# OPSINS in the DARK…

## Characterizing "light sensitive" genes in the Brazilian eyeless cavefish, *Phreatobius cisternarum*

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## What are **opsins**?

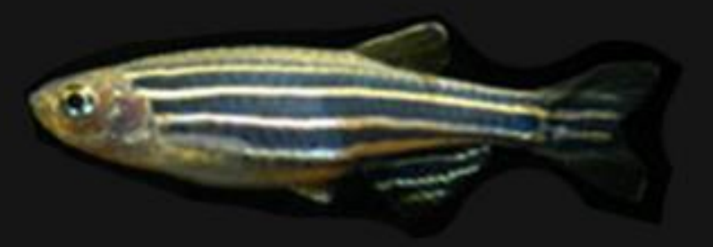
Opsins are light sensitive transmembrane proteins, with the best-known **visual opsins** found in rod and cone cells of the eyes. There are also **non-visual opsins** found in tissues not normally exposed to light such as the brain, kidney, and heart. Recent studies have reported opsins may have non-light functions such as **heat detection** in mammalian sperm[1] and **locomotion** in larval *Drosophila*[2].

## Hypothesis

To identify specific opsins with important roles other than light sensitivity, we hypothesize these genes would still be **expressed** in the blind eyeless subterranean fish (*Phreatobius cisternarum*) that have **evolved in the dark** for millions of years.

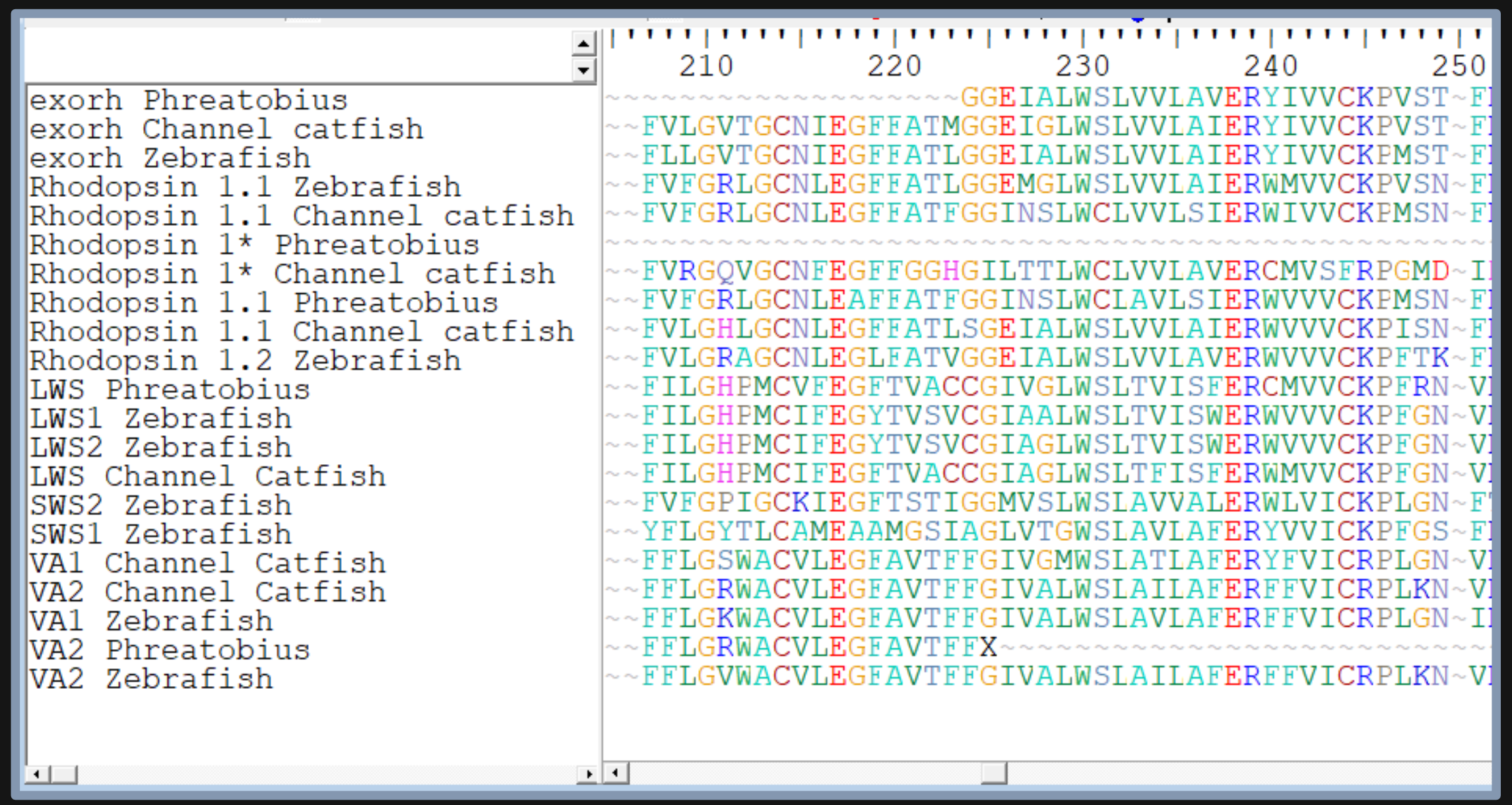
We also compare these genes to opsins of a surface relative, Channel catfish (*Ictalurus punctatus*), and Zebrafish (*Danio rerio*) by characterizing intron/exon boundaries and nucleotide substitutions.

## Methods



```
C:\Users\Pufferfish>blastall -p tblastx -i /home/taylorjs/data/Danio_rerio
_opsins_cds.fas -d ~/data/Phreatobius/data/Trinity_Phreatobius.fasta -e 1E
-6 -b 10 -v 10 -o /home/taylorjs/OUTPUT_for_Phreatobius_opsins.xml -m 7
```

PBS script of a blast search using 42 Zebrafish opsins as query sequences against the *Phreatobius* transcriptome. The same was done on a surface relative, the Channel Catfish transcriptome as comparison.



```
                               210       220       230       240       250
exorh Phreatobius              ~~~~~~~~~~~~~~~~~~GGEIALWSLVVLAVERYIVVCKPVST~F
exorh Channel catfish          ~~FVLGVTGCNIEGFFATMGGEIGLWSLVVLAIERYIVVCKPVST~F
exorh Zebrafish                ~~FLLGVTGCNIEGFFATLGGEIALWSLVVLAIERYIVVCKPMST~F
Rhodopsin 1.1 Zebrafish        ~~FVFGRLGCNLEGFFATLGGEMGLWSLVVLAIERWMVVCKPVSN~F
Rhodopsin 1.1 Channel catfish  ~~FVFGRLGCNLEGFFATFGGINSLWCLVVLSIERWIVVCKPMSN~F
Rhodopsin 1* Phreatobius       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Rhodopsin 1* Channel catfish   ~~FVRGQVGCNFEGFFGGHGILTTLWCLVVLAVERCMVSFRPGMD~I
Rhodopsin 1.1 Phreatobius      ~~FVFGRLGCNLEAFFATFGGINSLWCLAVLSIERWVVVCKPMSN~F
Rhodopsin 1.1 Channel catfish  ~~FVLGHLGCNLEGFFATLSGEIALWSLVVLAIERWVVVCKPISN~F
Rhodopsin 1.2 Zebrafish        ~~FVLGRAGCNLEGLFATVGGEIALWSLVVLAVERWVVVCKPFTK~F
LWS Phreatobius                ~~FILGHPMCVFEGFTVACCGIVGLWSLTVISFERCMVVCKPFRN~V
LWS1 Zebrafish                 ~~FILGHPMCIFEGYTVSVCGIAALWSLTVISWERWVVVCKPFGN~V
LWS2 Zebrafish                 ~~FILGHPMCIFEGYTVSVCGIAGLWSLTVISWERWVVVCKPFGN~V
LWS Channel Catfish            ~~FILGHPMCIFEGFTVACCGIAGLWSLTFISFERWMVVCKPFGN~V
SWS2 Zebrafish                 ~~FVFGPIGCKIEGFTSTIGGMVSLWSLAVVALERWLVICKPLGN~F
SWS1 Zebrafish                 ~~YFLGYTLCAMEAAMGSIAGLVTGWSLAVLAFERYVVICKPFGS~F
VA1 Channel Catfish            ~~FFLGSWACVLEGFAVTFFGIVGMWSLATLAFERYFVICRPLGN~V
VA2 Channel Catfish            ~~FFLGRWACVLEGFAVTFFGIVALWSLAILAFERFFVICRPLKN~V
VA1 Zebrafish                  ~~FFLGKWACVLEGFAVTFFGIVALWSLAVLAFERFFVICRPLGN~I
VA2 Phreatobius                ~~FFLGRWACVLEGFAVTFFX~~~~~~~~~~~~~~~~~~~~~~~~~~~
VA2 Zebrafish                  ~~FFLGVWACVLEGFAVTFFGIVALWSLAILAFERFFVICRPLKN~V
```

**Figure 1.** Alignment of opsins obtained from the blast search. Amino acids shown from opsins of Zebrafish, Channel Catfish, and *Phreatobius* in the sequence editing program BioEdit . Some *Phreatobius* sequences are fragmented (*Exorh*, *Rhodopsin 1.**, *VA2*) compared to the complete Zebrafish and Channel Catfish opsins. Intron and exon boundaries were described, and introns were removed from this amino acid alignment.

## Results

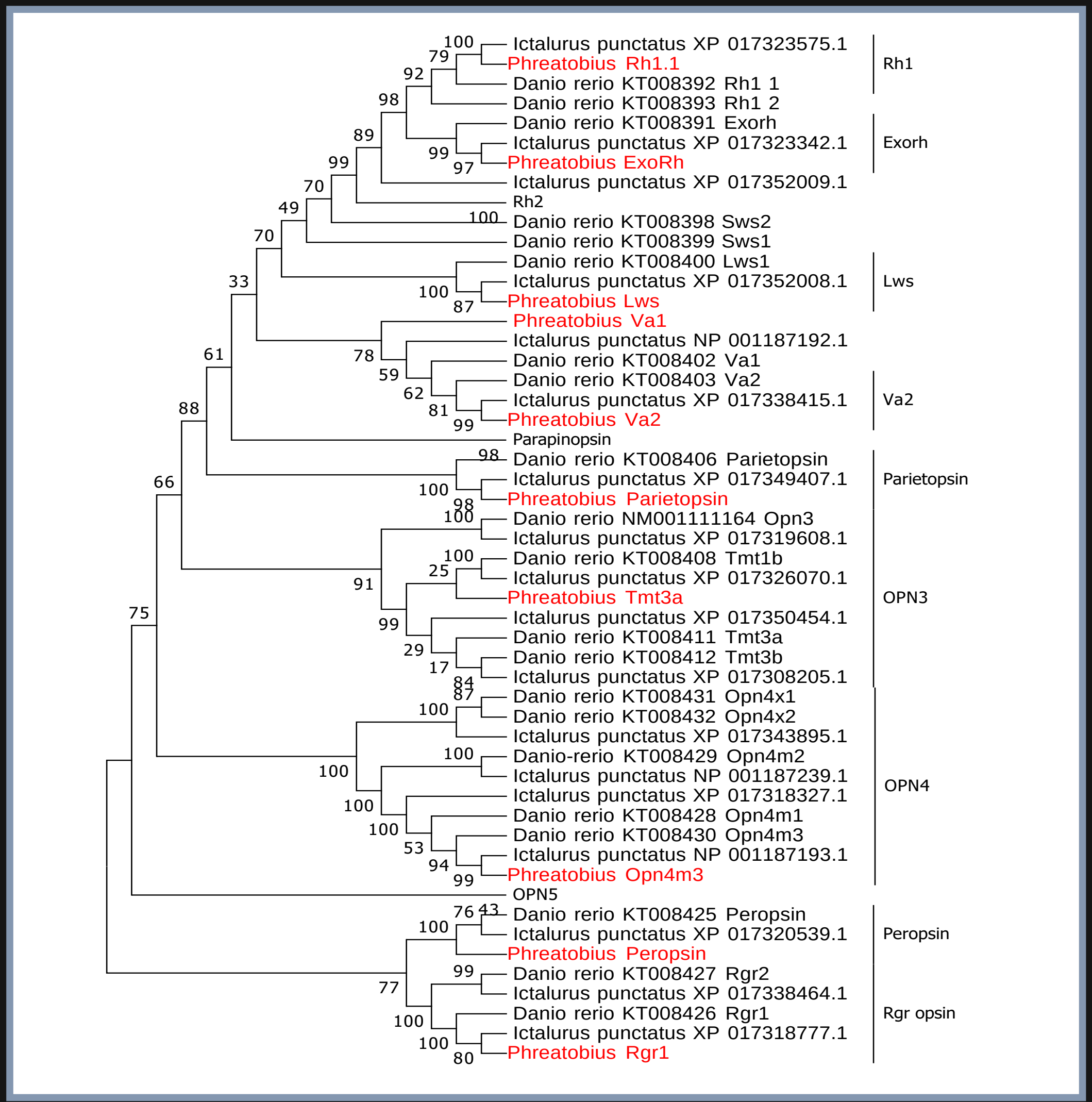


**Figure 2.** Opsins of Zebrafish, Channel Catfish, and *Phreatobius* shown in a phylogenetic tree (missing *Rh 1.**). The phylogeny shows, for example, that *Phreatobius* has an *Rgr1* gene which is sister to the Channel Catfish *Rgr1* and in a clade with the more distantly related *Danio rerio Rgr1*. *Phreatobius* lacks some opsins compared to Channel Catfish, and *Danio rerio*, for example *Rgr2*. Neighbour-joining tree from bootstrap method with 1000 replicates and evolutionary distances (P distance) using the program MEGA6.

**Table 1.** Comparison of opsins present in opsin families between three fish species and with +/- generalizing presence and absence of opsins found throughout the Mammalia taxa

| Current names | Rh1 | Rh1* | Exorh | Rh2 | Sws2 | Sws1 | Lws | Pinopsin | VA | Parietopsin | Parapinopsin | Opn 3 | Tmt 2 | Tmt 3 | Tmt 1 | Opn 4x | Opn 4m | Opn 5/9 | Opn 6a | Opn 6b | Opn 7a | Opn 7b(cd) | Opn 8 | Peropsin | Rgr | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Danio rerio* | 2 | 0 | 1 | 4 | 1 | 1 | 2 | 0 | 2 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 3 | 2 | 1 | 1 | 1 | 3 | 3 | 1 | 2 | 42 |
| *Ictalurus Punctatus* | 2 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 1 | 0 | 2 | 1 | 1 | 3 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 2 | 23 |
| *Phreatobius cisternarum* (head transcriptome) | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 11 |
| Mammalia | + | - | - | - | + | + | + | - | - | - | - | + | - | - | + | - | + | + | + | - | - | - | - | + | + | |

**Table 2.** Summary of *Phreatobius* opsin transcripts in regards to RNA splicing (Missing *Rh 1**). Percent % total indicates how much of the respective opsin gene the multiple contigs cover. Besides *Rh1.1* most of the other opsins are incomplete transcripts. 13 out of 18 transcripts show evidence of intron retention (IR), while 4 transcripts show evidence of both intron splicing and IR.

| | Contigs | Intron spliced | Intron present | Base pairs | % total |
|---|---|---|---|---|---|
| Rh1.1 | 1 | ✓ | | 1626 | 100 |
| Rh1.2 | 2 | ✓ | | 244 | 20 |
| Exorh | 3a | ✓ | | 317 | 41 |
| | 3b | | ✓ | 281 | |
| Lws | 4a | | ✓ | 348 | 80 |
| | 4b | ✓ | | 310 | |
| | 4c | | ✓ | 331 | |
| | 4d | | ✓ | 281 | |
| Parietopsin | 7 | ✓ | ✓ | 310 | 26 |
| Va1 | 5 | | ✓ | 241 | 7.5 |
| Va2 | 6a | | ✓ | 443 | 62 |
| | 6b | ✓ | ✓ | 393 | |
| Tmt3a | 8 | | ✓ | 887 | 20 |
| Opn4m3 | 9a | ✓ | ✓ | 238 | 37 |
| | 9b | ✓ | ✓ | 526 | |
| Peropsin | 10 | | ✓ | 491 | 32 |
| Rgr1 | 11a | | ✓ | 681 | 52 |
| | 11b | ✓ | | 514 | |

**Table 3.** Codon-based Test of Neutrality analysis between sequences of *Phreatobius* (PC), Zebrafish (DR) and Channel Catfish (IP). dS and dN represents synonymous and non-synonymous substitutions per site respectively. Negative values show evidence for purifying selection of the gene. Analysis used the Nei-Gojobori method with the program MEGA7.

| Opsin | Species | dN - dS | P-value |
|---|---|---|---|
| RH1.1 | DR-IP | -6.4 | 0.0 |
| | DR-PC | -5.9 | 0.0 |
| | IP-PC | -4.9 | 0.0 |
| ExoRh | DR-IP | -6.1 | 0.0 |
| | DR-PC | -3.1 | 0.003 |
| | IP-PC | -3.6 | 0.001 |
| LWS | DR1-DR2 | -4.5 | 0.0 |
| | DR1-IP | -6.3 | 0.0 |
| | DR1-PC | -6.1 | 0.0 |
| | DR2-IP | -6.2 | 0.0 |
| | DR2-PC | -6.0 | 0.0 |
| | IP-PC | -5.3 | 0.0 |
| VA1 | DR-IP | -3.6 | 0.001 |
| | DR-PC | 0.1 | n/c |
| | IP-PC | -1.6 | 0.111 |
| VA2 | DR-IP | -6.2 | 0.0 |
| | DR-PC | -4.6 | 0.0 |
| | IP-PC | -3.9 | 0.0 |
| Parietopsin | DR-IP | -3.0 | 0.004 |
| | DR-PC | -1.6 | 0.122 |
| | IP-PC | -2.8 | 0.005 |
| Tmt3A | DR-IP | -6.6 | 0.0 |
| | DR-PC | -2.8 | 0.005 |
| | IP-PC | -2.6 | 0.012 |
| Opn4m3 | DR-IP | -6.5 | 0.0 |
| | DR-PC | -5.3 | 0.006 |
| | IP-PC | -3.8 | 0.0 |
| Peropsin | DR-IP | -5.9 | 0.0 |
| | DR-PC | -1.6 | 0.004 |
| | IP-PC | -3 | 0.004 |
| RGR | DR-IP | -5.5 | 0.0 |
| | DR-PC | -2.7 | 0.007 |
| | IP-PC | -2.5 | 0.014 |

## Discussion

1. Many opsins have **gone missing** relative to the Channel Catfish
2. Present opsins are found mostly transcribed with **introns**
3. With one exception, opsins present are **incomplete** transcripts
4. The opsin sequences in *Phreatobius* compared to the two surface fish, do not show evidence of **neutral evolution**.

We found evidence of as many as 11 opsins that persist through evolution in the dark with the *Phreatobius* transcriptome. A prominent example, Rhodopsin (*Rh1*), is a highly expressed full length sequence known for light-independent functions such as a role in thermosensory signaling pathways[3].

Fragments of various sizes of the other 10 opsins were detected in the transcriptome with varying levels of expression and intron retention (Table 2). Even though these sequences are incomplete and contain introns, they show evidence against neutral molecular evolution and for **purifying selection**, in which opsins have selective pressure to retain their original function (Table 3).

Little is known about **intron retention**, but several studies have found transcripts with introns regulate translation of the those transcripts from the same gene that lack introns[4,5]. If it's true that these pieces may negatively regulate opsin translation, it's intriguing that we found them in a fish that lives in the dark.

This study is the first step in identifying opsins with non light-sensing roles. In future work we hope to analyze and compare our transcripts to the *Phreatobius* genome.

## References

*Phreatobius* header image: Muriel-Cunha, J., & de Pinna, M. 2005. New data on cistern catfish, *Phreatobius cisternarum,* from subterranean waters at the mouth of the Amazon River (Siluriformes, Incertae Sedis). **Pap. Avulsos Zool. 45(26): 327-339.**
Left Zebrafish from Fishbase, Right Channel Catfish from nature.mdc.mo.gov field guide: channel catfish

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