**Supplementary Files Description**

S1: All fluorescent and nonfluorescent species from new observations and literature search.

S2: All fluorescent species found from new observations with sample size and collection location.

S3: Genes used and accession numbers from GenBank.

S4: R code for time calibrations and converting maximum likelihood tree to ultrametric for Beast analysis.

S5: Maximum likelihood tree including all species. Error bars represent 95% HPD for time estimations at each node.

S6: R code for ancestral state reconstructions on the maximum likelihood tree.

S7: R code for ancestral state reconstructions on the Rabosky et al. 2018 tree.

S8: Rabosky et al. 2018 phylogeny with ancestral state reconstructions of biofluorescence absence and presence.

S9: Mean node ages and 95% HPD intervals of the maximum likelihood Actinopterygii phylogeny.

S10: Cumulated number of actinopterygian biofluorescent families (A) and species in families with biofluorescent representatives (B) over time. The dashed line marks the K-Pg boundary.

Gene Length

COI: 656 bp

Glyt: 883 bp

Myh6: 735 bp

Plagl2: 696 bp

Rag1: 1419 bp

Zic1: 905 bp

Cytb: 1149 bp

Iqtree code

/Users/emilycarr/Desktop/BiofluorTreeOgco\_New/iqtree2 -s AllGenes\_Ogco.fas -spp Partitions.nex -pre AllGenes\_Ogco.fas -m TEST -bb 1000

Time calibration in Beauti

<https://sites.google.com/view/sanchez-ramirez-s/programming/tutorial3>