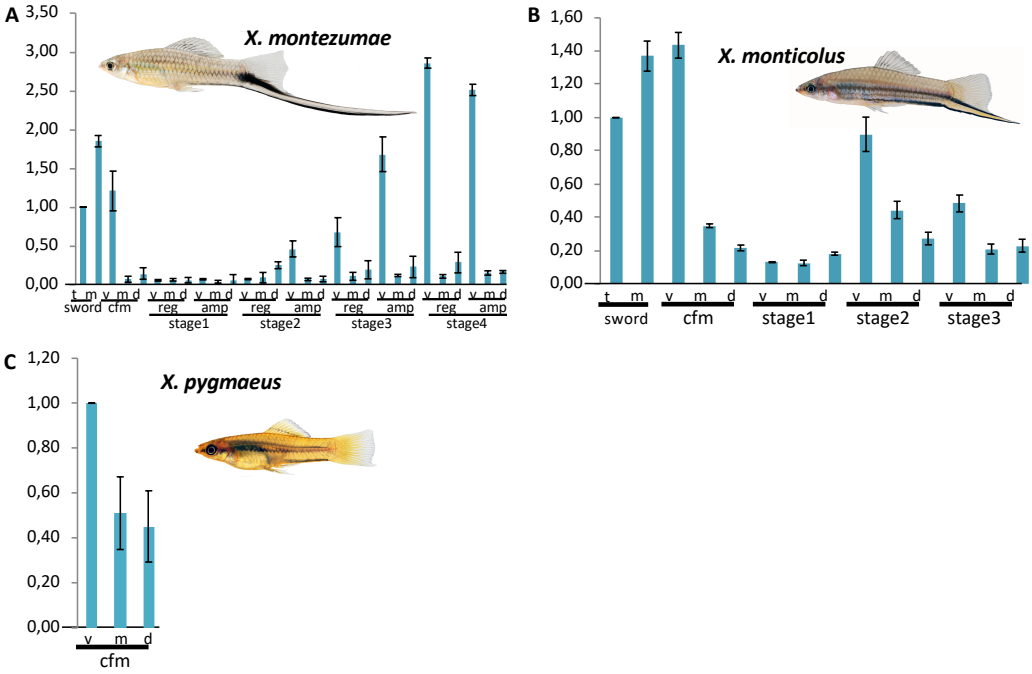
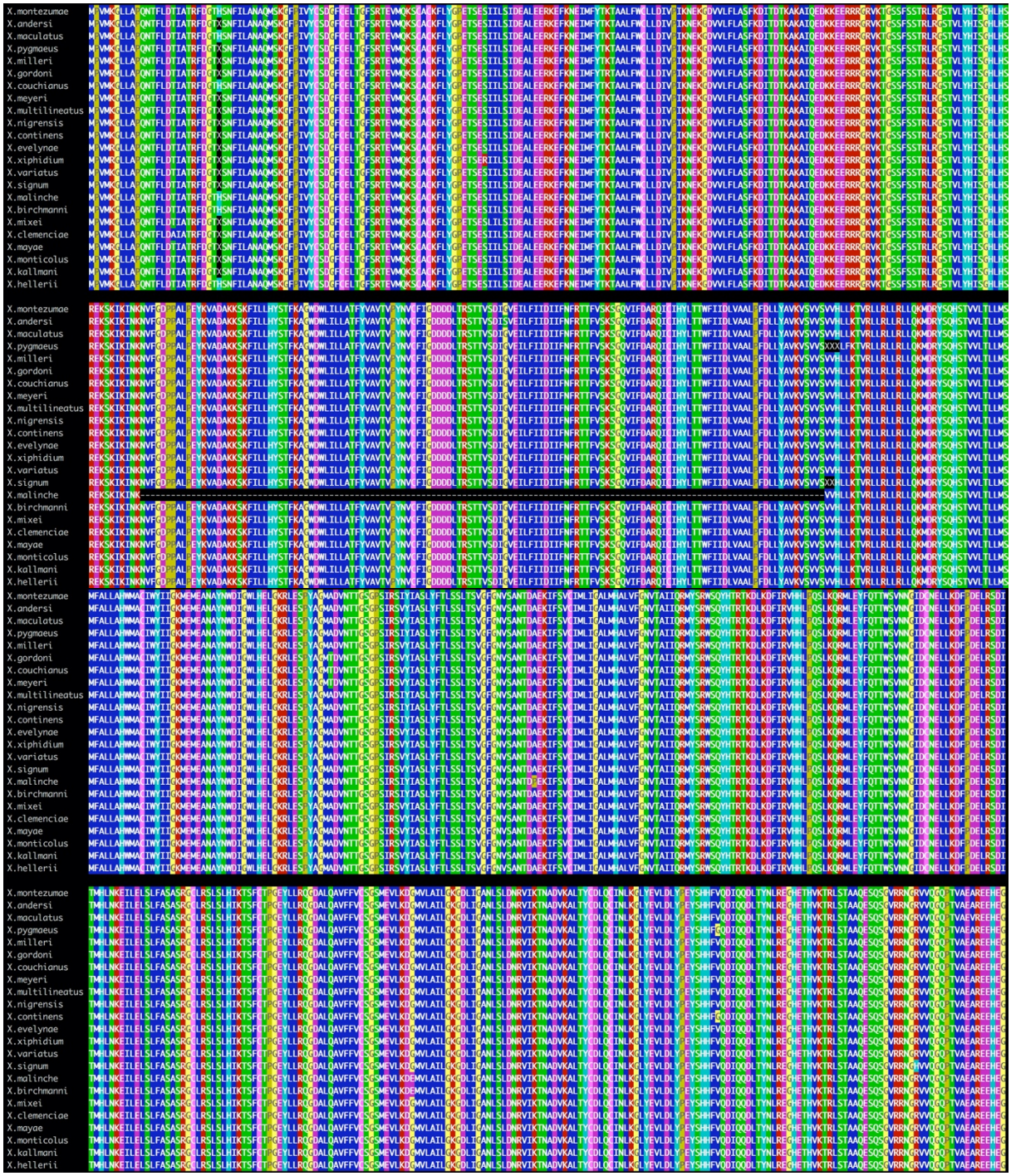


Figure S6: Spatial expression pattern of *kcnh8* in the caudal fin of *Xiphophorus* species, related to Figure 6 and STAR Methods section "qPCR expression analysis". Expression in the caudal fin margin of the tail fin (cfm) of adult *Xiphophorus montezumae* (**A**), *X. monticolus* (**B**), and *X. pygmaeus* (**C**) males, the median sector (m) and tip (t) of the sword and during sword regeneration (v, ventral, m, median, d, dorsal compartment). Vertical axis indicates fold change of expression normalized to sword, t (A), (B) and cfm, v (C). Data are represented as mean ± SEM.


X.montezumae
X.andersi
X.maculatus
X.pygmaeus
X.milleri
X.gordoni
X.couchianus
X.meyeri
X.multilineatus
X.nigrensis
X.continens
X.evelynae
X.xiphidium
X.variatus
X.signum
X.malinche
X.birchmanni
X.mixei
X.clemenciae
X.mayae
X.monticolus
X.kallmani
X.hellerii

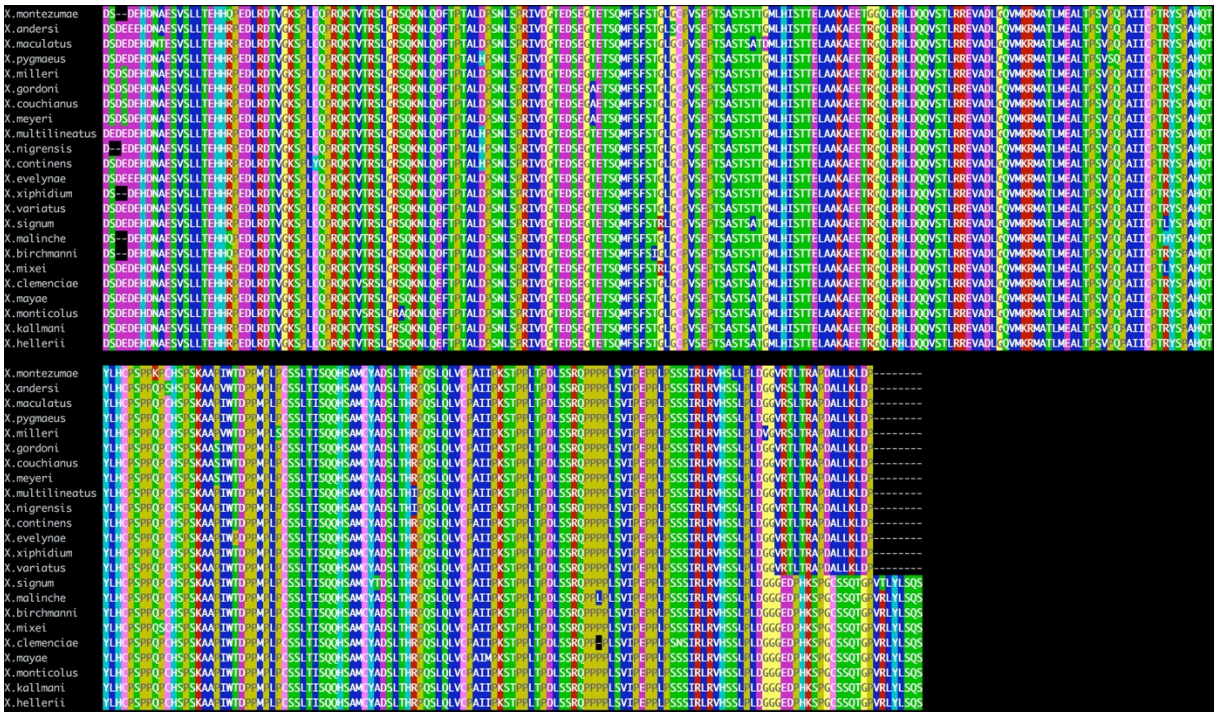


Figure S7: Alignment of protein sequences of Kcnh8 from 23 *Xiphophorus* species (of 26), related to Figure 6. The missing sequence from *X. malinche* (corresponding to one exon) is most likely due to a misassembly. - , missing aminoacid, X, ambiguous assembly position.