

Lighting Up Water: Characterizing the origin and diversification of the light organ in the bioluminescent ostracod *Vargula hilgendorffii*

Murad Jah¹, Lisa Mesrop¹ & Todd Oakley²

University of Southern California¹, University of California, Santa Barbara^{1,2}



This study characterizes the origin and diversification of transcription factors that differentiate secretory cell types in Metazoan organisms.

Background

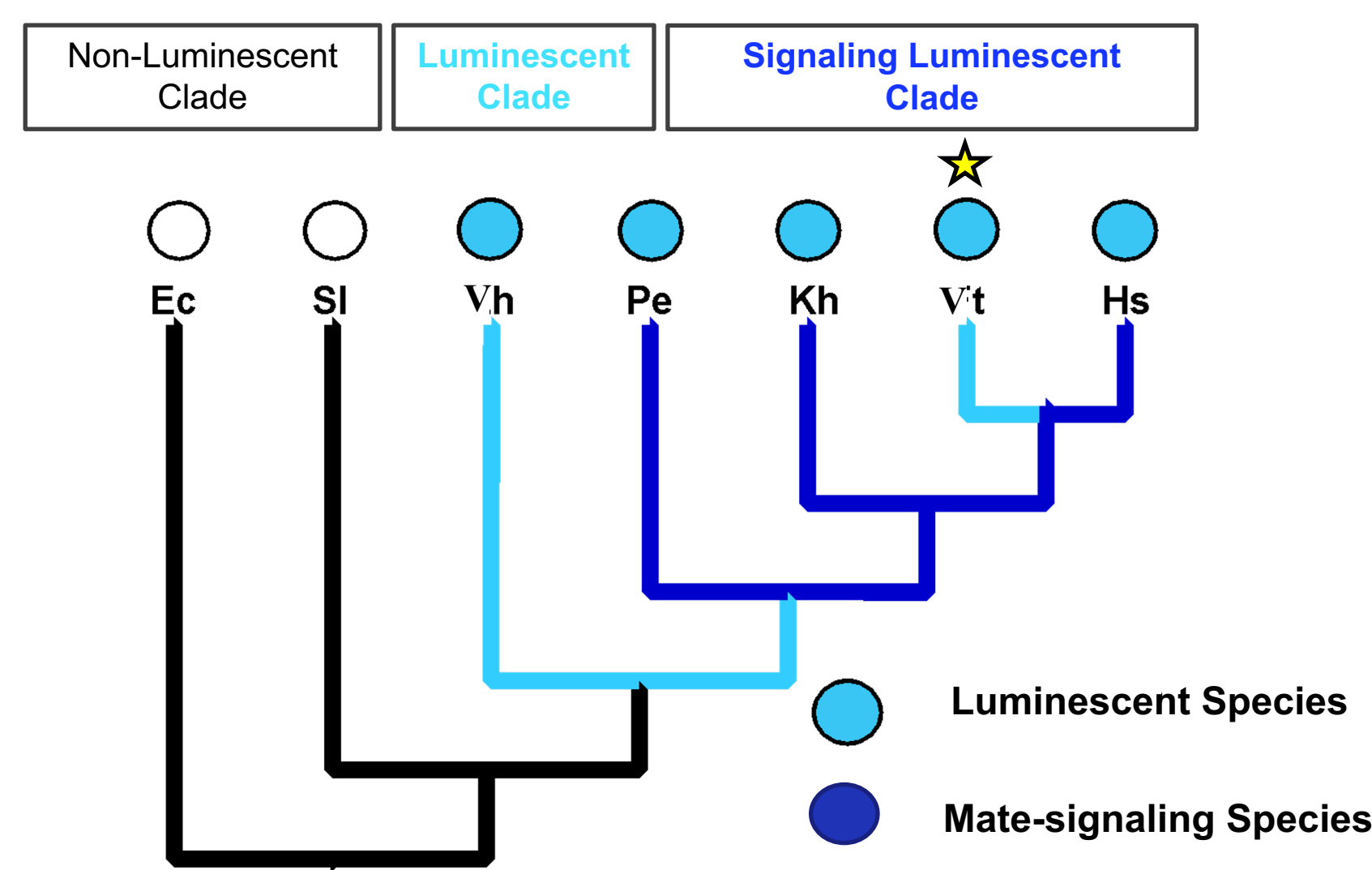
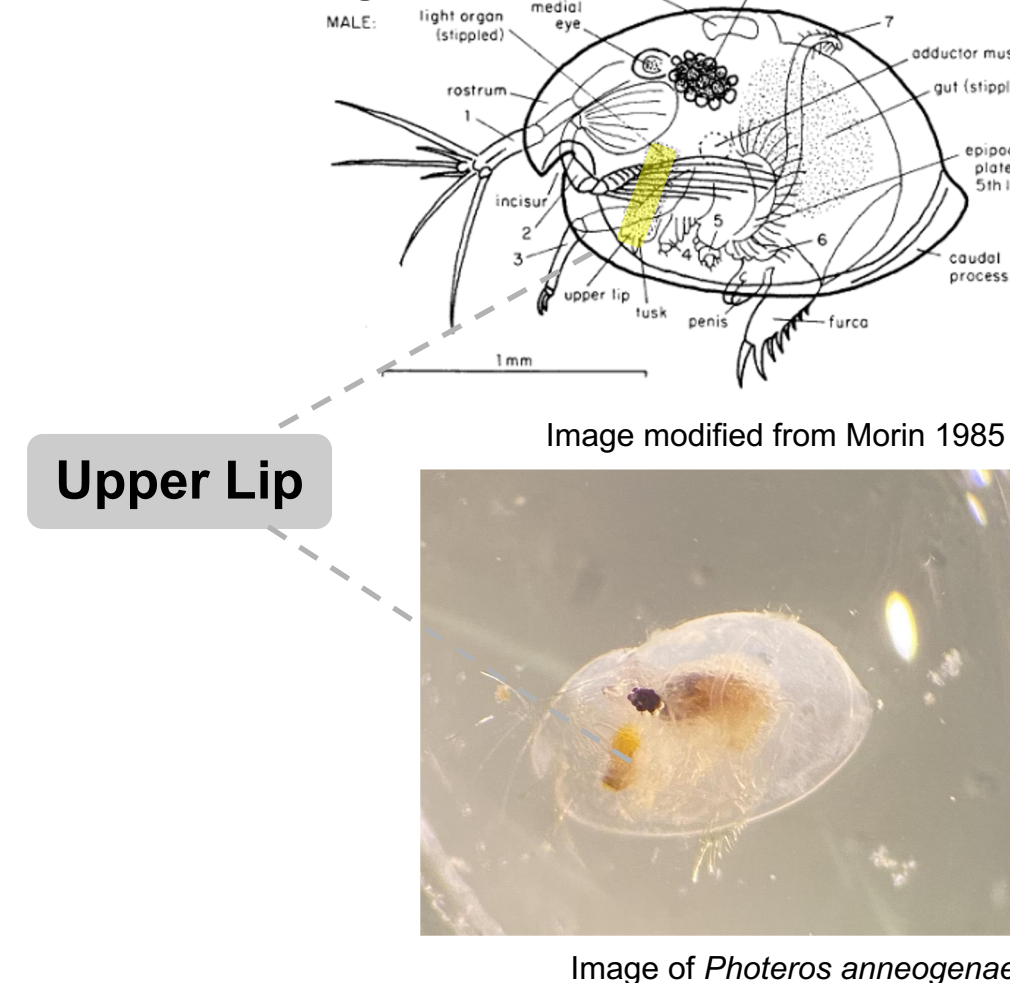


Figure 1: Ec, *Euphilomedes carcharodonta*; Sl, *Skosbergia lemeri*; Vh, *Vargula hilgendorffii*; Pe, *Photeros EGO*; Ft, *Vargula Tsujii*; Kh, *Kornickeria hastingsi*; Hs, H-group SVU. Study species.

- It is well known from developmental studies that transcription factors control cell differentiation.
- Transcription factors interact with one another and other regulatory elements such as cis-regulatory elements to regulate gene expression.
- The Gene Regulatory Network (GRN) determines the identity of the cell. The GRN is thought to be composed of transcription factors.
- Understanding the evolution of a novel complex trait such as the light organ requires first to identify the components of the GRN and their evolutionary history.



- Evolution of the upper lip in ostracods within the Cypridinidae family offers an opportunity to investigate cell-type origination.
- The upper lip is used for bioluminescence and luminous courtship signaling.
- Luminescent ostracods emit light producing compounds from a light organ within the upper lip.
- Light reaction involves a substrate, luciferin, and a luminescence enzyme, luciferase, each of which is synthesized in separate gland cells and discharged in a mucus-like substance from the upper lip¹.
- The mucus also contains digestive enzymes which may be secreted from gland cells in the upper lip or exported from the gut.

Research Question

- What are the identities of transcription factors that are responsible for the differentiation of exocrine secretory cells?
- What is the history of the transcription factors involved in secretory cell differentiation?
- How can this help us understand the origin of the light organ in the bioluminescent ostracod *Vargula hilgendorffii*?

Methods

- Performed a literature survey to identify transcription factors known to differentiate secretory cells or glands in Metazoan organisms.
- Downloaded the FASTA protein sequences of our transcription factors of interest, MIST1, SREBP1 and TBX19, from NCBI.
- Built a computational pipeline to run the BLAST software on the command line.
- Searched the protein sequence against our custom genome database that includes annotated genomes from animals and ostracod transcriptomes to determine if any orthologs of our transcription factor of interest is also present in the upper lip of ostracods. Species of ostracod transcriptomes in our study are highlighted with star in Figure 1.
- Using the homologs generated from the BLAST output, we will build a phylogenetic tree using the IQTree program.
- The tree will then be viewed and analysed in iTOL viewer.

Results & Conclusions

Developed a computational pipeline to use the BLAST software on the command line.

```
# Step1: Blast our transcription factor sequence against our custom database.
blastn -query Sex_comb_TF.fa -db
/home/lmesrop/secretory_cell_tfs/050520_mist1_genomedb_fixed/databaseBL
AST -evaluate 1e-10 -num_threads 4 -outfmt '6 sacc' | uniq >
sex_comb_TF_blast_e10.txt
```

```
#Step 2: Retrieving fasta sequences from our custom database.
blastdbcmd -db
/home/lmesrop/secretory_cell_tfs/050520_mist1_genomedb_fixed/databaseBL
AST -dbtype prot -entry_batch sex_comb_TF_blast_e10.txt -outfmt %f -out
sex_comb_TF_blast_e10_fasta.fasta
```

```
#Step 3: Replace the spaces in the fasta headers with an underscore; this will
be easier to annotate the tree later.
sed 's/ /_/' sex_comb_TF_blast_e10_fasta.fasta >
sex_comb_TF_blast_e10_fasta_underscore.fasta
```

```
#Step 4: Align the sequences using the mafft software.
mafft sex_comb_TF_blast_e10_fasta_underscore.fasta >
sex_comb_TF_blast_e10_fasta_underscore_mafft.fasta
```

```
#Step 5: Run the IQtree.
iqtree -s sex_comb_TF_blast_e10_fasta_underscore_mafft.fasta
```

```
#Step 6: Submit iqtree job.
sbatch iqtree_murad.sh
```

```
#Step 7: View our gene tree in iTOL.
```

Phylogenies of transcription factors known to differentiate secretory cell types.

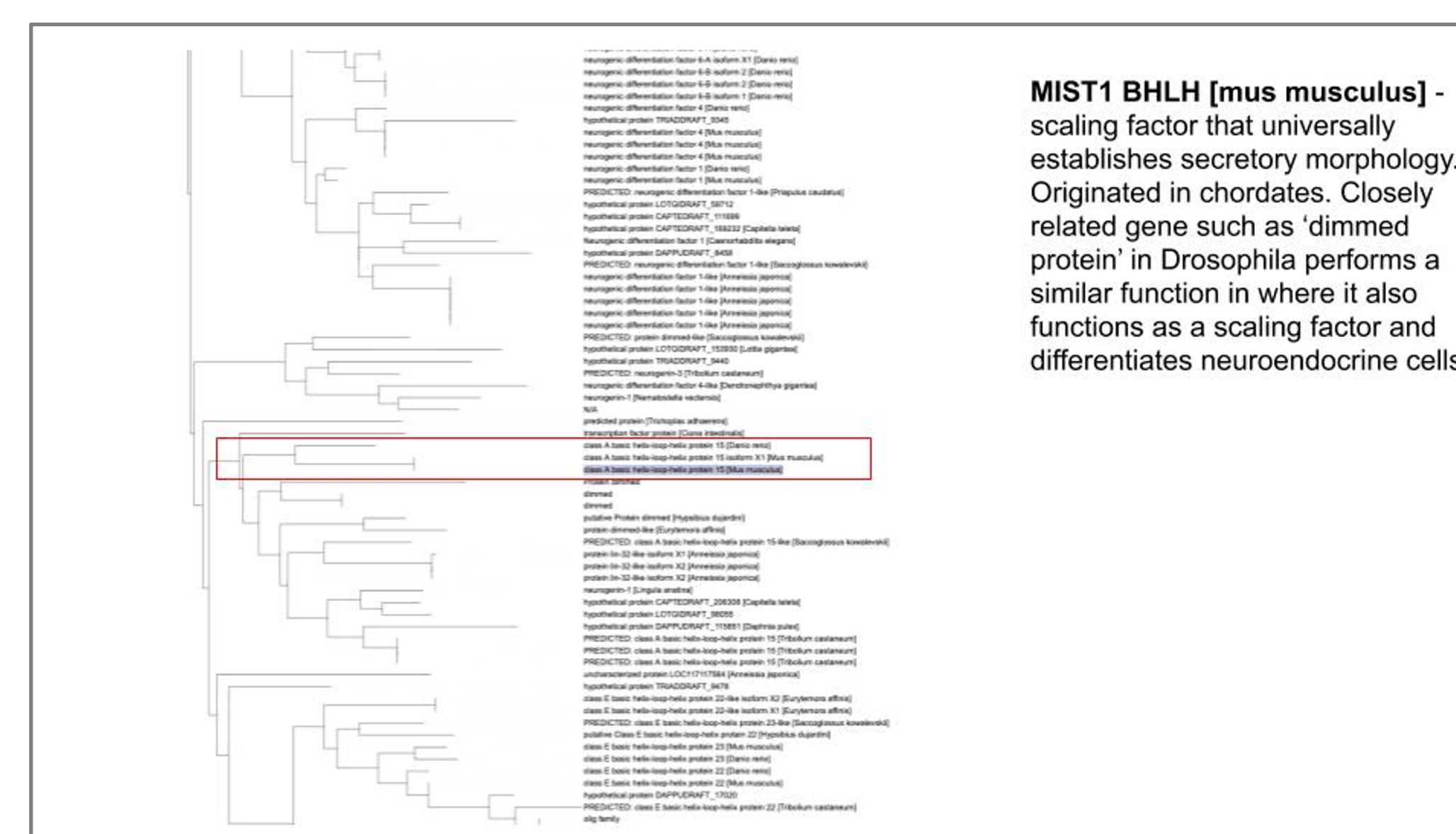


Figure 2: Phylogeny of the MIST1 transcription factor. MIST1 highlighted in purple. Red bracket indicates the clade that contains MIST1. MIST1 originated in Chordates.

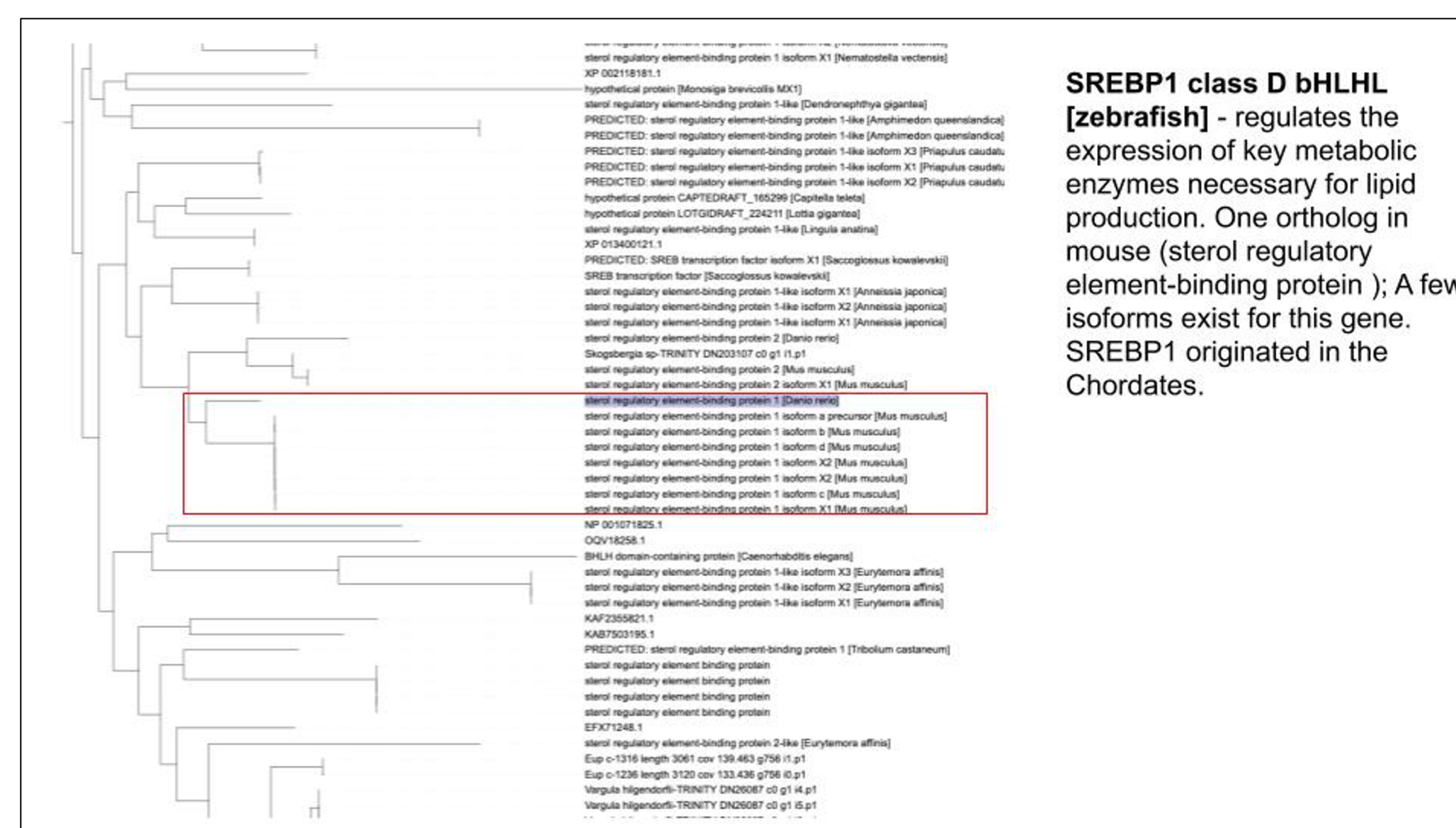


Figure 3: Phylogeny of the SREBP1 transcription factor. SREBP1 highlighted in purple. Red bracket indicates the clade that contains SREBP1. SREBP1 originated in Chordates.

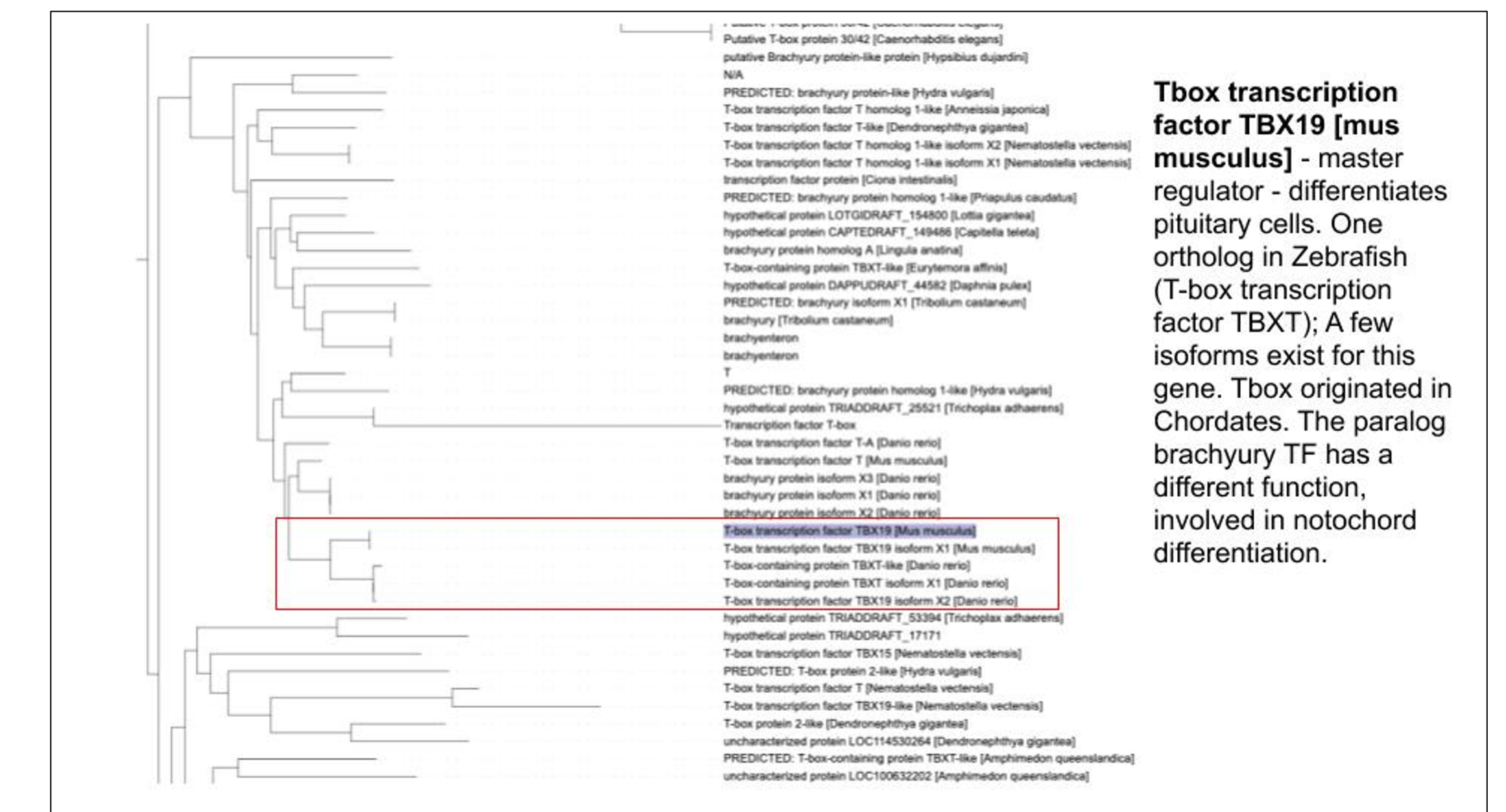


Figure 4: Phylogeny of the TBX19 transcription factor. TBX19 highlighted in purple. Red bracket indicates the clade that contains TBX19. TBX19 originated in Chordates.

- The MIST1, SREBP1 and TBX19 transcription factors are known to differentiate and establish the secretory phenotype of secretory cells and glands in well studied model systems such as mouse and zebrafish.
- Understanding whether the orthologs of these transcription factors are also present in the upper lip of ostracods will reveal important information about the diversification of secretory cell types in the light organ of bioluminescent Ostracods.
- The MIST1, SREBP1 and TBX19 transcription factors originated in vertebrates. We did not find the orthologs of these genes in other animals suggesting that the identity of transcription factors that differentiate and establish secretory cells in the light organ of ostracods might be different.

Future Work

What cell types are responsible for the production of luciferase and luciferin?

From what ancestral cell types did luciferase and luciferin producing cell types evolve from?

How did these bioluminescent cell types arise in evolution?

How can we apply this same framework to study the origin of other complex phenotypes such as eye vision in Mantis Shrimp.

References

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Acknowledgements

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