



## Sexual Dimorphism in Body Shape of *Hypseleotris Agilis* (Herre, 1927) from Lake Lanao, Philippines

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### Abstract

*H. agilis* is an endemic species of fish from Lake Mai-init, Surigao del Norte, and introduced to Lake Lanao of Lanao del Sur sometime in the 1960s. The principal objective of this study is to describe the variations in morphology of the species as well as determine its sexually dimorphic traits, using landmark-based geometric morphometrics. A total of 55 (25 females and 30 males) specimens were analyzed. Twenty landmark points were digitized from which relative warp scores were derived. Discriminant function analysis and thin-plate spline plot showed significant difference between body shape of the sexes with males exhibiting bigger head, along with elongated dorsal and anal fin bases. The bigger head of males relative to females could be a result of sexual selection, whereas, the elongated dorsal and anal fins could be a product of ecological/niche selection.

**Keywords:** Endemic, relative warp, thin-plate spline.

### Introduction

*Hypseleotris agilis* is a freshwater fish belonging to family Eleotridae. It is laterally compressed and is unusual among gobies in that they do not lie on the bottom or attached to bottom objects but instead swim freely in the water column<sup>1</sup>. It was first described by Herre<sup>1</sup> who considered it as a distinct species, but described as similar to the young of *Ophieleotris* (= *Ophiocara*) *aporos*. It is endemic to Lake Mainit<sup>2</sup> however, considered the species as synonym of *O. aporos*, a more widespread species in the Philippines) a lake from Southern Philippines, where it is considered the most abundant fish caught but recently recorded in reduced abundance<sup>3</sup>. The species may have been accidentally introduced to Lake Lanao, another lake from southern Philippines, sometime in the 1960s, together with the stocking of milkfish and tilapia fries in Lake Lanao<sup>4</sup>. Fish surveys have indicated that the species became an established fish in Lake Lanao<sup>4</sup>. The species was suspected to be partly responsible for the decline in the population of the endemic cyprinids in Lake Lanao either by direct competition or by predation<sup>4</sup>. The size range of specimens caught from Lake Lanao is between 70 to 156 mm.

Sexual shape variation between the sexes of this species has been described by several authors. Males of *A. agilis* were described as with a pronounced enlargement of the dorsal head/nape region<sup>2</sup>. Their description however was based on the specimens of *Ophieleotris aporos* (which they considered as a synonym of *A. Agilis*, as noted earlier) captured from Laguna de Bay, Northern Philippines. They also noted in the species the *Hypseleotris*-diagnostic sexual dimorphic character of elongation of the dorsal and anal fin rays of the males. Escudero and Demoral<sup>4</sup> using specimens from Lake Lanao,

Philippines noted another form of sexual shape dimorphism in breeding individuals; a prominent hump on the forehead of the male and a bulging belly of the females.

Darwin<sup>5</sup> was the first to postulate that sexual dimorphism can be explained by sexual selection. This concept predicts that differences in the reproductive roles between sexes may influence patterns of selection and could lead to sex differences in morphological attributes such as the shape of its body<sup>6</sup>. However, sexual dimorphism could also be a functional adaptation for different habits of the two sexes<sup>7,8</sup>, especially in terms of shape. In fact the aquatic environment is recognized as a powerful force in modelling the morphology of an organism during ontogeny. Thus, shape variation between the sexes could also be explained by ecological or niche selection and may be a reflection of the different habitat preference of the two sexes.

This study re-examines and quantifies sexual shape dimorphism in *Hypseleotris agilis* using landmark-based Geometric Morphometrics. One major advantage of this approach over traditional morphometrics is that the result of the analyses could be visualized as configurations of landmarks back in the original space of the organism and could be represented as actual shapes or shape deformations rather than only as statistical scatter plots<sup>9</sup>.

### Material and Methods

**Sampling and Study area:** Lake Lanao (8° 00' N, 123° 50' E) is the largest (357 km<sup>2</sup>) lake in Mindanao with an altitude of 702 m, a mean depth of 60.3 m and an altitude of 702 m. Five major river systems drain into the lake from the surrounding watershed areas, while Agus River is the one and only river draining the

Lake on its north side, towards Iligan Bay. Fresh specimens were bought from fishermen fishing on the shores of a village named "barangay Tuka" of Marawi City in the morning of September 15, 2010. Marawi City is one of the areas bordering the northern coast of Lake Lanao (figure-1). The fresh specimens were transported inside a styropor box and immediately brought to the laboratory for processing. The specimens range in size from 10-12 cm in standard length.

**Processing and Digitization of the Fish specimens:** Digital images from the left side of the samples were prepared from these selected specimens. These images were produced by a flat-bed scanner at a resolution of 600 dpi. From among 71 of the images, 55 (25 females and 30 males) were chosen for their excellent quality and included in this study. Sexing by examination of the gonads was done after scanning.

Twenty landmarks (equivalent to 20 X and 20 Y Cartesian coordinates) were digitized on the images using the tpsDig (version 2.12)<sup>9</sup> as shown in figure - 2. These landmarks were selected to provide a comprehensive summary of the morphology of the fish specimens. Digitization was done in triplicates for each fish sample.

**Shape Analysis.** The quantified data from the landmark points that were digitized on the specimens contain both shape and non-shape components of variation<sup>10</sup>. Since this study is focused on shape analysis, the non-shape components need to be removed before shape analysis could proceed. These non-shaped components were removed by General Procrustes Analysis (GPA) using tpsRelw ver. 1.36<sup>11</sup>. GPA aligned all the specimens in morphospace, eliminating size and rotational/translational differences. It proceeded as follows: first, the landmark specimens were translated to a common centroid at the origin of the reference coordinate system at point (X=0, Y=0). Then the set of landmark coordinates of each fish sample were scaled to unit centroid size thereby removing size differences and permitting analysis of body shape. Finally, the landmark configurations of all fishes were rotated to minimize the sum of squared distances between corresponding landmarks<sup>12</sup>. This step removed residual translational and rotational differences in the way the fishes were oriented in the flat-bed scanner when the digital images were taken.

Using the thin-plate spline (tps) equation and the standard formula for uniform shape components, a weight matrix (containing uniform and non-uniform shape components) from the aligned specimens were generated<sup>12</sup>. Variability in body shapes was then examined via relative warp (RW) analysis of the set of uniform and non-uniform components of shape using tpsRelw<sup>11</sup> generating new variables in the form RW scores. The RW scores were then subjected to discriminant function analysis (DFA) to test for differences of body shape between sexes using the PAST software (Paleontological Statistics, version 1.27<sup>13</sup>. In

DFA the relative warp scores were treated as independent variables and a multivariate function was defined such that males and females were maximally discriminated.

To visualize the differences between body shapes of the sexes, the software tps Spline, version 1.20<sup>14</sup> was used. It produces transformation grids that show the shape change from a grid with square cells superimposed onto the average landmark configuration to a grid that is deformed to fit a target configuration. This comparison is based on Procrustes distance ( $d^2$ ), which is the standard measure for the magnitude of shape differences used in geometric morphometrics<sup>12</sup>.

## Results and Discussion

Patterns of shape variation of the species is shown and is summarized via frequency histograms of the RW scores together with the grid plots of the landmark points showing the extremes of shape variation along the first six RW axes (figure-3) which explain 74% of the total shape variation. These variations occur in the head region (lengthening and broadening) and the body region (broadening and narrowing of the body; elongation and shortening of the fin bases) as shown in the plots representing the extreme shape configurations in the six relative warps.

Much of these variations can be explained by sexual dimorphism. Figure - 4 shows the DFA of the relative warp scores between sexes, which shows that body shape of male *H. agilis* is different from that of the female ( $p < 0.05$ ).

The visible pattern of major body shape variation differentiating the sexes is shown in tps Spline plot (figure - 5), and occurs in two sets: i those of the head region, and ii those of the trunk/abdominal region. In the head region, the male, is with elongated jaw and a deeper head region (expansion of the grid between landmarks 1 to 2, 12 and 13). In the trunk region, the female is shown with a deeper and elongated belly region (between landmarks 2, 4 and 10, 11) and the males with longer second dorsal fin base (expansion of the grids between landmarks 3 and 5), longer anal fin base (expansion of the grids between landmarks 9 and 10).

The bigger head of the males relative to females in this study conforms with the observation describing males of *A. agilis* as with a pronounced enlargement of the dorsal head/nape region<sup>2,4</sup>. Other fish species also have been shown to exhibit such trait in males<sup>7,15,16</sup>. While the deeper and elongated belly region agrees with the observation of Escudero and Demoral<sup>4</sup>.

An elongated dorsal and anal fin rays of males are *Hypseleotris*-diagnostic sexual dimorphic character<sup>2</sup>. Height of dorsal and anal fins distinguishes male from females in in the Mossambique tilapia (*Oreochromis mossambicus*)<sup>15</sup>. Longer anal and dorsal fin of three-spines Stickleback (*Gasterosteus*

*aculeatus*) was also noted in males<sup>7</sup>. Perhaps, this is a common sexual dimorphic character in fishes. This study did not specifically measure the height of the fins but did observe the elongated bases of the second dorsal and anal fins.

Darwin<sup>5</sup> was the first to postulate that sexual selection is one main mechanism driving sexual dimorphism and that sexual selection acting on males will favour exaggeration of the targeted male traits if individuals with larger traits achieve higher mating success. Hypothetically the bigger head and bodies of males could be explained by sexual selection as it can be used in agonistic behaviour to threaten potential rivals and in actual fighting and winning against rivals<sup>16-20</sup>.

Alternatively, sexual dimorphism can evolve by ecological selection acting differentially on both sexes and thus favouring both dimorphic niches and, as a consequence, dimorphic trophic structures<sup>7,21</sup>. Ecological factors may provide additional selective pressures for shape differences between sexes<sup>7</sup>. It was observed that specimens from large lakes exhibit increased shape dimorphism and decreased in small and shallow ponds. Thus, such traits may be functional adaptations to illustrate different ecological niches between sexes<sup>7</sup>. The same suggestion was made when wild-caught females of *Poecilia reticulata* showed smaller heads and deeper caudal peduncles than wild-caught males compared with laboratory-reared specimens which showed no such difference<sup>8</sup>. Such selective pressure may be presumed to accentuate or suppress sexual shape dimorphism, depending on the diversity of the environment which could accommodate the varying habitat preference of each sex.

The result of the fin elongations in males of *H. agilis* observed in this study would be an increase in the span of the fins from the leading edge to the tip of the trailing edge and a change in its aspect ratio. These fin elongations can be argued as triggered by sexual selection, as it can be effective in agonistic behaviour to threaten rivals. A characteristic of this behaviour as recorded in *Tilapia mossambicus* is broadside displays with full erection of the fins<sup>20</sup>.

The fact however, remains that fins exist first as functional structures enabling fishes to manoeuvre in an aquatic medium. Any change therefore in its form and total surface area would first impact on the fish capacity to manoeuvre. The natural selection pressure therefore, for the fins, is towards a fish becoming more efficient on its preferred behaviour and habit while conserving energy to enhance its fitness. Any other function that maybe ascribed to these fins should be treated as secondary and may either accentuate or attenuate such traits<sup>22-24</sup>.

The dorsal and anal fins in fishes have been shown to act as stabilizers by inhibiting roll movements during steady swimming and turning, and at the same time to contribute to thrust production of the caudal fin<sup>25-27</sup>. The effect elongation of

these fins, as observed in *H. agilis* of the present study, would result into an increase of the fin's total surface area, imparting on the sexes differing capabilities to manoeuvre. This anal and dorsal fin difference between them may have been promoted by their differing preferred habits. It was observed that the young and juveniles of *H. agilis* in Lake Lanao are usually found feeding close to the shoreline and in shallow, well lighted regions of the lake, while the adults tend to thrive in the deeper limits of the littoral zone<sup>28</sup>. On the other hand, it was also observed that the species requires no specific type of water bottom and is conspicuously found everywhere around the lake<sup>4</sup>. Such differing habits between the sexes of the species if any, could be easily accommodated in the wide expanse of the shallow well lighted in Lake Lanao, especially the northern side (see figure-1) where the limit of the depth range between 10m to 60m is several kilometres away from the shore. Hypothetically then, the environment of Lake Lanao could provide such potential between males and females of *H. agilis* to diversify in their habits and lead to sexual dimorphism. This argument supports the concept of natural selection by that may explain sexual dimorphism<sup>29</sup>.

## Conclusion

Relative warp analysis has shown sexual dimorphism in the body shape of *H. Agilis*. The environment of Lake Lanao is hypothesized to have contributed to the diversification of the fish in their habits leading to sexual dimorphism.

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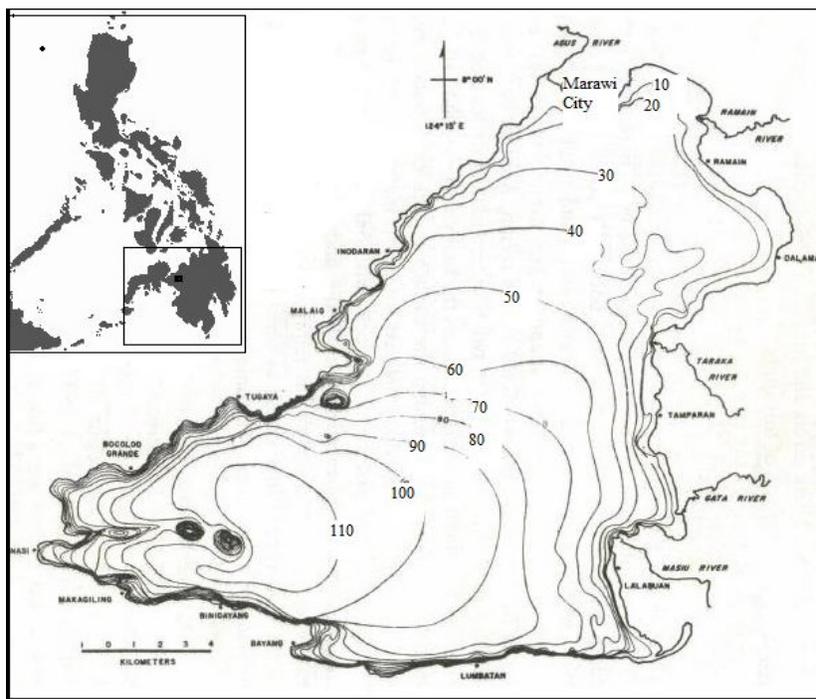


Figure - 1

Map of the Philippines showing the approximate location of Lake Lanao (black box) in the island of Mindanao (boxed). Figure also shows the bathymetric map of Lake Lanao (after Frey, 1974). Contour interval is 10 m below the mean lake water level. It shows that the lake is shallowest on the northern side, becoming progressively deeper going south. It is being drained to the north by way of Agus River and towards Iligan Bay (inset)

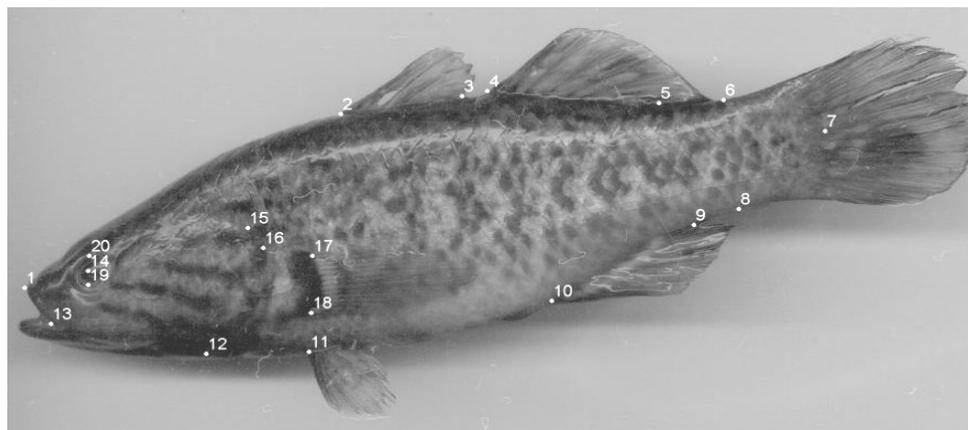


Figure - 2

A scanned image of *H. agilis*, digitized with the 20 landmarks as follows: (1) snout tip; (2) and (3) anterior and posterior insertion of the first dorsal fin; (4) and (5) anterior and posterior insertion of the second dorsal fin (6) and (8) dorsal and ventral region of the caudal peduncle where there is the greatest curvature; (7) posteriormost body extremity; (9) and (10) posterior and anterior insertion of the anal fin; (11) insertion of the pelvic fin; (12) insertion of the operculum on the lateral profile; (13) posterior extremity of premaxillar; (14) centre of the eye; (15) superior insertion of operculum; (16) point of maximum extension of operculum on the lateral profile; (17 and 18) superior and inferior insertion of the pectoral fin; (19) and (20) superior and inferior margin of the eye

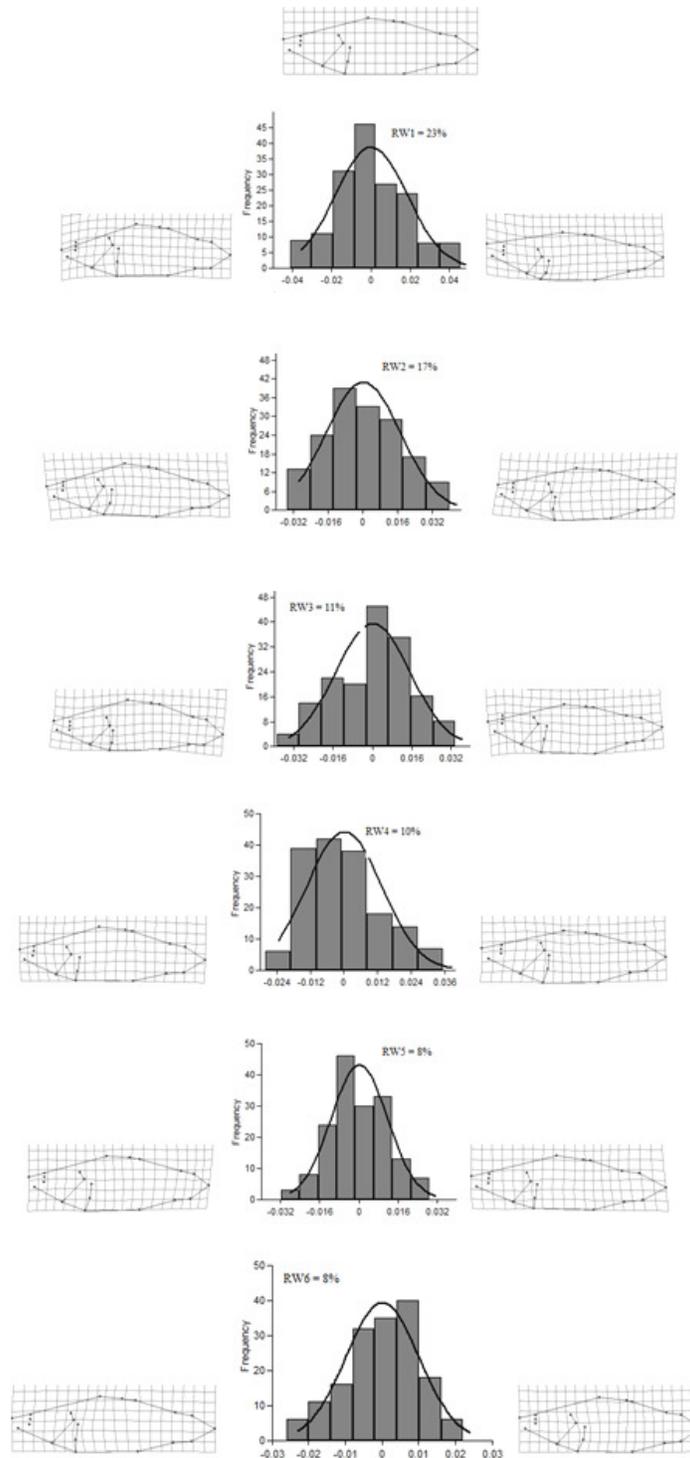


Figure – 3

Summary of geometric morphometric analysis of body shape among *H. agilis*. It shows the frequency histograms for each of the relative warp axis, and the extremes of their variation in body shape (plots to the left and right of the histograms) produced by the first six relative warps explaining 74% of the total shape variation (RW1 = 22.99%, RW2 = 17.09%, RW3 = 11.20%, RW4 = 9.65%, RW5 = 7.84%, RW6 = 5.17%). The uppermost plot is the consensus morphology

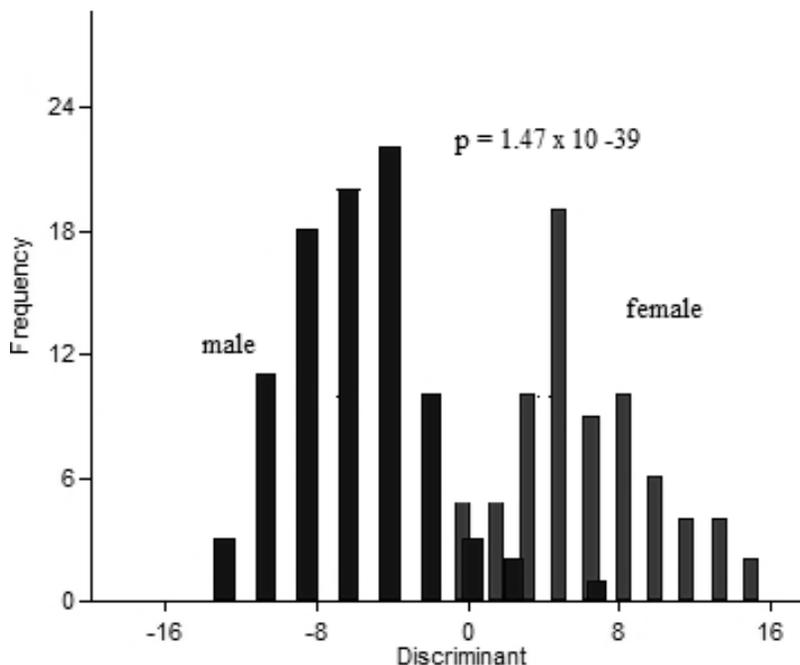


Figure - 4

DFA graph of the frequency and distribution of relative warp scores of body shape variation between male and female of *H. agilis* with the difference between sexes being highly significant ( $p << 0.05$ )

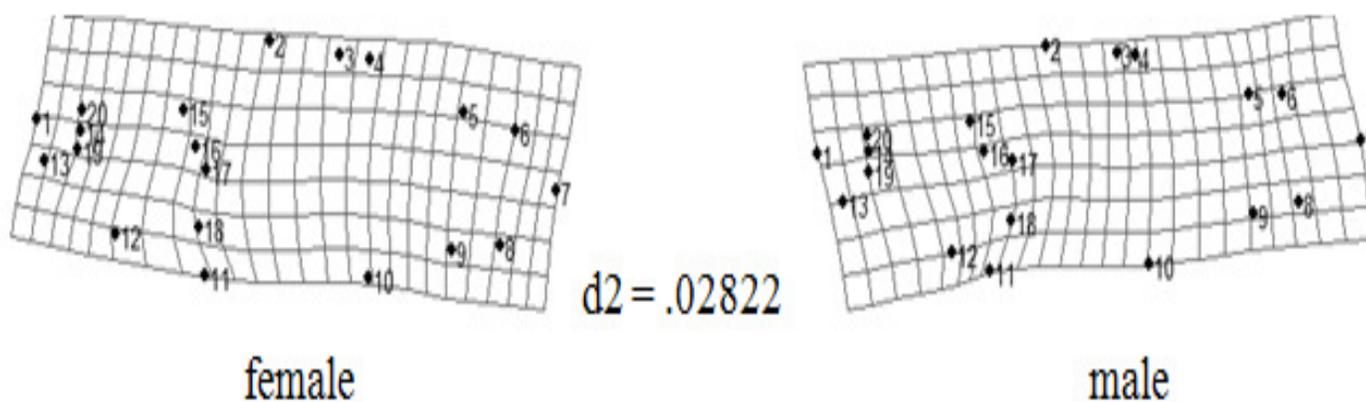


Figure - 5

Thin Plate Spline plot of both sexes of *H. agilis* plotted against each other. It shows the males with a deeper head and a bigger mouth, a narrower body with the bases of the dorsal and anal fins being more elongated