Developmental Biology 493 (2023) 13–16

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

# Reinterpreting the work of Charles Breder: Sensory neuromasts and orbital skeleton variation in eyeless *Astyanax* cavefish

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ARTICLE INFO

*Keywords:*
Mexican tetra
Intramembranous bones
Lacrimal
Jugal
Synostosis

ABSTRACT

Charles Breder, a pioneering researcher of blind Mexican cavefish was the first to note extreme variation in the facial skeleton of this intriguing subterranean-dwelling organism. Using a system of polar coordinate plots, he identified substantial dysmorphic changes affecting bones of the orbital skeleton. A complication of his landmark publication from 1944 was an error in the number of orbital bones depicted for this species. Intriguingly, however, he proposed an unknown "organizing force" likely influences final bone position and associated dysmorphia. At the time this was merely hypothetical. Roughly eight decades since its publication, however, insights into sensory influences on facial bone development may explain dysmorphia and variation in bone numbers for *Astyanax* cavefish. A morphological association between mechano-sensory neuromasts of the lateral line and dermal bones of the facial skeleton had been appreciated in the classical literature, but the polarity of this interaction has long remained unclear. Here, we propose that sensory-skeletal integration between sensory neuromasts and bones explain the incomplete numbers of bones, and dysmorphic features such as fusion between neighboring elements. We propose that in closely-related surface fish (and most teleost fish) this developmental coupling enables the sensory and skeletal systems to become integrated into a functional unit over the course of life history. In this opinion article, we discuss the relevance of this (poorly understood) phenomenon as a potential evolutionary source of variation in the facial bone structures of taxa across deep geologic time. We provide three potential explanations for the error in Breder's drawings, that may be explained by natural developmental variation documented in other related species. Moreover, we argue that the natural variation in this "evolutionary" model system is useful for explaining diverse cranial features by uniting aberrations occurring during embryogenesis with long-term adult dysmorphia.

## 1. Introduction

As Head Curator of Ichthyology at the American Museum of Natural History, Charles Breder began studying a curious species in the early 1940s, *Anoptichthys jordani* – commonly called the "blind Mexican cavefish". Although considered a distinct genus at the time, genetic similarity (Avise and Selander, 1972; Ornelas-García et al., 2008; Warren et al., 2021) and the ability to interbreed with the Mexican tetra (*Astyanax mexicanus*) (Jordan, 1946; Sadoglu, 1955, 1957), led to loss of the *Anoptichthys* designation and collapse of *mexicanus* into a single species comprising surface- and cave-dwelling morphs. Among his diverse interests included anatomical variation between cave and surface morphs, particularly of the craniofacial complex. In 1944, he published a landmark description of variation in the facial skeleton, specifically around the orbit of the eye (Breder, 1944). These intramembranous "suborbital" (also called "infraorbital") bones are deeply conserved across vertebrate evolution (Long, 2016) and frequently comprise six bones named according to their positions from anterior to posterior. Although generically named in fish, these bones are homologous to deeply conserved vertebrate facial bones; namely, suborbital 1 ("SO1") = lacrimal bone, SO2 = jugal bone; SO3 = post-orbital bone; and SO6 = dermatosphenoid bone.

At the time, the skeletal impact of evolved eye loss was incompletely understood, so the objective of this work was to describe changes to the cranial complex arising as the consequence of an absent visual system. To understand impacts on the orbital skeleton, Breder (1944) created 'polar coordinate plots' based on cleared-and-stained cranial tissue. These plots showed the shifting positions of bony landmarks in eyeless cavefish relative to surface-dwelling morphs (which have completely normal eyes

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https://doi.org/10.1016/j.ydbio.2022.11.004
Received 28 September 2022; Received in revised form 25 October 2022; Accepted 4 November 2022

and skulls). Consequently, this work underscored the dramatic transformations in position, shape and size of the suborbital bones encircling the eye.

In the decades since Breder's work, the Mexican tetra has emerged as an important model for several human-relevant disorders including insulin resistance/obesity (Riddle et al., 2018), sleep dysregulation (Duboué et al., 2012), autism (Yoshizawa et al., 2018), and craniofacial asymmetry (Powers et al., 2017). Facial asymmetry in this species is mediated, in part, by aberrations of the suborbital bones that Breder first described (Gross and Powers, 2015). Interestingly, however, the number of suborbital bones depicted by Breder was incorrect. Rather than six suborbital bones, he only depicted the presence of five bones within this complex. Given the discrepancies between the orbital skeletal structure described by Breder, and contemporary research findings, a reexamination of his landmark 1944 paper is warranted.

### 1.1. *Expeditions to the natural cave environment*

In 1940, Breder led an expedition to the Chica cave – the first locality discovered for *Astyanax* cavefish. The research group *remained inside the cave* for 15 straight days (Breder, 1942). On a second expedition in 1942, Breder traveled to the second cave locality discovered at Sabinos (Breder, 1944). These trips allowed for a comparative analysis of specimens from two distinct cavefish localities and surface-dwelling fish. By comparing camera lucida drawings of cleared and stained specimens, he examined morphological features of the eye rudiment and associated modifications of the skull – particularly in the orbital skeleton.

Breder concluded that modifications to the orbit were a direct consequence of eye regression. Among his observations included a mildly affected Chica specimen with “new centers of ossification” caused by the shrinkage of the eye diameter (Breder, 1944), a “closed in” appearance of the entire circumorbital series, and fragmentations impacting the second and third suborbital bones. Based on a specimen with more extreme cave-associated characteristics, Breder (1944) reported diversity in the fragmentation pattern of the third suborbital bone, reduced mineralization (thinner bones), and a tight crowding of the circumorbital series of bone around the vacant eye orbit.

### 1.2. *“The force of some general regulating control”*

Breder argued that the degree of orbital skeleton regression was a function of eye regression. He wrote that there was “a general shifting around of all the head bones in more or less minor fashion, as they evidently contract to fit the new conditions imposed by the removal of the eye.” However, for the suborbital bones themselves, he made a remarkably astute prediction by commenting that the circumorbital bones were “moving and rearranging themselves under the force of some general regulating control”.

To decipher the origin of this “regulating force”, he performed a polar coordinate analysis in which the origin (center point) was placed directly on the eye (or putative eye position) of camera lucida drawings of multiple specimens. This allowed a direct comparison of morphological changes across populations. He concluded that surface fish represented the *stereotypical* arrangement of circumorbital bones, Chica showed mild variations, and Sabinos cavefish showed severe variations.

### 1.3. *An anatomical error*

In the original drawings, the suborbital bone series was depicted as having five, rather than six, intramembranous constituent bones. In his publication, Breder (1944) explained that his depictions assumed *Astyanax* harbors a ‘generalized cranial form’, similar to *Cheirodon* – a South American genus of freshwater fish (note that this genus is also classified as *Paracheirodon* and *Hyphessobrycon*).

Interestingly, however, fish of the genus *Cheirodon* – from which his depictions were based - are known to have *six* circumorbital bones. The SO1 and SO2 bones are elongated and define the antero-ventral boundary of the orbital skeleton. The largest suborbital bone, SO3, occupies a large region of the lateral cheek. A “chain” of three smaller bones, SO4 – 6, create the postero-dorsal rim of the circumorbital chain (Fig. 1D). These latter three bones represent the source of a discrepancy between Breder's description and contemporary osteological descriptions. Breder depicted this chain as having two (rather than three) bones - resulting in a total of five suborbital bones. It is our opinion that this discrepancy may be explained by one of three possibilities.

### 1.4. *Explanation 1: A prior historical inaccuracy*

The published anatomical description of surface morphs from Breder (1944) was based on the cranial complex of a “generalized tetra”, such as *Hyphessobrycon*. However, contemporary osteological descriptions of *Hyphessobrycon compressus* by Carvalho and Malabarba (2015) report the presence of six suborbital bones – not five bones as depicted by Breder (Carvalho and Malabarba, 2015). So why did Breder claim the presence of five, rather than six, suborbital bones? One explanation may be historical. Breder based his anatomical description on the work of Gregory (1933) and Gregory and Conrad (1938) – two seminal osteological descriptions of the Characiform skull (Gregory, 1933; Gregory and Conrad, 1938). Gregory (1933) presented a composite representation of the “typical” characin skull complex - for which he assigned homology to the bones of the circumorbital series (Fig. 1A). Interestingly, this depiction represented *only five bones* of the suborbital complex. One possibility is that Breder adopted this schema for his representation of the *Astyanax* orbital skeleton. Based on contemporary studies of suborbital bone numbers and positions (Gross and Powers, 2015), we feel the depictions by Breder (1944) and Gregory (1933) represent the SO4 and SO5 bones as a single bone – when in fact they should be depicted as two separate bones.

### 1.5. *Explanation II: A “missing” SO6 bone*

A second possible explanation of this historical anatomical inaccuracy impacts on *bona fide* natural variation. *Hyphessobrycon* fish, for example, harbor variability in suborbital bone numbers, specifically impacting the SO6 bone. In a recent study, Carvalho and Malabarba (2015) noted that, “infraorbitals 4, 5, and 6 [were] variable in form and presence: IO4, IO5, and IO6 [was] sometimes absent.”

This finding is consistent with a recent study by Powers et al. (2018) which found the same aberration can be present in *Astyanax* fish (Powers et al., 2018). Interestingly, however, absence of the SO6 bone was exclusively found in cave morphs, never in surface morphs (Fig. 2B). Thus, the cavefish specimens collected by Breder may have lacked an SO6 suborbital bone, reflecting normal natural variation. These fish may very well have only harbored 5 suborbital bones in his collection, given the frequency of SO6 absence reported in contemporary specimens (Powers et al., 2018). This explanation, however, does not account for the depiction of five suborbital bones in surface morphs. Perhaps surface morphs were depicted for consistency based on the natural variation clearly present in cave morphs.

### 1.6. *Explanation III: Fused bones in the suborbital chain*

A form of variation in the cavefish suborbital chain are fusions (synostoses) between neighboring bones. A two-bone fusion would result in a reduction of the number of bones within the orbital complex. Suborbital chain fusions have been described in *Hyphessobrycon*, the reference taxon for Breder's depictions. Carvalho and Malabarba (2015) reported that the SO4 and SO5 “may be fused and separate from” the SO6 bone. Notably, these fusions impact the anatomical region of inaccuracy in Breder's 1944 publication.

Interestingly, these fusions are frequently present in *Astyanax*. SO4 and SO5 fusions, in particular, are among the most commonly fused in

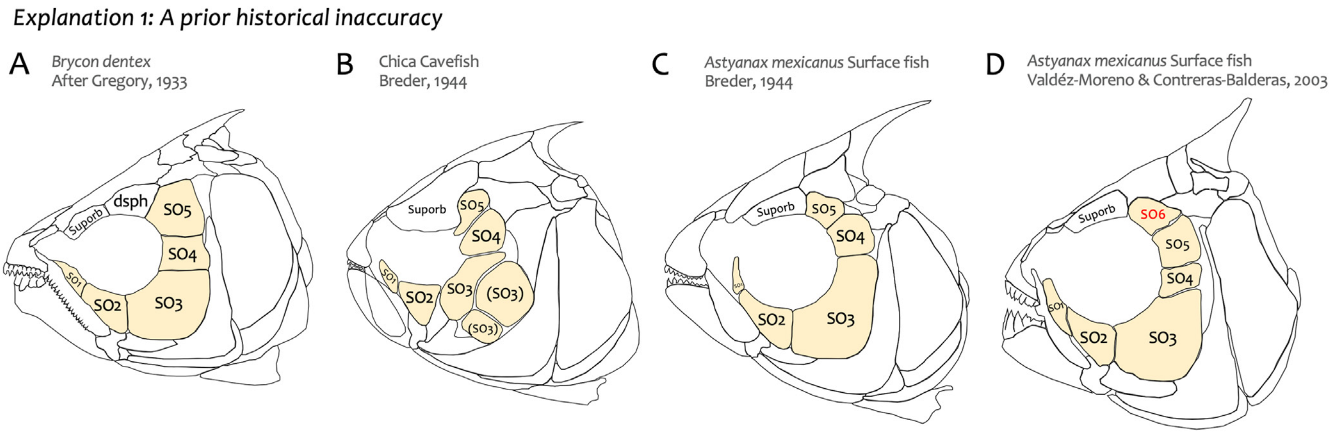

**Fig. 1.** The discrepancy in the number of suborbital bones may have originated from historical anatomical descriptions. Modified after Gregory (1933), *Brycon dentex* harbors 5 bones in the suborbital chain including the dermosphenoid at the end of the chain that may represent the position of the SO6 (A). Tracings made of Breder's original drawings of a Chica cavefish (B) and surface fish (C) demonstrate an inaccuracy in the number of suborbital bones compared to a contemporary drawing of *A. mexicanus* (D) from Valdéz-Moreno and Contreras-Balderas (2003 (Valdéz-Moreno and Contreras-Balderas, 2003);). Suporb = supraorbital; dsph = dermosphenoid; SO = suborbital.

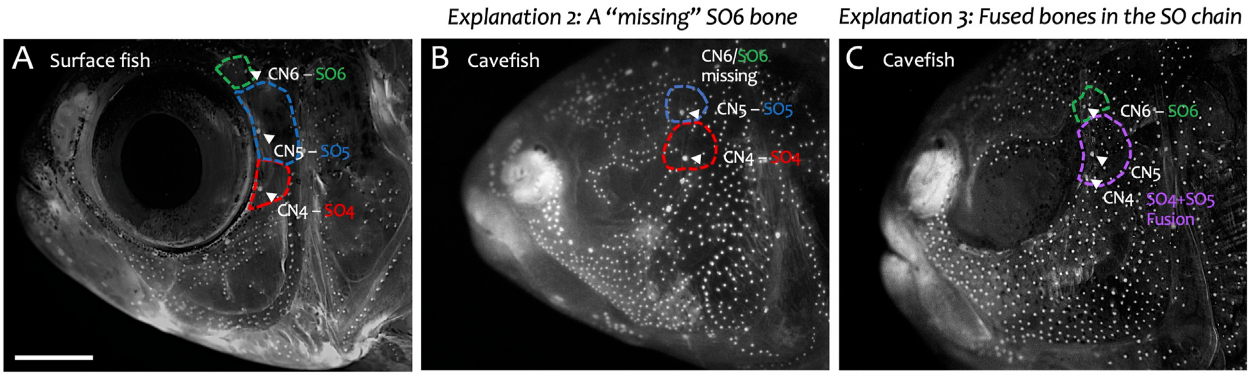

**Fig. 2.** Evidence for sensory-bone integration within the cranium. Modified from Powers et al., 2018), Surface fish display a stereotypical pattern of canal neuromasts (CNs) and suborbital bones (A). A representative cavefish from the Pachón lineage demonstrates that when the last CN in the chain is absent, the SO6 bone fails to form (B). A representative cavefish from the Chica lineage shows that when CNs are patterned closely together, there is likely to be a fusion event (C). CNs are denoted by white arrows. The SO4 bone is outlined in red, the SO5 in blue and the SO6 in green. The SO4+5 bone fusion is outlined in purple. Scale bar = 1 cm.

the suborbital series of *Astyanax* (Fig. 2C). This fusion event has a genetic basis mediated by a cave-associated allele and is present more often on the right side of the head (Gross et al., 2014). Thus, it may be that the SO4 and SO5 bones were fused in the cavefish specimens collected and analyzed by Breder.

This scenario is perhaps most likely for two reasons. First, Powers et al. (2018) reported that SO4/SO5 fusions occur in ~35% of the cavefish individuals they analyzed. Although this report did not examine specimens from the Sabinos locality (like Breder), or if fusions were present across multiple different cave localities, including Chica, Pachón and Tinaja. Second, the anatomical position represented by Breder (1944) points to the presence of an SO6 bone (incorrectly identified by Breder (1944) as the SO5 bone). Based on contemporary osteology (Valdéz-Moreno and Contreras-Balderas, 2003), it appears that Breder (1944) represented the SO3 and SO4 as a fused bone, with the SO4 incorrectly named the SO5 (compare Fig. 1C and D). An alternate explanation is that Breder (1944) merged the SO4 and SO5 bones into a single bone. Based on the bone positions represented in Valdéz-Moreno and Contrera-Banderas (2003), we feel this is a less likely explanation (Fig. 1). An important caveat, however, is that these fusions have not been reported in surface-dwelling fish - leaving open the question of why Breder depicted surface morphs as having five bones despite unreported instances of fusion for these bones.

### 1.7. *Canal neuromasts as the "general organizing force" of facial bone structure and variation*

Although not known at the time, recent studies suggest that variation in form and structure of the orbital skeleton is likely rooted in developmental variation. A longitudinal analysis of neuromast and bone development provides a potential mechanistic explanation (Powers et al., 2018). First, canal neuromasts co-localize precisely to the position of primary ossification centers for the circumorbital bones (Powers et al., 2018). These neuromasts arrive to their final position long before the appearance of bone, suggesting they may induce bones to form early in development. If neuromasts are osteoinductive, then changes in their positions across development may explain two key morphological aberrations observed in cave-dwelling morphs.

First, if the canal neuromast associated with a particular bone, e.g., SO4, is positioned too closely to a neighboring canal neuromast, e.g., SO5, then this may render the bones vulnerable to fusion (Fig. 2C). This phenomenon has been hypothesized in humans born with certain forms of craniosynostosis. Namely, isolated (non-syndromic) forms of craniosynostosis impacting the coronal and metopic sutures appear to have ossification centers set abnormally close to one another (Mathijssen et al., 1996). If canal neuromasts mark sites of the primary ossification center, and if positioned too closely to one another, skeletogenic mesenchyme between areas of ossification may merge – resulting in fusion. In support of this, Powers et al. (2018) observed that the positions of canal neuromasts positioned abnormally close to one another resulted in fusion of neighboring bones, a feature found for numerous bone pairs around the suborbital chain. These fusions were commonly observed in cave-dwelling morphs, perhaps as a consequence of variable collapse of the orbit which led to the repositioning of canal neuromasts. Consistent with this notion, surface morphs were found to display invariant positioning of canal neuromasts, and no resultant facial bone fusions.

A second aberration possibly explained by this putative mechanistic relationship concerns the presence of the most dorsal canal neuromast, associated with the SO6 bone. Powers et al. (2018) noted that when the associated canal neuromast was absent – the bone does not appear (Fig. 2B). This aberration occurred ~20% of the time in their analyzed specimens. This is consistent with the notion that neuromasts are osteoinductive and ensure the correct placement and position of specific bones of the orbital complex. This feature is unlikely to be idiosyncratic to *Astyanax*, since the same aberrations present themselves in the closely-related *Cheirodon* (*Hyphessobrycon*) lineage. Thus, two forms of morphological aberration, originally discovered and reported by Breder (1944) are likely explained by developmental aberrations impacting on a form of sensory-skeletal integration deeply conserved across teleost fish lineages. The fusion of neighboring bones (e.g., SO4/SO5) and the absence of a particular bone (SO6) may ultimately be rooted in developmental positioning that is more often present in *Astyanax* cave morphs (compared to surface morphs) owing to loss of the visual system in this cave-adapted species.

## 2. Conclusions

Charles Breder, a pioneer in ichthyology and cave biology, published a seminal paper in 1944 providing the first description of cranial aberrations in *Astyanax* cave-dwelling morphs. This important work first noted aberrations of the *Astyanax* cranial complex, which is receiving renewed attention as a model for human-relevant disease and aberrations of the skull. However, this work included an error in the depiction of suborbital bone numbers in surface fish, specifically one less than the correct number. Cavefish, however, frequently harbor only five suborbital bones. This opinion article seeks to provide three potential explanations Breder's drawings, which may have resulted from an historical inaccuracy, or reflect natural variation that has been noted in several other species. Contemporary work suggests this variation is rooted developmental aberrations and an intriguing example of sensory-skeletal integration – specifically, wherein canal neuromasts serve as a source for osteoinductive signaling. Breder correctly, and presciently, envisaged a general organizing force – which we interpret as the osteoinductive nature of canal neuromasts. The source of these aberrations is sensory organ repositioning resulting from eye loss in this species – a feature correctly predicted by Breder (1944). Future work is anticipated to clarify the molecular basis for this intriguing phenomenon and provide deeper insight to the question of how sensorineural systems serve to organize the induction, positioning and number of facial bones.

## Data availability

No data was used for the research described in the article.

## Acknowledgments

This paper is dedicated to Charles Breder, a pioneer in the field of *Astyanax* biology, whose fundamental insights and contributions laid the rich foundation for contemporary research into this fascinating system. The authors wish to thank members of the Gross lab for helpful discussions of this topic, especially Shane Kaplan, Tyler Boggs and Daniel Berning. JBG is supported by a grant from the National Science Foundation (IOS-2234685).

## References

Avise, J.C., Selander, R.K., 1972. Evolutionary Genetics of Cave-Dwelling Fishes of the Genus *Astyanax*, pp. 1–19. https://doi.org/10.2307/2406978. Evolution.

Breder, C.M., 1942. Descriptive ecology of La Cueva Chica, with especial reference to the blind fish, *Anoptichthys*. New York Zoological Society, pp. 7–15.

Breder, C.M., 1944. Ocular anatomy and light sensitivity studies on the blind fish from Cueva de los Sabinos, Mexico. The American Museum of Natural History, pp. 131–144.

Carvalho, F.R., Malabarba, L.R., 2015. Redescription and osteology of *Hyphessobrycon compressus* (Meek)(Teleostei: Characidae), type species of the genus. Neotrop. Ichthyol. 13, 513–540. https://doi.org/10.1590/1982-0224-20140173.

Duboué, E.R., Borowsky, R.L., Keene, A.C., 2012. β-adrenergic signaling regulates evolutionarily derived sleep loss in the Mexican cavefish. Brain Behav. Evol. 80 (4), 233–243. https://doi.org/10.1159/000341403.

Gregory, W.K., 1933. Fish skulls: A study of the evolution of natural mechanisms. Trans. Am. Phil. Soc. 23, 75–481.

Gregory, W.K., Conrad, G.M., 1938. The phylogeny of the characin fishes. Zoologica 23, 319–360.

Gross, J.B., Powers, A.K., 2015. The Evolution of the Cavefish Craniofacial Complex: Biology and Evolution of the Mexican Cavefish.

Gross, J.B., Krutzler, A.J., Carlson, B.M., 2014. Complex craniofacial changes in blind cave-dwelling fish are mediated by genetically symmetric and asymmetric loci. Genetics 196 (4), 1303–1319. https://doi.org/10.1534/genetics.114.161661.

Jordan, C.B., 1946. Bringing in the new cavefish, *Anoptichthys jordani*. Aquarium 15, 203–204.

Long, J.A., 2016. The first jaws: A fossil fish helps to explain how jaws first evolved. Science 354, 6310. https://doi.org/10.1126/science.aai8828.

Mathijssen, I., Vaandrager, M., van der Meulen, J., Pieterman, H., Zonneveld, F.W., Kreiborg, S., Vermeij-Keers, C., 1996. The role of bone centers in the pathogenesis of craniosynostosis. Plast. Reconstr. Surg. 98 (1), 17–26. https://doi.org/10.1097/00006534-199607000-00004.

Ornelas-García, C.P., Domínguez-Domínguez, O., Doadrio, I., 2008. Evolutionary history of the fish genus *Astyanax* Baird & Girard (1854)(Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. BMC Evol. Biol. 8 (1), 1–17. https://doi.org/10.1186/1471-2148-8-340.

Powers, A.K., Davis, E.M., Kaplan, S.A., Gross, J.B., 2017. Cranial asymmetry arises later in the life history of the blind Mexican cavefish, *Astyanax mexicanus*. PLoS One 12 (5), e0177419. https://doi.org/10.1371/journal.pone.0177419.

Powers, A.K., Boggs, T.E., Gross, J.B., 2018. Canal neuromast position prefigures developmental patterning of the suborbital bone series in *Astyanax* cave-and surface-dwelling fish. Dev. Biol. 441 (2), 252–261. https://doi.org/10.1016/j.ydbio.2018.04.001.

Riddle, M.R., Aspiras, A.C., Gaudenz, K., Peuß, R., Sung, J.Y., Martineau, B., Peavey, M., Box, A.C., Tabin, J.A., McGaugh, S., Borowsky, R., 2018. Insulin resistance in cavefish as an adaptation to a nutrient-limited environment. Nature 555 (7698), 647–651. https://doi.org/10.1038/nature26136.

Sadoglu, P., 1955. A Mendelian gene for albinism in natural cave fish. Experientia 13, 394–395.

Sadoglu, P., 1957. Mendelian Inheritance in the Hybrids between the Mexican Blind Fishes and Their Overground Ancestor. Verh. Dtsch. Zool. Ges. Graz., pp. 432–439, 1957.

Valdéz-Moreno, M.E., Contreras-Balderas, S., 2003. Skull osteology of the characid fish *Astyanax mexicanus* (Teleostei: Characidae). Proc. Biol. Soc. Wash. 116 (2), 341–355.

Warren, W.C., Boggs, T.E., Borowsky, R., Carlson, B.M., Ferrufino, E., Gross, J.B., Hillier, L., Hu, Z., Keene, A.C., Kenzior, A., Kowalko, J.E., 2021. A chromosome-level genome of *Astyanax mexicanus* surface fish for comparing population-specific genetic differences contributing to trait evolution. Nat. Commun. 12 (1), 1–12. https://doi.org/10.1038/s41467-021-21733-z.

Yoshizawa, M., Settle, A., Hermosura, M.C., Tuttle, L.J., Cetraro, N., Passow, C.N., McGaugh, S.E., 2018. The evolution of a series of behavioral traits is associated with autism-risk genes in cavefish. BMC Evol. Biol. 18 (1), 1–16. https://doi.org/10.1186/s12862-018-1199-9.