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Comparative Shape of Two Recently Diverged Species of Pacific Rockfish: *Sebastes ciliatus* and *S. variabilis*

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Abstract: Species delimitation can be based on the consideration of several different criteria, including the differentiation of ecological or functional traits. Two species of Pacific rockfish, the dark rockfish (*Sebastes ciliatus*) and the dusky rockfish (*Sebastes variabilis*), appear to represent recently divergent evolutionary lineages. We evaluate evidence for the differentiation of these two species in somatic shape using geometric morphometrics at two locations in the northeast Pacific where they occur in sympatry. The somatic shape was significantly different between species, but the species' shape did not vary between the two locations. *Sebastes ciliatus* had an upturned and relatively smaller head, eye, and jaw, and an elongated midbody, whereas *S. variabilis* had a downturned and larger head, eye, and jaw, and a shorter midbody. These results suggest that *S. ciliatus* and *S. variabilis* are morphometrically differentiated in a similar way in both locations. The somatic shape differentiation between these two sympatric species is similar to genus-wide patterns of somatic shape differentiation.

Keywords: speciation; species delimitation; rockfish; geometric morphometrics; somatic shape

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1. Introduction

Under a unified species concept, the only necessary property of a species is that it exists as a separately evolving metapopulation lineage [1]. Any type of data that provide evidence of a separately evolving metapopulation lineage may be useful for species delimitation. Due to the ease of DNA sequencing, many studies focus on genetic data for species delimitation [2,3]. Though genetic data provide evidence for species delimitation, ecological and functional trait data are important, especially for shallowly derived species that may show little genetic differentiation [4,5].

Two species of Pacific rockfish, the dark rockfish (*Sebastes ciliatus)* and the dusky rockfish (*Sebastes variabilis*), appear to represent separately evolving metapopulation lineages that are recently divergent [6,7]. The Pacific rockfish genus *Sebastes* is the most speciose genus in the family *Scorpaenidae*, having experienced apparent adaptive radiation that has produced at least 110 currently recorded species worldwide [7]. Diversification within the genus *Sebastes* has made classification and identification complicated [7] and suggests the need for corroborating ecological and functional trait data to determine species delimitation. The taxonomic history of *S. ciliatus* and *S. variabilis* has involved uncertainty and revision [6–9]. *Sebastes ciliatus* was regarded as one species with two color morphs [10] before its eventual designation as two separate species [6], with the lighter color variant being assigned the name *Sebastes variabilis*. The basis for the separate descriptions of these species relied heavily on differences in color and depth of occupied habitat. Subsequent studies show low genetic divergence between *S. ciliatus* and *S. variabilis* [7,11]. These two species occur in sympatry across most of their geographic range; thus, information on the divergence between these two species in sympatric locations in ecological and functional traits would provide important corroboration for species delimitation.

Variation in somatic shape has been linked to variation in the ecology and environment of many fishes in both freshwater and marine systems [12–16]. Because of this tight linkage between body shape and ecology, quantifying morphometric shape variation can be an important avenue of research for determining species delimitation. Landmark-based geometric morphometrics provides a quantitative way to characterize and compare shape variation [17–19] and has been shown to accurately and reliably detect patterns of shape variation among populations and species [20–24].

To characterize the evolutionary differentiation between these two shallowly diverged rockfish species, we measured and compared somatic shape using geometric morphometrics in two different locations where these species are sympatric. By using two separate locations where the two species are sympatric, we could test whether shape differs consistently between the two species and whether the shape of both species differs between locations. If shape variation between species is consistent between the two locations (i.e., location is not a significant predictor of shape variation), this suggests a general selective driver of shape divergence as opposed to location-specific conditions.

2. Methods

For this study, we collected *S. ciliatus* and *S. variabilis* via hook-and-line fishing in Frederick Sound near the north end of Kuiu Island, Alaska, in late June of 2016, and in Icy Strait, Alaska, in June of 2023 (160 km straight-line distance between the two sampling locations; Figure 1). We used samples of both species collected in both locations for comparison of divergence in somatic shape. We used a total of 105 specimens: 60 *S. ciliatus*, 34 from Kuiu (18 males, 16 females) and 26 from Icy Strait (15 males, 11 females); 45 *S. variabilis*, 17 from Kuiu (8 males, 9 females), and 28 from Icy Strait (12 males, 16 females). Both species are considered sexually monomorphic [25], and we had a relatively even mix of both sexes in each sample. Specimens for both species ranged from 30 to 47 cm in both locations.

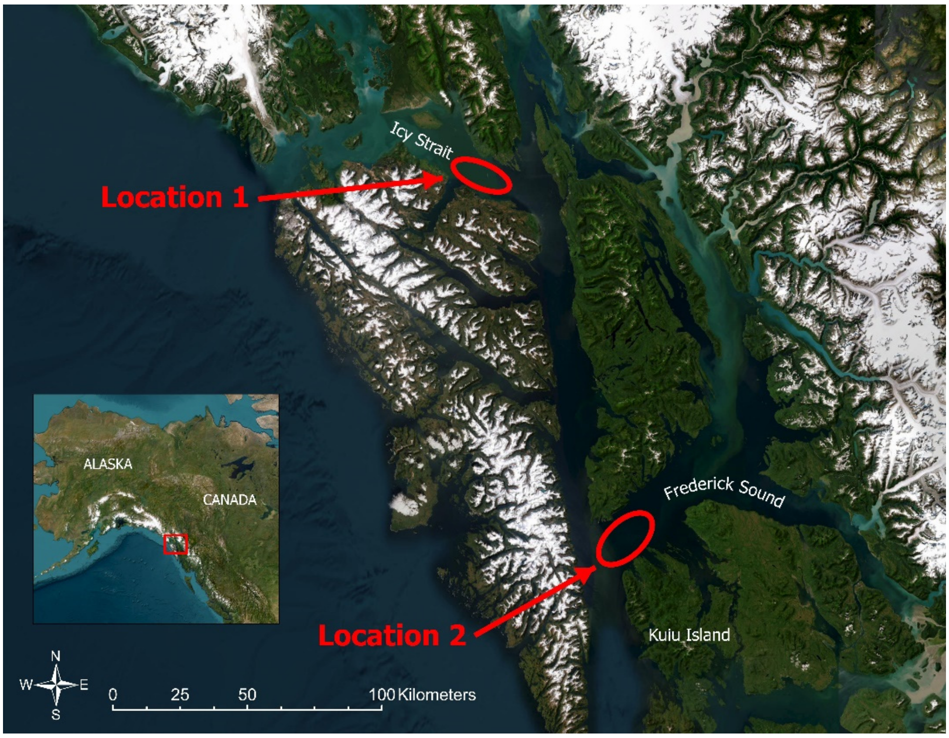

Figure 1. Collection locations (red circles) for comparison of *S. ciliatus* and *S. variabilis*. Inset shows location relative to western North America.

Specimens were photographed within a few minutes after capture to ensure that photos reflected fresh, relaxed, and natural shape variation. For each individual in all samples, we photographed the right lateral view for shape analysis, and we digitized

13 landmarks (Figure 2) using tpsDig Version 2.32 [26]. Landmarks were as follows: (1) anterior tip of the lower jaw (i.e., symphyseal knob); (2) anterior extent of the eye; (3) posterior extent of the eye; (4) posterior extent of the operculum; (5) posterior, ventral extent of the maxilla; (6) anterior insertion of the dorsal fin on body outline; (7) posterior extent of scalation at the midline on the caudal peduncle; (8) anterior insertion of anal fin; (9) anterior insertion of pelvic fin; (10) dorsal outline vertical of landmark 3; (11) dorsal outline halfway between landmark 6 and 7; (12) dorsal outline at smallest width of caudal peduncle; (13) ventral outline at smallest width of caudal peduncle. Landmarks 2, 3, 11, 12, and 13 were sliding semilandmarks. All specimens were landmarked by one researcher without respect to predictor variables. The landmarked images were then independently inspected by another researcher to confirm homologous and consistent placement of landmarks. This procedure resulted in reduced errors in digitizing [27]. We used tpsRelW Version 1.74 [28] to align specimens via a generalized Procrustes analysis (a superimposition method to remove non-shape variation via rotation, translation, and scaling) [28]. We generated shape variables as partial warps and uniform components comprising the weight matrix (i.e., **W**). The weight matrix is used as input for principal component analysis, and the resulting principal components, termed relative warps [29], are used as shape variables for the subsequent analysis. Like all principal components, relative warps are ordered by the amount of variation they individually explain. Typically, we used all relative warps that account for >1% of the variation. In this study, we used the first 10 relative warps (that collectively explain 97.1% of shape variation) to characterize shape.

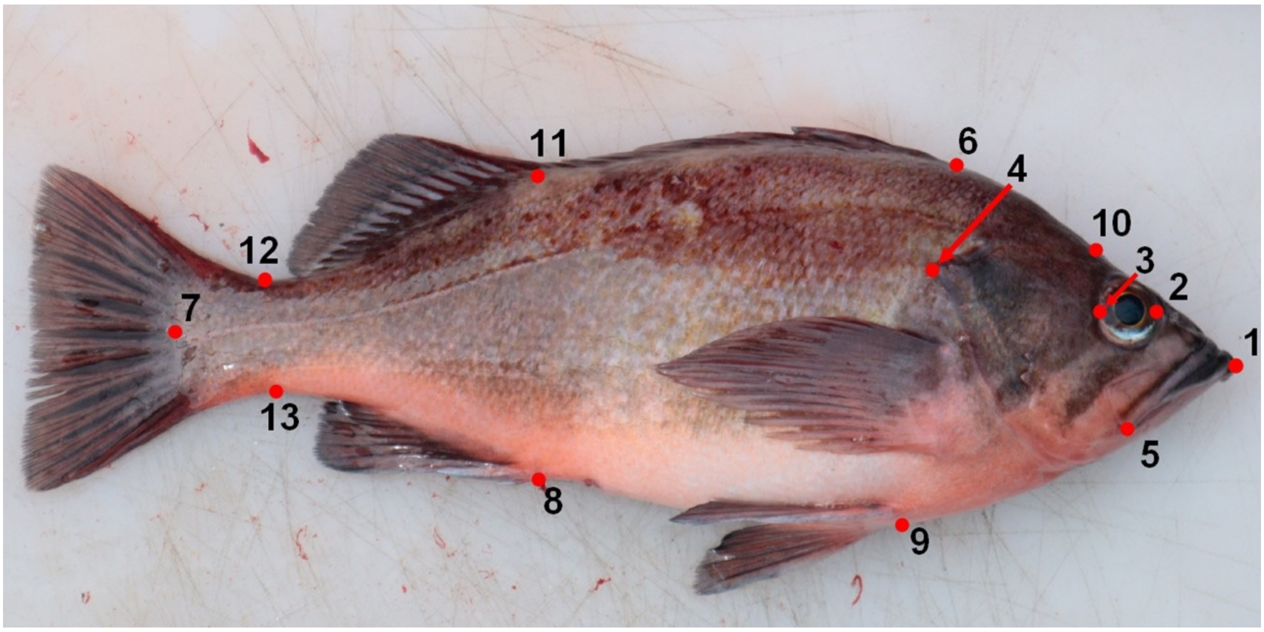

Figure 2. Photograph of *S. variabilis* collected in Icy Strait, AK, indicating location of 13 landmarks used to characterize somatic shape variation. The same landmarks were used for specimens of *S. ciliatus*. Red dots indicate location of landmarks, and numbers correspond to landmark numbers in the text.

We used a multivariate linear mixed model to determine if shape differed between *S. ciliatus* and *S. variabilis* or between locations. The response variable was shape as represented by the first 10 relative warps. The predictor variables were species (*S. ciliatus* or *S. variabilis*), location (Kuiu or Icy Strait), centroid size (a multivariate measure of size), and the index variable (indexing relative warps 1–10, see explanation below). We also included all two-way and three-way interactions with the index variable and other predictor variables. Body size commonly influences shape among many species of fishes [30–34]. Although our samples varied little in size, we included centroid size as a covariate to avoid confounding shape differences caused by size with shape differences between species because of potential sampling differences.

A multivariate linear mixed model assumes a univariate response variable, so we vectorized the shape variables such that each row represented one response variable, but each specimen was represented by multiple rows of data [35]. Thus, the first row represented relative warp 1 for the first specimen, the second row represented relative warp 2 for the first specimen, and so forth until all relative warps were represented in successive rows for the first individual. The same pattern was repeated for all individuals, each with 10 rows. The index variable preserved the order of the relative warps such that comparisons between groups (e.g., *S. ciliatus* and *S. variabilis*) were made by matching each relative warp to the same relative warp in each group (e.g., relative warp 1 in one species was compared to relative warp 1 in the other species). Our main goal was to determine how shape differed between species and if shape was different within species in different locations; thus, it was the two-way interaction of species and the index variable and the three-way interaction of species and location and index variable that tested our hypothesis of interest. Main effects by themselves (without the interaction with the index variable) tested only for an average effect across all relative warps. Because relative warps are principal components, they have a mean of 0; and, more importantly, they have an arbitrary ordination. Thus, a single individual may have a positive score on some relative warps and a negative score on other relative warps so that their mean score across all relative warps may be near 0. It was only by matching relative warps in the same order (by using the index variable as a predictor) that we could accurately test the hypothesis of interest. Specifically, the hypothesis of interest is whether shape differs between species or locations on at least some relative warps (i.e., shape variables). This vectorization of the multivariate response variables allows parametric testing of multiple and complex interaction effects and has been used successfully to test for shape variation in a variety of systems, among populations, and species [5,22,23,30–34,36]. We estimated degrees of freedom using the Kenward and Roger method [37]. We used Proc MIXED in SAS to run this analysis (SAS version 9.4; SAS Institute Inc., Cary, NC, USA).

To visualize the differences between species, we plotted mean scores by species for each of the 10 relative warps (i.e., shape variables). In addition, we calculated a divergence vector between *S. ciliatus* and *S. variabilis* across all 10 relative warps using methods from Langerhans [38] and Langerhans and Makowicz [39]. To create the divergence vector, we performed a principal components analysis (PCA) of the least-squares means for each species derived from the multivariate linear mixed model output. We multiplied the values of the first eigenvector (from the PCA) by each of the corresponding relative warp values for each individual and then summed these to create the individual divergence score. We then used the vector of divergence scores as the regressor variable in tpsRegr Version 1.49 [40] and the original tps landmark file as the response variable to generate thin-plate spline visualizations of species' shapes. These visualizations represent the overall shape divergence between species across all 10 relative warps simultaneously.

3. Results

The species differ significantly in shape (as evidenced by the species by index interaction), but the shape does not differ between locations, with centroid size, or between species in each location (three-way interaction; Table 1). The species differ in relative warps 1–4 but not in relative warps 5–10 (Figure 3). In general, *Sebastes ciliatus* had a relatively smaller eye, shorter jaw, and upturned, smaller head, but an elongated midbody, whereas *S. variabilis* had a larger eye, longer jaw, and a downturned, larger head, but a shorter midbody (Figure 4).

Table 1. Multivariate analysis of covariance effects for body shape differences between *S. ciliatus* and *S. variabilis* in two separate locations. Significant *p*-values are bolded.

Effect	Degrees of Freedom Num/Den	F-Value	*p*-Value
Species	1/479	41.35	**<0.0001**
Location	1/479	1.39	0.2390
Species × Location	1/479	2.05	0.1524
Centroid Size (CS)	1/479	0.28	0.5996
Index	9/397	1.11	0.3524
Species × Index	9/397	7.40	**<0.0001**
Location × Index	9/397	1.28	0.2456
CS × Index	9/397	1.24	0.2708
Species × Location × Index	9/397	1.13	0.3429

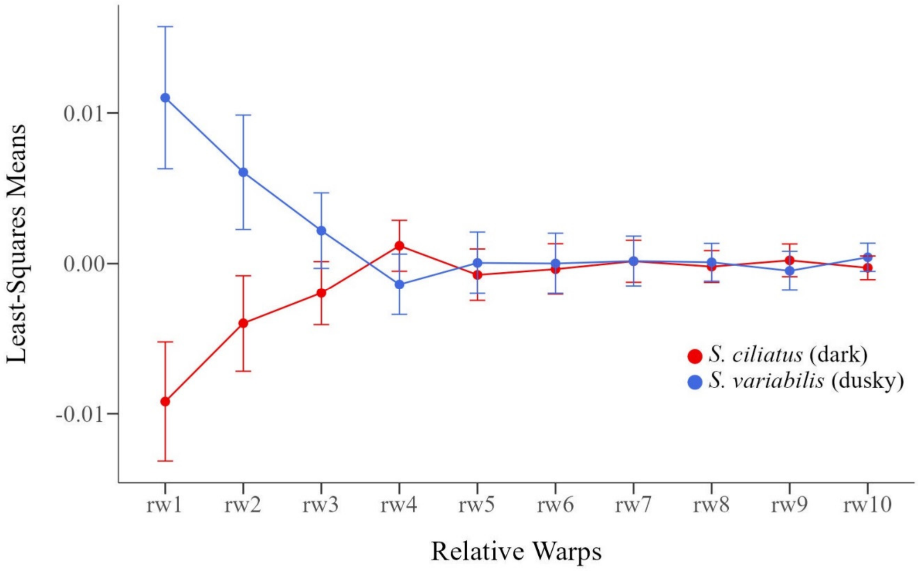

Figure 3. Least-squares means (and 95% confidence interval of the mean) of each relative warp for each species. Species differ in relative warps 1–4 but not in relative warps 5–10 based on overlap of 95% confidence intervals and means. Shape did not differ between locations.

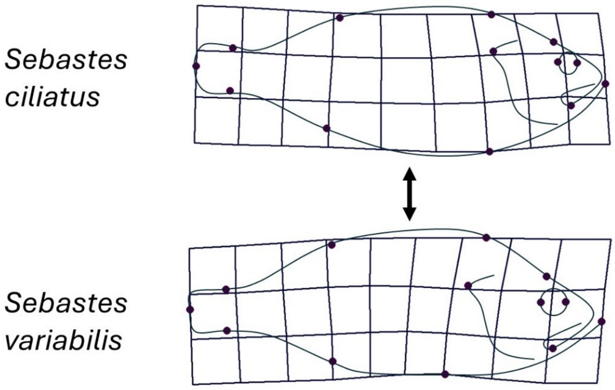

Figure 4. Visualization of shape differentiation across all 10 relative warps combined between *S. ciliatus* and *S, variabilis*. Species-specific shape did not differ significantly between the two locations.

4. Discussion

Our objective was to quantify the difference in somatic shape between *S. ciliatus* and *S. variabilis* at two locations where they are found in sympatry. We found consistent and significant somatic shape variation between the species at both locations. Because shapes did not differ between locations, we suggest that the selective driver of divergence is not location-specific but rather represents a common selective factor of differentiation across the sympatric range of both species (e.g., [41,42]). This clear divergence in somatic shape between sister species suggests a mechanism of ecological character displacement [43]. Character displacement occurs when species in sympatry experience divergence of one or more characters in response to interspecific competition [36,44]. Selection for reduced competitive overlap can drive the ecological and accompanying morphological divergence in regions where the species are sympatric [45,46]. However, in allopatric speciation, we would expect shape divergence between sister species to exhibit adaptation to conditions in the occupied locations, not in response to the shape of the other species [43,47–49]. Divergence in somatic shape between species represents strong evidence for the existence of separately evolving metapopulation lineages of both *S. ciliatus* and *S. variabilis*, providing additional corroboration for previous studies that have suggested recognition of these two lineages as valid species [6,7].

The axis of divergence we observed between these two sister species is characterized by changes in the proportion of the head to body and position of the head. This axis of divergence is consistent with adaptation to the depth of habitat occupied, identified by Ingram [41] across the genus *Sebastes*. Our example of coordinated shape variation between species provides greater detail to describe this axis of shape variation. Along with larger relative eye size and smaller relative body depth, we add the relative size of the midbody (shorter in *S. variabilis*), relative size of the head (larger in *S. variabilis*), position of the head (upturned versus downturned), and relative jaw size (larger in *S. variabilis*). This pattern suggests that these two closely related species are exhibiting a pattern of character displacement where *S. ciliatus* is evolving toward a shallow, pelagic ecology, and *S. variabilis* may be evolving toward a deep-water, benthic ecology [41,45,47,49]. In addition, the larger eyes found in *S. variabilis* and other species closer to the benthic side of the continuum are consistent with expected adaptation to low-light conditions in deeper water [45,50].

In addition to implications for the evolutionary divergence of *Sebastes* species, this study points out a relatively simple metric for use in the field to differentiate the two species. In general, the ratio of the length of the head (anterior extent of the symphyseal knob to the posterior extent of the operculum) to the midbody (posterior extent of the operculum to the vent) differs between the two species. If this ratio of head/midbody is >1, then the species is usually *S. variabilis*; however, if the ratio of head/midbody is <1, then the species is usually *S. ciliatus* (Figure 5). There is substantial variation in shape within each species, and the shape differences observed between species include more patterns than just this simple ratio. Thus, there will inevitably be exceptions to this general rule, but it can be a useful tool, especially if combined with differences in the coloration of fresh-caught specimens.

Our study serves as a model for evaluating recent divergence through geometric morphometric analysis of somatic shape and presents a clear direction for future research to amplify the results and implications of our work. We were limited in our scope of inference in this study because we were only able to compare two locations of sympatry. Replicating this work with sampling from multiple locations across the sympatric range as well as allopatric ranges could confirm our suggestions of ecological character displacement between *S. ciliatus* and *S. variabilis.* Such a study would provide insight into the process of sympatric ecological speciation in species with wide geographic ranges. Quantification of additional functional and ecological traits between species across broad geographic ranges would allow insight into the rate and coordination of evolution among traits during the course of speciation (e.g., [51–54]).

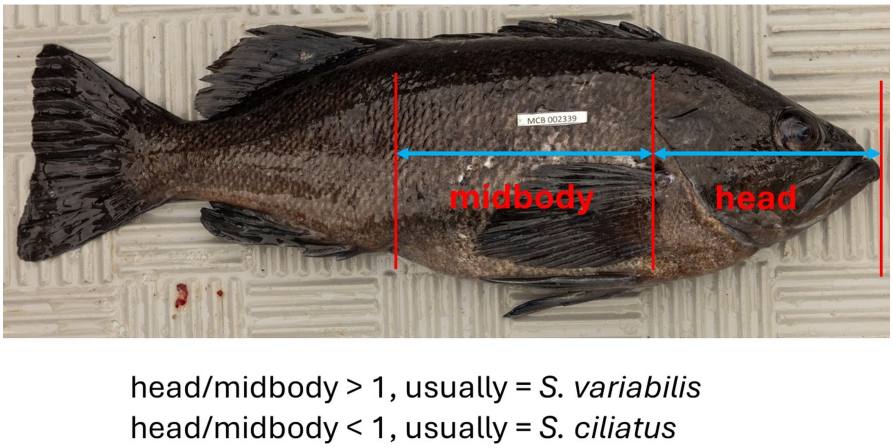

Figure 5. Photograph of *S. ciliatus* collected in Icy Strait, AK, illustrating the simple ratio of length of head/midbody that can be used in the field to differentiate between the two species. Red lines indicate boundaries of head and midbody regions, and blue arrows indicate the respective lengths of head and midbody.

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References

1. De Queiroz, K. Species concepts and species delimitation. *Syst. Biol.* **2007**, *56*, 879–886. [CrossRef]
2. Hubert, N.; Hanner, R. DNA Barcoding, species delineation and taxonomy: A historical perspective. *DNA Barcodes* **2015**, *3*, 44–58. [CrossRef]
3. Wilkins, J.S.; Zachos, F.E.; Pavlinov, I.I.A. (Eds.) *Species Problems and Beyond: Contemporary Issues in Philosophy and Practice*; CRC Press: Boca Raton, FL, USA, 2022.
4. Crandall, K.A.; Bininda-Emonds, O.R.P.; Mace, G.M.; Wayne, R.K. Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **2000**, *15*, 290–295. [CrossRef]
5. Belk, M.C.; Schaalje, G.B. Multivariate heritability of shape in June sucker (*Chasmistes liorus*) and Utah sucker (*Catostomus ardens*): Shape as a functional trait for discriminating closely related species. *Dev. Genes Evol.* **2016**, *226*, 197–207. [CrossRef] [PubMed]
6. Orr, J.W.; Blackburn, J.E. The dusky rockfishes (Teleostei: Scorpaeniformes) of the North Pacific Ocean: Resurrection of *Sebastes variabilis* (Pallas, 1814) and a redescription of *Sebastes ciliatus* (Tilesius, 1813). *Fish. Bull.* **2004**, *102*, 328–348.
7. Hyde, J.R.; Vetter, R.D. The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). *Mol. Phylogenetics Evol.* **2007**, *44*, 790–811. [CrossRef] [PubMed]
8. Kendall, A.W. Systematics and identification of larvae and juveniles of the genus *Sebastes*. In *Rockfishes of the Genus Sebastes: Their Reproduction and Early Life History*; Boehlert, G.W., Yamada, J., Eds.; Springer: Dordrecht, The Netherlands, 1991; pp. 173–190.
9. Kendall, J. An Historical Review of *Sebastes* Taxonomy and Systematics. *Mar. Fish. Rev.* **2000**, *62*, 1–23.

10. Kurbanov, Y.K.; Novikov, R.N. The Finding of the Dark Dusky Rockfish (Tilesius, 1813) (Sebastidae) in the Commander Archipelago with Notes on its "Captures" in Near-Surface Waters of the Northern Kuril Islands. *Russ. J. Mar. Biol.* **2022**, *48*, 521–524. [CrossRef]
11. Searle, P.C.; Kokkonen, A.L.; Campbell, J.R.; Shiozawa, D.K.; Belk, M.C.; Evans, R.P. Phylogenetic relationships of three rockfish: *Sebastes melanops*, *Sebastes ciliatus* and *Sebastes variabilis* (Scorpaeniformes, Scorpaenidae) based on complete mitochondrial genome sequences. *Biodivers. Data J.* **2023**, *11*, e98167. [CrossRef]
12. Moreira, C.; Froufe, E.; Vaz-Pires, P.; Triay-Portella, R.; Correia, A.T. Landmark-based geometric morphometrics analysis of body shape variation among populations of the blue jack mackerel, *Trachurus picturatus*, from the North-East Atlantic. *J. Sea Res.* **2020**, *163*, 101926. [CrossRef]
13. Reis-Júnior, J.; Bertrand, A.; Frédou, T.; Vasconcelos-Filho, J.; Aparecido, K.C.; Duarte-Neto, P.J. Community-scale relationships between body shape and trophic ecology in tropical demersal marine fish of northeast Brazil. *J. Fish Biol.* **2023**, *102*, 1017–1028. [CrossRef] [PubMed]
14. Walker, J.A. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.* **2008**, *61*, 3–50. [CrossRef]
15. Blasina, G.; Molina, J.; Cazorla, A.L.; de Astarloa, J.D. Relationship between ecomorphology and trophic segregation in four closely related sympatric fish species (Teleostei, Sciaenidae). *Comptes Rendus. Biol.* **2016**, *339*, 498–506. [CrossRef] [PubMed]
16. Haas, T.C.; Heins, D.C.; Blum, M.J. Predictors of body shape among populations of a stream fish (*Cyprinella venusta*, Cypriniformes: Cyprinidae). *Biol. J. Linn. Soc.* **2015**, *115*, 842–858. [CrossRef]
17. Rohlf, F.J.; Marcus, L.F. A revolution in morphometrics. *Trends Ecol. Evol.* **1993**, *8*, 129–132. [CrossRef]
18. Foster, K.; Bower, L.; Piller, K. Getting in shape: Habitat-based morphological divergence for two sympatric fishes. *Biol. J. Linn. Soc.* **2015**, *114*, 152–162. [CrossRef]
19. Kerschbaumer, M.; Sturmbauer, C. The utility of geometric morphometrics to elucidate pathways of cichlid fish evolution. *Int. J. Evol. Biol.* **2011**, *2011*, 290245. [CrossRef]
20. Roy, D.; Docker, M.F.; Hehanussa, P.; Heath, D.D.; Haffner, G.D. Genetic and morphological data supporting the hypothesis of adaptive radiation in the endemic fish of Lake Matano. *J. Evol. Biol.* **2004**, *17*, 1268–1276. [CrossRef] [PubMed]
21. De La Cruz-Agüero, J.; Vergara-Solana, F.J.; García-Rodríguez, F.J. Geometric morphometrics support the proposed molecular taxonomy for three *Eucinostomus* species (Perciformes: Gerreidae) along the coasts of Mexico. *Zoomorphology* **2015**, *134*, 125–134. [CrossRef]
22. Ingley, S.J.; Billman, E.J.; Belk, M.C.; Johnson, J.B. Morphological divergence driven by predation environment within and between species of *Brachyrhaphis* fishes. *PLoS ONE* **2014**, *9*, e90274. [CrossRef]
23. Mercer, M.; Searle, P.C.; Cifuentes, R.; Habit, E.; Belk, M.C. Morphometric Response of *Galaxias maculatus* (Jenyns) to Lake Colonization in Chile. *Diversity* **2020**, *12*, 219. [CrossRef]
24. Langerhans, R.B.; Layman, C.A.; Langerhans, A.K.; DeWitt, T.J. Habitat-associated morphological divergence in two Neotropical fish species. *Biol. J. Linn. Soc.* **2013**, *80*, 689–698. [CrossRef]
25. Love, M.S.; Yoklavich, M.; Thorsteinson, L.K. *The Rockfishes of the Northeast Pacific*; University of California Press: Berkeley, CA, USA, 2002; ISBN 978-0-520-23438-3.
26. Rohlf, F.J. *tpsDig2: Digitize Landmarks and Outlines, Version 2.32*; Department of Ecology and Evolution, State University of New York at Stony Brook: Stony Brook, NY, USA, 2021.
27. Moccetti, P.; Rodger, J.R.; Bolland, J.D.; Kaiser-Wilks, P.; Smith, R.; Nunn, A.D.; Adams, C.E.; Bright, J.A.; Honkanen, H.M.; Lothian, A.J. Is shape in the eye of the beholder? Assessing landmarking error in geometric morphometric analyses on live fish. *PeerJ* **2023**, *11*, e15545. [CrossRef] [PubMed]
28. Rohlf, F.J. *tpsRelw64: Relative Warps Analysis, Version 1.74*; Department of Ecology and Evolution, State University of New York at Stony Brook: Stony Brook, NY, USA, 2021.
29. Zelditch, M.; Swiderski, D.L.; Sheets, H.D. *Geometric Morphometrics for Biologists: A Primer*; Academic Press: New York, NY, USA, 2012.
30. Meyers, P.J.; Belk, M.C. Shape variation in a benthic stream fish across flow regimes. *Hydrobiologia* **2014**, *738*, 147–154. [CrossRef]
31. Williams, T.J.; Johnson, J.B.; Belk, M.C. Interaction between predation environment and diet constrains body shape in Utah chub, *Gila atraria* (Cypriniformes: Cyprinidae). *Biol. J. Linn. Soc.* **2017**, *122*, 147–156. [CrossRef]
32. Hassell, E.M.; Meyers, P.J.; Billman, E.J.; Rasmussen, J.E.; Belk, M.C. Ontogeny and sex alter the effect of predation on body shape in a livebearing fish: Sexual dimorphism, parallelism, and costs of reproduction. *Ecol. Evol.* **2012**, *2*, 1738–1746. [CrossRef] [PubMed]
33. Searle, P.; Mercer, M.; Habit, E.; Belk, M. Ontogenetic shape trajectory of *Trichomycterus areolatus* varies in response to water velocity environment. *PLoS ONE* **2021**, *16*, e0252780. [CrossRef]
34. Wesner, J.S.; Billman, E.J.; Meier, A.; Belk, M.C. Morphological convergence during pregnancy among predator and nonpredator populations of the livebearing fish *Brachyrhaphis rhabdophora* (Teleostei: Poeciliidae). *Biol. J. Linn. Soc.* **2011**, *104*, 386–392. [CrossRef]
35. Anderson-Cook, C.M. An Introduction to Multivariate Statistical Analysis (3rd ed.). *J. Am. Stat. Assoc.* **2004**, *99*, 907–909.
36. Roth-Monzón, A.J.; Belk, M.C.; Zúñiga-Vega, J.J.; Johnson, J.B. Beyond pairwise interactions: Multispecies character displacement in Mexican freshwater fish communities. *Am. Nat.* **2020**, *195*, 983–996. [CrossRef]

37. Kenward, M.G.; Roger, J.H. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* **1997**, *53*, 983–997. [CrossRef] [PubMed]
38. Langerhans, R.B. Trade-off between steady and unsteady swimming underlies predator-driven divergence in Gambusia affinis. *J. Evol. Biol.* **2009**, *22*, 1057–1075. [CrossRef] [PubMed]
39. Langerhans, R.; Makowicz, A. Shared and unique features of morphological differentiation between predator regimes in Gambusia caymanensis. *J. Evol. Biol.* **2009**, *22*, 2231–2242. [CrossRef] [PubMed]
40. Rohlf, F.J. *tpsRegr: Multivariate Regression of Shape onto One or More Independent Variables, Version 1.50*; Department of Ecology and Evolution, State University of New York at Stony Brook: Stony Brook, NY, USA, 2021.
41. Ingram, T. Speciation along a depth gradient in a marine adaptive radiation. *Proc. R. Soc. B-Biol. Sci.* **2011**, *278*, 613–618. [CrossRef]
42. Hyde, J.R.; Kimbrell, C.A.; Budrick, J.E.; Lynn, E.A.; Vetter, R.D. Cryptic speciation in the vermilion rockfish (*Sebastes miniatus*) and the role of bathymetry in the speciation process. *Mol. Ecol.* **2008**, *17*, 1122–1136. [CrossRef]
43. Brown, W.L.; Wilson, E.O. Character Displacement. *Syst. Zool.* **1956**, *5*, 49–64. [CrossRef]
44. Grant, P.R. Convergent and divergent character displacement. *Biol. J. Linn. Soc.* **2008**, *4*, 39–68. [CrossRef]
45. Gaither, M.R.; Gkafas, G.A.; de Jong, M.; Sarigol, F.; Neat, F.; Regnier, T.; Moore, D.; Gröcke, D.R.; Hall, N.; Liu, X.; et al. Genomics of habitat choice and adaptive evolution in a deep-sea fish. *Nat. Ecol. Evol.* **2018**, *2*, 680–687. [CrossRef]
46. Martin, R.A.; Pfennig, D.W. Disruptive selection in natural populations: The roles of ecological specialization and resource competition. *Am. Nat.* **2009**, *174*, 268–281. [CrossRef]
47. Rincon-Sandoval, M.; Duarte-Ribeiro, E.; Davis, A.M.; Santaquiteria, A.; Hughes, L.C.; Baldwin, C.C.; Soto-Torres, L.; Acero, P.A.; Walker, H.J.; Carpenter, K.E.; et al. Evolutionary determinism and convergence associated with water-column transitions in marine fishes. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 33396–33403. [CrossRef]
48. Kautt, A.F.; Machado-Schiaffino, G.; Meyer, A. Lessons from a natural experiment: Allopatric morphological divergence and sympatric diversification in the Midas cichlid species complex are largely influenced by ecology in a deterministic way. *Evol. Lett.* **2018**, *2*, 323–340. [CrossRef] [PubMed]
49. Puebla, O. Ecological speciation in marine v. freshwater fishes. *J. Fish Biol.* **2009**, *75*, 960–996. [CrossRef] [PubMed]
50. Warrant, E.J.; Adam Locket, N. Vision in the deep sea. *Biol. Rev.* **2004**, *79*, 671–712. [CrossRef] [PubMed]
51. Rocha, L.A.; Robertson, D.R.; Roman, J.; Bowen, B.W. Ecological speciation in tropical reef fishes. *Proc. R. Soc. B Biol. Sci.* **2005**, *272*, 573–579. [CrossRef]
52. Faulks, L.; Svanbäck, R.; Eklöv, P.; Östman, Ö. Genetic and morphological divergence along the littoral–pelagic axis in two common and sympatric fishes: Perch, *Perca fluviatilis* (Percidae) and roach, *Rutilus rutilus* (Cyprinidae). *Biol. J. Linn. Soc.* **2015**, *114*, 929–940. [CrossRef]
53. Gillespie, G.; Fox, M. Morphological and life-history differentiation between littoral and pelagic forms of pumpkinseed. *J. Fish Biol.* **2003**, *62*, 1099–1115. [CrossRef]
54. Belk, M.C.; Ingley, S.J.; Johnson, J.B. Life history divergence in livebearing fishes in response to predation: Is there a microevolution to macroevolution barrier? *Diversity* **2020**, *12*, 179. [CrossRef]