

# Morphology and reproductive strategies of cave fish of genus *Trichomycterus* in Torotoro National Park (Potosí, Bolivia)

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## Abstract

Fishes of the genus *Trichomycterus* (Siluriformes) show hypogean and epigean populations in Torotoro National Park (Andes, Bolivia). The geographic isolation makes possible a comparative study of morphology and reproductive strategies among populations from different habitats. External morphology and brain measurements were considered. Significant reduction of eyes diameter and surface of optic lobes are registered, as well as an increase in the surface of the telencephalon (olfactory lobes and brain) and cerebellum. On the contrary, the number of chromatophores is not reduced, as it would be expected, as well as the barbels length that is not larger in the cave populations. In the case of reproduction, the subterranean populations have larger eggs in smaller number in comparison with the epigean populations. We discuss the morphological and reproductive changes between hypogean and epigean populations as product of the influence of the environment in the embryonic and post-embryonic development.

## Resumen

**Morfología y estrategias reproductivas de peces cavernícolas del género *Trichomycterus* en el Parque Nacional Torotoro (Potosí, Bolivia).** En el Parque Nacional de Torotoro (Andes, Bolivia) existen poblaciones cavernícolas y epigeas de peces del género *Trichomycterus* (Siluriformes). El aislamiento geografico hace posible un estudio comparativo de la morfología y estrategias reproductivas entre poblaciones de diferentes habitats. Al nivel morfologico se ha considerado medidas externas y del cerebro. Se ha encontrado reducción significativa del diámetro de los ojos y superficie de los lóbulos ópticos e incremento en la superficie del telencéfalo (lóbulos olfativos y cerebro) y del cerebelo. El número de cromatóforos no es reducido como se esperaba, ni la longitud de las barbillas es mayor en las poblaciones cavernícolas. Al nivel reproductivo, las poblaciones cavernícolas poseen huevos grandes y en poca cantidad frente a las epigeas que poseen huevos pequeños y gran cantidad. En discusion se consideran los cambios morfológicos y reproductivos como producto de la influencia del medio en el desarrollo embrionario y post-embrionario.

## Introduction

*Trichomycterus chaberti* (Durand, 1968) is an endemic cave fish of Torotoro National Park (Bolivia), only known in the Umajalanta cave. Other populations of epigean *Trichomycterus* (possibly *T. aff. fassli* or *T. aff. barbouri*) inhabit rivers from canyons and valleys of the same area. These populations are geographically isolated and recent genetic study (GAZEL, 1999) considered that the three species could be separated, corresponding to the three main kind of habitat: valley, canyon and subterranean rivers.

The geographic isolation makes possible a comparative study among close species or populations at the phylogenetic sense but living independently in very different environmental conditions (cave vs. superficial rivers). This possibility is rare in the case of fishes adapted to cave life. The aim of this study is to compare morphological parameters, known to be affected by cave life adaptation, and reproductive strategies among 8 populations from Torotoro National Park area which inhabit rivers in the three main type of habitat: valley, canyon and subterranean rivers.

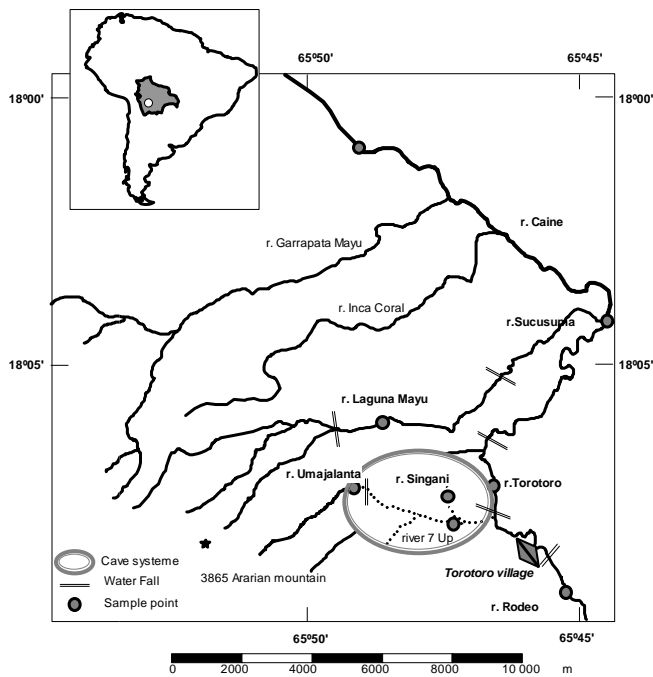
## Methods

### Study area

Torotoro National Park is located in the Bolivian Andes (18°15'S, 65°45'W) between 2700 and 3500 meters of elevation. It corresponds to a small massif of Cretaceous calcareous marked by karstic phenomena like caves and canyons. The hydrographic network is altered by waterfalls and then presents rivers in different environments (cave, canyon and valley). From a biological point a view this situation allows only up-down migrations of the individuals and then the watershed heads could be considered as independent.

### Fish sampling

Fishes were collected with a portable electro-fishing gear delivering a current between 300 and 600 Volts. It corresponds to an adequate method for this kind of habitat and rivers and for studies of fragile or small populations. Samples of 30 individuals from each population were preserved in buffered formaldehyde (4%) and transported to the laboratory.



**Figure 1.** Hydrographic network of Torotoro National Park. The points present the fish samples populations. The circle corresponds to the subterranean part of the network in Umajalanta cave (rio Umajalanta and rio Singani)

### Morphological measures

Four morphological attributes, known to be affected by cave life adaptation (GINET & DECOU, 1977; CULVER, 1982), were considered. Length of maxillary barbels were measured from insert until tip and expressed as a ratio with the standard length of the individual. Diameters of left and right eyes were measured horizontally and expressed as a ratio between the left and right diameters average and the standard length of the individual. Pigmentation level was estimated by a mean number of chromatophores per  $\text{mm}^2$  after counting chromatophores on  $1 \text{ mm}^2$  in three body parts (right post cephalic region, region of the lateral line at the tip of pectoral and pelvic fins). Surface of 4 main encephalon parts (smell lobes, optic lobe, oblong medulla and cerebellum) were estimated by measuring the maximum length and width and considering they had an ovoid form. An analysis of variance was performed to determine the differences of attribute values among the populations.

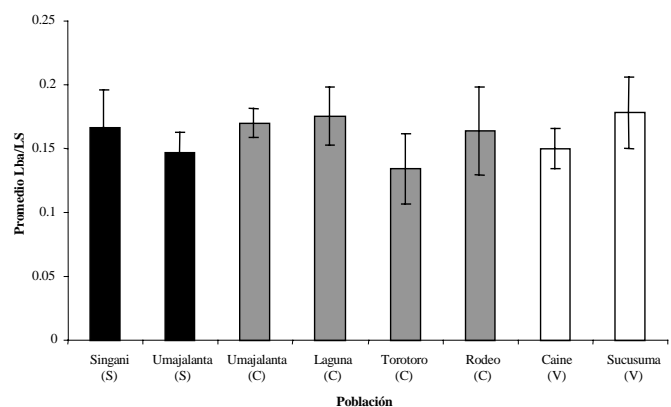
### Reproductive strategy

Fecundity and eggs diameter were considered as an estimation of reproductive strategy. They were determined from mature females (corresponding in stage 5 of BAGENAL's categorization (1978). For each mature female, after the extraction of the entire gonads, eggs were counted in totality as a estimation of fecundity. Then, diameter of 30 randomly selected eggs was measured with a micrometric scale under a microscope. An analysis of variance was performed to determine the differences in number and eggs diameter among the populations.

## Results

### Morphology

**Barbels.** Significant differences existed in maxillary barbels length among the 8 populations ( $F = 9,039$ ,  $p < 0.001$ ). However the differences did not correspond to an environmental or a spatial pattern (Fig 2). The populations of Laguna and of the superficial parts of Umajalanta and subterranean river Singani showed the largest barbels whereas the populations of the Torotoro canyon and from the Caine river showed the smallest one.

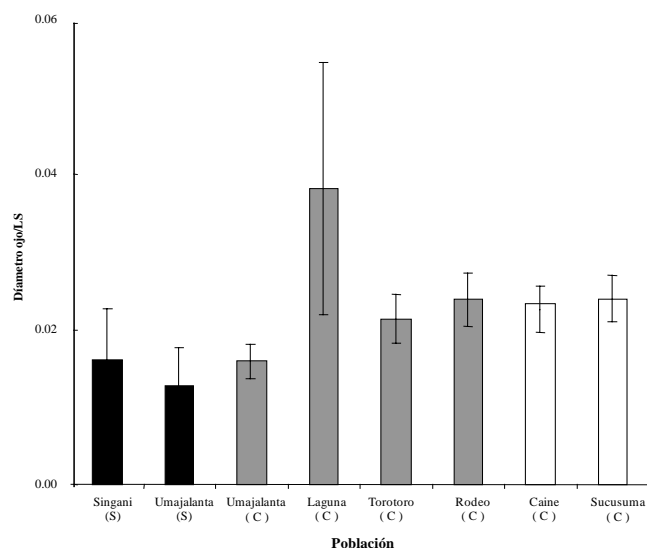


**Figure 2.** Mean and standard deviation of maxillary barbels length of 8 *Trichomycterus* populations from different habitats: subterranean rivers (S), canyon (C) and valley (V).

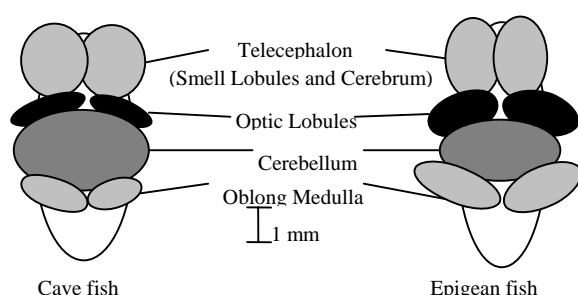
**Eyes.** There was a significant difference ( $F = 24.770$  and  $p < 0.001$ ) in eyes diameter between the cave and the epigeal populations. Populations of the Umajalanta system showed a reduction of eyes diameter in comparison with the other populations (Fig 3).

**Pigmentation.** The number of chromatophores was also significantly different among the 8 populations ( $F = 4.837$  and  $p < 0.001$ ). As in the case of barbel length there is no clear pattern with an environmental or a spatial interpretation. The smallest number of chromatophores was recorded in the hypogean population of Umajalanta river, but the maximum value was observed also in the hypogean populations of Singani river (Table 1).

**Encephalon.** Optic lobes areas, telencephalon and oblong medulla areas were significantly different among the populations (respectively  $F = 3.097$  and  $p < 0.05$ ;  $F = 11.665$  and  $p < 0.001$ ; and  $F = 2.672$  and  $p < 0.05$ ). Optical lobes appeared smaller in cave populations than in superficial populations (Table 1, Fig. 4) but conversely the telencephalon (which corresponds to smell lobes and brain) appeared larger in cave populations than in superficial populations. Cerebellum areas did not show significant differences among the populations ( $F = 1.693$ ;  $p < 0.163$ ), however as for oblong medulla there was a tendency of smaller values in the canyon populations and higher and similar values in cave and valley populations.



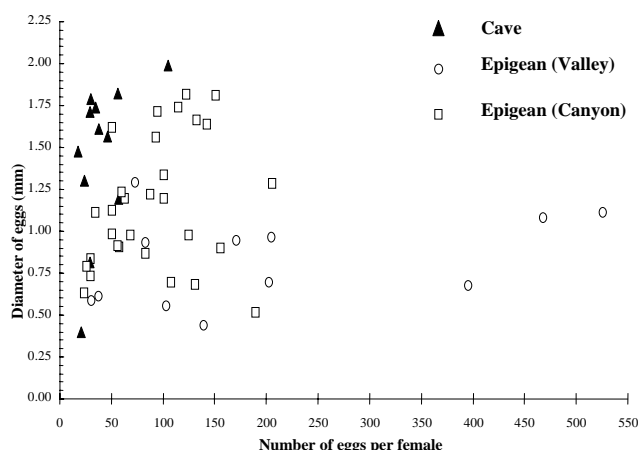
**Figure 3.** Mean and standard deviation of eyes diameter of 8 *Trichomycterus* populations from different habitats: subterranean rivers (S), canyon (C) and valley (V).



**Figure 4.** Comparison scheme of encephalon of epigeal and hypogean *Trichomycterus* (based on surface calculation of each parts of the encephalon in 30 individuals)

## Reproduction

The two parameters, fecundity and eggs diameter, were significantly different among the 8 populations ( $F = 3.273$  and  $p = 0.007$ ;  $F = 102.566$  and  $p = 0.000$ ; respectively). The results showed a trend from valley to canyon and subterranean rivers, which corresponds to a reduction of the fecundity and an augmentation of eggs size (Fig. 5)



**Figure 5.** Relationships between eggs diameter and fecundity in 53 mature females from different habitat rivers: subterranean rivers, canyon and valley

## Discussion

Eye reduction is without any doubt the most obvious adaptation of cave animals (EIGENMANN, 1909; POULSON, 1969; MITCHEL *et al.*, 1975; CULVER, 1982). The regression process begin first by the reduction in size of the optic apparatus (eyes and brain optic lobes) which result in a partial or a total blindness. According to the isolation time of the population, the regression process can evolve so far as the total loss of the optic apparatus (anophthalmia) and, in case of fishes, to deep modifications in skull structure (BREDER, 1944). Eyes of *Trichomycterus chaberti* of Umajalanta cave are still present but show a significant diameter reduction in comparison with superficial populations. The eye diameter reduction is related to a similar reduction of optic lobes in cave fish brain, showing as evident the first step of a regression process.

As compensation to the loss of vision, cave animals develop other types of sensory perceptions like chemical or tactile ones (CULVER, 1982). *Trichomycterus chaberti* do not show any evidence of barbels modifications as an adaptation to cave life. In particular we did not observed the expected pattern of an increased barbel length in the hypogean populations. Because of their behavior (nocturnal life, deep and obscure habitat use) the Trichomycteridae, like the majority of Siluriformes fishes, present in general long barbels as a pre-adaptation to cave life (BERTIN, 1958). An interpretation for the lack of pattern in barbel length could be that these organs are sufficiently long to sustain an increase of activity. This hypothesis could be supported by the significant variations observed in brain size. Telencephalon (smell lobes and brain) reached a significantly larger size in cave populations in relation to the epigeal populations. Therefore, this result supports the hypothesis of an increase of smell and tactile activity in compensation of loss of vision.

Another important adaptation of fishes to cave life mentioned in the literature is the considerable reduction of pigmentation (CULVER, 1982; GINET & DECOU, 1977). In our results from the *Trichomycterus* of Torotoro the density of chromatophores was highly variable and in addition did not correspond to the expected pattern of density reduction in the cave populations. The population of *Trichomycterus chaberti* that inhabit the river Singani of Umajalanta cave possesses the highest density of chromatophores. However, during sampling we observed a real appearance of depigmentation, which seems to be produced by the concentration of melanin in the central part of chromatic cells. We also observed that this appearance was clearly reversible by exposing some individuals to sunlight during a few minutes. Therefore, for this characteristic, we can conclude that there is no evidence of an evolution of the cave population in comparison with the epigeal populations.

Fecundity and eggs size modifications result in a change of the reproductive strategy of the populations, which could be classically related to the environmental conditions of the rivers. *Trichomycterus chaberti* of Umajalanta cave shows larger eggs and in reduced number

Population	Habitats	N	Eye diameter / SL		Maxillary Barbels/SL		Chromatophores /mm <sup>2</sup>		Optic lobe /SE		Telencephalon/SE		Cerebellum /SE		Medulla/SE	
			A	S. D.	A	S. D.	A	S. D.	A	S. D.	A	S. D.	A	S. D.	A	S. D.
Singani	Cave	15	0.0161	0.007	0.1662	0.03	88	37	0.0917	0.018	0.2341	0.017	0.4806	0.106	0.1015	0.013
Umajalanta	Cave	15	0.0127	0.005	0.1467	0.016	47	14	0.0913	0.014	0.2512	0.024	0.4948	0.092	0.1032	0.035
Umajalanta	Canyon	7	0.0159	0.002	0.1699	0.012	59	5	---	---	---	---	---	---	---	---
Laguna	Canyon	20	0.0382	0.016	0.1757	0.023	75	44	0.1233	0.019	0.149	0.01	0.3307	0.05	0.0676	0.014
Torotoro	Canyon	30	0.0214	0.003	0.134	0.028	77	25	0.1266	0.018	0.1652	0.025	0.3519	0.072	0.0728	0.026
Rodeo	Canyon	30	0.0239	0.004	0.1637	0.035	55	29	0.1418	0.04	0.1587	0.032	0.3855	0.137	0.1065	0.043
Caine	Valley	30	0.0234	0.003	0.15	0.016	83	32	0.1296	0.014	0.1717	0.046	0.4575	0.118	0.1267	0.021
Sucusuma	Valley	30	0.024	0.003	0.178	0.028	85	33	0.1452	0.046	0.1555	0.021	0.4395	0.131	0.1231	0.034
F			24.77		9.039		4.837		3.097		11.665		1.693		2.672	
p			<0.001		<0.001		<0.001		<0.02		<0.001		<0.163		<0.037	

**Table 1. Averages (A) and standard deviations (S.D.) of morphological measurements in cave and epigeal *Trichomycterus* in 8 populations of National Park of Torotoro (Bolivia). SL: Standard leng, SE: Surface encephalon.**

(K strategy trend), while the superficial populations spread to have small eggs and in great quantity (r strategy trend). The same tendency of modification has been observed by POULSON (1963) in the Amblyopsidae fish family. It is likely that in subterranean habitats the environmental conditions are more stable than in superficial habitats and that organisms like fishes did not support any predators. These conditions could explain the modification of the reproductive strategy of populations adapted to cave life. Food rarity is another condition of the subterranean habitats. The increase of egg size could give a better autonomy at the beginning of larval stage and then could be favorable for the development of the population.

## Conclusion

In conclusion, from all the morphological and biological parameters considered in our study, we observed different levels of modification between the hypogean and the epigeal populations of *Trichomycterus* from the Torotoro National Park. The optic perception (eyes diameter and surface of optic lobes) and the two parameters of reproduction show a clear tendency of adaptation to cave life. Pigmentation, generally affected by an adaptation to cave life, did not present any evidence of modification. Chemical and tactile perceptions (barbel length and telencephalon surface) show an intermediate situation with a nervous modification but without morphological changes. It is then likely that the populations of *Trichomycterus chaberti* of Umajalanta cave are the result of a recent incursion to the subterranean habitat and that they are in process of adaptation. It would be benefit to work more precisely on these populations to determine how the cave populations have genetically derived from the superficial ones and which part of the observed modifications is due only to the interaction with the environment in the embryonic and post-embryonic development.

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