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Heterochrony in the Longjaw Mudsucker (Gobiifomres: Gillichthys mirabilis)

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Author
Milan, Jimjohn Duhinog

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UNIVERSITY OF CALIFORNIA SAN DIEGO

Heterochrony in the Longjaw Mudsucker (Gobiifomres: *Gillichthys mirabilis*)

A Thesis submitted in partial satisfaction of the requirements

for the degree Master of Science

in

Marine Biology

by

Jimjohn D. Milan

Committee in charge:

      Professor Philip A. Hastings, Chair
      Professor Brice X. Semmens
      Professor Jennifer R. Taylor

2020

The Thesis of Jimjohn D. Milan is approved and it is acceptable in quality and from for publication on microfilm and electronically:

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Chair

University of California San Diego

2020

DEDICATION

This thesis is dedicated to my family and friends who have supported me in my journey to become a scientist.

This thesis is also dedicated to my high school teacher, Mrs. Lynda Tran, and my community college professor, Professor Jason Bram, who both re-ignited my passion for Marine Biology when I was lost in my career path.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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Many thanks to my friend and collection manager, Ben Frable, who constantly inspired me with curious and fascinating fishes and taught me that collection work can be very stimulating. I will forever be grateful for your guidance in my many attempts to learn techniques used in collection work.

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Lastly, I would like to thank my family for their support and encouragement on my decision to pursue a career in marine science.

ABSTRACT OF THE THESIS

Heterochrony in the Longjaw Mudsucker (Gobiifomres: *Gillichthys mirabilis*)

by

Jimjohn D. Milan

Master of Science in Marine Biology

University of California San Diego, 2020

Professor Philip A. Hastings, Chair

Studying evolutionary change with respect to the development of morphological traits in an organism can give insight on the evolutionary development and diversification of those traits, as well as valuable information about how these characteristics contribute to behavior. The Longjaw Mudsucker (*Gillichthys mirabilis*, Gobiidae) has been studied extensively for its ability to occupy low oxygen environments, yet few studies research the ontogeny of its elongate jaw as it relates to a unique gaping display behavior. Members of the *Gillichthys* genus exhibit this territorial defense behavior during mating months and *G. mirabilis* possess exceptionally long maxillae that are laterally flared during its gaping display. The elongate maxilla and associated

buccopharyngeal membrane amplifies the aggressive gaping display of *G. mirabilis* and also increases surface area for gas exchange during aerial respiration. In this study, the maxillae of *G. mirabilis*, *Gillichthys seta*, and the outgroup species *Eucyclogobius newberryi* were examined and analyzed in an ontogenetic size series using digitized landmarks and caliper measurements. With these features, principal component analysis was used to study evolution in the maxilla of these three species under the heterochrony framework. Sexual dimorphism in the maxilla and body shape-space were also investigated to observe sexual trait selection and body shape correlation. The results show the maxilla of *G. mirabilis* evolved via acceleration (increased growth rate) and hypermorphosis (continued growth), two forms of peramorphosis. These results give us a start in understanding the evolutionary progression of the Longjaw Mudsucker and the potential connection of its elongated maxillae with its gaping display.

Introduction

Gaping behavior, better known as "gaping display", is a common behavior found in fish where an open mouth is presented toward other individuals for aggression or defense (Lindquist, 1975, Ritter, 2008). One fish known for its exaggerated gaping display is the Sarcastic Fringehead (*Neoclinus blanchardi*). The elongated maxilla of the fringehead was found to amplify its gaping display (Hongjamrassilp et al., 2018). However, aside from the Sarcastic Fringehead, no other studies have been conducted on fish who also exhibit this extreme form of a common behavioral display.

Like the Sarcastic Fringehead, the Longjaw Mudsucker (*Gillichthys mirabilis*) also possesses elongated maxillae and exhibits a gaping display (Weisel, 1947). *G. mirabilis* is an iconic fish, well known for its ability to occupy low oxygen environments in coastal California and within the Gulf of California by taking gulps of air into the branchial cavity where gas exchange occurs (Barlow, 1961a,b; Todd and Ebeling, 1966). Its common name derives from the prolonged maxilla that, in large individuals, extends to or beyond the gill opening. A member of the "Bay Goby" lineage (Ellison et al., 2014; Thacker, 2015), the genus *Gillichthys* includes two additional species restricted to the northern Gulf of California: the Shortjaw Mudsucker (*Gillichthys seta*), found in the rocky intertidal, and the Delta Mudsucker (*Gillichthys detrusus*), restricted to soft sediments in the Colorado River delta region (Barlow, 1961a; Swift et al., 2011).

Members of the genus *Gillichthys* are known to show a gaping display for territorial or nest defense during mating intervals that is especially pronounced in *G. mirabilis* because of its prolonged jaws (Weisel, 1947; Miles, 1985; Crabtree, 1985). This study examines the role of heterochrony in the development and evolution of the prolonged jaws of *G. mirabilis*.

Heterochrony is a useful tool to quantify the evolution of developmental processes through the characterization of relationships between the growth and shape of a trait over time. Any genetically controlled difference in duration or timing of an organism's developmental process can be compared to its inferred ancestor by comparison with closely related species. The two main types of heterochrony are paedomorphosis, in which descendants retain juvenile features of ancestors, and peramorphosis, in which they develop traits beyond those present in ancestors (McNamara 2012).

Barlow (1961a), in a detailed systematic study on *Gillichthys,* noted that *G. seta* and *G. mirabilis* have similar growth trajectories and comparable sizes. However, larger individuals of *G. mirabilis* are unique compared to *G. seta* in several features, most notably the length of the upper jaw. Barlow hypothesized that *G. seta* is paedomorphic, having evolved from *G. mirabilis* by a reduction in body size associated with the transition to their rocky intertidal habitat. This study tests this hypothesis by documenting the ontogeny of the jaw of these two species in a phylogenetic context and comparison with an appropriate outgroup species, *Eucyclogobius newberryi*. In this study, I examined (1) the role of heterochrony in the evolution of the maxilla in *Gillichthys* using morphometrics based on a truss network established from landmarks and additional measurements, (2) the presence of sexual dimorphism in the maxilla of *Gillichthys* species, and (3) the overall body shape space of *Gillichthys* species.

Materials and Methods

Heterochrony compares size or shape of morphological features with developmental time. However, the age of preserved specimens could not be determined, so body size was used as a proxy for age (Klingenberg, 1996; Hongjamrassilp et al., 2018). Preliminary morphometric data on preserved specimens of *Gillichthys mirabilis* (Cooper, 1864) and *Gillichthys seta* (Ginsburg, 1938) from the Scripps Institution of Oceanography Marine Vertebrate Collection (MVC) were obtained from an unpublished study (Table 1). Additional *G. mirabilis* specimens were obtained from the Natural History Museum of Los Angeles County (LACM, supporting information Table S1) to expand the sample size range. Specimens from LACM were stored in 70% ethanol and were selected based on larger size class individuals. Specimens of *Eucyclogobius newberryi* (Girard, 1856) preserved in 50% isopropanol were obtained from the MVC (Table 1). Specimens were selected based on a balanced size range and high preservation quality.

The sex of the specimens from all species was identified with a dissecting scope based on genital papilla morphology. Females have a rounded papilla, while the papilla of males is more pointed. Sex was confirmed in a subsample of specimens through dissection and examination of the gonads. Those too small to be confidently identified to sex were considered juveniles.

The preliminary heterochrony study focused on small individuals, which comprised 26 specimens of *G. mirabilis* (13♂, 9♀, 4 juveniles: Standard length: 20.3 - 130.4 mm) and 28 specimens of *G. seta* (10♂, 13♀, 5 juveniles: SL 18.3 - 63.4 mm). The size range of sampled *G. mirabilis* specimens in this study was increased using 18 large specimens (6♂, 12♀: SL 96 - 147 mm) from LACM. To further expand the heterochrony framework, 30 specimens of *E. newberryi* (12♂, 12♀, 6 juveniles: SL 10.5 - 46 mm) were included as an outgroup to the genus *Gillichthys*

3

(Gong et al. 2018; Zang and Shen 2019). Only specimens collected above 34° latitude were included to ensure the specimens were of the northern species and not of the recently described species of *Eucyclogobius* occurring only in Southern California (Swift et al., 2016).

Individual specimens were photographed in lateral view using a camera with a scale bar so traditional truss distances could be taken from the photographs. The truss distances included the following seven landmarks (Figure 1): 1) anterior tip of nasal bone, 2) first dorsal-fin spine insertion, 3) insertion of first element of second dorsal fin, 4) upper tip of dorsal hypural plate, 5) lower tip of ventral hypural plate, 6) first anal-fin spine insertion, and 7) pelvic-fin spine base. These landmarks were indicated by pins in each specimen prior to photographing. Before digitizing the landmarks, the image editing package "magick" was used to sharpen the images in R-Studio. The function "digitize2d" in the package "geomorph" was then used to load in the images onto R-studio and landmarks were digitized into each image, creating a TPS file. The "dist" function was used on the TPS file to produce 13 truss distances using the digitized landmarks. Six additional measurements were taken directly from each preserved specimen using digital calipers. The additional measurements included the following (Figure 2): 1) head length from snout to farthest posterior point of the operculum (HLL), 2) head length from snout to dorsal operculum insertion (HLI), 3) total upper jaw length (TJL), 4) free maxilla length or the posterior portion of the maxilla that is not attached to the head (FML), 5) maximum maxilla depth (MD), and 6) body depth at the origin of the anal fin (BD).

The truss measurements and additional measurements were analyzed using principal component analysis (PCA) under the covariance matrix using the function "princomp" in R-Studio. Allometric-Burnaby transforms for shape space analysis were performed in PAST 4.0 before processing data into R-Studio. This technique in PAST log transforms the 19

4

measurements and orthogonally projects them to the first principal component (Hammer et al., 2001). The first few principal components from a PCA usually explain most of the variance in the data. Since PC1 is representative of body size (Fitzgerald et al., 2002), the PC1 scores were plotted against log transformed free maxilla length and total jaw length. The results were analyzed using ANCOVA in R-studio to compare slope differences between the three species.

Results

From the PCA, PC1 accounts for 97.44% of the variation in the 13 truss distances and seven additional measurements. PC1 loadings (Table 1) are all positive, thus PC1 score is used as a representation of body size (Strauss and Bookstein, 1982). The highest loadings along PC1 were jaw measurements, more specifically FML, MD, and TJL (Table 1). Positive correlation is seen between PC1 and both free maxilla length and jaw length in all regression lines (Figure 3). An analysis of covariance (ANCOVA) was used and the growth rate of free maxilla length and total jaw length, inferred by the slopes of the regression lines in Figure 3, were shown to have significant differences in the three species ($p < 0.001$). Post-hoc tests reveal the growth rate of free maxilla length differs between each of the three species ($p < 0.001$). Growth rate of total jaw length significantly differs between *G. mirabilis* and *E. newberryi* ($p < 0.001$) and between *G. seta* and *E. newberryi* ($p = 0.041$) but does not differ between *G. mirabilis* and *G. seta* ($p = 0.164$).

With PC1 representing body size, sexual dimorphism within the maxilla is not seen in *G. mirabilis* total jaw length and free maxilla length (Figure 4). ANCOVA tests show no difference in the slopes between male and female *G. mirabilis* (TJL: β (male) = 0.131, β (female) = 0.132, p = 0.6036; FML: β (male) = 0.177, β (female) = 0.167, p = 0.6587). The tests also show that sexual dimorphism is not clearly seen in *G. seta* total jaw length (β (male) = 0.1, β (female) = 0.124, p = 0.3761), and in *E. newberryi* total jaw length and free maxilla length (TJL: β (male) = 0.131, β (female) = 0.115, p = 0.2345; FML: β (male) = 0.147, β (female) = 0.114, p = 0.1623). Sexual dimorphism is only statistically seen in *G. seta* free maxilla length (β (male) = 0.145, β (female) = 0.121, p = 0.0198).

After adjusting the measurement data of mature specimens with the Burnaby transformation, PC1 accounts for 25.8% of variation while PC2 accounts for 20.7% of variation (Figure 5; Table 2). The highest positive loadings along PC1 were TR10 (4-5): the vertical length of the caudal peduncle and TR13 (6-7): the length from the anal fin insertion to the pelvic fin insertion, while the highest negative loadings were jaw measurements: FML and MD (Table 2). *G. seta* had the lowest range of scores along PC1 while *E. newberryi* showed the widest range. The variables that loaded highest along PC2 were all jaw measurements: TJL and FML were positive loadings, while MD was a negative loading. Both species of *Gillichthys* on average scored more positive values along PC2 than *E. newberryi* with *G. seta* showing the highest range among the three. A multivariate analysis of variance (MANOVA) test reports the transformed data shows significant differences between species ($p < 0.001$).

A phylogenetic interpretation of the evolutionary pattern of heterochrony in jaw length (Fig. 6) was created using the inferred developmental pattern in the immediate ancestor of the genus *Gillichthys*.

Discussion

The ontogenetic trajectories for jaw length and free jaw length for the two species of *Gillichthys* do not differ significantly from one another, but both differ from that of *Eucyclogobius* . In addition, adults of the two species of *Gillichthys* occupy similar shape space compared to *Eucylogobius* . This is consistent with Barlow's (1961a) report that individuals of the two species of *Gillichthys* of the same size are similar and that mature individuals of *G. seta* resemble premature individuals of *G. mirabilis*. Given this similarity it is worth noting that these two species occur in distinctly different habitats. As the name implies, the Longjaw Mudsucker is found on mud bottoms in sheltered bays (Weisel, 1947), while the Shortjaw Mudsucker, *G. seta*, occurs in rocky tide pools in the upper intertidal zone of the northern Gulf of California (Barlow, 1961a; Thomson et al., 2000).

Sexual dimorphism is not clearly seen in our data on *Gillichthys* , despite previous studies that reported that males have longer jaws than females in *G. mirabilis* (Crabtree, 1985; Weisel, 1947). Several possibilities may account for this discrepancy. First, and most importantly, Crabtree (1985) sampled far more males and females than we did (n = ~130; ~80 males, ~50 females compared to our n = 35). Second, our estimate of body size (PC1) is based on multiple features instead of just standard length (SL) used by Crabtree. This possibility was quickly investigated by plotting SL with total jaw length (TJL) and free maxilla length (FML). The resulting ANCOVA supports the initial finding that sexual dimorphism is not seen in the maxilla of *G. mirabilis* (TJL: $F(1, 1) = 0.0003$, p = 0.98615; FML: $F(1, 1) = 0.3864$, p = 0.5388). Third, while Crabtree sampled a single population, our samples came from multiple sites over multiple years and patterns of dimorphism may differ as populations experience different selective

pressures and evolve differently (Sol et al., 2020). Although the ANCOVA found no statistical difference between the slopes of male and female *E. newberryi,* our study found a significant difference in the overall length of the jaw between sexes, which is consistent with previous reports that both species of *Eucyclogobius* are dimorphic in jaw length (Swift et al. 2016).

This study indicates that the extraordinarily long jaws of *G. mirabilis* evolved via two separate steps of peramorphic heterochrony. Compared to the outgroup *Eucyclogobius*, the upper jaw and especially the free posterior jaw extension of two species of *Gillichthys* grow more rapidly in length with increasing body size, a pattern known as acceleration (McKinney, 2013). Within *Gillichthys*, *G. mirabilis* grows to a considerably larger body size than *G. seta* (maximum size 147 vs. 66 mm SL; Barlow, 1961a), and as a consequence of the positive allometry of jaw growth, it has a relatively longer jaw and free posterior extension, a pattern known as hypermorphosis (McKinney, 2013).

Our finding that G. mirabilis jaws evolved peramorphically is in contrast to the conclusion of Barlow (1961a) that *G. seta* evolved via paedomorphosis by a reduction in body size. From a non-evolutionary developmental perspective, mature *G. seta* closely resembles premature, similarly-sized individuals of *G. mirabilis* and thus may be considered developmentally paedomorphic. However, from an evolutionary perspective, it is clear that the unique elongate jaw morphology of *G. mirabilis* (and *G. detrusus*, see below) evolved via peramorphosis as a result of their increase in body size compared to other members of the Bay Gobies. Peramorphic heterochrony is poorly documented in gobies, which are more often known to evolve via paedomorphosis (Kon & Yoshino, 2002). The typical evolutionary trend among gobies is a reduction in body size (Miller, 1979), while the increase in body size seen in *G. mirabilis* compared to other gobies appears to be relatively rare. It must be noted that this study

9

only demonstrates peramorphosis in the jaw of *Gillichthys*. Further research is needed to observe the pattern of heterochrony in other traits, such as head length and body depth. A third species of *Gillichthys* (*G. detrusus*) is morphologically similar and closely related to *G. mirabilis,* with which it was confused for decades (Swift et al., 2011). It grows nearly as large as *G. mirabilis* (maximum SL 111 mm; Swift et al., 2011), thus its pattern of heterochrony in the jaw is predicted to be similar to that of the Longjaw Mudsucker.

The *Gillichthys* genus is included in the so-called "Bay Goby" lineage (Ellison et al., 2014; Thacker, 2015) and, based on these and other recent phylogenetic studies, its closest relatives are within the monophyletic eastern Pacific clade that includes *Lepidogobius, Clevelandia, Quietula, Illypnus, Evermannia, Lethops, Typhlogobius,* and *Eucyclogobius*. The latter was included in this study as an outgroup based on the phylogenetic hypothesis of Gong et al. (2018) and Zang and Shen (2019). Members of the Bay Goby lineage tend toward relatively long jaws compared to many other lineages of gobies. While in some members, such as *Lepidogobius,* the maxilla ends at mid-obit (Gill, 1863), in others, most notably *Quietula,* the maxilla extends past the posterior margin of the orbit, but typically not to the level of the preopercular margin (Kindermnn et al., 2007). While it extends beyond the preopercular margin in *G. seta*, in no other member of the "Bay Gobies" does the maxilla extend past the posterior margin of the operculum as it does in large individuals of *G. mirabilis* (and *G. detrusus*). Jaw growth in *Gillichthys* species is clearly accelerated compared to that of *Eucyclogobius*. Inclusion of the ontogenetic trajectory of additional species of Bay Gobies would provide increased confidence in the ancestral condition

in *Gillichthys* and may affect the inferred point on the phylogeny that acceleration of jaw growth occurred. However, *G. mirabilis* grows to a larger size than other Bay Gobies (maximum 147 mm SL; LACM 8897-2 Female #4), so the inferred hypermorphosis event leading to the Longjaw Mudsucker is unlikely to change.

The finding of peramorphosis leading to an especially elongate jaw in *G. mirabilis* is similar to that reported for the Sarcastic Fringehead, *Neoclinus blanchardi* (Blenniiformes), except that both heterochronic changes (acceleration and hypermorphosis) were reported to have occurred along the same branch leading to that species (Hongjamrassilp et al., 2018). Similar to the Sarcastic Fringehead, the elongate jaw of *G. mirabilis* appears to function to amplify the aggressive gaping display in which the mouth is opened widely, flared laterally, and presented to conspecifics (Weisel, 1947; Crabtree, 1985). In the Sarcastic Fringehead, the lateral flaring of the maxilla is facilitated by a unique notch in the first infraorbital bone (lacrimal) permitting lateral movement that is constrained in related blennies by a straight, rigid lacrimal (Hongjamrassilp et al., 2018). However, the lacrimal of gobies, including members of the Bay Gobies, is reduced (Kindermann et al., 2007) and apparently does not obstruct lateral movement of the maxilla.

Using CT-scan and cleared-and-stained specimens, the posterior extension of the Sarcastic Fringehead was shown to comprise uncalcified bone (Hongjamrassilp et al., 2018). Preliminary analysis of *G. mirabilis* CT-scan, x-ray, and cleared-and-stained specimens show possible reduced ossification in the free maxilla. This reduced ossification may play a role in extending the maxilla of *G. mirabilis* and may have evolved to facilitate the exaggerated gaping display of *G. mirabilis,* but further images are necessary to confirm this hypothesis.

The Sarcastic Fringehead also has an unusually large buccopalatal membrane that assists the considerable lateral movement of the maxillae during its gaping display (Hongjamrassilp et al., 2018). The membrane is brown in color, but the edge is a bright yellow and may visually maximize the apparent size of the gape during display, yet it can also be seen with its mouth closed. *G. mirabilis* has no distinct coloration on its maxilla, which is not surprising given the reduced water clarity of its natural habitat of murky mud bottoms. The rich vascularization of the buccopalatal membrane of *G.mirabilis* has been shown to serve as an accessory respiratory apparatus in its low oxygen environment (Weisel, 1947; Todd and Ebeling, 1966). This may be the primary selective pressure leading to the evolution of long jaws of this species, but this feature also serves to enhance its gaping display. While the gaping display of the Sarcastic Fringehead is used in territorial defense throughout the year, especially in male-male interactions (Hongjamrassilp et al., 2018), in the Longjaw Mudsucker, it may be more commonly used during reproductive periods (Weisel, 1947; Miles, 1975). Other species of Bay Gobies, including *G. seta* and *E. newberryi,* perform a similar aggressive gaping display (Miles, 1975; Swenson, 1997), but it is not known if these involve a lateral flaring of the maxilla that effectively amplifies the display in *G. mirabilis* and *N. blanchardi*.

Figures
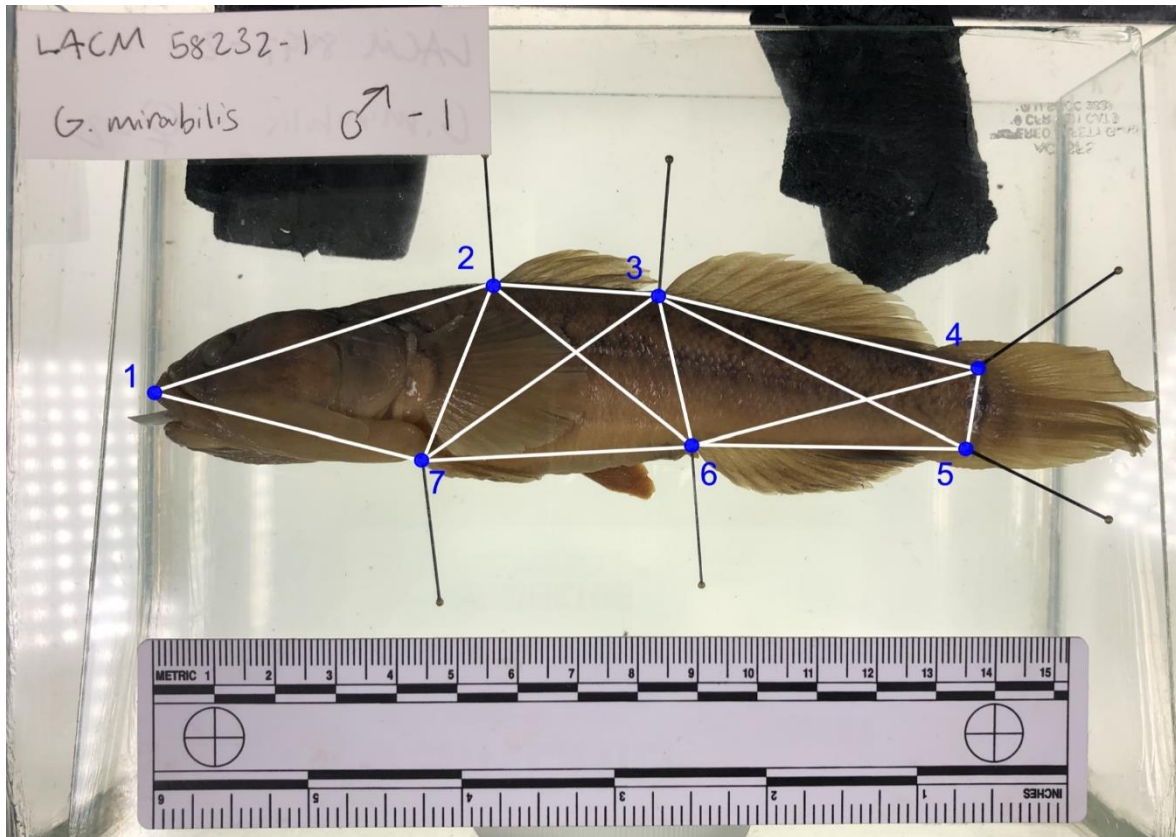
Figure 1: Landmarks used in geometric morphometric analysis: (1) anterior tip of nasal bone, (2) first dorsal-fin spine insertion, (3) insertion of first element of second dorsal fin, (4) upper tip of dorsal hypural plate, (5) lower tip of ventral hypural plate, (6) first anal-fin spine insertion, and (7) pelvic-fin spine base
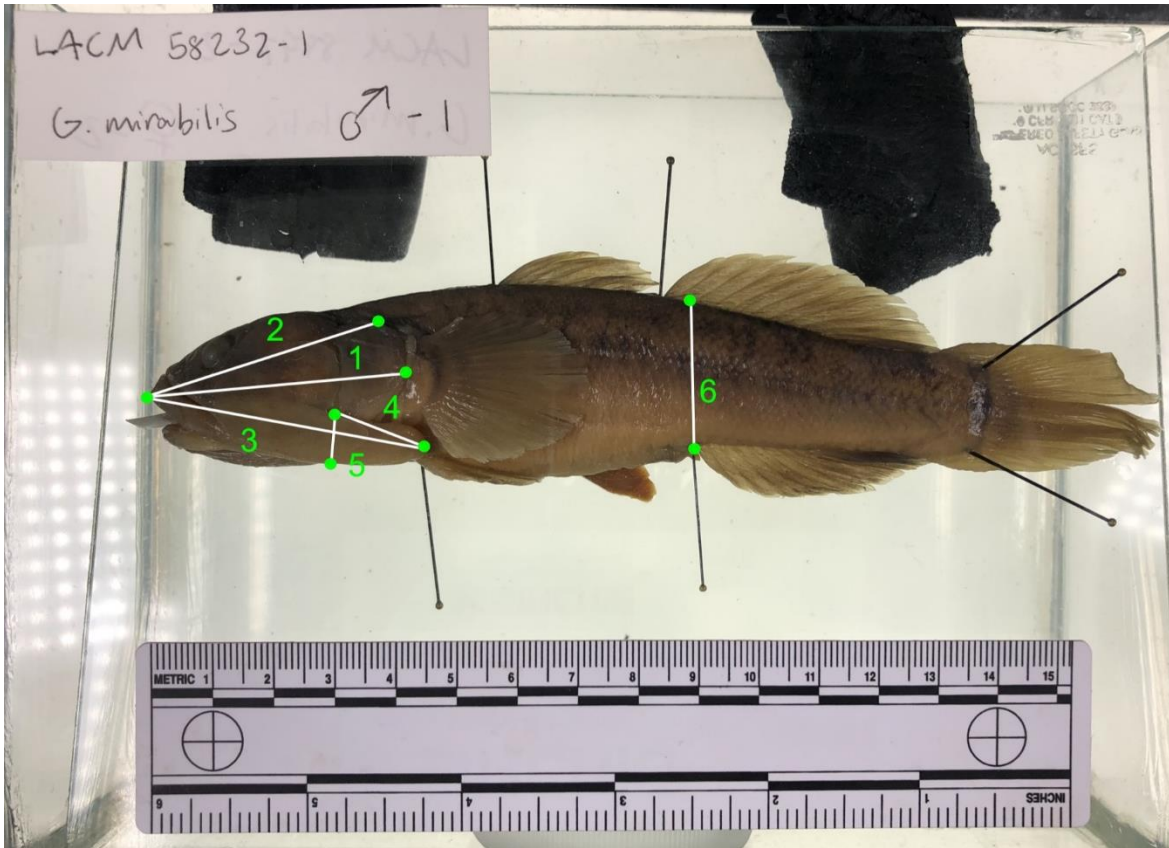
Figure 2: Additional measurements used in geometric morphometric analysis: (1) head length from snout to farthest posterior point of the operculum, (2) head length from snout to dorsal operculum insertion, (3) total upper jaw length, (4) free maxilla length or the posterior portion of the maxilla that is not attached to the head, (5) maximum maxilla depth, and (6) body depth at the origin of the anal fin
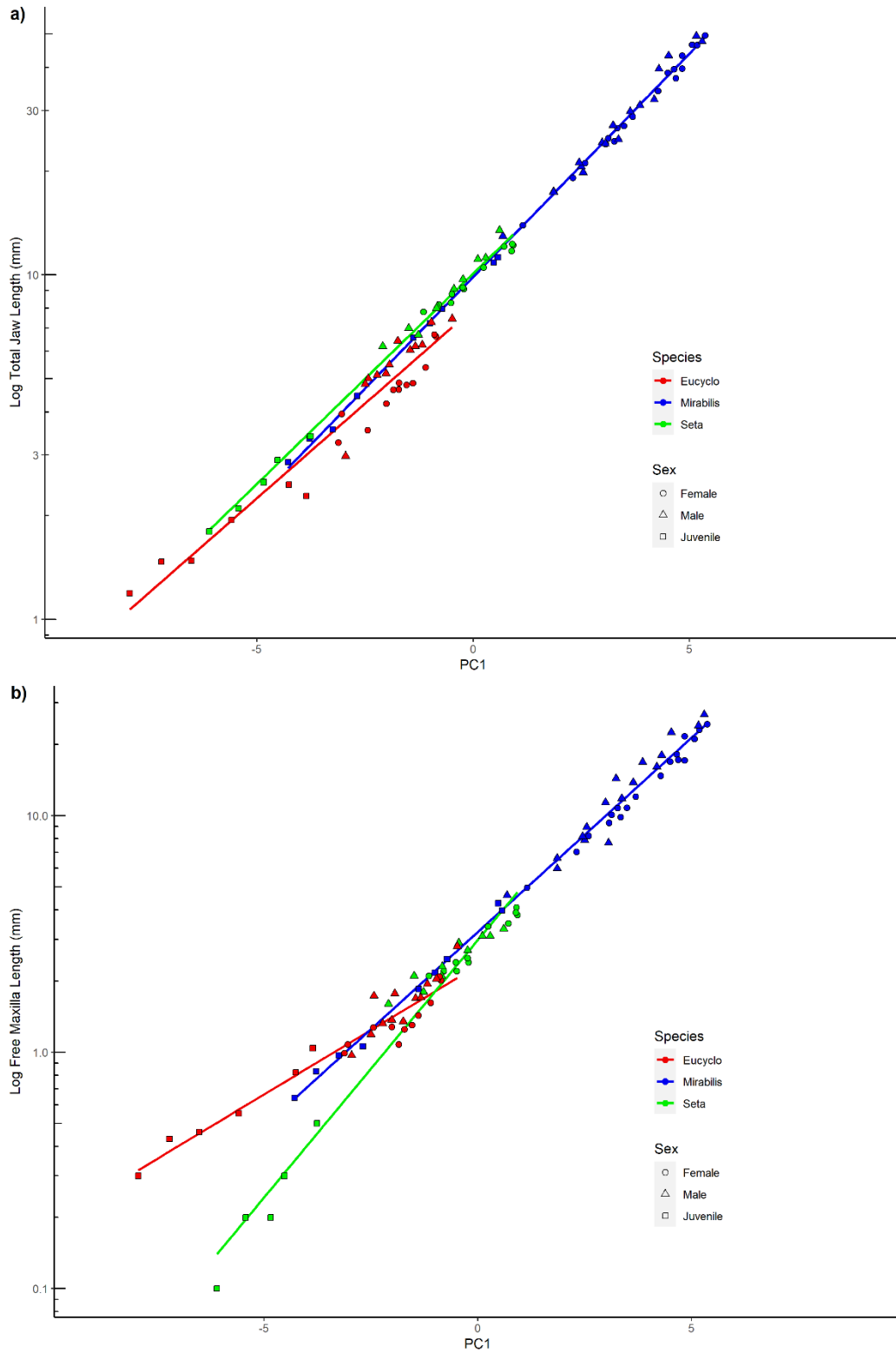
Figure 3: Scatter plot of principal component 1 score (representative of body size) for all three species against a) log-transformed total jaw length and b) log-transformed free maxilla length. Linear trendline shown as lines matching the colors for each species. Sex is shown with different shapes for each species
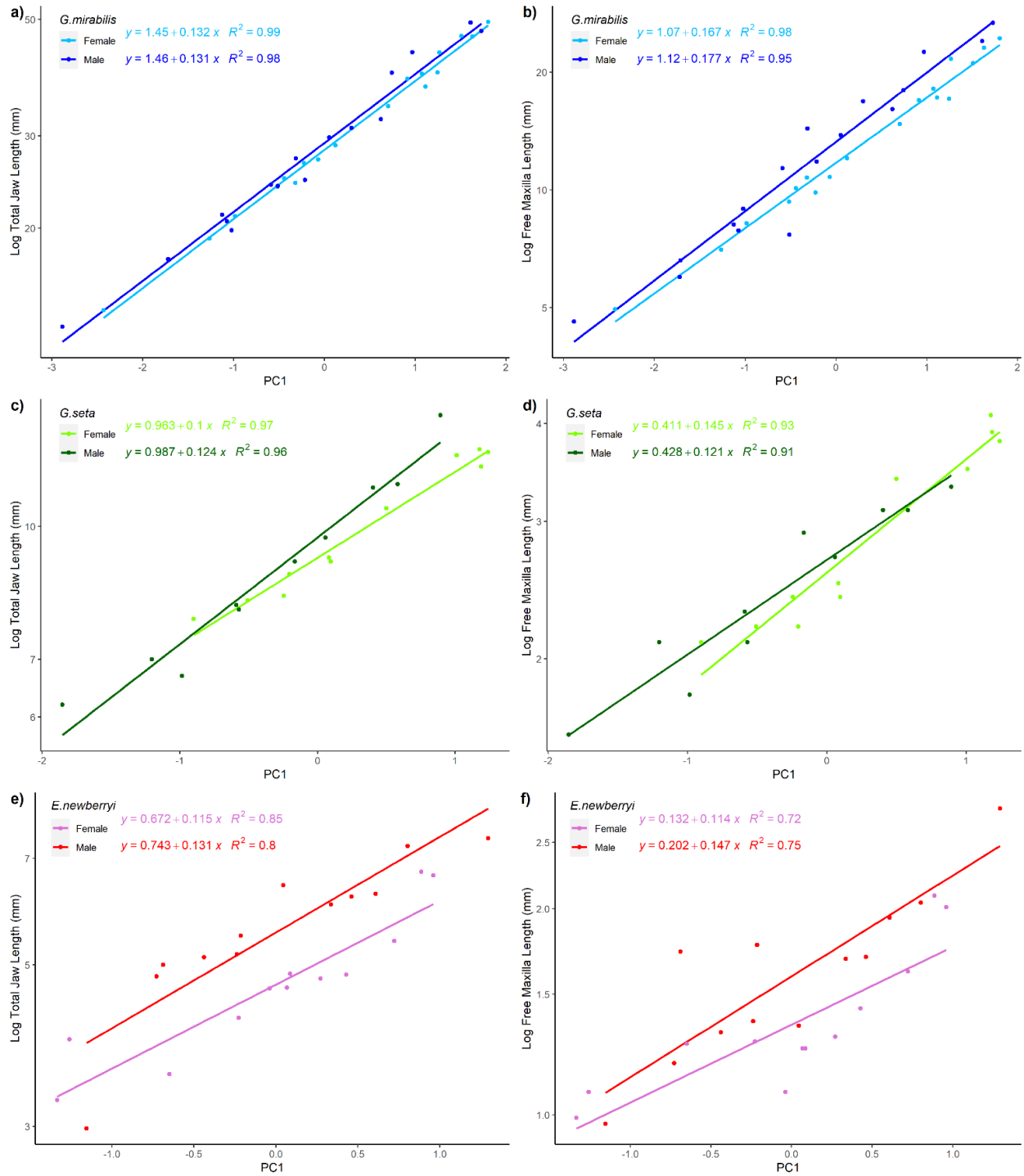
Figure 4: Scatter plot of principal component 1 score (representative of body size) for mature a) *G. mirabilis* against log-transformed total jaw length, b) *G. mirabilis* against log-transformed free maxilla length, c) *G. seta* against log-transformed total jaw length, d) *G. seta* against log-transformed free maxilla length, e) *E. newberryi* against log-transformed total jaw length, f) *E. newberryi* against log-transformed free maxilla length. Linear trendlines shown with corresponding equations matching the colors for each sex.
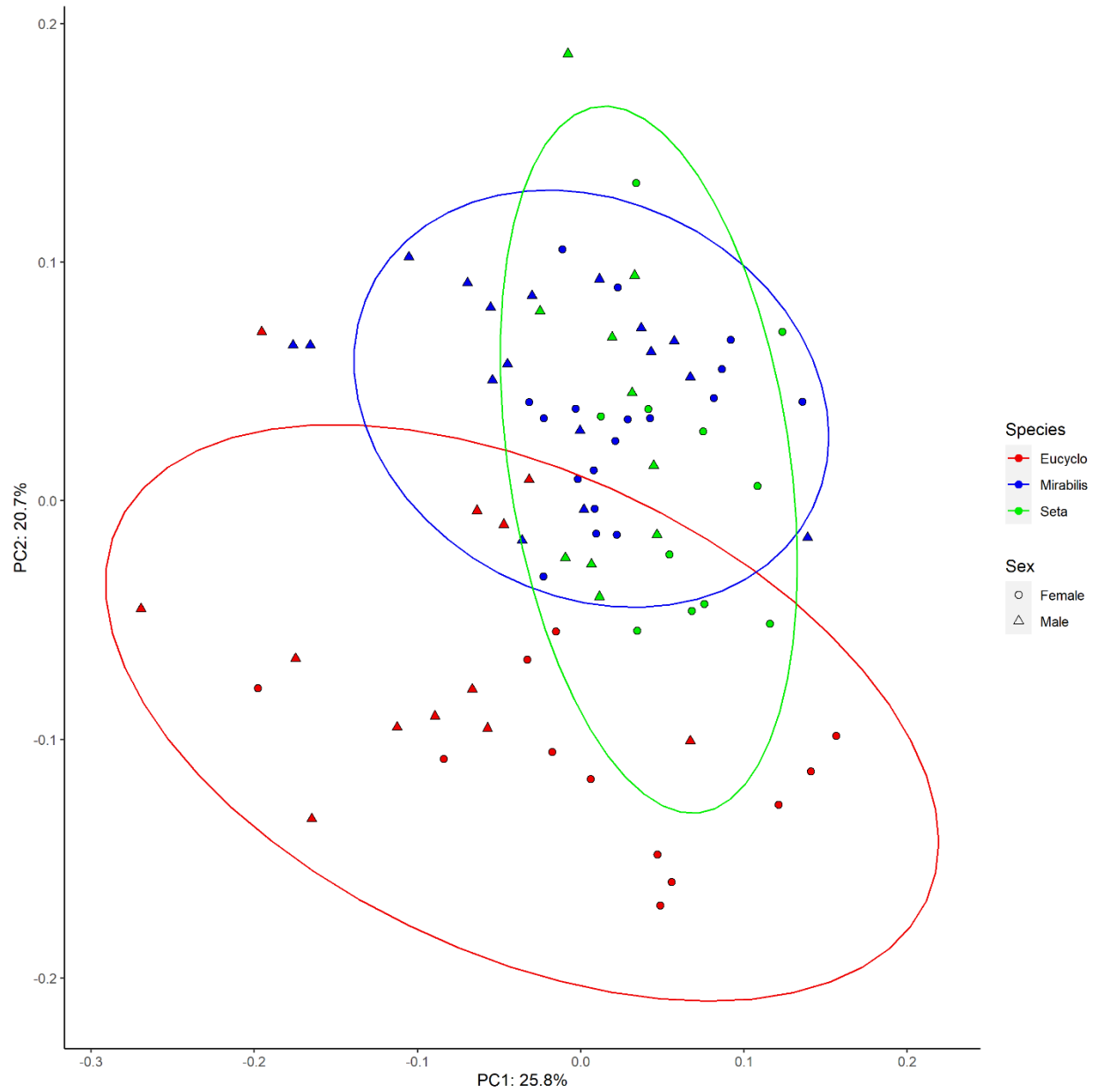
Figure 5: Scatterplot of principal component 1 and 2 from a principal component analysis of sexually mature *G. mirabilis*, *G. seta*, and *E. newberryi* (morphometric data adjusted using allometric-Burnaby transformation). Ellipses represent 95% confidence intervals

Figure 6: Hypothesized phylogeny and inferred patterns of heterochrony between Eucylcogobius and Gillichthys

Tables

Table 1: List of specimens used in this study. SIO is from Scripps Institution of Oceanography Marine Vertebrate Collection and LACM is from the Natural History Museum of Los Angeles. SL = standard length

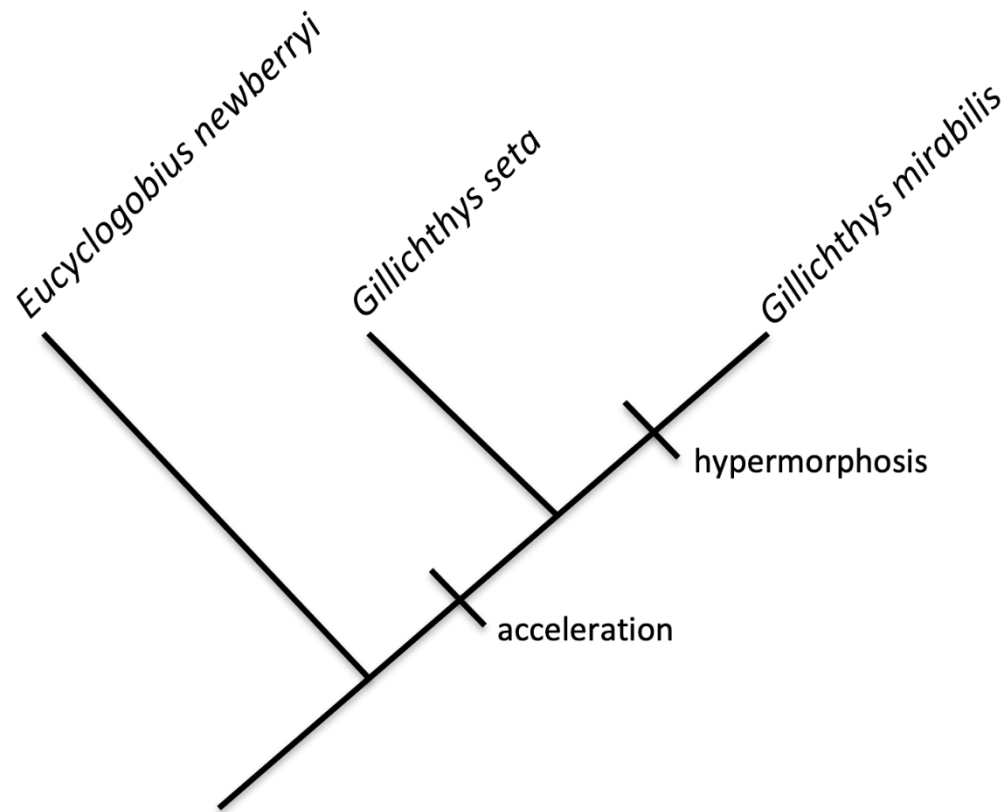|	Catalog number	Species	Sex	ID #	SL
1	SIO 48-205	*G.mirabilis*	Female	1	130.4
2	SIO 48-205	*G.mirabilis*	Female	2	119.9
3	SIO 48-205	*G.mirabilis*	Female	3	96.5
4	SIO 48-205	*G.mirabilis*	Female	4	93.6
5	SIO 48-205	*G.mirabilis*	Female	5	85.1
6	SIO 48-205	*G.mirabilis*	Female	6	78.4
7	SIO 64-279	*G.mirabilis*	Female	1	66.4
8	LACM 50366-1	*G.mirabilis*	Female	1	135.98
9	LACM 50366-1	*G.mirabilis*	Female	2	123.38
10	LACM 50366-1	*G.mirabilis*	Female	3	113.58
11	LACM 50366-1	*G.mirabilis*	Female	4	104.97
12	LACM 58232-1	*G.mirabilis*	Female	1	136.6
13	LACM 58232-1	*G.mirabilis*	Female	2	97.9
14	LACM 8897-2	*G.mirabilis*	Female	1	144.4
15	LACM 8897-2	*G.mirabilis*	Female	2	134.35
16	LACM 8897-2	*G.mirabilis*	Female	3	141.09
17	LACM 8897-2	*G.mirabilis*	Female	4	147.34
18	LACM 8897-2	*G.mirabilis*	Female	5	104.64
19	SIO 48-205	*G.mirabilis*	Male	1	102.8
20	SIO 48-205	*G.mirabilis*	Male	2	101.6
21	SIO 48-205	*G.mirabilis*	Male	3	96.4
22	SIO 48-205	*G.mirabilis*	Male	4	89.1
23	SIO 48-205	*G.mirabilis*	Male	5	80.1
24	SIO 48-205	*G.mirabilis*	Male	6	80.7
25	SIO 48-205	*G.mirabilis*	Male	7	79.9
26	SIO 48-205	*G.mirabilis*	Male	8	73.6
27	SIO 48-205	*G.mirabilis*	Male	9	70.8
28	SIO 64-279	*G.mirabilis*	Male	1	59.2
29	LACM 50366-1	*G.mirabilis*	Male	1	125.3
30	LACM 50366-1	*G.mirabilis*	Male	2	100.19
31	LACM 58232-1	*G.mirabilis*	Male	1	141.2
32	LACM 58232-1	*G.mirabilis*	Male	2	96
33	LACM 58232-1	*G.mirabilis*	Male	3	123
34	LACM 6622-2	*G.mirabilis*	Male	1	146.76
35	LACM 6622-2	*G.mirabilis*	Male	2	123.22
36	SIO 64-279	*G.mirabilis*	Juvenile	1	57.8

	Catalog number	Species	Sex	ID #	SL
37	SIO 68-695	*G.mirabilis*	Juvenile	1	44.6
38	SIO 68-695	*G.mirabilis*	Juvenile	2	53.9
39	SIO 68-695	*G.mirabilis*	Juvenile	3	37.8
40	SIO 68-695	*G.mirabilis*	Juvenile	4	37.1
41	SIO 68-695	*G.mirabilis*	Juvenile	5	29.8
42	SIO 68-695	*G.mirabilis*	Juvenile	6	25.3
43	SIO 68-695	*G.mirabilis*	Juvenile	7	23.2
44	SIO 68-695	*G.mirabilis*	Juvenile	8	20.3
45	SIO 00-66	*G.seta*	Female	1	62.8
46	SIO 00-66	*G.seta*	Female	2	58
47	SIO 86-55	*G.seta*	Female	1	63.4
48	SIO 86-55	*G.seta*	Female	2	43.3
49	SIO 86-55	*G.seta*	Female	3	41.7
50	SIO 86-58	*G.seta*	Female	1	60.5
51	SIO 87-185	*G.seta*	Female	1	53.7
52	SIO 87-185	*G.seta*	Female	2	45.2
53	SIO 88-1	*G.seta*	Female	1	49.5
54	SIO 88-1	*G.seta*	Female	2	48.6
55	SIO 88-1	*G.seta*	Female	3	47.7
56	SIO 00-66	*G.seta*	Male	1	54.2
57	SIO 86-55	*G.seta*	Male	1	46.7
58	SIO 86-55	*G.seta*	Male	2	41.8
59	SIO 86-55	*G.seta*	Male	3	40.8
60	SIO 86-55	*G.seta*	Male	4	38.1
61	SIO 86-58	*G.seta*	Male	1	57.2
62	SIO 87-185	*G.seta*	Male	1	33.1
63	SIO 88-1	*G.seta*	Male	1	51.4
64	SIO 88-1	*G.seta*	Male	2	45.9
65	SIO 88-1	*G.seta*	Male	3	36.9
66	SIO 47-55	*G.seta*	Juvenile	1	26.3
67	SIO 47-55	*G.seta*	Juvenile	2	23.7
68	SIO 47-55	*G.seta*	Juvenile	3	21.7
69	SIO 47-55	*G.seta*	Juvenile	4	19.6
70	SIO 47-55	*G.seta*	Juvenile	5	18.3
71	SIO 10-189	*E.newberryi*	Female	1	27
72	SIO 10-189	*E.newberryi*	Female	2	28.5
73	SIO 10-189	*E.newberryi*	Female	3	45
74	SIO 10-189	*E.newberryi*	Female	4	44
75	SIO 11-334	*E.newberryi*	Female	1	36.5
76	SIO 62-192	*E.newberryi*	Female	1	31
77	SIO 62-192	*E.newberryi*	Female	2	38.5

	Catalog number	Species	Sex	ID #	SL
78	SIO 62-192	*E.newberryi*	Female	3	39
79	SIO 72-87	*E.newberryi*	Female	1	41
80	SIO 72-87	*E.newberryi*	Female	2	36.5
81	SIO 72-88	*E.newberryi*	Female	1	35
82	SIO 72-88	*E.newberryi*	Female	2	39
83	SIO 10-189	*E.newberryi*	Male	1	28
84	SIO 10-189	*E.newberryi*	Male	2	32
85	SIO 10-189	*E.newberryi*	Male	3	33
86	SIO 10-189	*E.newberryi*	Male	4	36
87	SIO 10-189	*E.newberryi*	Male	5	40
88	SIO 10-189	*E.newberryi*	Male	6	41
89	SIO 10-189	*E.newberryi*	Male	7	42
90	SIO 10-189	*E.newberryi*	Male	8	44
91	SIO 10-189	*E.newberryi*	Male	9	46
92	SIO 11-334	*E.newberryi*	Male	1	37
93	SIO 72-87	*E.newberryi*	Male	1	33
94	SIO 72-87	*E.newberryi*	Male	2	31.5
95	SIO 62-192	*E.newberryi*	Juvenile	1	23
96	SIO 72-87	*E.newberryi*	Juvenile	1	12.5
97	SIO 72-87	*E.newberryi*	Juvenile	2	16.5
98	SIO 72-87	*E.newberryi*	Juvenile	3	14
99	SIO 72-88	*E.newberryi*	Juvenile	1	10.5
100	SIO 72-88	*E.newberryi*	Juvenile	2	20

Table 2: PC1 scores of each character from *Gillichthys* and *Eucyclogobius* specimens from Figure 1. SL = standard length, HLL = head length from snout to farthest posterior point of the operculum, HLI = head length from snout to dorsal operculum insertion, TJL = total upper jaw length, FML = free maxilla length or the posterior portion of the maxilla that is not attached to the head, MD = maximum maxilla depth, BD = body depth at the origin of the anal fin

Characters	PC1
Percent variance explained	97.4443
TR1(1-2)	0.20587
TR2(1-7)	0.20321
TR3(2-3)	0.18681
TR4(2-6)	0.20274
TR5(2-7)	0.20306
TR6(3-4)	0.19458
TR7(3-5)	0.19076
TR8(3-6)	0.19641
TR9(3-7)	0.19317
TR10(4-5)	0.19019
TR11(4-6)	0.18074
TR12(5-6)	0.17632
TR13(6-7)	0.20543
SL	0.19674
HLL	0.21319
HLI	0.21641
TJL	0.29788
FML	0.38430
MD	0.30667
BD	0.21297

Table 3: PC1 and PC2 scores of each character from *Gillichthys* and *Eucyclogobius* specimens from Figure 3. SL = standard length, HLL = head length from snout to farthest posterior point of the operculum, HLI = head length from snout to dorsal operculum insertion, TJL = total upper jaw length, FML = free maxilla length or the posterior portion of the maxilla that is not attached to the head, MD = maximum maxilla depth, BD = body depth at the origin of the anal fin

Characters	PC1	PC2
Percent variance explained	25.7885	20.6920
TR1(1-2)	0.12537	0.12351
TR2(1-7)	- 0.11505	0.01293
TR3(2-3)	0.17031	0.08483
TR4(2-6)	0.26730	- 0.08190
TR5(2-7)	0.20615	- 0.04200
TR6(3-4)	- 0.12299	- 0.22683
TR7(3-5)	- 0.06289	- 0.19638
TR8(3-6)	0.22435	- 0.19439
TR9(3-7)	0.21166	- 0.02981
TR10(4-5)	0.50409	0.26920
TR11(4-6)	- 0.12874	- 0.08772
TR12(5-6)	- 0.14731	- 0.10217
TR13(6-7)	0.30921	- 0.11676
SL	- 0.03192	- 0.05855
HLL	0.00711	0.05162
HLI	0.03253	0.08303
TJL	- 0.15295	0.43083
FML	- 0.43749	0.45769
MD	- 0.31536	- 0.53174
BD	0.09753	- 0.21260

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