Acta Limnologica Brasiliensia

Publication of the Brazilian Association of Limnology

Original Articles
Acta Limnologica Brasiliensia, 2025, vol. 37, e15
https://doi.org/10.1590/S2179-975X4124
ISSN 2179-975X on-line version

Morphological variation between two fish populations – phenotypic radiation in a rare case of geographical isolation

Variação morfológica entre duas populações de peixes – radiação fenotípica em um raro caso de isolamento geográfico

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Cite as: Saluceste, F.B. et al. Morphological variation between two fish populations – phenotypic radiation in a rare case of geographical isolation. *Acta Limnologica Brasiliensia*, 2025, vol. 37, e15. https://doi.org/10.1590/S2179-975X4124

Abstract: Aim: We examined the morphology of two populations of the Neotropical Characidae *Psalidodon* aff. *fasciatus* from two distinct environments with different selective pressures. One is the single fish population from an isolated lake, hence is deprived of interaction with any other fish species for countless generations. The other shares life-history with several fish. **Methods:** We obtained 10 linear body measurements from 294 specimens from both populations, calculated and compared the ecomorphological indices for each population. **Results:** We found significant distinct morphometry between populations, primarily attributed to the high level of isolation observed in individuals from the isolated population. This population exhibited greater morphological variation, likely due to reduced selection pressure and limited ecological interactions (e.g., absence of other fish species). Conversely, the non-isolated population displayed less morphological variation, possibly as a result of more intense intra- or interspecific interactions, such as competition and predation. **Conclusions:** Considering that allopatry and major factors such as "isolation time" and "ecological interactions" are crucial drivers of evolution, this study highlights a rare case of natural isolation and provides insights for evolutionary investigations on isolated populations, allopatric speciation, and the role of ecological interactions in phenotypic intrapopulation variation.

Keywords: polymorphism; ecotypes; allopatry; interaction; isolation.

Resumo: Objetivo: Examinamos a morfologia de duas populações do Characidae Neotropical *Psalidodon* aff. *fasciatus* de dois ambientes distintos com diferentes pressões seletivas. Uma é a população única de peixes de um lago isolado, portanto, privada de interação com qualquer outra espécie de peixe por inúmeras gerações. A outra compartilha história de vida com vários peixes. **Métodos:** Obtivemos 10 medidas lineares do corpo de 294 espécimes de ambas as populações, calculamos e comparamos os índices ecomorfológicos para cada população. **Resultados:** Encontramos morfometria significativamente distinta entre as populações, principalmente atribuída ao alto nível de isolamento observado nos indivíduos da população isolada. Esta população exibiu maior variação morfológica, provavelmente devido à reduzida pressão seletiva e às limitadas interações ecológicas (por exemplo, ausência de outras espécies de peixes). Por outro lado, a população não isolada apresentou menor variação morfológica, possivelmente como resultado de interações intra ou interespecíficas mais intensas, como competição e predação. **Conclusões:** Considerando que a alopatria e fatores importantes como "tempo de isolamento" e "interações ecológicas" são impulsionadores cruciais da evolução, este estudo destaca um caso raro de isolamento natural e fornece insights para investigações evolutivas sobre populações isoladas, especiação alopátrica e o papel das interações ecológicas na variação fenotípica intrapopulacional.

Palavras-chave: polimorfismo; ecótipos; alopatria; interação; isolamento.

1. Introduction

Ecomorphology investigates the relationship between phenotype and environmental or ecological traits, especially in relation to natural selection and evolution (Breda et al., 2005). Natural allopatric events offer unique opportunities to test hypotheses related to the ecomorphological paradigm, particularly when geographic barriers separate populations and subject them to distinct environmental pressures (Lande, 1980; Shibatta & Artoni, 2005; Torres-Dowdall & Meyer, 2021). Over time, these differences can lead to the accumulation of morphological and molecular divergences, resulting in speciation.

Although geographic isolation is an important evolutionary mechanism, other forms of reproductive isolation, such as temporal, behavioral, and mechanical isolation, can also trigger evolutionary processes that favor the formation of new species (Meffe, 1994; Leroux et al., 2022). Reduced gene flow and exposure to new environmental conditions select for distinct phenotypic traits. However, the presence of other species can moderate these effects. For example, Scharnweber et al. (2013) demonstrated that, even in environments with similar selective pressures, the presence of predators can shape the morphology of prey organisms, promoting body shapes that facilitate escape, but do not necessarily result in greater intrapopulation morphological diversity.

Complex biological interactions, such as competition and predation, play a central role in determining the morphological traits of species. Studies show that competitors reduce niche diversity (Hall & Kingsford, 2016), while predators can regulate competitive pressure, allowing greater diversification (Arribas et al., 2018). Thus, geographic isolation, combined with the absence of predators, can lead to divergent adaptive trajectories.

In South America, a region with high freshwater fish biodiversity (Pelicice et al., 2017), the Characidae family stands out for its diversity, including the genus Psalidodon, with 34 species (Fricke et al., 2022). This genus presents high phenotypic plasticity, allowing its survival in different environmental conditions (FEOW, 2023). Among the Psalidodon species, some are endemic to isolated basins, such as the Iguaçu River, which has an endemism rate of 96% (Baumgartner et al., 2012), reinforcing the importance of geographic isolation in phenotypic divergence and speciation.

This study investigated two populations of *Psalidodon* aff. *fasciatus* (Cuvier, 1819) from environments with rare geological configurations that promote different degrees of isolation. The isolated population inhabits a lagoon with no connection to other drainage systems and faces severe restrictions on gene flow (Artoni & Almeida Matiello, 2003; Kerniske et al., 2021). In contrast, the connected population coexists with other fish species, including predators, and maintains gene flow with the main basin (Artoni et al., 2006).

Considering that biotic pressures, such as competition and predation, significantly influence evolutionary processes, especially when abiotic conditions are similar, this study sought to investigate how geographic isolation influences the morphometry of these populations. We hypothesize that the isolated population has a greater potential for phenotypic radiation due to the absence of competitors and predators, while the connected population exhibits more homogeneous ecomorphological patterns, resulting from more intense biotic interactions.

2. Material and Methods

This research was conducted under permits issued by the Ministry of the Environment (MMA / ICMbio No. 15115-1) and the Environmental Institute of Paraná (IAP Protocol 15.190.528-5; Authorization 15.18). All procedures involving animal subjects were approved by the Research Ethics Committee on the Use of Animals of the Universidade Tecnológica Federal do Paraná (CEUA No. 2018-025/2018).

2.1. Study area

Vila Velha State Park is a conservation unit in southern Brazil (25°14'09"S, 50°00'17"W) that features unique geological formations from the Siluro-Devonian period (Guimarães et al., 2007), with steep craters, locally known as furnas, which are Furnas that shelter and separate lakes (Figure 1). The studied environments are the Furna and the Lagoa Dourada. The Furna is a slope of landslides (or a ground cavity) formed from sandy soil collapse which hosts a natural and completely isolated 50 m deep lake, with an approximate diameter of 80 m and steep walls of 60 m until the surface of the lake, completely deprived of connection with other water bodies (Artoni et al., 2006; Campos & Dalcomune, 2011). Two kilometers far from the Furna, a non-isolated population inhabit the Lagoa Dourada, which is described as a silted furna in its terminal stage. It takes sediments from other environments and is connected to the Tibagi Basin through a small stream (Melo & Giannini, 2007). The isolated population of lambaris in the Furna has been deprived of interaction with other fish species for countless generations (Artoni & Almeida Matiello, 2003; Shibatta & Artoni, 2005; Kerniske et al., 2021, 2023), while the population in Lagoa Dourada coexists with several other fish species, including predators and competitors (Lévêque et al., 2008).

2.2. Sampling

Fish were sampled monthly between February and October 2019 from the Furna (isolated population) and Lagoa Dourada (non-isolated population), using trawls and minnow traps. After capture, individuals were transported to the Aquatic Ecology Laboratory of the Federal Technological University of Paraná, where they were anesthetized with benzocaine and subjected to morphological measurements (Alves et al., 2021).

The measurements followed the linear measures used in fish taxonomy (according to Taphorn & Lilyestrom, 1984). Ten body measurements were taken using a digital caliper (precision of 0.01 mm): standard length, head length, body height, anal fin length, dorsal fin length, pectoral fin length, pelvic fin length, snout length, orbital diameter, and inter-orbital space (Figure 2).

2.3. Statistical analyses

The number of sampled individuals reflects the ecological and methodological characteristics of the studied populations. The isolated population naturally presented fewer individuals due to spatial confinement and the lack of predation pressure from fish, which made their capture more efficient. Furthermore, differences observed between sites are linked to the sampling methods used: in the confined environment of the Furnas, only the target species was captured, whereas in Lagoa Dourada,

Figure 1. Furna 2 (A) and Lagoa Dourada (B) at Vila Velha State Park, Paraná, Brazil. The Furna 2 is isolated from any other water body while Lagoa Dourada is connected to the Tibagi basin.

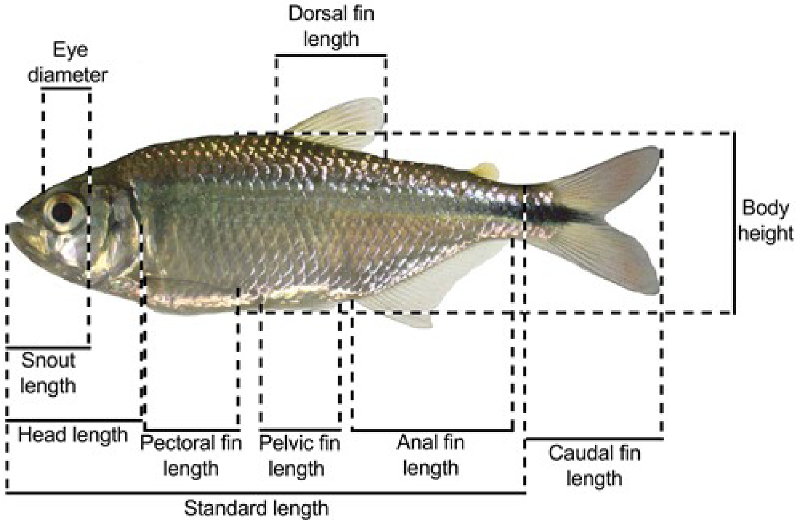

Figure 2. General outline of an individual of *Psalidodon* aff. *fasciatus* indicating the morphological measures (infraorbital space is not indicate) (Image modified from the original source: Luz Elena de La Ossa Guerra.)

the same sampling effort resulted in the capture of additional species, thereby reducing the proportion of the target species in the total collected.

From the linear measurements, ecomorphological indices were calculated following methods outlined by Winemiller (1991) and Oliveira et al. (2010). The use of morphological indices were adopted to control the effect of the size on the analysis (Peres-Neto, 1999). Then indices representing the head length, body height, pectoral fin length, pelvic fin length, anal fin length, dorsal fin length and caudal fin length were standardized with standard length; snout length and eye diameter were standardized by the head length, and infraorbital distance standardized by body width.

To summarize the ecomorphological characteristics of the populations, a Principal Coordinate Analysis (PCoA) was conducted based on the euclidean distance matrix. To complement the PCoA a Principal Component Analysis was performed (PCA), to obtain the eigenvectors of each morphological index according to the ordination axis. The number of retained axes for interpretation followed the Kaiser-Guttman criterion (eigenvalues > 1.0) (Kaiser, 1961). The multidimensional distribution obtained from the PCoA's axes allowed for the calculation of the ecomorphological space for each population using the functional richness (Fric) method (Petchey & Gaston, 2002). The resulting area represents the intraspecific ecomorphological range and variability exhibited by the isolated and non-isolated populations.

Additionally, a Permutation Multivariate Analysis of Dispersion (PERMDISP) was performed based on the euclidean distance matrix. It is a parametric test that considers the coordinates of each individual on the multivariate ordination and compares the mean distance of the individuals in the group and between the groups (Anderson, 2006). The considered groups are formed by individuals of the different populations (isolated and non-isolated).

Also, a non-parametric multivariate variance analysis (MRPP - Multi-Response Permutation Procedure) was employed to detect differences in the ecomorphological indices between the *Psalidodon* aff. *fasciatus* populations (McCune et al., 2002). This analysis was chosen due to the data not meeting the assumptions of normality.

To ensure replication of the methods, the description of the statistical analyses was detailed, including the use of Euclidean distances to calculate the ecomorphological space, the application of PERMDISP to test for differences in dispersion among groups, and the execution of MRPP to verify significant nonparametric differences. The analyses were conducted using R software (R Core Team, 2024), with the "ade4," "vegan," and "FD" packages (Thioulouse et al., 2018; Oksanen et al., 2025; Laliberté et al., 2014). The MRPP analysis was performed using the PC-Ord software.

To assess differences in body size between the populations of *Psalidodon* aff. *fasciatus* from Furnas (isolated population) and Lagoa Dourada (non-isolated population), we performed an analysis of variance (ANOVA). Initially, we applied Levene's test to verify the homogeneity of variances between the groups for the variables standard length (SL) and total length (TL). The test was performed considering a significance level of 0.05. Next, we used one-way analysis of variance (ANOVA) to compare the means of SL and TL between the populations. Additionally, we included boxplots to illustrate morphological differences between groups, providing a visual representation of the observed variations. This approach allowed us to assess whether the observed differences were statistically significant.

3. Results

A total of 294 individuals from the Furnas and 102 from Lagoa Dourada were analyzed, the Principal Component Analysis (PCA) identified three main axes (Table 1), which together explained 58.4% of the cumulative variance of the data. The first axis, responsible for 30.53% of the variance, was strongly associated with head length, body height, and fin length (anal, dorsal, and pelvic) (Figure 3). The second axis, explaining 17.6%, stood out for its association with snout length and infraorbital distance. In both axes, the Furnas population presented greater morphological amplitude compared to Lagoa Dourada, indicating greater variability in specific metrics (Table 1). Functional richness (FRic) corroborated this trend, with the isolated Furnas population presenting a FRic of 355.15, while the connected Lagoa Dourada population registered a FRic of 52.97, evidencing a lower functional diversity in the latter environment.

Multivariate permutation dispersion analysis (PERMDISP) did not indicate statistically significant differences in morphological dispersion among populations ($p = 0.413$; Table 1), suggesting that variability within groups is greater than between groups. On the other hand, the multi-response permutation procedure (MRPP) revealed significant differences in overall ecomorphological patterns between the isolated (Furna) and connected (Lagoa Dourada) populations of *Psalidodon aff. fasciatus*, ($T = -19.304$, $A = 0.026$, $p < 0.05$) which. The negative T value indicates a clear separation between the groups, while the A value suggests moderate heterogeneity, meaning that the populations differ significantly in their ecomorphological profiles without being completely dissimilar or random in structure. These results confirm that the observed differences between the populations are statistically significant and reflect meaningful ecological divergence.

Statistical differences in ecomorphological patterns between populations were confirmed by nonparametric testing ($p < 0.05$) (Table 2). To avoid spurious patterns caused by collinearity between morphometric variables, we incorporated body length as a covariate in a nonparametric analysis of covariance.

Analyses considering body size confirmed significant differences between the isolated (Furnas) and non-isolated (Lagoa Dourada) populations. Levene's test indicated homogeneity of variances for standard length (SL) and total length (TL) between the groups ($p = 0.0643$), allowing the use of ANOVA (Figure 4).

ANOVA revealed highly significant differences for both variables analyzed. For standard length (SL), the results were $F(1, 205) = 300.5$; $p < 2e\text{-}16$. For total length (TL), $F(1, 205) = 289.5$; $p < 2e\text{-}16$. Thus, individuals from the Furnas population

Table 1. Eigenvectors for each ecomorphological index of *Psalidodon* aff. *fasciatus*, according to the PCA axis and eigenvalues and cumulative variation for the axis.

Ecomorphological Index	PC1	PC2	PC3
Relative Head Length (RHdL)	-0.41	0.27	-0.39
Relative Body Height (RBdH)	-0.37	0.12	-0.18
Relative Pectoral Fin Length (RPtL)	-0.32	-0.04	-0.04
Relative Pelvic Fin Length (RPvL)	-0.41	-0.15	0.10
Relative Anal Fin Length (RAL)	-0.38	0.00	0.22
Relative Dorsal Fin Length (RDL)	-0.41	-0.14	0.24
Relative Caudal Fin Length (RCdL)	-0.32	-0.10	0.27
Relative Snout Length (RSL)	-0.05	-0.63	-0.06
Relative Eye Diameter (RED)	-0.07	-0.33	-0.77
Relative Infraorbital Distance (RIOD)	0.11	-0.59	0.16
Eigenvalues	3.05	1.77	1.02
Cumulative Variance Percentage	30.53%	48.19%	58.43%

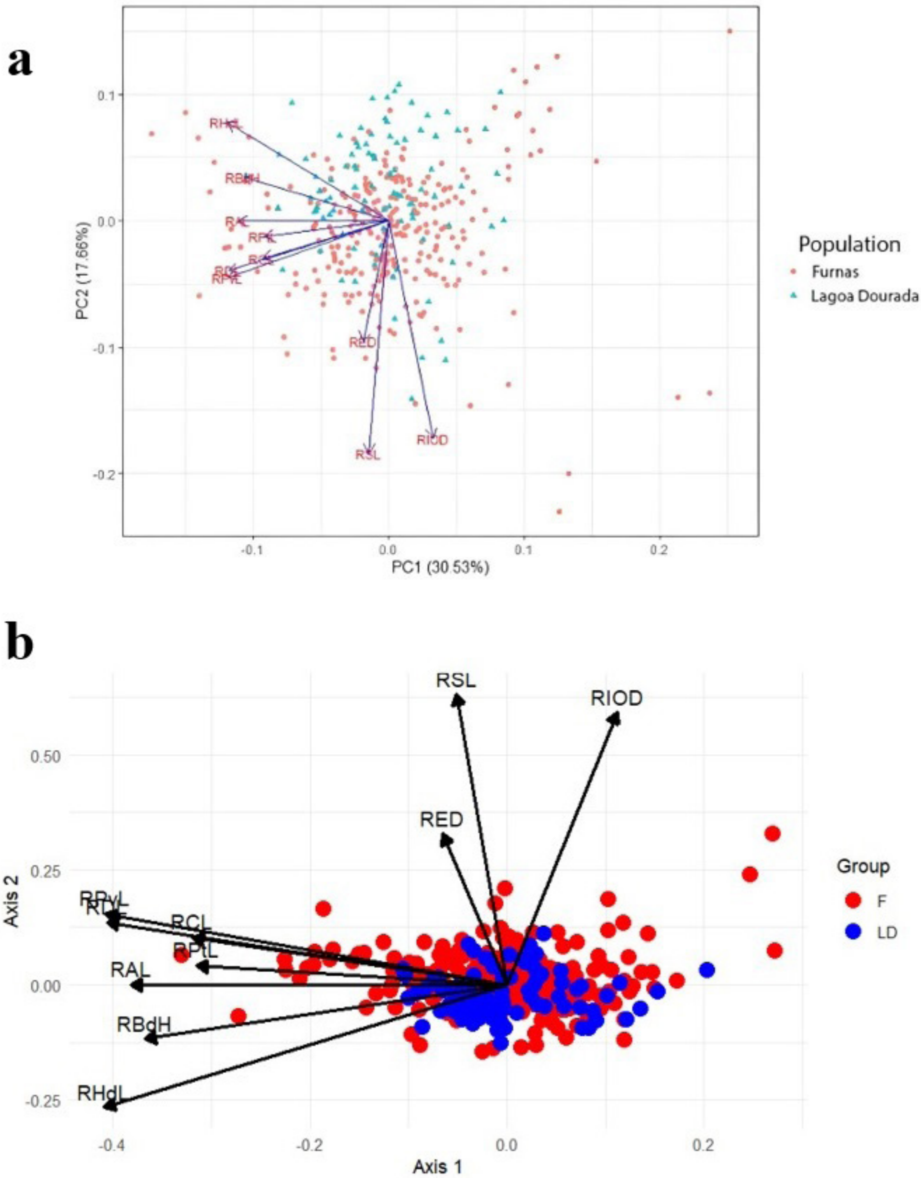

Figure 3. Multivariate ordination analyses of morphological variation in *Psalidodon* aff. *fasciatus* populations from an isolated (Furnas, F) and a connected (Lagoa Dourada, LD) environment at Tibagi River. (a) Principal Component Analysis (PCA) showing individual scores and vector loadings of ecomorphological indices; (b) Principal Coordinate Analysis (PCoA) based on Euclidean distance, illustrating the multivariate distribution of individuals and associated morphological vectors (see Table 1).

Table 2. Permutation Multivariate Analysis of Dispersion (PERMDISP) results, comparing morphological variability in isolated and non-isolated populations of *Psalidodon* aff. *fasciatus*.

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)
Groups	1	0.002	0.002	0.669	0.413
Residuals	367	1.138	0.003		

presented, on average, greater standard and total length compared to those from Lagoa Dourada (Figures 4a and 4b). This variation aligns with the greater functional richness and morphological range observed in the PCA data. At the same time, the lower variability in Lagoa Dourada reflects the more homogeneous patterns associated with intense biotic interactions.

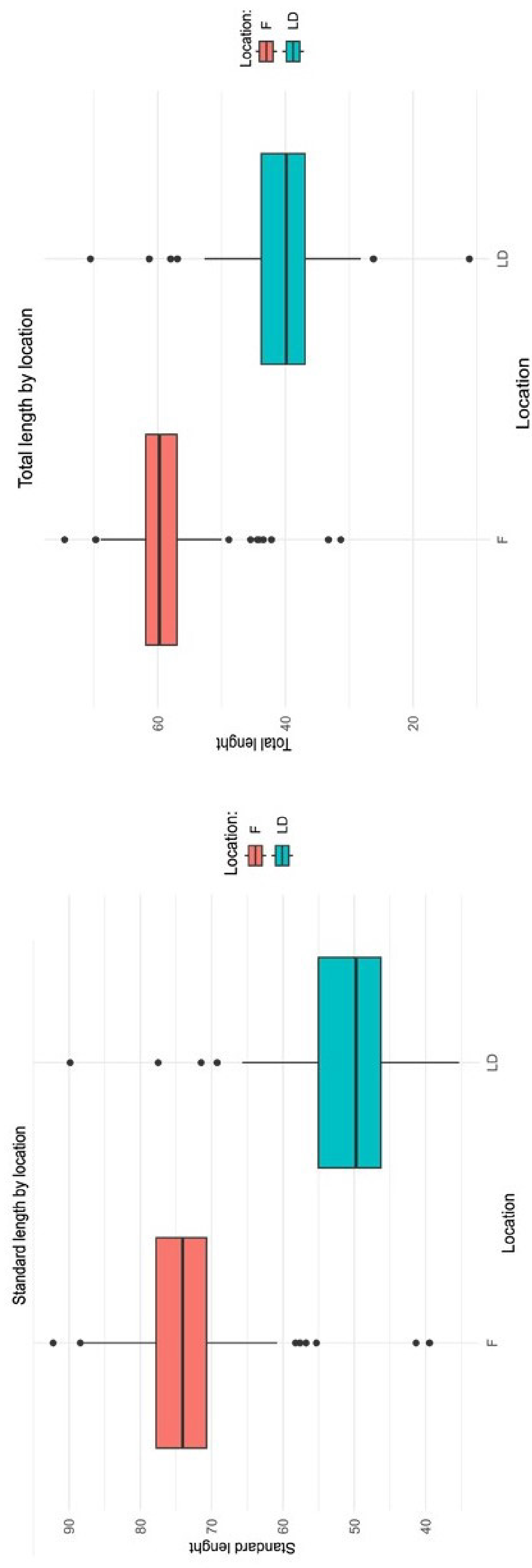

Figure 4. Morphometric variation in *Psalidodon* aff. *fasciatus* from the isolated (F) and connected (LD) populations: (a) Standard Length (SL); (b) Total Length (TL).

4. Discussion

Proportion-based indices provide an effective approach to detecting differences in body shape, minimizing size-related effects while illuminating the ecological traits of the studied populations (Winemiller, 1991; Breda et al., 2005). The analysis revealed greater phenotypic variability in the isolated population compared to the non-isolated one, likely due to differing selective pressures in their respective environments. This morphological diversity may stem from natural phenotypic plasticity or genetic factors, such as congenital abnormalities (Kerniske et al., 2021), although other unexamined influences may also contribute to ecomorphological variation. The indices used in the study were designed to control for size effects, but the size and sex of individuals could still influence the observed morphological patterns. While these variables were not specifically analyzed in the present study, this limitation should be addressed in future research.

The studied environments exhibit significant ecological differences. Lagoa Dourada, connected to a stream, hosts various fish species, including predators like Hoplias sp., which prey on small Characidae such as lambaris. Predation is a well-known selective pressure influencing morphology (Lima & Dill, 1990; Langerhans & Makowicz, 2009; Ahti et al., 2020). In this non-isolated environment, selective pressures appear more stringent, resulting in reduced morphological variability compared to the isolated Furna population, which experiences little to no interaction with other species or predators (Kerniske et al., 2023). The absence of these biotic interactions in Furna likely contributes to its significant morphological variability, suggesting that intraspecific competition plays a critical role in shaping ecological interactions within the population (De Santis et al., 2021). Greater morphological variation can reduce competition between individuals (Svanbäck et al., 2008), and traits such as caudal peduncle size and eye diameter showed notable variation in this environment.

Standardizing the ecomorphological indices removed the effects of individual size, allowing observed differences between groups to be attributed to selective pressures and environmental variation. The indices contributing most to the differences between populations were relative head length, relative body height, and anal and dorsal fin length. In the Furna population, the greater variability of these traits may be linked to the absence of biotic interactions, enabling greater exploration of ecological niches and increased phenotypic flexibility (Svanbäck et al., 2008; Robinson & Wilson, 1994).

Eye diameter also showed significant variation in Furna, potentially reflecting adaptations to low-light conditions. Individuals with larger eyes might have advantages in detecting prey at greater depths, while smaller eyes may be favored due to lower energy demands in an environment where vision is less critical (Kowalko, 2020; Sifuentes-Romero et al., 2023). Conversely, in Lagoa Dourada, the lower variability in eye diameter appears to be associated with intense selective pressures imposed by interactions with other species, including predators such as Hoplias sp. (Lima & Dill, 1990; Langerhans & Makowicz, 2009). The depth of Furna, which reaches around 50 meters with minimal light penetration below 15 meters, creates conditions reminiscent of cave habitats. In such environments, variation in eye size could reflect adaptations to different light levels within the water column, potentially minimizing intraspecific competition. Personal observations of *Psalidodon* aff. *fasciatus* at depths of 18 meters further support the hypothesis that this variation is linked to ecological niche differentiation. This process, known as character release, has been documented in other species, where isolated populations show greater differentiation in traits associated with resource use (Robinson & Wilson, 1994).

Character release can promote individual specialization within a population, described as an adaptive eco-evolutionary process (Bolnick et al., 2011). These dynamics may result in disruptive selection and speciation, as observed in African cichlids (Shaw et al., 2000), sticklebacks (Bolnick, 2004), and Poeciliidae in Central America (Poeser, 1998). The increased morphological variation observed in Furna suggests heightened intraspecific competition, which could drive speciation over time, as seen in specialized cichlids from African lakes (Langerhans et al., 2007).

The contrasting results of the PERMDISP and MRPP analyses highlight important aspects of morphological variability between Furna and Lagoa Dourada populations. While PERMDISP did not indicate statistically significant differences in dispersion, MRPP revealed significant differences in general morphological patterns. These findings suggest that despite a lack of evidence for variability differences within groups, the morphological divergence between populations is pronounced. The greater variability observed in Furna may reflect its geographic isolation, allowing a broader range of phenotypic expression. Conversely, the reduced variability in Lagoa Dourada could be interpreted as a subset of Furna's variability, shaped by the specific selective pressures and environmental conditions present in Lagoa Dourada.

Non-isolated populations, such as those in Lagoa Dourada, are subject to stronger selective pressures due to interspecific competition and predation, which may result in reduced trait variability. This phenomenon aligns with "inverse selection," where isolated populations lose traits due to relaxed selective pressures (Wund et al., 2015). The natural reduction of selective pressures in Furna may explain its greater morphological diversity.

This study highlights how isolation and differing selective pressures influence morphological variability, suggesting that environmental factors play a significant role in shaping phenotypic patterns. Additionally, the analysis was limited to two populations due to the unique nature of the studied system, where one population represents a natural isolated environment (Furnas) and the other a non-isolated natural lagoon (Lagoa Dourada). This specific comparison was designed to provide insight into how contrasting ecological and geographical contexts influence morphological variability. Despite the small sample size (N = 2), the focused approach emphasizes the distinctiveness of this system and its potential to serve as a model for understanding the interplay between isolation, selective pressures, and phenotypic evolution. Future studies should explore these questions by integrating molecular and morphological approaches and incorporating additional populations from diverse ecological contexts (Anderson, 2006; Petchey & Gaston, 2002).

5. Conclusion

This study revealed significant morphological differences between isolated and connected populations of *Psalidodon* aff. *fasciatus*, highlighting the impact of geographic isolation and ecological interactions on phenotypic variation. The greater variability observed in the isolated population of Furnas can be attributed to the absence of intense biotic interactions, such as predation and competition, favoring the release of traits and allowing greater exploration of ecological niches. On the other hand, the lower variability in Lagoa Dourada reflects more stringent selective pressures, which promote stabilizing selection and limit phenotypic diversity, especially in environments with high species density.

The results reinforce the importance of geographic isolation in phenotypic diversification and suggest that populations subject to relaxed selective pressures may have greater potential for specialization and, eventually, speciation. Variability in specific traits, such as eye diameter and caudal peduncle length, is aligned with adaptations to particular environmental conditions, such as low light in deep environments. The dynamics observed in the studied populations illustrate eco-evolutionary processes that act at different scales, including intraspecific competition and character release.

From an evolutionary perspective, the study contributes to the understanding of how biotic and abiotic factors influence the morphology of isolated and connected populations, with important implications for conservation biology. Maintaining isolated populations, such as that of Furnas, is essential to preserve phenotypic and functional diversity, especially in scenarios of environmental change and habitat fragmentation.

Furthermore, understanding how selective pressures act in connected populations, such as that of Lagoa Dourada, can assist in the sustainable management of species that coexist in denser and more competitive environments. However, some limitations of the study should be highlighted. The lack of molecular data made it impossible to distinguish between differences attributable to phenotypic plasticity and those resulting from genetic divergence. Future studies should integrate morphological and genetic approaches to clarify the relative influence of these factors. Furthermore, expanding the analysis to include multiple populations in different ecological contexts may provide a more comprehensive view of the evolutionary processes and adaptive responses of *Psalidodon* aff. *fasciatus*.

Finally, this work highlights how geographic isolation, combined with varying selective pressures, can significantly influence patterns of morphological variation. This integrative and data-driven approach contributes to the field of ecomorphology and offers perspectives for biodiversity conservation in aquatic environments

Acknowledgements

We thank the support of Universidade Tecnológica Federal do Paraná and Universidade Estadual de Ponta Grossa for supporting the research and sample collection. We also thank colleagues at the Aquatic Ecology Laboratory who indirectly helped to carry out this research and Vinicius Abilhoa for making his laboratory available for training to learn how to perform the first morphological analyses. IPA thanks CNPq (443256/2023-3) for a postdoc grant. FBS thanks CAPES (88887.080649/2024-00) for the master's scholarship.

Data availability

All data analyzed in this research are available in the GitHub repository: https://github.com/fernandasaluceste/furnas. Access is open, and the data can be accessed directly via the link.

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Received: 09 May 2024
Accepted: 17 June 2025

Associate Editor: Ronaldo Angelini