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Parallel Evolution of Allometric Trajectories of Trophic Morphology between Sympatric Morphs of Mesoamerican *Astyanax* (Characidae)

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Abstract: Parallel evolution of the body shape and trophic-related traits has been detected between sympatric pairs of lake-dwelling characin fishes in Mesoamerica. Here, we evaluated the variation in and the ontogenetic allometric trajectories of trophic morphology between sympatric *Astyanax* morphs (elongate and deep-body) in two geographic systems, Lake Catemaco (Mexico) and San Juan River basin (Nicaragua and Costa Rica). Using geometric morphometrics, we determined the shape variation and disparity in the premaxillary bone, and the patterns of allometric trajectories between morphs in each system. We found a higher differentiation and disparity in the premaxilla shape between morphs from San Juan River basin than between the Lake Catemaco ones. We found shared (parallel evolution) patterns of divergence between systems, which included allometric trajectories showing a positive correlation between the premaxilla shape and log centroid size, as well as trajectories being extended in the elongated-body morph (truncated in the deep-body morph). Regarding the unique patterns of divergence, we recovered parallel allometric trajectories between morphs from Lake Catemaco, while the San Juan River basin morphs showed divergent trajectories. Our results are congruent with the hypothesis that divergence in trophic morphology can be considered a triggering factor in the divergence in the genus *Astyanax* from Mesoamerica.

Keywords: premaxilla shape; late ontogenetic trajectories; ecological divergence; geographical cline; geometric morphometrics

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1. Introduction

Body size influences many traits in animals [1–5]. Allometry is defined as the variation in morphometric variables or other features of organisms associated with size. Thus, ontogenetic, static, and evolutionary allometries are recognized based on whether this relationship is over the development of individuals, across individuals at a similar developmental stage, or across species, respectively [2,6]. Ontogenetic allometry could contribute to evolutionary diversification, as has been described in studies of morphological diversification associated with ontogenetic allometric trajectories at intra- and interspecific levels (e.g., [7–12]). According to the latter idea, changes in ontogenetic trajectories could have an adaptive basis, being useful to the study of how different selective pressures imposed by ecological factors could affect development [10,12,13].

The fish genus *Astyanax* Baird and Girard, 1854, distributed in North and Central America, shows ideal features for the study of ontogenetic allometric trajectories associated with trophic adaptation in an ecological divergence context. These fishes are known to adapt to a very wide range of ecological conditions, the cave-adapted morphs being the most conspicuous example [14–17]. Interestingly, previous works have found evidence of the parallel evolution of sympatric morphs associated with trophic morphology and body shape divergence in different Mesoamerican lakes inhabited by lineages of *Astyanax*, which evolved independently [18–22]. This has been shown in two distant geographical systems, the San Juan River basin in Central America (which includes the Lakes Managua and Nicaragua), and the Lake Catemaco system in Mexico [20,23,24]. The lacustrine morphs, consisting of an elongate-body and a deep-body morph (Figure 1), show major morphological differences, which prompted their original consideration as different genera (i.e., *Astyanax* and *Bramocharax*, [25,26]). Some of the morphological differences distinguishing these morphs correspond to the body depth and the head length, as well as to their trophic morphology (i.e., number of maxillary teeth and of cuspids on the anterior tooth of premaxilla, see [21,22]), which are associated with alternative trophic habits. Additionally, a geographical cline (i.e., a differentiation gradient between north and south systems) in morphological differentiation between morphs was suggested between the Lake Catemaco and San Juan River systems [22], and, recently, we explored the genetic and ecological differentiation between sympatric morphs of *Astyanax* in these systems, finding evidence of a geographical cline also in the genetic differentiation [23,24]. The morphs from the San Juan River system show greater genetic differentiation in contrast to the lack of thereof between morphs in Lake Catemaco, suggesting that both systems represent different stages of divergence between sympatric *Astyanax* morphs pairs [23,24].

Previous studies have shown that size is an important factor determining body shape variation in *Astyanax* morphs from the Lake Catemaco and San Juan River systems [19,21]. Particularly, there are differences in body size between morphs in Lake Catemaco [19], where the elongate-body morph reaches a larger size than the deep-body morph. Despite the possible relevance of size in trophic morphology variation and its potential role on the diversification in the *Astyanax* genus [21,22] and in Characiforms [27], it remains to be explored how trophic morphology varies throughout ontogeny and if it can be considered as a mechanism promoting morphological diversification. Here, we focused on determining the ontogenetic allometric trajectories of trophic morphology between sympatric morphs of *Astyanax* in two different geographic systems, by analyzing the shape of a relevant trophic structure, the premaxillary bone.

We have a two-fold objective. First, we aimed to compare the variation in the premaxilla shape and the disparity (i.e., morphological diversity, [28]) between morphs inside each system. Second, we aimed to describe the patterns of ontogenetic allometric trajectories of the premaxillary bone between sympatric morphs of *Astyanax* in each system and to elucidate shared (parallel evolution) and unique patterns of divergence between morphs for each system.

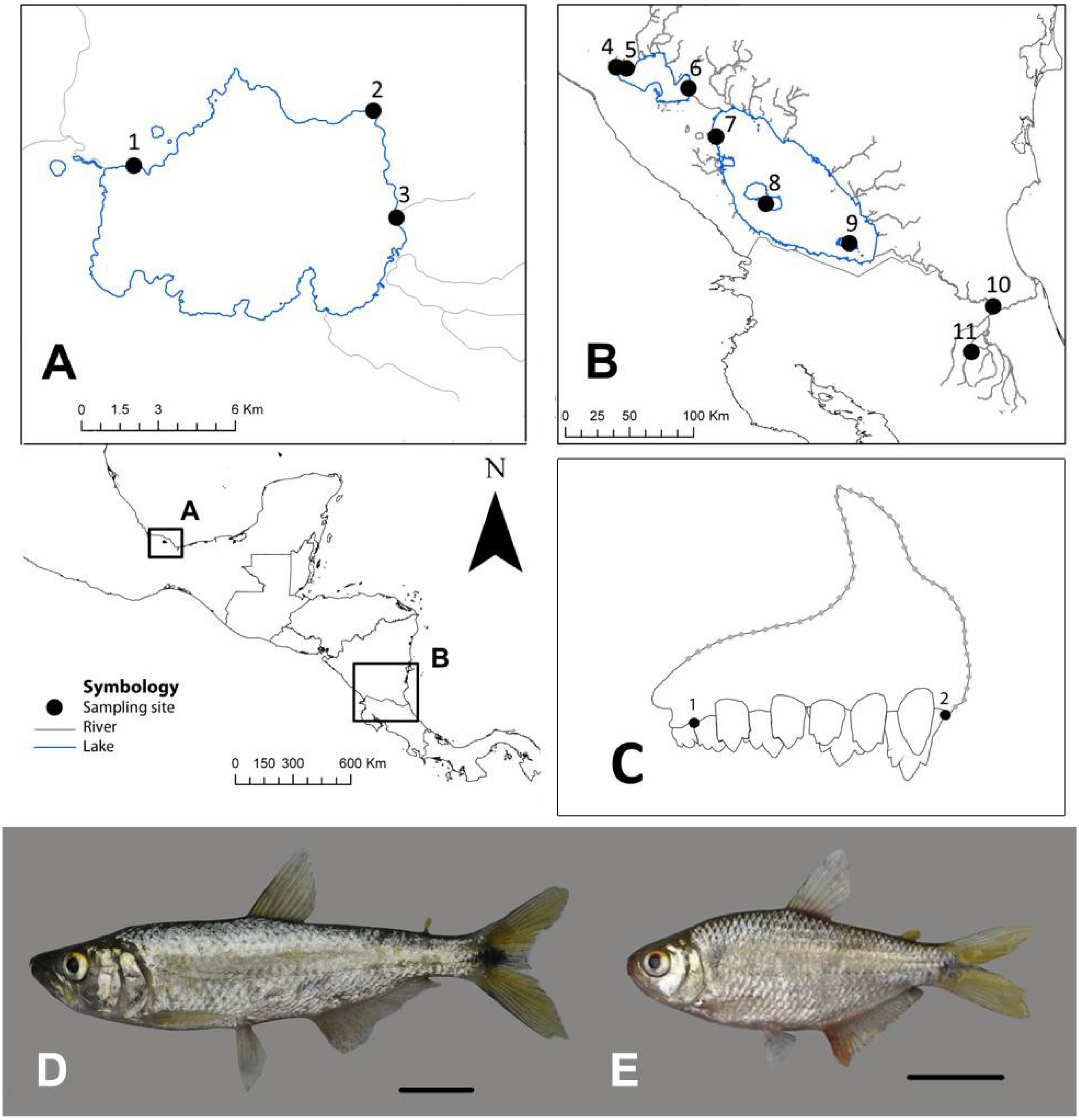

Figure 1. Sampling sites and morphs included in this study. (**A**) Lake Catemaco system (1–3) and (**B**) San Juan River system (4–11). (**C**) Landmarks (black) and 50 semilandmarks (grey) in the premaxilla. Examples of (**D**) elongate-body and (**E**) deep-body morphs from San Juan River system. Scale bar in (**D**,**E**) = 2 cm.

2. Methods

2.1. Sampling Collection

We collected *Astyanax* fish of the two different morphs from two geographical systems: in México, from Lake Catemaco (belonging to Papaloapan basin); and in Central America, from San Juan River basin, which includes Lake Managua, Lake Nicaragua, and the Sarapiquí River, which flows into the San Juan River, the outlet of the two mentioned lakes (Figure 1). We focused on late ontogenetic stages (i.e., post-embryonic development), as is usual in allometric trajectories studies (see [29]). We analyzed a total of 218 specimens

(130 deep-body and 88 elongate-body) collected in 11 sampling sites in both systems between November 2011 and January 2017 (Table 1, Figure 1). We used cast nets and gill nets to collect the fish and assigned specimens to morphs based on their external morphology [25,26]. Fish were euthanized in iced water and voucher specimens were deposited at the Colección Nacional de Peces, IBUNAM, México.

Table 1. Number of specimens of both morphs by sampling sites and geographical system. Site numbers according to Figure 1. Size range of specimens (standard length), Lake Catemaco: deep-body 49.4–89.5 mm, elongate body 6.70–135.7 mm, San Juan River: deep-body 45.8–102.1 mm, elongate body 42.9–132.5 mm.

System	Lake/River	Sampling Site	Site Number	Deep-Body	Elongate-Body
Lake Catemaco	Lake Catemaco	Market	1	-	7
		Coyame	2	22	14
		Tebanca	3	17	16
San Juan River	Lake Managua	Puerto Momotombo	4	1	1
		South of Momotombo volcano	5	7	7
		Isla Escondida	6	6	2
	Lake Nicaragua	Isletas de Granada	7	23	9
		Ometepe	8	19	6
		Solentiname	9	1	1
	Sarapiquí River	Tambor	10	18	24
		Tirimbina	11	16	1

2.2. Premaxilla Shape Variation

For each specimen, we dissected the premaxillary bone and cleaned it with 1 M of KOH solution for 15 min. We took a picture of each bone with a stereoscope (Zeiss Stemi305) and the shape of the premaxilla was quantified using a 2D geometric morphometric approach. We digitalized two landmarks and 50 semilandmarks (Figure 1C) using TPSDig2, version 2.31 [30]. We performed a Procrustes superimposition and obtained relative warps (RWs) and the centroid size (measured as the square root of the summed, squared distances of all landmarks from their geometric center, which is considered a proxy of size in geometric morphometric studies) using the program TPS Relw, version 1.69 [30]).

To describe the variation in the shape of the premaxilla between morphs in each system, we plotted RW1 vs. RW2 and compared the disparity in shape (Procrustes variance) between morphs by system. We used the Geomorph package in R (R Core Team, 2013, version 4.02: http://cran.r-project.org, accessed on 22 June 2020) to perform the disparity analysis. The Procrustes superimposition was carried out using the gpagen function, and comparisons of Procrustes variance (morphol.disparity function) were made between sympatric morphs. Additionally, to graphically represent the differences in the shape of premaxilla between morphs, we calculated the consensus shape of Procrustes coordinates of morphs by system (mshape function) and plotted the shape differences using the deep-body morph for reference (plotRefToTarget function, magnification = 2) for each system.

2.3. Ontogenetic Allometric Trajectories

In order to analyze the late allometric ontogenetic trajectories, we first regressed RW1 and RW2 against the log centroid size for each morph in both systems. We then performed ANCOVA models for each system to test for differences in the shape of premaxilla (RW1 and RW2 of premaxilla: response variables) related to log centroid size (covariate), morph, and the interaction morph*log centroid size (to test for differences in slopes between morphs). We included a biplot of the RW1 and RW2 to represent the overall shape of the premaxilla. ANCOVA models and regression analyses were performed in R.

3. Results

3.1. Premaxilla Shape Variation

We found a shared pattern of divergence in the premaxilla shape (parallel evolution) between morphs in the two systems analyzed (Figure 2). RW1 was related to the width and the angle of the ascending process, with positive values showing a narrower ascending process and a sharper angle corresponding to the elongate-body morph in both systems, with opposite patterns found in the deep-body morph. RW2 showed variation related to the width of the premaxilla and length of the ascending process, with morphs showing no differentiation based on this relative warp. RW1 and RW2 together explained most of the variation (80.2%) of the shape of the premaxilla (Supplementary Material Table S1A for explained variation of all relative warps). In the northern system, Lake Catemaco, morphs showed some overlap in premaxilla shape (Figure 2A), contrasting with the southern morphs from the San Juan River system, which showed a larger divergence, with almost no overlap in premaxilla shape between morphs (Figure 2B), depicting a geographical cline, increasing north to south, in the premaxilla shape differentiation between the morphs.

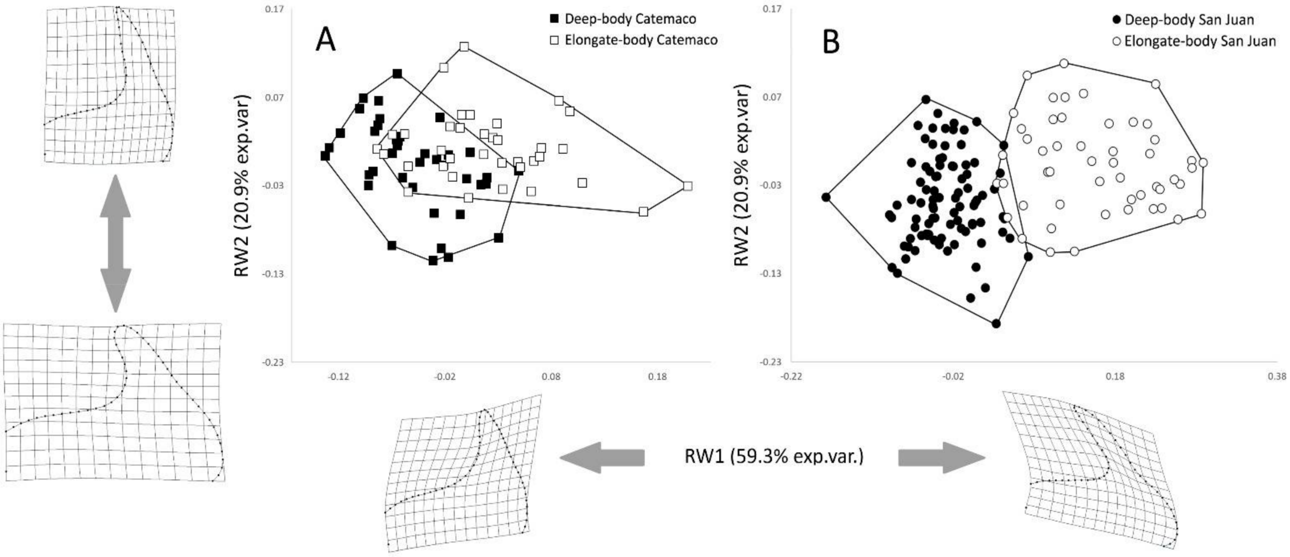

Figure 2. Premaxilla shape variation for (**A**) Lake Catemaco system and (**B**) San Juan River system by morph. Deformation grids show shape variation along RW1 (*X* axis) and RW2 (*Y* axis).

The analyses of the premaxilla shape revealed no differences in disparity between morphs in the Lake Catemaco system (Procrustes variance in the deep-body morph: 0.008 vs. the elongate-body morph: 0.01, $p = 0.21$), while in the San Juan River system, the elongate-body morph showed a two-fold higher disparity of premaxilla shape than the deep-body morph (Procrustes variance in the elongate-body morph: 0.015 vs. the deep-body morph: 0.009, $p < 0.001$). We can appreciate these differences in the graphical representation in the vectors' length toward the elongate-body morph, where the morphs from the San Juan River showed a larger differentiation (Figure 3B,D) than the morphs from the Lake Catemaco system (Figure 3A,C). Thus, we observed a parallel pattern of premaxilla shape evolution in the two systems when plotting the shape differences using the deep-body morph for reference.

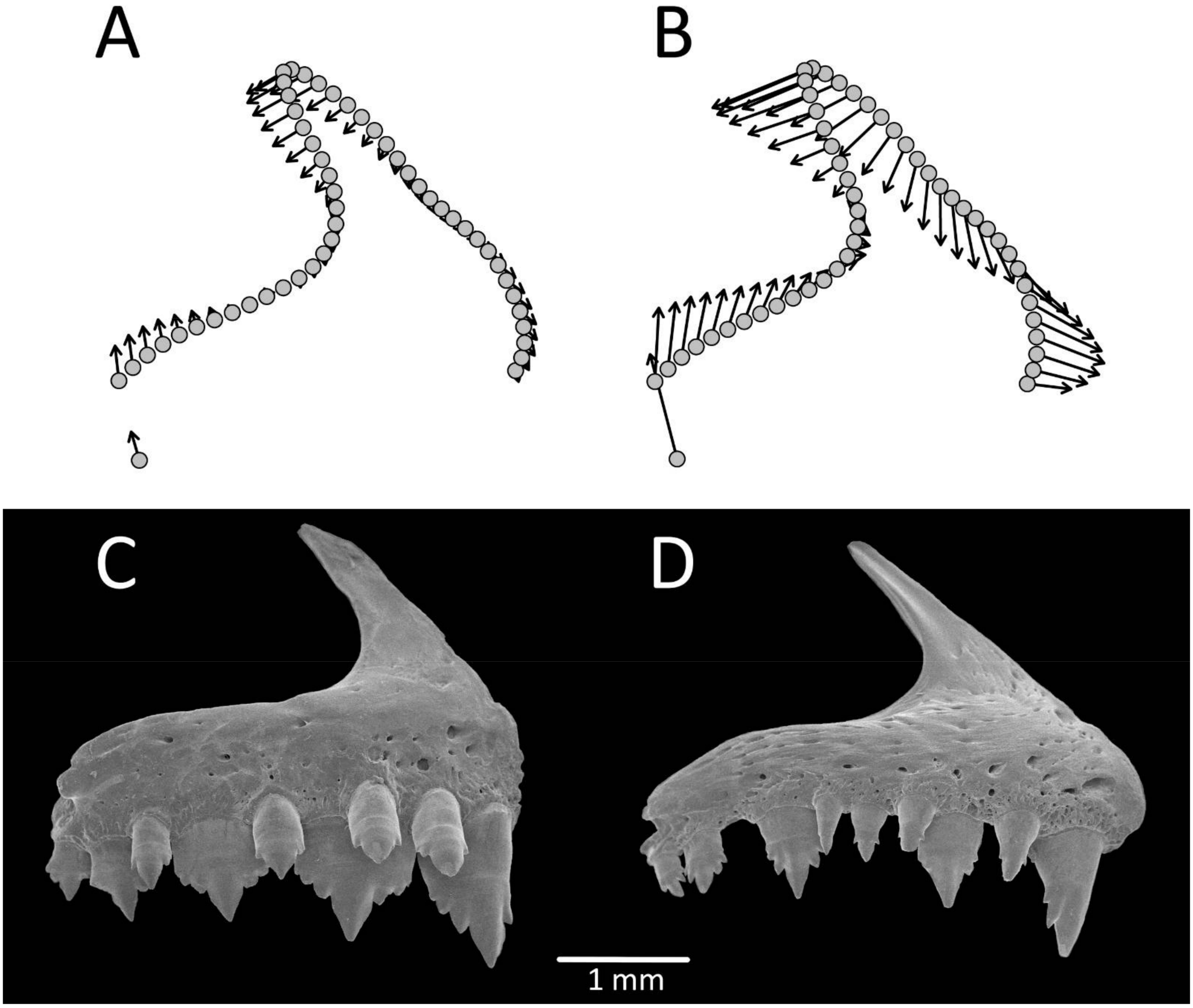

Figure 3. Differences in the consensus shape of premaxilla between morphs for (**A**) Lake Catemaco and (**B**) San Juan River systems. Gray dots in the shapes in (**A**,**B**) represent the deep-body morph used for reference; change vectors in black show the deformations toward the sympatric elongate-body morph. Examples of premaxilla of the elongate-body morph for (**C**) Lake Catemaco and (**D**) San Juan River systems.

3.2. Ontogenetic Allometric Trajectories

We found a positive correlation between RW1 and log centroid size in the regression analysis for both morphs in the two systems (Table 2, Figure 4A,B). This indicates that specimens with a longer premaxilla also had a sharper angle and narrower ascending processes. For RW2, only the regression analysis for the deep-body morph from the San Juan River system showed a positive association with log centroid size (Table 2, Figure 4C,D). In this case, as the log centroid size increases, fish showed narrower premaxilla and longer ascending processes (Figure 4D).

Table 2. Regression analysis for RW1 and RW2 on log centroid size for Lake Catemaco and San Juan River systems. Bold *p* values indicate statistically significant results.

System/Morph	RW1			RW2		
	Slope	R^2	*p*	Slope	R^2	*p*
Lake Catemaco						
Deep-body	0.19	0.13	**0.02**	0.03	0.004	0.69
Elongate-body	0.33	0.26	**0.001**	0.00001	<0.001	0.99
San Juan River						
Deep-body	0.17	0.09	**0.002**	0.21	0.09	**0.003**
Elongate-body	0.42	0.52	**<0.001**	0.004	<0.001	0.94

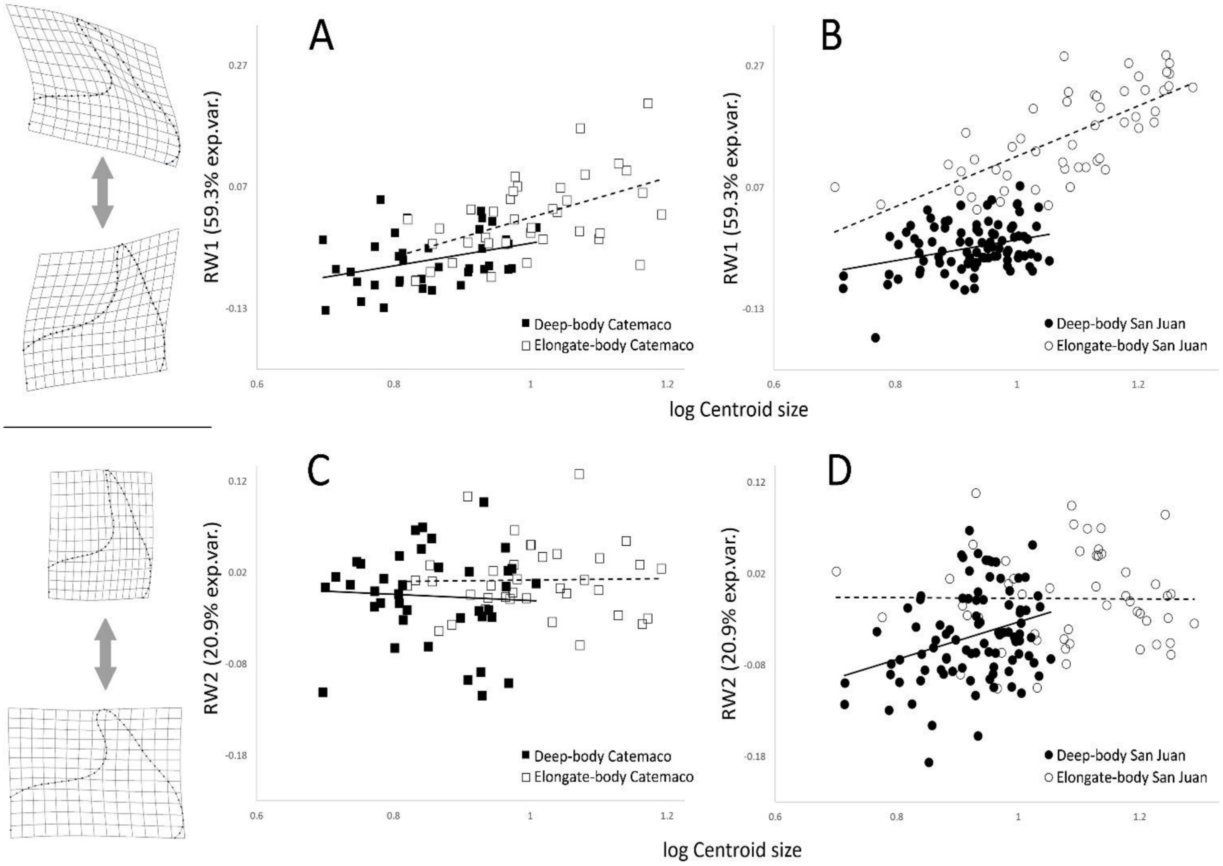

Figure 4. Late allometric ontogenetic trajectories of the shape of premaxilla. RW1 and RW2 on log centroid size for (**A**,**C**) Lake Catemaco system and (**B**,**D**) San Juan River system by morph. Deformation grids on the left show shape variation along RW1 (*Y* axis) in (**A**,**B**), and RW2 (*Y* axis) in (**C**,**D**).

In the late ontogenetic allometric trajectories analysis of the shape of premaxilla for RW1, we found differences between morphs in both systems as revealed by the ANCOVAs (morph term, Table 3). We found differences in the slopes (indicating different direction of allometric trajectories) between morphs in the San Juan system, while in Lake Catemaco, the slopes did not differ statistically, suggesting parallel allometric trajectories (interaction term morph*log Centroid, Table 3). The elongate-body morph showed a parallel pattern of an extended ontogenetic trajectory when compared with its sympatric deep-body morph in both systems (Figure 4A,B). However, in the San Juan River system, the elongate-body morph showed a steeper slope (Figure 4B, Table 2). For the RW2, allometric trajectories of the shape of premaxilla showed contrasting patterns between systems. In the ANCOVA for the Lake Catemaco system, no factor showed a significant effect (Table 3), and there was no difference between morphs and premaxilla shape in relation to size (Figure 4C). Morphs from the San Juan River system showed convergent allometric trajectories for RW2. In the ANCOVA, we found differences in the shape of premaxilla (RW2) between morphs and found differences in slopes (interaction Morpho*log Centroid size was significant, Table 3). As the centroid size increased, the shapes of the premaxilla of both morphs became more similar (Figure 4D).

Table 3. ANCOVA models for RW1 and RW2 for Lake Catemaco and San Juan River systems. Bold *p* values indicate statistically significant effects. All factors have one degree of freedom.

Factor	RW1			RW2		
	Sum of Squares	**F**	***p***	**Sum of Squares**	**F**	***p***
Lake Catemaco						
Log Centroid size	0.13	53.41	**<0.001**	0.001	0.58	0.44
Morph	0.011	4.68	**0.03**	0.004	2.01	0.16
Morph*log Centroid size	0.003	1.25	0.26	0.0002	0.11	0.73
San Juan River						
Log Centroid size	0.89	459.9	**<0.001**	0.046	18.86	**<0.001**
Morph	0.367	188.95	**<0.001**	0.016	6.66	**0.01**
Morph*log Centroid size	0.019	10.15	**0.001**	0.013	5.44	**0.02**

4. Discussion

In this study, we characterized the degree of variation and disparity of the premaxilla shape and the late ontogenetic allometric trajectories of the premaxillary bone between sympatric morphs of *Astyanax* in two lacustrine systems, Lake Catemaco in México and San Juan River basin in Nicaragua and Costa Rica. We found parallel evolution in the divergence of the premaxilla shape (i.e., a narrower ascending process and a sharper angle corresponding to the elongate-body morph in both systems) and common patterns in the late ontogenetic allometric trajectory between the morphs in both systems. Interestingly, these patterns have not evolved completely in the same manner in both systems, while there are shared (parallel) but also unique patterns of diversification of the ontogenetic trajectories in each *Astyanax* setting analyzed. Our results suggest evolvability of the ontogenetic allometric trajectories in *Astyanax*, supporting the general evidence of the relevance of change in allometric trajectories for biological diversification [13].

4.1. Premaxilla Shape Divergence between Morphs and Systems

The analysis of the premaxilla shape (RW1-RW2 biplot, disparity analysis, and differences in the consensus shape) showed a geographical cline of differentiation between systems. The results are consistent with the patterns of body and head shape divergence previously described [21,22], which showed a larger morphological divergence in body shape, trophic ecomorphology, and dentition between morphs in the San Juan River system than between morphs from the Lake Catemaco system. In addition, the pattern of divergence in premaxilla shape between morphs in the San Juan River system have been characterized [24], and in the present study, we went one step further and compared those patterns with yet another geographically distant system, Lake Catemaco, allowing further understanding of the evolution of the premaxilla shape.

The two systems analyzed belong to independent evolutionary lineages of the genus *Astyanax* in Mesoamerica, differing in time of divergence, as Lake Catemaco is inhabited by a younger lineage than the lineage inhabiting the Río San Juan system [18]. Time of divergence has been considered an important factor explaining morphological divergence and speciation [27,31,32]. Thus, as the lineage from the Río San Juan system is older than that in the Lake Catemaco system, this longer time of divergence could be associated with the more pronounced morphological divergence and disparity in shape of premaxilla found for this system. A similar pattern of skull shape disparity was found when analyzing 87 cichlids fish species [33] endemic to the Great African lakes Tanganyika, Malawi, and

Victoria, where fishes from the youngest of these lakes (L. Victoria) exhibited the lowest amount of skull shape disparity, while the oldest lake (L. Tanganyika) showed the largest disparity [33].

4.2. Patterns of Allometric Ontogenetic Trajectories between Morphs and Systems

Our results showed differences between sympatric morphs in both systems in the late allometric ontogenetic trajectories, although these patterns have not evolved in the same way in both systems. Klingenberg [28] noted that allometric trajectories can potentially change direction, shift sideways through lateral transposition, or be extended or truncated. In addition, Sheets and Zelditch [34] summarized patterns of variation in ontogenetic trajectories based on changes in direction, magnitude (length of trajectory), and outset shape of the trajectory, which could produce parallel, divergent, or convergent patterns of such ontogenetic trajectories. For the RW1 (which accounted for 59.3% of the explained variation and allowed morph discrimination), in Lake Catemaco, we did not find statistical differences in slopes of the allometric trajectories between morphs, suggesting parallel ontogenetic trajectories. However, fitted lines in Figure 4A showed a slightly divergent pattern, with a faster shape change in the elongate-body morph. In the San Juan River System, allometric trajectories for RW1 were clearly divergent [34] between morphs. There was a clear difference in the direction of the ontogenetic trajectories between morphs (as shown by the heterogeneity of slopes); as the size of the premaxilla increased, the elongate-body morph showed a steeper slope, suggesting a faster change in shape than the deep-body morph. In the San Juan River system, the shape of the premaxilla between morphs in the outset of the trajectories is markedly different, arguing also for a larger divergence between San Juan system morphs than between morphs from Lake Catemaco.

A shared pattern for both systems corresponds to the extended allometric trajectory in the elongate-body morphs, corresponding to parallel evolution when compared with their sympatric deep-body morphs. This corresponds to changes in the length of trajectories and, hence, to changes in magnitude [34]; consequently, the elongate-body morph can form a longer premaxilla, while the deep-body morph showed a truncated trajectory. In addition, in agreement with the role of size in *Astyanax* diversification, Ornelas-García et al. [19] reported in Lake Catemaco a longer body size in the elongate-body morph when compared with the deep-body morph, which also corresponded to an extended trajectory for the elongate-body morph. The patterns of the late ontogenetic allometric trajectories for RW2 were contrasting with the patterns for the RW1. As evidenced by the regression analysis, only the deep-body morph from the San Juan system showed a positive correlation between RW2 and log centroid size, resulting in convergent allometric ontogenetic trajectories for morphs in this system. The shape variation described by the RW2 (width of the premaxilla and length of ascending process) is not clearly associated with morph differentiation in both systems, and therefore, we hypothesize that these premaxilla features are probably not associated with the trophic specialization of morphs.

Several studies have shown an association between the ecology and variation in ontogenetic allometric trajectories in invertebrates [8] and vertebrates [9,10,19,35–38], with some cases related to morphological diversification or speciation in fishes [7,11,39,40]. In this study, the differences in allometric trajectories between morphs are consistent with the divergent patterns in trophic ecology associated with trophic level considering ecological data (diets and stable isotopic ratios of nitrogen), with the elongate-body morph occupying a higher trophic level in both systems [20,24]. In addition, the shape of the premaxilla showing a sharper angle in the ascending process in the elongate-body morph resembles the shape of premaxilla in another predator characiform, the genus *Oligosarcus* [41]. Other lacustrine fish groups, such as Eurasian perch (*Perca fluviatilis*) and Arctic charr (*Salvelinus alpinus*), also present sympatric morphs showing differences in ontogenetic allometric trajectories, which are associated with contrasting ecological adaptations of diet (piscivores vs. omnivore) and habitat use (littoral and pelagic zones or littoral and profundal zone) [7,11].

Differences in premaxilla shape in the outset of the late allometric trajectories in the range of size analyzed can explain, at least in part, the differentiation between morphs in the two systems studied; however, information is lacking about early embryonic development and early allometric trajectories of the morphs. In other *Astyanax* populations, some studies have addressed the early development of trophic morphology. For example, in *Astyanax mexicanus*, Trapani et al. [42] described the early ontogenetic transition from unicuspid to multicuspid oral dentition, and Atukorala and Franz-Odendaal [43] studied the development of pharyngeal dentition. However, the generalization of these patterns to other species or populations of *Astyanax* is unknown, and a detailed description of patterns associated with size is still lacking. Future studies on early ontogenetic allometric trajectories between *Astyanax* morphs from San Juan River and Lake Catemaco could shed light on the first stages of divergence between morphs (i.e., embryonic, or post-hatching development).

5. Conclusions

This study provides additional support to the relevance of trophic morphology in the diversification of *Astyanax* fish in different Mesoamerican lakes, and suggests evolvability of the allometric ontogenetic trajectories associated with the ecological divergence. In recent years, the genus *Astyanax* has built up as an important model in evolutionary biology [44]. However, to our knowledge, this study is the first analysis of ontogenetic allometric trajectories in *Astyanax*, and gaining knowledge on these trajectories will surely prove very useful to our understanding of morphological evolution in this complex system.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11178020/s1, Table S1. Percentage of variance explained by the relative warps in the premaxilla shape variation analysis (A), Procrustes coordinates (B) and relative warps scores (C).

Author Contributions: Conceptualization, C.A.G.-A. and C.P.O.-G.; methodology, C.A.G.-A. and C.P.O.-G.; software, C.A.G.-A.; validation, C.A.G.-A. and C.P.O.-G.; formal analysis, C.A.G.-A.; data curation, C.A.G.-A.; writing—original draft preparation, C.A.G.-A.; writing—review and editing, C.A.G.-A. and C.P.O.-G.; supervision, C.P.O.-G.; project administration, C.P.O.-G.; funding acquisition, C.P.O.-G. Both authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in Supplementary Materials Table S1B,C.

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Conflicts of Interest: The authors declare no conflict of interest.

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