Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(2): 429 – 440 (2024)
www.ejabf.journals.ekb.eg

Sexual Dimorphism of Ambigous Hulu'u Fish (*Giuris* sp. Limboto) from Limboto Lake, North Sulawesi

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ARTICLE INFO

Article History:
Received: Jan. 27, 2024
Accepted: March 8, 2024
Online: April 2, 2024

Keywords:
Endemism,
Intersexual competition,
Evolution,
Population dynamics,
Domestication

ABSTRACT

Intraspecific sexual dimorphism between males and females is tied to sexual selection, such as body features and color patterns. Sexual selection pressure in the genus *Giuris* may occur in either or both sexes. The present study aimed to analyze the sexual dimorphism of Hulu'u fish (*Giuris* sp. Limboto) from Limboto Lake, Gorontalo Province, North Sulawesi. 272 individuals of *Giuris* sp. from Lake Limboto, were used with a male to female ratio of 1:1. The mean total length (TL) of male and female fish was 11.5± 2.1 and 12.2± 2.8cm, respectively. A total of 17 morphometric characters were analyzed using the morphometric truss method (dividing using TL) and analyzed by Z-test. Characters with significant differences between males and females were then analyzed using principal component analysis (PCA). The study found that male fish have longer fin sizes than females, including the length of the first dorsal, pectoral, pelvic, anal and caudal fin. In addition, male fish have longer heads than female fish. PCA analysis of the first principal component (C1) consisted of several characteristics: the length of the first dorsal, pectoral, and caudal fin length. The characteristics of head length, pelvic fin length, and anal fin length are associated with the second principal component (C2). The first and second main components could explain 78.3% of the data diversity. Furthermore, the male individuals had a conical genital papillae shape, contrasting with female individuals with bulbous genital papillae shape. In conclusion, males and females of *Giuris* sp. from Lake Limboto can be distinguished by their fins, heads, and papillae.

INTRODUCTION

Studies of cryptic species are closely related to proper taxonomic classification and successful conservation of the species. Issues such as high morphological similarity within taxa lead to misclassification interference with the conservation status

categorization of the two or more species involved **(Ekanayake *et al.*, 2021)**. The Eleotridae family has high and complex morphological similarities among its members. Some morphological characters such as morphometrics often overlap, causing ambiguities **(Keith *et al.*, 2020)**. *Giuris* is one of the genera in the Eleotridae family with high ambiguity. No systematic identification can be used as a reference in identifying *Giuris* species, especially for Hulu'u fish (*Giuris* sp. from Lake Limboto). *Giuris* as a genus originally only had one single described species, *Giuris margaritacea,* a.k.a *Giuris margaritaceus,* a.k.a *Ophieleotris aporos* **(Kottelat, 2013)**, making the identification of *Giuris* frequently misidentified with their wide distribution. *Giuris* sp. from Lake Limboto has some differences from the meristic characters of *G. margaritaceus, Giuris aporocephalus, Giuris tolsoni, Giuris viator,* and *Giuris laglaizei*, especially in the number of pectoral fin rays (P) (14- 16), and the number of second dorsal fin rays (D2) (1.8- 9). These two meristic characteristics ranged in number exceeding that of the five comparative species reported to occur in Sulawesi **(Ndobe *et al.*, 2023; Lamadi *et al.*, 2023)**.

Giuris genus has sexual dimorphism, which has not been systematically studied, but *G. margaritaceus*, *G. viator,* and *G. tolsoni* generally have conical urogenital papillae in males, while females have bulbous urogenital papillae, as well as the male body coloration pattern is more colorful than females **(Keith *et al.*, 2020)**. Morever, sexual dimorphism has been reported in males of *G. laglaizei*, which tend to have larger mean sizes than females **(Ndobe *et al.*, 2023)**. Furthermore, sexual and stadia-level dimorphism can be both taxonomic and biological conundrums, with extreme cases such as members of the family Cetomimidae, which was previously separated into three different families, Mirapinnidae for larval stadia, Megalomycteridae for male adult stadia and Cetomimidae for female adult stadia **(Johnson *et al.*, 2009)**. Systematic studies on the sexual dimorphism are necessary to avoid ambiguity in the morphological characteristics of a species, especially in this study considering the population of *Giuris* sp. from Lake Limboto.

Sexual dimorphism is the difference in intersexual secondary characteristics within a species **(Garcia & Zuanon, 2019)**. Sexual dimorphism is the result of sexual selection, one of the basic concepts introduced by Charles Darwin. Certain sexes develop some characteristics to increase reproductive success by two general mechanisms. The first mechanism is character development in intrasexual competition, commonly performed by males in the population. The second mechanism is mate selection, commonly performed by females. Morphological differences can occur in size, color, shape, and behavior **(Mieno & Karino, 2017)**. Given that several sexual dimorphisms in *Giuris* spp. are fin elongation and prominent coloration in males, these characters can potentially develop through domestication. Male eye-catching characters developed by artificial selection can increase the economic value in ornamental fish markets. However, the potential of indigenous fish as aquaculture commodities is still lacking **(França *et al.*, 2021)**.

Furthermore, common coloration patterns such as sexual dimorphism may change, and the most extravagant colors generally appear during the reproductive periods as nuptial coloration. Nuptial coloration is generally prominent in males, as the positive correlation between mating success and display characters. Display characters perform strongly only during reproductive interactions but not otherwise **(Olsson *et al.*, 2022)**.

Further observation on differences between common coloration and nuptial coloration is also essential, as the genus Hypseleotris, also from the Eleotridae family, was confirmed to display nuptial coloration during the breeding season. Males of Hypseleotris will display brighter dorsal and anal fin colors during the mating season to attract mates **(Thacker *et al.*, 2022)**. To the best of our knowledge, there is no research on sexual dimorphism of male and female *Giuris* sp. Therefore, this study aimed to investigate the sexual dimorphism of male and female Hulu'u fish (*Giuris* sp.) from Limboto Lake, Gorontalo Province, North Sulawesi. Studies of sexual dimorphism in *Giuris* sp. from Lake Limboto can be used as a foundation for future taxonomic and biological discussions, especially in conservation efforts and its domestication as the potential commodity for ornamental fish markets.

MATERIALS AND METHODS

A total of 272 *Giuris* sp. specimens, consisting of 136 males and 136 females, were collected from Lake Limboto from June 2022 to May 2023 (Fig. 1). Limboto Lake is in Gorontalo Province, Indonesia (0°35'35.71"N 122°59'26.37"E).

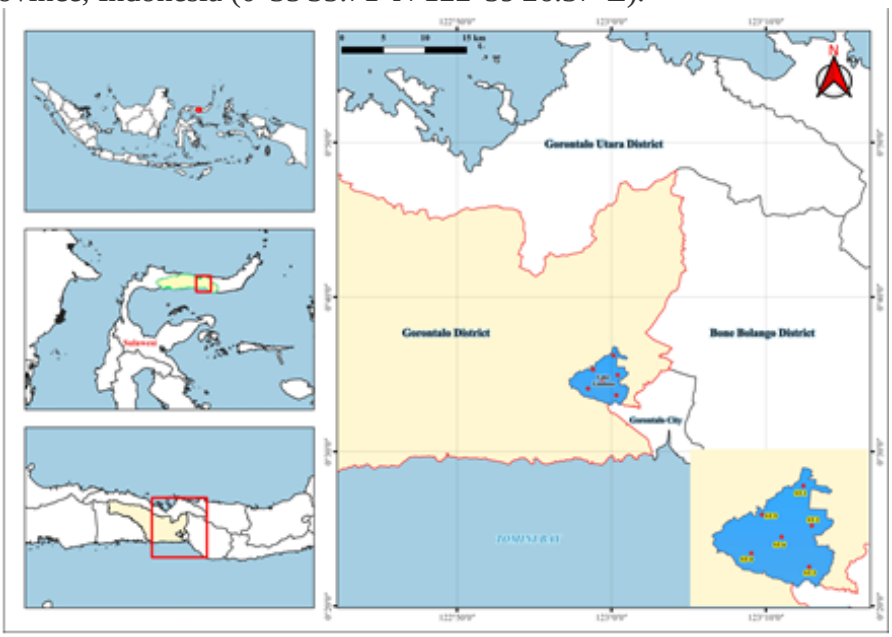

Fig. 1. Research location conducted in Limboto Lake, Gorontalo Province, located in the western side of Sulawesi Island (red box)

Morphometric character measurements were carried out to determine the physical differences between male and female fish. The fish's left side was photographed using a measurement scale (cm), and then the photo was processed with the help of TpsDig2 software version 2.32 (Institute of Data Analysis and Visualization (IDAV) and the University of California, Davis, USA) to determine the size of each character. The 17 morphometric characters measured and the description of the measurements can be seen in Fig. (2). After morphometric measurements, the specimen's abdominal cavity was opened to identify sex by examining the gonads.

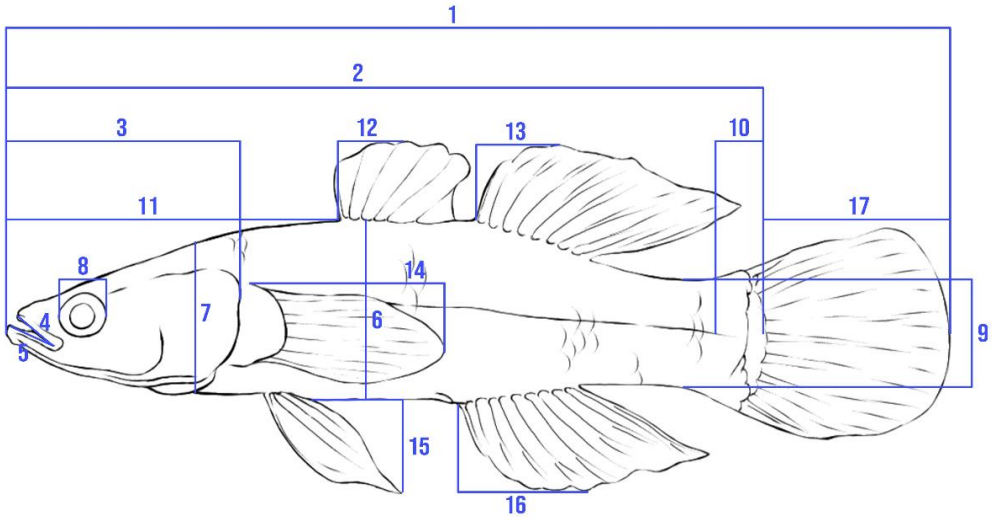

Fig. 2. Landmarks of 17 morphometric characters measurement: 1-TL (Total length), 2-SL (Standard length), 3-HL (Head length from snout tip to operculum margin), 4-UJ (Maxillary length), 5-LJ (Lower jaw length), 6-BD (Maximum body height), 7-HH (Head height), 8-ED (Eye diameter), 9-CP (Height of tail peduncle), 10-LP (Length of caudal peduncle), 11-SD (Snout tip to fin base), 12-DA (Length of the first anterior dorsal fin), 13-DP (Length of the first posterior dorsal fin), 14-PF (Length pectoral fin), 15-VP (pelvic fin length), 16-AL (anal fin length), and 17-CL (caudal fin length)

RESULTS AND DISCUSSION

G. margaritaceus has a cylindrical body shape, a small, slightly flat head like a snake's head, a superior mouth position, two dorsal fins, pectoral fins, pelvic fins, anal fins, and a round tail fin (Fig.3). Male fish have parallel black stripes running across their bodies, while females have orange and green dots on their bodies.

Sexual characteristics in fish can be determined through various morphometric parameters, meristic, and the relationship between length and weight **(Vodounnou *et al.*, 2017)**. Geometric morphometrics can analyze the body shape and identify the sexual dimorphism in monomorphic fish **(Uba, 2019)**. A total of 272 Hulu'u fish, consisting of 136 males and 136 females, were subjected to morphometric measurements. The total length of male Hulu'u fish ranges from 7.6- 17.9cm, with an average of 11.5± 2.1cm, while female *Giuris* sp. range from 6.5- 20.9cm, with an average of 12.2± 2.8cm. The results of truss morphometric measurements are presented in Table (1).

The characters that characterize the differences between males and females (SL, HL, DA, PF, VP, AV, and CL) were tested using the principal component analysis (PCA). PCA results on the data correlation matrix of seven morphometric characters from 272 *Giuris* sp. specimens (Fig. 4) produced variations in the main components. In the graph of the results of the morphometric character analysis, a grouping of the male (red circle) and female (yellow ring) Hulu'u fish populations was detected. Differences in morphological characteristics of each sex cause this grouping. The first main component (C1) consists of DA, VP, and CL with an Eigenvalue of 4.12 and can explain data variations of 58.9% of

the total variation. The second principal component (C2) consists of HL, PF, and AL with an Eigenvalue of 1.35 and can explain data diversity of 19.4%. The first and second main components could explain 78.3% of the data diversity.

Fig. 3. Whole body documentation of **(A)** male and **(B)** female *Giuris* sp. from Lake Limboto individual. Males have brighter coloration than females with longer several fins

Table 1. Truss morphometric ratio measurement results of 272 *Giuris* sp. from Lake Limboto individuals with TL as divider

Code	Truss morphometric ratio (%TL)		Z test
	Male n = 136	Female n = 136	
SL	77.3 ± 2.0	77.9 ± 1.6	Significant
HL	24.4 ± 1.5	24.0 ± 1.4	Significant
UJ	10.4 ± 1.5	10.2 ± 1.5	Non-significant
LJ	6.0 ± 1.3	5.8 ± 1.1	Non-significant
BD	17.7 ± 1.2	17.8 ± 1.4	Non-significant
HH	14.8 ± 1.5	14.6 ± 1.4	Non-significant
ED	4.3 ± 0.8	3.8 ± 0.5	Non-significant
CP	17.4 ± 2.2	16.3 ± 1.6	Non-significant
LP	10.4 ± 0.9	10.4 ± 0.9	Non-significant
SD	35.8 ± 1.6	35.9 ± 1.3	Non-significant
DA	7.3 ± 1.2	6.9 ± 1.2	Significant
DP	9.1 ± 1.3	8.5 ± 1.6	Non-significant
PF	16.5 ± 1.2	16.1 ± 1.2	Significant
VP	14.3 ± 1.5	13.8 ± 1.3	Significant
AL	10.0 ± 1.5	9.6 ± 1.3	Significant
CL	19.1 ± 1.8	18.6 ± 1.5	Significant

Note: SL: Standard length; HL: Head length from snout tip to operculum margin; UJ: Maxillary length; LJ: Lower jaw length; BD (Maximum body height), HH: Head height; ED: Eye diameter; CP: Height of tail peduncle, LP: Length of caudal peduncle; SD: Snout tip to fin base; DA: Length of first anterior dorsal fin; DP: Length of first posterior dorsal fin; PF: Length pectoral fin; VP: Pelvic fin length; AL: Anal fin length; CL: Caudal fin length.

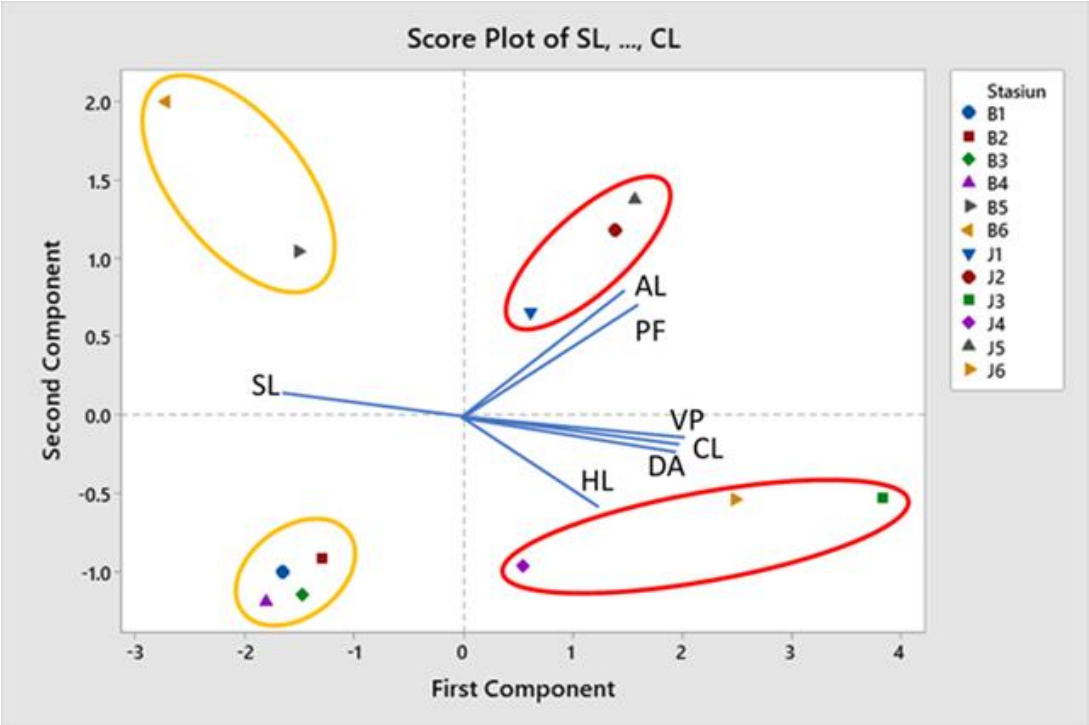

Fig. 3. Principal component analysis of morphometric characters.
Note: B (Female, n= 136, yellow circle), J (Male, n= 136, red circle), 1- 6 (Observation station), SL: Standard length, HL: Head length from snout tip to operculum margin, DA: Length of the first anterior dorsal fin, CL: Caudal fin length, VP: Pelvic fin length, PF: Length pectoral fin, and AL: Anal fin length

Correlation analysis aims to determine the relationship between one variable and other variables. In correlation analysis, other variables can be influenced if one variable changes. The correlation value (r) ranges from 1 to -1. A value closer to 1 or -1 indicates that the relationship between two variables is getting stronger, whereas a value closer to 0 means that the relationship between two variables is getting weaker. Positive values indicate a unidirectional relationship (X increases, then Y increases), and negative values indicate an inverse relationship (X increases, then Y decreases) **(Subandriyo, 2020)**. Based on the discriminant analysis results of the morphometric characters from six observed stations, there were positive and negative variations and a lack of correlation. The highest positive correlation value in *Giuris* sp. from Lake Limboto population was obtained between VP and CL, with a correlation of 0.886. While, the lowest positive correlation was obtained between HL and AL, with a correlation of 0.084. The highest negative correlation value was obtained between SL and VP, with a correlation of -0.676, and the lowest negative correlation was between SL and AL, with a correlation of -0.345 (Table 2).

A positive correlation value indicates an unidirectional relationship between two characters. If the length of one character increases or decreases, then the character positively correlated with that character will also increase or decrease in length. Meanwhile, a negative correlation value indicates an opposite relationship between the two correlated characters. If one of the characters experiences an increase in length, then the character negatively correlated with that character will experience a reduction in

length, and vice versa **(Subandriyo, 2020)**. **Passos *et al.* (2023)** stated that the closeness of positive or negative correlations can indicate that the character can be represented by one of the characters that is highly correlated. According to **Kvaestad *et al*. (2022)**, a positive correlation indicates that some characters are closely related or depend on another character.

Table 2. Correlation between each morphometric character

	SL	HL	DA	PF	VP	AL
HL	-0.425					
DA	-0.477	0.753				
PF	-0.365	0.112	0.589			
VP	-0.676	0.551	0.601	0.458		
AL	-0.345	0.084	0.416	0.846	0.436	
CL	-0.434	0.577	0.752	0.499	0.886	0.439

Note: 0.81- 1.00 = Very strong correlation, 0.61- 0.80 = Strong correlation, 0.41- 0.60 = Moderate correlation, 0.21- 0.40 = Weak correlation, 0, 00- 0.20 = Mo correlation, negative (-) = Negative correlation. SL: Standard length, HL: Head length from snout tip to operculum margin, DA: Length of the first anterior dorsal fin, CL: Caudal fin length, VP: Pelvic fin length, PF: Length pectoral fin, and AL: Anal fin length.

Differences in morphometric characters can be influenced by several factors, including water temperature, dissolved oxygen, ammonia, salinity, photoperiod, competition, quantity and quality of food, age, mortality rate, and the interaction of these factors. These characters, collectively, can significantly influence fish growth **(Yunita *et al*., 2020)**. Each species will have a different absolute size from each other. **Nurmadinah (2016)** stated that fish size differences are caused by age, gender, and environmental factors such as food, temperature, pH, and salinity. Morphometric parameters in fish greatly determine differences between the same species from various geographic regions **(Khanom *et al*., 2020)**.

The results of truss morphometric calculations show differences in sexual dimorphism between male and female *Giuris* sp. from Lake Limboto. Male individuals have longer fin sizes than females, namely the length of the first dorsal fin, length of the pectoral fin, length of the pelvic fin, length of the anal fin, and length of the caudal fin. Male individuals also have longer heads than females. Meanwhile, females have a higher ratio of standard length to total length than males. These results align with those reported in several previous researches, which stated that male fish generally have longer fins than females. **Mieno and Karino (2017)** found that male *Puntius titteya* have longer fins than females, and male marine medaka (*Oryzias dancena*) fish have longer dorsal and anal fins than females. An electric fish such as *Hypopomus pinnicaudatus* also has striking sexual dimorphism in its tail filaments, with males having longer tails than females **(Hopkins *et al*., 1990)**. Furthermore, in marine species, results from **Uba (2019)**, found that male *Decapterus macrosoma* has a wider dorsal fin and caudal fin than females, while females have a deeper body height and a wider abdominal area.

The differences between male and female fish can vary depending on the fish species. Some common differences that can be found include body shape and size, color, and fin shape **(Dang & Kienzler, 2019)**. Sexual dimorphism in fish is usually caused by

differences in sexual selection and reproductive roles between males and females. For example, males have brighter colors or certain physical features that attract females for mating purposes. On the other hand, female fish are larger or have a certain body shape to make spawning easier and protect their eggs **(Stanislas *et al.*, 2023)**. Furthermore, **Ronco *et al.* (2019)** related the sexual dimorphism in pipefish size to the natural and sexual selection, where larger females have higher fecundity and are preferred by males. Sexual selection is the process by which certain characteristics become more common in a population; such characteristics can increase the mating success of individuals with those characteristics. In this case, male fish may develop longer fins to attract females or compete with other male fish. Apart from that, this difference could also be caused by the different roles of male and female fish in the reproductive process **(Welsh & Fuller, 2015)**. The difference between male and female *Giuris* sp. fish can also be observed through different color patterns; male individuals have brighter body colors than females. Near spawning, male fish look brighter than females, especially the posterior dorsal fin, anal fin, and caudal fin of male fish looks bright orange. In line with the research of **Thacker *et al.* (2022)**, which stated that *Hypseleotris acropinna* is included in the Eleotridae family; the fins of the fish show bright colors during the breeding season. Differences in fin morphology often serve as secondary sexual markers and can affect mate selection.

The genital papilla (Fig. 5) is a body structure located near the anus, which functions as an outlet for sperm or eggs during spawning **(Ribeiro *et al.*, 2017)**. *Giuris* sp. from Lake Limboto has genital papillae that protrude posteriorly from the anus, genital papillae can be used to differentiate the gender. The genitals of female individuals are wider than male fish. Upon approaching spawning, the tip of the genital papillae is more reddish in both males and females. In general, males of *Giuris* sp. have round or triangular urogenital papillae with rounded distal ends.

Meanwhile, females have bulbous urogenital papillae with fimbriate projections around the distal opening **(Keith *et al.*, 2021)**. Male fish usually have longer, narrower and often sharper genital papillae, contrasting with females with shorter, wider, and rounder genital papillae **(Welsh & Fuller, 2015)**. Genital papillae are secondary sexual morphological structures for releasing sperm in males and eggs in females. The size of the male genital papillae depends on androgen hormones, hence their morphology is directly related to the stage of sexual maturity of the fish. The genital papillae are located behind the anus, and their morphology can provide an idea of the stage of sexual maturity **(Neves *et al.*, 2019)**. The current study revealed the difference in sexual dimorphism between males and females of *Giuris* sp. from Lake Limboto. Therefore, males and females of *Giuris* sp. from Lake Limboto can be differentiated based on fins, head and papillae.

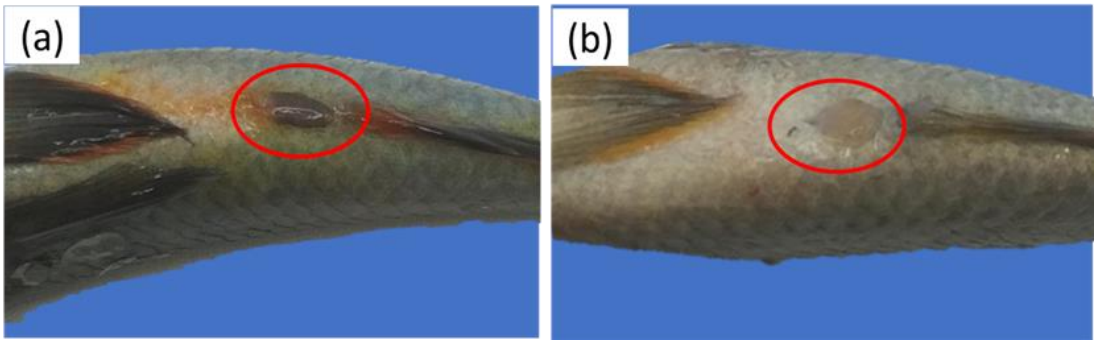

Fig. 5. Genital papillae in **(a)** male and **(b)** female

CONCLUSION

The present study demonstrated that male and female *Giuris* sp. from Lake Limboto can be distinguished by their fins, heads, and papillae. Male fish have longer fin sizes than females, namely the length of the first dorsal fin, length of the pectoral fin, length of the pelvic fin, length of the anal fin, and length of the tail fin. Male fish also have longer heads than females. Meanwhile, female fish have a longer standard length compared to males. Male individuals have a conical genital papillae shape, unlike female individuals with rounded genital papillae shape. Studies of sexual dimorphism in *Giuris* sp. from Lake Limboto can be a foundation for future taxonomic and biological discussions, especially in conservation efforts and the potential domestication of this species, as a commodity for ornamental fish markets.

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