



Nota

Ecomorphological variations of *Orestias* sp. (*gr agassizii*, Cyprinodontiformes, Cyprinodontidae) from Eastern slope of the Andes

Variaciones ecomorfológicas de *Orestias* sp. (*gr agassizii*, Cyprinodontiformes, Cyprinodontidae) de la Vertiente Oriental de los Andes

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Andes mountains in Bolivia are a water divider that gives rise to the Amazon basin in the Eastern and the Altiplano basin in the Western slope. *Orestias* (Cuvier & Valenciennes 1846) is one of three native genera of endemic teleost fish of the Altiplano (Parenti 1984, Villwock 1986, Vila *et al.* 2007) and is distributed from southern Peru, western Bolivia and northeast Chile, with Lake Titicaca as its main distribution area (Parenti 1984, Guerlesquin 1992, Lauzanne 1992). More than 40 species were described in the Altiplano (Parenti 1984, Vila *et al.* 2007, Sarmiento *et al.* 2014), although there is some divergence on species validity based on genetic traits (Lüssén *et al.* 2003, Vila *et al.* 2007, Garrigos *et al.* 2013, Ibañez *et al.* 2014, Takahashi & Moreno 2015). The distribution of this native genus has been a consequence of geological events that occurred during the Holocene and late Pleistocene, which have developed small disconnected basins along the Altiplano in the Western slope, where *Orestias* maintains small populations that have remained isolated in an arid and extreme climate (Parker & Kornfield 1995, Vila *et al.* 2010, 2013). The wide variety of ecological conditions in the distribution area has led to morphological differences with local adaptations and a particular phenotypic expression (Lauzanne 1982, Maldonado *et al.* 2009). For instance, *Orestias agassizii*, the species with the largest distribution of the genus, inhabits different aquatic ecosystems from the north to the south of the Altiplano, and shows high intra-specific variation (Arratia 1982, De la Barra *et al.* 2009, Flores 2013, Takahashi & Moreno 2015).

Although *Orestias* species has been described only for Western slope, there are small little-known and well

established populations of these fish in the headwaters from Eastern slope (Sarmiento & Barrera 1997, Identidad Madidi & SERNAP 2017), characterized by an abrupt relieve with pronounced slopes, deep and enclosed alluvial valleys, that provide new geomorphologic and ecological conditions to which this native fish could adapt (e.g. increased turbidity and water flow). *Orestias* populations of agassizii group reported for the Puina Valley, at the headwaters of the Tuichi River on the Eastern slope were introduced about 30 to 40 years ago. They are nowadays well established and correspond to the same group, although their identity is still unknown (Identidad Madidi & SERNAP 2017). The aim of this study was to analyse and compare the ecomorphological variation among the *Orestias* sp. (*gr agassizii*) populations from the Puina Valley on the Eastern slope and from Western slopes of the Andes mountains.

We compared ecomorphological measurements of 130 specimens corresponding to four *Orestias* sp. (*gr agassizii*) populations. Western slope populations were collected from Hichu Kotha (*CorOc1*: 28 specimens, -68.380019°; -16.174192°; 4,350 m), and Kara Kotha (*CorOc2*: 8 specimens, -68.376306°; -16.150344°; 4,450 m) lagoons from Hichu Khota Valley, northwest of La Paz city, in the “glacial cordillera” hydro-ecoregion (Wasson *et al.* 2002). Eastern slope populations were collected from Celeste (*CorOr1*: 63 specimens, -69.163199°; -14.650412°; 4,200 m), and “Siembra” (*CorOr2*: 31 specimens, -69.089377°; -14.617934°; 4,300 m, Figure 1) lagoons in Puina Valley. Nine ecomorphological attributes were evaluated (Table 1). Measurements were made to the nearest 0.1 mm using digital calliper (absolute digimatic digital callipers,

Mitutoyo, Japan). A one-way MANOVA analysis (Willks lambda test) was performed to determine the significant differences on ecomorphological attributes among populations ($p < 0.05$). When MANOVA was significant, corresponding one-way ANOVAs followed of Dunnett's test were subsequently performed for each

ecomorphological character to evaluate its potential contribution to differences among the populations. A Linear Discriminant Analysis (LDA) was carried out using R (package MASS; Ripley *et al.* 2020) to determine how accurately the morphology of specimens corresponded to their respective populations.

Table 1. Attributes used in the ecomorphological analysis of *Orestias* sp. (*gr agassizii*) populations studied.

Ecomorphological attributes	Measures taken	Ecological explanation
Relative body height (RHB)	Body height divided by standard length	Inversely related to the speed of the animal and directly related to its ability to make vertical spins (Gatz 1979b)
Relative length of caudal peduncle (RLCP)	Length of caudal peduncle divided by the standard length	Long peduncles indicate good swimmers with good resistance to sites with higher turbulence (Watson and Balon 1984)
Compression index of caudal peduncle (CICP)	Height of caudal peduncle divided by its width	More compressed peduncles indicate fish with lower speed (Gatz 1979a)
Relative area of pectoral fin (RaPF)	Area of pectoral fin divided by body surface area	High values are found in slower individuals who use fins for breaking and manoeuvres, or in individuals who inhabit waters with greater flow, using the fins as current deflectors in order to remain close to the bottom (Watson & Balon 1984)
Relative area of caudal fin (RaCF)	Caudal fin area divided by body area	Caudal fins with larger relative areas are important for acceleration (Webb 1977)
Configuration of the caudal fin (CCF)	Ratio of the square of the height of the caudal fin by its area	High values indicate active and continuous swimmers (Gatz 1979b)
Relative head length (RHL)	Head length divided by standard length	Relative to the size of prey consumed. High values may indicate fish that feed on relatively larger prey (Gatz 1979b)
Relative width of the mouth (RWM)	Mouth width divided by standard length	Relative to the size of prey consumed. High values may indicate fish that feed on relatively larger prey (Gatz 1979b)
Relative eye size (DO)	Diameter of the eye divided by the standard length	(Gatz 1979a)

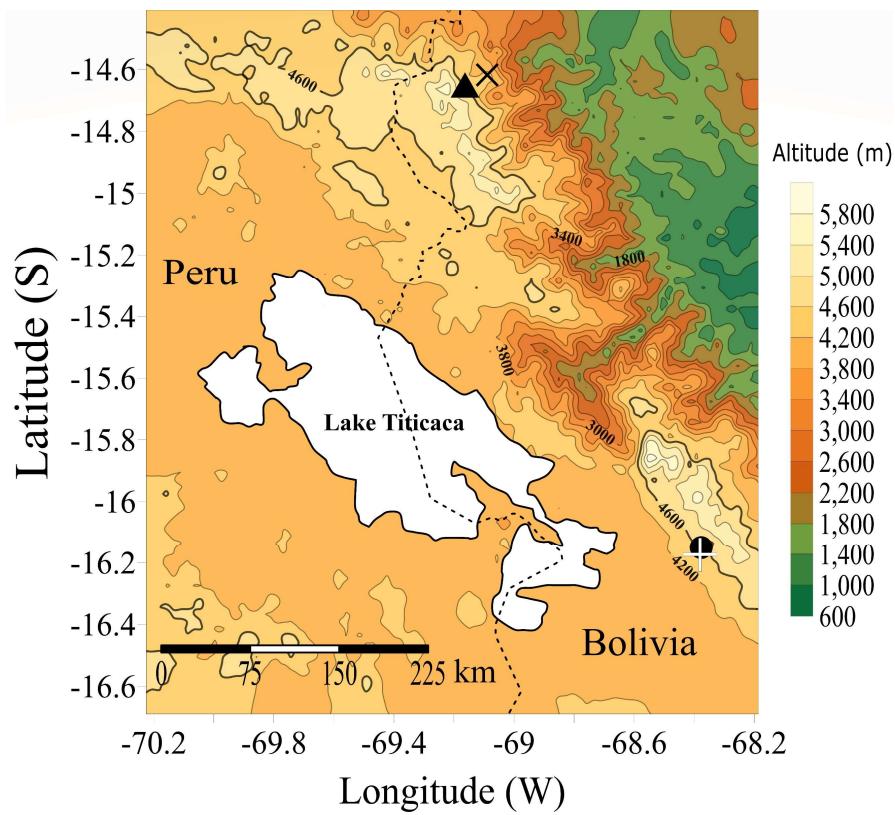


Figure 1. Map of sampling lagoons of *Orestias* sp. (*gr agassizii*) populations in the slopes of the Andes. Western slope: CorOr1 = black circle (●), CorOr2 = white cross (+); Eastern slope: CorOr1 = black triangle (▲), and CorOr2 = ex symbol (X).

Length and weight data including ranges and means are shown in Table 2. The MANOVA revealed that studied populations showed globally significant ecomorphological differences ($F=31.4$, Wilk's $\lambda = 0.08$, $p < 0.001$). Most ecomorphological attributes differentiated the populations from each mountain slope, with the exception of the caudal fin configuration (CCF, Table 3). Indeed, LDA analysis showed that the three populations were correctly discriminated according to their ecomorphological attributes (>95% of correct classification, Fig. 2, Table 4 and 5). The linear discriminant coefficients revealed that the first function (explaining 84.44% of the variation) differentiated populations principally according to eye size (DO) and relative body height (RHB). The second

function (12.23% of the variation) differentiated populations according to relative width of the mouth (RWM) and the compression index of caudal peduncle (CICP).

Living conditions in high altitude lagoons (> 3,500 m) are extreme, with high exposure to solar radiation, which can even be up to 20% higher than that recorded at sea level at the same latitude (Aceituno 1997). Moreover, the valleys located on both sides of the Andes slopes are exposed to intense climate warming caused by the combined effects of tropical location (16°S) and altitude, projecting twice the planet average warming towards 2100 (Bradley 2006, Valdivia *et al.* 2013, Rangecroft *et al.* 2016).

Table 2. Descriptive data of *Orestias* sp. (*gr agassizii*) populations sampled from Eastern and Western slope of the Andes.

Population	n	Total length (mm)		Weight (g)	
		(min-max)	(mean±SD)	(min-max)	(mean±SD)
<i>CorOc1</i>	28	41.16-133.10	75.10 ± 25.39	1.56-40.66	9.17 ± 10.77
<i>CorOc2</i>	8	141.28-169.67	152.03 ± 9.22	43.25-63.91	53.73 ± 7.12
<i>CorOr1</i>	63	23.30-52.51	32.87 ± 7.85	0.08-1.30	0.34 ± 0.30
<i>CorOr2</i>	31	23.25-75.27	36.70 ± 10.13	0.08-4.78	0.65 ± 0.84

Table 3. Descriptive statistics of univariate ANOVA of *Orestias* sp. (*gr agassizii*) populations based on ecomorphological characters. a= There are differences among both Eastern populations with Western population.

Ecomorphological attributes	F value	P value	Populations differences
RHB	74.952	<0.001	a
RLCP	18.173	<0.001	a
CICP	19.451	<0.001	<i>CorOc1,2 – CorOr2</i>
RaPF	8.634	<0.001	a
RaCF	2.649	0.051	Non
CCF	0.5302	0.66	Non
RHL	16.903	<0.001	a
RWM	9.768	<0.001	<i>CorOc1,2 -CorOr1</i>
DO	160.01	<0.001	a

Table 4. Coefficients of the LDA analysis of the *Orestias* sp. (*gr agassizii*) populations studied.

Ecomorphological attributes	LD1	LD2
RHB	-33.791409	24.015487
RLCP	1.171206	-3.788746
CICP	-1.750943	-21.066398
RaPF	-4.164207	6.825065
RaCF	-17.999487	-7.083884
CCF	26.624066	3.202326
RHL	8.114130	29.788974
RWM	-6.838686	34.106515
DO	95.567962	23.944461
Proportion	0.8444	0.1223

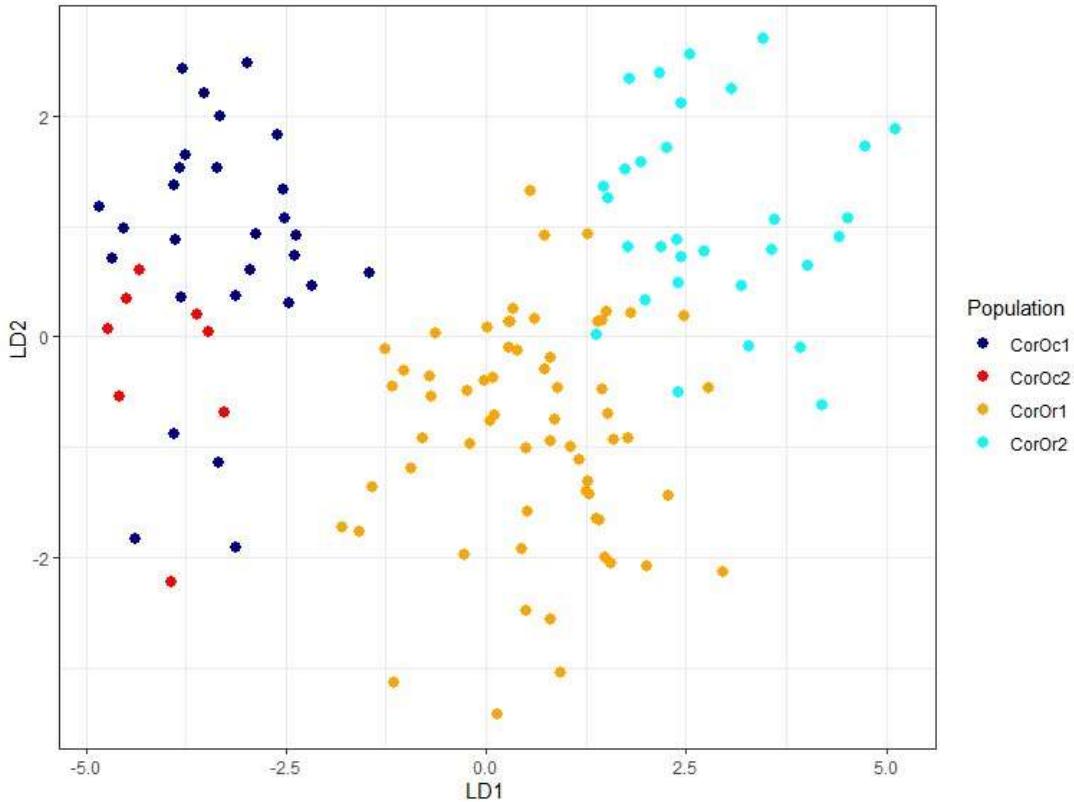


Figure 2. LDA analysis of the *Orestias* sp. (*gr agassizii*) populations studied. Western slope population: *CorOc1* and *CorOc2*; Eastern slope populations: *CorOr1* and *CorOr2*.

Table 5. Classification matrix of the LDA analysis of the *Orestias* sp. (*gr agassizii*) populations studied.

	<i>CorOc1</i>	<i>CorOc2</i>	<i>CorOr1</i>	<i>CorOr2</i>	Correct (%)
<i>CorOc1</i>	26	2	0	0	92.9
<i>CorOc2</i>	0	8	0	0	100
<i>CorOr1</i>	0	0	60	3	96.8
<i>CorOr2</i>	0	0	2	29	93.5
Total	26	10	62	32	95.8

The formation of these lagoons on the western slope corresponds to the evaporation of large paleo-lakes (Keller & Soto 1998, Placzek *et al.* 2006), unlike the lagoons on the Eastern slope, which have a glacial origin. The retreat of the waters of paleo-lakes caused a fragmentation, leaving small lagoons scattered in the Altiplano, many of them without any connection between them.

Although, the Hichu Khota and Puina Valleys are located in the “glacial cordillera” hydro-ecoregion on the humid mountains region (Wasson *et al.* 2002), they have some differences, caused by the geography of each slope. The waters that flow into the Hichu Khota Valley come from melting glaciers that run through a series of low-slope moraine hills (7-10%), that arrives to a fluvio-glacial foothill, to finally cross the Altiplano and end up in the Lake Titicaca (Marín 1989). In contrast, the waters that

run through the Puina Valley, on the Eastern slope, flow on steep slopes (> 20%), descending rapidly on sedimentary rock and presenting high turbidity values caused by the natural instability of the substrate (Identidad Madidi & SERNAP 2017), finally flowing into the Tuichi River in the ANMI-Madidi in the Amazon basin.

Indeed, morphological variations can be influenced by the habitat, the search for food resource or environmental stress conditions (Webb 1984, Parsons & Robinson 2007). Environmental differences between both slopes of the Andes could influence the body shape of the *Orestias* populations studied, as for example the length of the peduncle (RLCP), adapting to a greater water flow in the Eastern slope. Likewise, this change in water flow could also influence the macroinvertebrates composition, the main food resource of *Orestias*, which could explain the

differences found related to the head shape (head size, mouth width and eye diameter).

It is evident that the presence of *Orestias* in the Eastern slope was not entirely natural (Identidad Madidi & SERNAP 2017), despite the presence of aquatic environments with similar characteristics to those inhabited by *Orestias* (De la Barra *et al.* 2009). Conversely, the wide distribution of *Orestias* in the Altiplano is attributed to a very extensive hypothetical ancestral distribution (Lüssen *et al.* 2003), which accompanied the evaporation processes of the paleo-lakes. This fragmentation caused changes to accumulate separately, and evolve into different species that diversified through allopathic speciation (Northcote 2000). This process was observed in *Orestias* from north of Chile (Vila *et al.* 2013), even going so far as to describe a new genre (Arratia *et al.* 2017).

The presence of *Orestias* in the Eastern slope of the Andes is not well documented (Sarmiento & Barrera 1997). Furthermore, many aspects of the hydrology (e.g. water quality, flow characteristics) of the headwaters from Amazon basin remain unknown. In conclusion, significant differences between *Orestias* populations from each Andean slope were found, based on peduncle length, head size, width mouth and eye diameter. These results highlighted the need for further studies in order to identify these “Eastern *Orestias*”, applying genetic analysis or DNA barcodes, which would allow us to confirm or exclude the hypothesis that these Eastern populations would be geographical variations of a single species.

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