

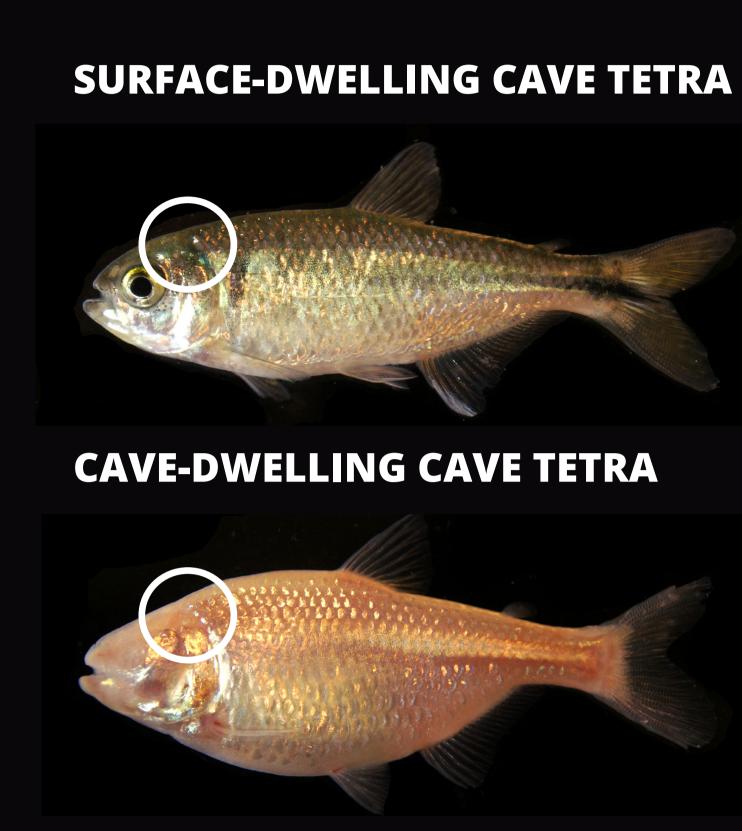
Animals living in well-lit environments utilize optical stimuli for detecting visual information, regulating the homeostatic pacemaker, and controlling patterns of body pigmentation. In contrast, many subterranean animal species living without optical stimuli have evolved regressed binocular eyes and body pigmentation.

Interestingly, some fossorial and cave-dwelling animals with regressed eyes still respond to light. These light-dependent responses may simply be evolutionary residuals or they may be adaptive, in which case negative phototaxis helps animals avoid predator-rich surface environments. However, the relationship between these non-ocular light responses and the underlying light-sensing Opsin proteins has not been fully elucidated.

METHODS

To indicate how opsins may function in a blind subterranean animal, we used the Mexican cave tetra to investigate opsin gene expression in the eyes and several brain regions of both surface- and cavedwelling adults.

We performed database surveys, expression analyses by quantitative reverse transcription PCR (RT-qPCR), and light-dependent locomotor activity analysis using pinealectomized fish.





RESULTS

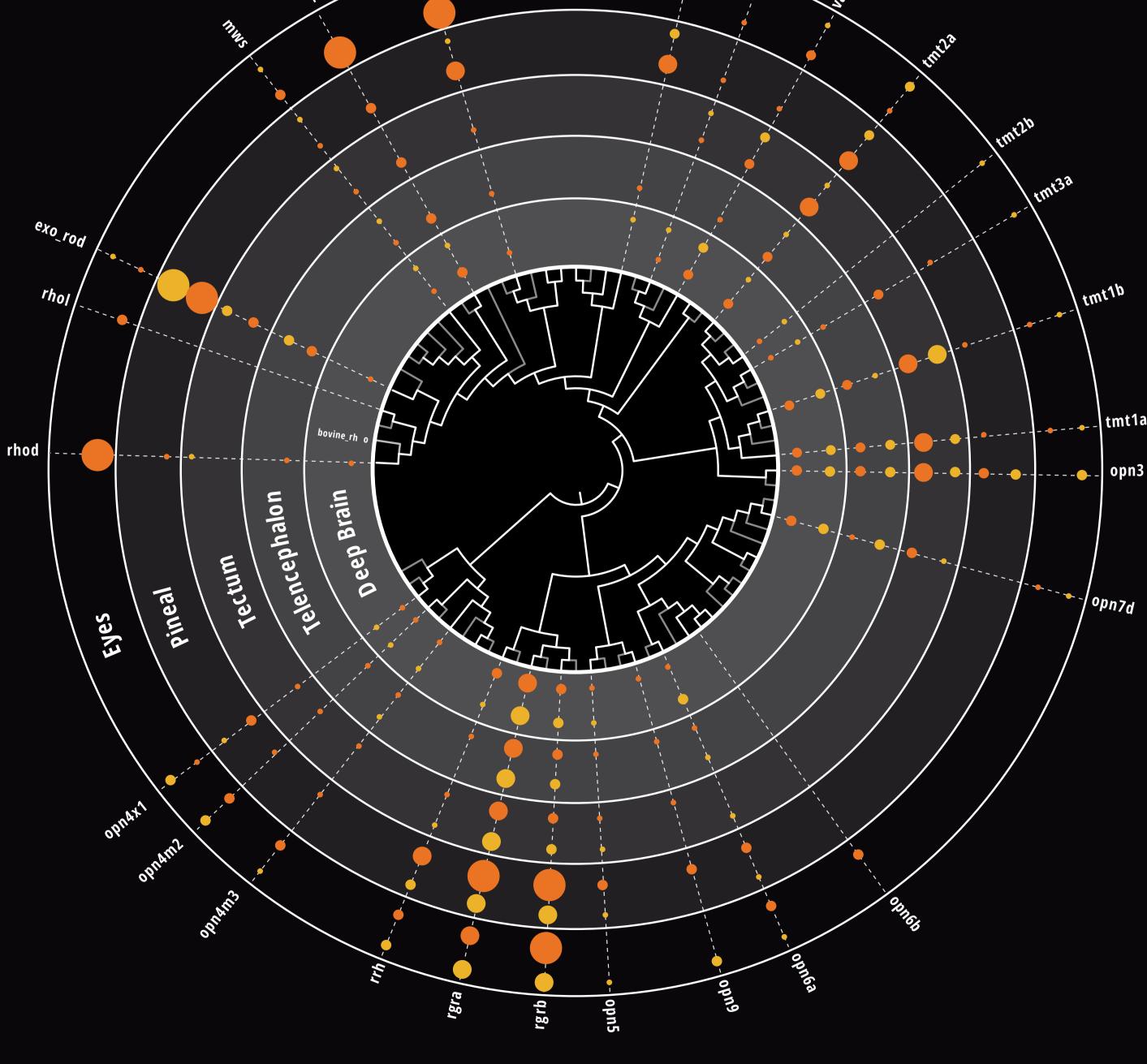
Based on conservative criteria, we identified 33 opsin genes in the cavefish genome. Surveys of available RNAseq data found 26 of these were expressed in the surface fish eye and 24 were expressed in cavefish extraocular tissues, 20 of which were expressed in the brain.

RT-qPCR of 26 opsins in the eye and brain tissues showed that the highest opsin-expressing tissue in cavefish was the pineal organ, which expressed exo-rhodopsin at 72.7 % of the expression levels in surface fishes' pineal. However, a pinealectomy did not result in a change to the light-dependent locomotor activity in juvenile cavefish and surface fish.

ASTYANAX OPSINS This phylogeny illustrates the evolutionary relationships among

PHYLOGENY AND RELATIVE EXPRESSION LEVELS OF

This phylogeny illustrates the evolutionary relationships among cave tetra (white branches) and zebrafish (grey branches) opsins, and includes bovine rhodopsin (indicated) for reference. The relative expression levels in cave (yellow) versus surface (orange) cave tetra tissues for the 26 opsins investigated are CAVE **SURFACE** displayed in expanding circles around the phylogeny. Each opsin transcript is labelled on the outermost ring, with a dashed line connecting the expression for that gene in each tissue to the transcript's placement in the phylogeny. 100-999 10-99 1-9 0.1-0.9 exo_rod tmt1b rhol tmt1a



CONCLUSION

We conclude that, after 20,000 or more years of evolution in darkness, cavefish's light-dependent basal activity is regulated by a non-pineal extraocular organ.



