

# Morphological And Genetic Features of *Oxyurichthys Ophthalmonema* Distributed in Central Vietnam

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## ABSTRACT:

Goby (*Oxyurichthys ophthalmonema* Bleeker, 1856) is a species of the Gobiidae family with high commercial and nutritional value, making it a promising candidate for development in Vietnam. A total of 408 fish samples were collected at 9 sites in 5 study areas in central Vietnam from October 2019 to September 2020. The research results showed that the average fish size ranges from 67.70 - 190.20 mm, corresponds to a mass of 4.70 - 29.25g, and the correlation between standard length and body mass is proportional to each other. Compared to 23 morphological criteria, it was shown that the farther the distribution distance, the greater the difference in morphological criteria ( $p < 0.05$ ). 19 total DNA samples of *O. ophthalmonema* in 5 study areas were extracted, in which 14 samples were amplified and sequenced for the COI gene region; the results obtained the sequences that had differences at different positions compared to the COI gene region of *O. ophthalmonema*. The phylogenetic tree construction of 14 fish samples with COI sequences of *O. ophthalmonema* and 5 species of the genus *Oxyurichthys* showed that the fish samples belonged to *O. ophthalmonema* species with a 100% bootstrap value. However, their genetic distances were far apart, and there were also differences between the samples.

**KEYWORDS:** Central region, Genetic diversity, Goby, Morphological characteristics, *O. ophthalmonema*.

## INTRODUCTION

*Oxyurichthys ophthalmonema* (Bleeker, 1856) belongs to the family Gobiidae, in the order Gobiiformes, which is mainly distributed in lagoons and estuaries of coastal areas. This fish has good resistance, health and high nutritional value, so it is favored by many people. Currently, *O. ophthalmonema* is one of the fish species with high consumption. However, the main supply source of this fish still comes from small-scale fishing with rudimentary tools such as small mesh nets, gill nets, etc., making the fish species currently declining in numbers and decreasing day by day. Therefore, the cultivation of this fish is an urgent issue to reduce the pressure of overfishing and provide enough for the market's demand. However, studies of this species around the world, as well as in our country, are very limited. In the world, the most remarkable research is the work of Remys Mohan & Sherly Williams (2018) on the nutritional and reproductive characteristics of this species in Lake Ashtamudi, India. In Vietnam, only a few studies have been recorded at Tam Giang - Cau Hai Lagoon in Hue, Rao Cai river in Ha Tinh and Mai Giang river in Nghe An (Thai & Dang 2015, Nguyen, 2005). Nowadays, there is very little research on this species, and there is no connection between the previous studies. Therefore, we conducted a study on morphological and genetic characteristics of Goby (*Oxyurichthys ophthalmonema* (Bleeker, 1856)) to provide scientific data for research and teaching at universities, as well as to regulate fishing and to conserve the existence of this species in central Vietnam.

## MATERIALS AND METHODS

### *Sample collection locations*

A total of 408 samples at 9 sites in 5 study areas in central Vietnam were collected from the beginning of October 2019 to the end of September 2020 (table 1). Samples were randomly collected and repeated at the site by direct fishing together with local people, or ordering samples from fishermen in the study area.

Table 1. Collection sample

No.	Location	Collection time	Number of samples
1	Water area in Quang An commune, Quang Dien district, Thua Thien Hue province	Oct, 2019 – Jan, 2020	51
2	Water area of Thuan An town, Thua Thien Hue province	Jan, 2019 – May, 2020	50
3	The brackish water area of Quang Ngan commune, Quang Dien district, Thua Thien Hue province	Sept, 16 <sup>th</sup> , 2020	11
4	The brackish water area of Dien Hai commune, Quang Dien district, Thua Thien Hue province	Aug, 30 <sup>th</sup> , 2020	1
5	Trieu An commune, Trieu Phong district, Quang Tri province (at the market)	Feb, 22 <sup>th</sup> , 2020	39
6	Trieu Phuoc Commune, Trieu Phong District, Quang Tri Province (at the market)	July, 22 <sup>th</sup> , 2020	71
7	Nhat Le River Estuary, Dong Hoi City, Quang Binh province	March, 2020 – Sept, 2020	115
8	River door Sot, Nghen river, Ha Tinh province	Sept, 2020	69
9	Ben Thuy river, Nghe An province	Sept, 2020	1

Morphological study: samples were treated when the fish was still fresh by fixing them with a 40% formol solution; After imaging, the sample was transferred to a 4% formol solution. After that, the fish samples underwent morphological characterization using the fish morphological description guidelines provided by Pravdin (1973) and Nguyen (2005).

Genetic study: fish samples were cut for pectoral fin samples and stored in eppendorf tubes containing 96% alcohol, and stored at -20°C until analysis.

Table 2. Total DNA extraction fish samples

Province	Sample number	Sample symbol
Thua Thien Hue	4	TTHTG002 (MZ798204), TTHTG006 (MZ798208), TTHTG033 (MZ798216), TTHTG034 (MZ798217)
Quang Tri	3	QT004 (MZ798206), QT007 (MZ798209), QT008 (MZ798210)
Quang Binh	7	QB005 (MZ798207), QB009 (MZ798211), QB010 (MZ798212), QB036, QB037, QB041, QB042
Ha Tinh	4	HT003 (MZ798205), HT011 (MZ798213), HT012 (MZ798214), HT009

**DNA Barcoding:** DNA extraction, PCR amplification and decoding. Total DNA was extracted from the pectoral fins of each individual fish using the "GeneJET Genomic DNA Purification Kit DNA Extraction Kit" according to the manufacturer's instructions. The 683 bp mitochondrial COI was amplified with the primer pairs Fish F1 (5'- TCAACCAACCACAAAGACATTGGCAC-3') and Fish R1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'). PCR reactions were performed with a total volume of 60 µl solution consisting of: 2 µl of total DNA (~50ng), 30 µl of Go Taq (2X), 3 µl of each primer (10pmol/µl) and 22 µl of Kit distilled water. The PCR machine (Esco) was run according to the following temperature program: DNA denaturation: 95°C/2 mins; (2). Amplification: 35 cycles: (94°C/3 mins; 54°C/3 mins; 72°C/1 mins); final 72°C/10 mins. PCR products were electrophoresed on a 0.8% agarose gel and the DNA bands were visualized under a UV projector. The results were recorded using the GelDoc image analysis system.

**DNA barcoding and phylogenetic analysis:** sequences were initially aligned using the BioEdit sequence editing program. After that, the research sequences were compared with similar sequences on Genbank using BLAST (<https://blast.ncbi.nlm.nih.gov>). The phylogenetic trees were built by Clustal W. and constructed by the Test Maximum Likelihood method using MEGA X software (Kumar *et al.* 2018) with the bootstrap values repeated 1000 times for the sample.

## RESULTS AND DISCUSSION

### *Morphological characteristics of Oxyurichthys ophthalmonema*

#### *External appearance*

From morphological characteristic analysis combined with the COI gene barcoding results, the goby species has been identified as having the scientific name *Oxyurichthys ophthalmonema* (Bleeker, 1856). The study samples have the Vietnamese names "goby" or "goby van eye fish".



Figure 1. Morphology of the Goby *Oxyurichthys ophthalmonema*

Based on 25 measurement criteria and 5 count criteria, 408 samples collected in 5 research areas in central Vietnam (only one sample in Nghe An) have been identified as a fish species named *Oxyurichthys ophthalmonema* (Bleeker, 1856) with the following main features: antennae, upper lip retraction at the anterior jaw joint, upper jaw has a single row of teeth, nape has a thin membrane (crest), has 2 dorsal fins, 1st dorsal fin with filamentous, not elongated spines and 2nd dorsal fin with 1 starting spine and posterior rays, with the last ray branching at the base (Figs 1 and 2). According to Betancur *et al.* (2017), this species belongs to the genus *Oxyurichthys*, family Gobiidae, order Gobiiformes, class Actinopterygii, phylum Chordata.

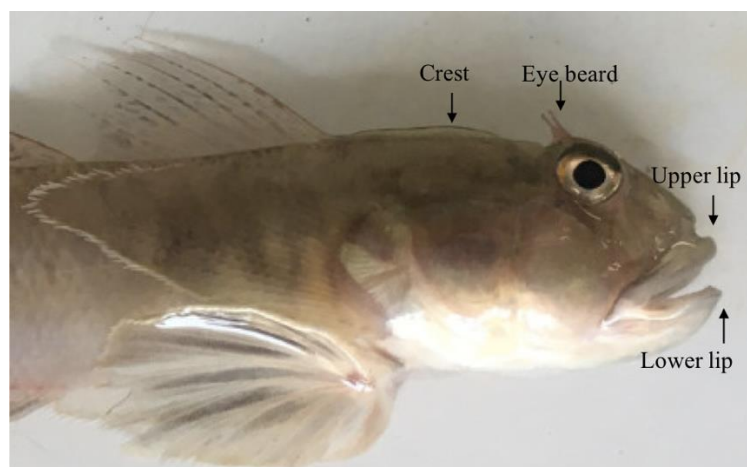


Figure 2. Head part of *O. ophthalmonema*

The results showed that this species has an average size ranging from 67.70 - 190.20 mm, corresponding to a weight of 4.70-29.25g; the number of spines and dorsal fin rays 1 is 6.0; the number of spines and dorsal fin rays 2 is 1-2, 12-14; the number of spines and pectoral fin rays is 1-5, 14-22; the number of spines and ventral rays is 2.10; the number of spines and pelvic fin rays is 2-11, 12-19; the number of spines and rays of anal fin is 1.13–14.

The external morphological characteristics of the population of *O. ophthalmonema* goby fish distributed in central Vietnam have many similar characteristics, which can be distinguished by the naked eye. They have a diamond-shaped body; their bodies are elongated and flat, covered with comb scales; the back is straight; the belly is slightly curved; the head is short; the mouth of the fish is inclined at an angle of about 45° to its body axis; the mouth is at the tip of the snout, facing forward; the arc is quite wide; the muzzle is long and slightly protruding; the developed muzzle covers the upper lip; the upper lip constricts; a wide gill membrane is attached to the gill waist (Figure 2). The fish's eyes are slightly convex laterally, favoring the top of the head and forming a crest on the dorsal surface. The distance between the eyes is narrow, and there is especially the presence of an eye antenna extending above the back of the eye.

The color of the body varies from ventral to dorsal; dark gray back, white gray belly. When the fish is alive, its body color is slightly pinkish gray, and its pelvic and anal fins are opaque gray. The dorsal, pectoral, and anal fins are quite clear. The tips of the caudal and dorsal fins are red. The pelvic and pectoral fins are bordered with thin white edges.

#### Size and body mass of *O. ophthalmonema*

The standard length and body mass of *O. ophthalmonema* in different regions were carefully studied. The results showed that both the largest mass and the longest standard length were from the samples collected in Quang Binh province (table 3).

Table 3. Body mass and standard length of *O. ophthalmonema* (Bleeker, 1856) distributed in the central Vietnam

Targets	Location	Thua Thien Hue	Quang Tri	Quang Binh	Ha Tinh	Medium
1.	Min-					
Weight	Max	8.60-29.25	4.70-20.80	7.60-24.35	5.39-11.01	4.70-29.25
(g)	M± SD	15.05±4.04	9.54±3.42	16.55±4.59	8.07±1.45	<b>12.80±5.10</b>

2.	Min-	80.00-	67.70-	82.00-	70.11-	67.70-
Standar	Max	109.65	190.20	180.11	84.52	190.20
d length						
(mm)	M± SD	93.83±7.65	84.45±13.9	101.81±13.3	77.66±4.2	<b>90.82±14.0</b>
			1	0	6	<b>8</b>

The morphological parameters of *O. ophthalmonema* in the central area were carefully studied, indicates that: the length of fish in Thua Thien Hue ranged from 80.00-109.65 mm, average 93.83± 7.65, Quang Tri fish ranged from 67.70-190.20 mm, average 84.45±13.91, Quang Binh fish ranged from 82.00-180.11 mm, average 101.81± 13.30 mm and Ha Tinh fish from 70.11 to 84.52 mm, average 77.66±4.26. Measures have variation among individuals in the same province. There are significant differences between provinces in a number of criteria.

The lengths of other body parts relative to the head length were measured. The results showed that the head width reaches 57.04% of the head length, while the figures for the height of the head, the distance between the eyes, the eye diameter and the width of the mouth are 68.57%, 18.81%, 23.79% and 48.96% respectively (table 4).

Table 4. The lengths of other body parts relative to the head length (%HL)

Location		Thua Hue	Thien	Quang Tri	Quang Binh	Ha Tinh	Medium
Compared to	HL						
1.	Min-	45.45 - 70.83	47.16 - 71.43	34.13 - 68.04	50.14 - 56.42	34.13 - 71.43	
Head	Max						
Width	M ± SD	56.73 ± 4.53	58.38 ± 4.38	57.44 ± 4.24	54.74 ± 1.14	<b>57.04 ± 4.18</b>	
2.	Min-	42.31 - 80	43.04 - 82.00	39.02 - 78.95	58.78 - 67.03	39.02 - 82.00	
Head	Max						
Height	M ± SD	70.25 ± 4.96	68.88 ± 4.95	69.72 ± 5.19	63.37 ± 2.59	<b>68.57 ± 5.29</b>	
3.	Min-	13.64 - 27.89	11.76 - 28.12	12.00 - 26.02	16.62 - 21.78	11.76 - 28.12	
Distan	Max						
ce							
betwe	M ± SD	21.08 ± 3.04	18.43 ± 3.09	17.56 ± 3.04	17.87 ± 1.43	<b>18.81 ± 3.18</b>	
en							
eyes							
4.	Min-	14.29 - 27.81	15.00 - 38.59	14.75 - 30.8	21.49 - 26.65	14.29 - 38.59	
Eye	Max						
Diame							
ter	M ± SD	22.76 ± 3.33	23.66 ± 3.69	25.19 ± 2.49	23.39 ± 1.7	<b>23.79 ± 3.13</b>	
5.	Min-	23.69 - 74.20	32.41 - 95.83	27.74 - 58.58	23.78 - 45.66	23.69 - 95.83	
Mouth	Max						
width	M ± SD	54.68 ± 9.62	48.56 ± 9.43	47.60 ± 5.30	42.53 ± 3.78	<b>48.96 ± 8.73</b>	

Besides the measurement criteria, the count criteria for the species have also been studied. The analysis and comparison for counting indicators found that fish species in the central area of Vietnam has the following characteristics: number of spines and dorsal fin rays 1 is 6 and 0; number of spines and dorsal fin rays 2 is 1 - 2 and 12 - 14; number of spines and pectoral fin rays is 1 - 5 and 14 - 22; number of spines and ventral rays is 2 and 10; number of spines and pelvic fin rays is 2 - 11 and 12 - 19; number of spines and rays of anal fin is 1 and 13 - 14 (table 5).

Table 5. Comparison of the counting parameters of *O. ophthalmonema*

Location			Thua Hue	Thien Hue	Quang Tri	Quang Binh	Ha Tinh
Targets							
1. Number of pectoral fin spines (P)	Min-Max		1 – 5		1 – 4	2 - 4	3
	M ± SD		2.55 ± 0.83		2.67 ± 0.62	2.75 ± 0.62	3 ± 0
2. Number of pectoral fin rays	Min-Max		17 – 22		14 – 20	18 - 20	18 - 20
	M ± SD		18.62 ± 1.21		18.64 ± 0.89	18.66 ± 0.87	19.07 ± 0.9
3. Number of caudal fin spines	Min-Max		2 – 10		4 – 11	5 - 10	7 - 8
	M ± SD		6.56 ± 2.88		7.93 ± 1.13	6.98 ± 1.16	7.17 ± 0.38
4. Number of caudal fin rays	Min-Max		13 – 17		12 – 19	13 - 18	14 - 14
	M ± SD		14.45 ± 0.78		14.68 ± 1.26	14.34 ± 0.77	14.0

Through the analysis of measurements and counts, the average size of *O. ophthalmonema* ranges from 67.70 - 190.20 mm; average  $90.82 \pm 14.08$  mm; the head length is about 1/5 of the standard length while body height is about 1/4 of the standard length; the average weight is  $12.80\text{g} \pm 5.10$  g. These results are similar to the previous descriptions of Pezold and Larson (2015) and Nguyen and associates (2020) on goby fish collected in Thua Thien Hue province.

Table 6. Mean values ( $\pm$  standard deviation) of parameters compared to standard length (SL) of *Oxyrichthys ophthalmonema*

Compared to SL (%)	Thua Hue	Thien Hue	Quang Tri	Quang Binh	Ha Tinh
1. Head length	$24,18 \pm 0,71^a$		$24,38 \pm 1,74^a$	$24,66 \pm 2,00^a$	$26,04 \pm 0,55^b$
2. Body height	$20,15 \pm 1,37^{ac}$		$19,14 \pm 1,73^b$	$19,66 \pm 1,88^{ab} \pm$	$20,33 \pm 0,95^{ac}$
3. Tail stalk length	$9,7 \pm 1,36^a$		$10,1 \pm 1,80^{ab}$	$9,91 \pm 1,75^a$	$10,43 \pm 0,35^b$
4. Tail stalk height	$10,9 \pm 0,75^a$		$10,12 \pm 0,67^b$	$10,67 \pm 1,49^{ac}$	$10,57 \pm 0,55^c$
5. Dorsal fin length 1	$19,37 \pm 1,98^a$		$19,02 \pm 2,08^a$	$19,14 \pm 2,25^a$	$19,33 \pm 3,28^a$
6. Dorsal fin length 2	$39,08 \pm 3,85^a$		$39,82 \pm 12,90^{ab} \pm$	$39,94 \pm 6,57^{ab} \pm$	$40,74 \pm 1,02^b$
7. Pectoral fin length	$28,90 \pm 2,84^a$		$29,83 \pm 3,66^{abc}$	$30,79 \pm 3,43^{bc} \pm$	$30,52 \pm 1,61^c$
8. Pelvic fin length	$22,98 \pm 1,63^a$		$23,06 \pm 2,12^a$	$23,59 \pm 2,32^{ab} \pm$	$23,97 \pm 1,07^b$

9. Tail fin length	45,45 ± 4,17 <sup>a</sup>	49,2 ± 7,46 <sup>b</sup>	47,74 ± 4,77 <sup>b</sup>	44,56 ± 3,34 <sup>a</sup>
10. Anal fin length	41,29 ± 1,66 <sup>a</sup>	39,53 ± 2,68 <sup>b</sup>	40 ± 2,98 <sup>b</sup>	39,87 ± 1,01 <sup>b</sup>
11. Dorsal fin height 1	14,19 ± 3,91 <sup>a</sup>	11,1 ± 2,44 <sup>b</sup>	12,56 ± 1,82 <sup>c</sup>	11,94 ± 1,40 <sup>c</sup>
12. Dorsal fin height 2	13,55 ± 5,20 <sup>a</sup>	10,37 ± 2,22 <sup>b</sup>	10,98 ± 3,35 <sup>b</sup>	9,34 ± 1,79 <sup>c</sup>
13. Pectoral fin height	18,05 ± 1,56 <sup>a</sup>	17,33 ± 2,43 <sup>a</sup>	18,96 ± 2,26 <sup>b</sup>	15,81 ± 0,97 <sup>c</sup>
14. Pelvic fin height	18,47 ± 2,70 <sup>a</sup>	18,32 ± 2,59 <sup>a</sup>	19,8 ± 2,55 <sup>b</sup>	18,28 ± 0,76 <sup>a</sup>
15. Tail fin height	21,06 ± 2,82 <sup>a</sup>	21,14 ± 3,4 <sup>a</sup>	23,16 ± 3,6 <sup>b</sup>	18,21 ± 1,47 <sup>c</sup>
16. Anal fin height	11,02 ± 3,51 <sup>a</sup>	8,91 ± 1,52 <sup>bc</sup>	9,49 ± 3,37 <sup>b</sup>	8,53 ± 0,94 <sup>c</sup>
17. Head length behind eyes	12,32 ± 1,70 <sup>a</sup>	13,13 ± 3,16 <sup>ab</sup>	13,01 ± 3,10 <sup>ab</sup>	13,6 ± 0,41 <sup>b</sup>
18. Muzzle length	11,7 ± 0,70 <sup>a</sup>	11,65 ± 1,15 <sup>a</sup>	12,56 ± 1,89 <sup>b</sup>	12,43 ± 0,39 <sup>b</sup>

Note: Values in the same row with the same letter are not significantly different ( $p>0.05$ ).

Table 7. Mean values (± standard deviation) of parameters compared to head length (HL) of *Oxyrichthys ophthalmonema*

Compared to HL (%)	Thua Thien Hue	Quang Tri	Quang Binh	Ha Tinh
1. Head Width	56,73 ± 4,53 <sup>a</sup>	58,38 ± 4,38 <sup>b</sup>	57,44 ± 4,24 <sup>ab</sup>	54,74 ± 1,14 <sup>c</sup>
2. Head Height	70,25 ± 4,96 <sup>a</sup>	68,88 ± 4,95 <sup>a</sup>	69,72 ± 5,19 <sup>a</sup>	63,37 ± 2,59 <sup>b</sup>
3. Distance between eyes	21,08 ± 3,04 <sup>a</sup>	18,43 ± 3,09 <sup>b</sup>	17,56 ± 3,04 <sup>b</sup>	17,87 ± 1,43 <sup>b</sup>
4. Eye Diameter	22,76 ± 3,33 <sup>a</sup>	23,66 ± 3,69 <sup>a</sup>	25,19 ± 2,49 <sup>b</sup>	23,39 ± 1,7 <sup>a</sup>
5. Mouth width	54,68 ± 9,62 <sup>a</sup>	48,56 ± 9,43 <sup>b</sup>	47,6 ± 5,3 <sup>b</sup>	42,53 ± 3,78 <sup>c</sup>

Note: Values in the same row with the same letter are not significantly different ( $p>0.05$ ).

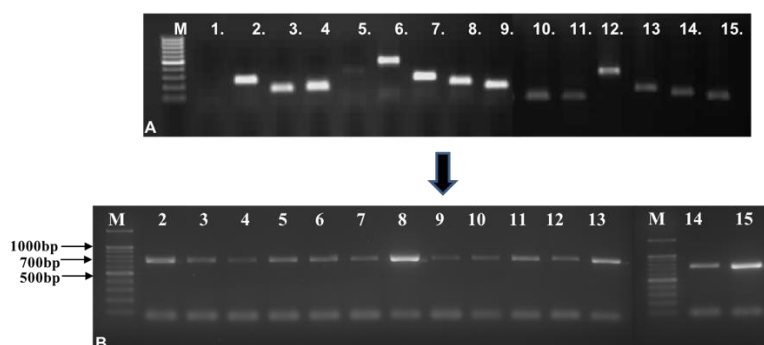
Among 23 surveyed criteria, the ratio of criteria has a significant difference ( $p<0.05$ ) between fish populations distributed in 4 provinces as follows: Thua Thien Hue and Quang Tri only differ in 11 criteria (48%), Thua Thien Hue and Quang Binh differ in 14 criteria (61%), Thua Thien Hue and Ha Tinh differ in 18 criteria (78%), Quang Tri and Quang Binh differ in 7 criteria (28%), Quang Tri and Ha Tinh differ in 13 criteria (57%), Quang Binh and Ha Tinh differ in 12 criteria (52%).

The research results indicated that if *O. ophthalmonema* in the provinces are close to each other, the average values of the measured criteria change little. However, if they are far apart, the mean values of those criteria have more changes between the two criteria. Among all the fish samples collected in Nghe An, we conducted analysis and determined that only one of them was *O. ophthalmonema* species, with morphological characteristics similar to those of Goby in the remaining 4 study areas. However, in such small quantities, it is not possible to compare the measurement and count criteria with other study areas.

#### Genetic diversity of goby fish

##### Total DNA extraction, PCR performance

Total DNA samples were extracted with good quality, 0.8% gel electrophoresis, and the electrophoresis images showed clear dark bands (Figure 4A).



**Figure 4. Total DNA electrophoresis (A): (M, Marker 1, HT009; 2, HT003 (MZ798205); 3, HT011 (MZ798213); 4, HTT012 (MZ798214); 5, QT004 (MZ798206); 6, QT007 (MZ798209); 7, QT008 (MZ798210); 8, NA013 (MZ798215); 9, TTHTG002 (MZ798204); 10, TTHTG006 (MZ798208); 11, QB005 (MZ798207); 12, QB009 (MZ798211); 13, QB010 (MZ798212); 14, TTHTG033 (MZ798216); 15, TTHTG034 (MZ798217). Results of PCR product electrophoresis (B): (M, Marker 2, HT003 (MZ798205); 3, HT011 (MZ798213); 4, HTT012 (MZ798214); 5, QT004 (MZ798206); 6, QT007 (MZ798209); 7, QT008 (MZ798210); 8, NA013 (MZ798215); 9, TTHTG002 (MZ798204); 10, TTHTG006 (MZ798208); 11, QB005 (MZ798207); 12, QB009 (MZ798211); 13, QB010 (MZ798212); 14, TTHTG033 (MZ798216); 15, TTHTG034 (MZ798217)**

The 14 genomic DNA samples were amplified by PCR reaction using 2 pairs of primers FishF1 & FishR1. The PCR products were analyzed using a 0.8% agarose gel and the results showed that their sizes were about 650 - 750 bp (Figure 4B). The size of the PCR products of 14 fish samples in 5 areas is consistent with the theoretical size of the target *COI* gene fragment.

#### *COI* gene sequence analysis results

After sequencing and editing with MEGA X software, all research samples were compared with similar sequences on Genbank using BLAST. As a result, the fish samples had a high similarity with other species (not *O. ophthalmonema*) while the remaining 14 samples had a high similarity rate with the *O. ophthalmonema* species.

The nucleotide sequence of each fish sample (14 samples) was compared with the *COI* gene sequence of *O. ophthalmonema* on the Genbank. The sequence with code MZ798204 was selected for detailed analysis for nucleotide differences, other sequences not shown here. The fish sample MZ798204 has a 93.43% similarity with *O. Ophthalmonema (JX193749.1)*, with 58 nucleotide positions difference (Figure 5). Nucleotide frequencies of 14 samples were T 32.3%; C 25.3%; A 24.1%; G 8.2%.



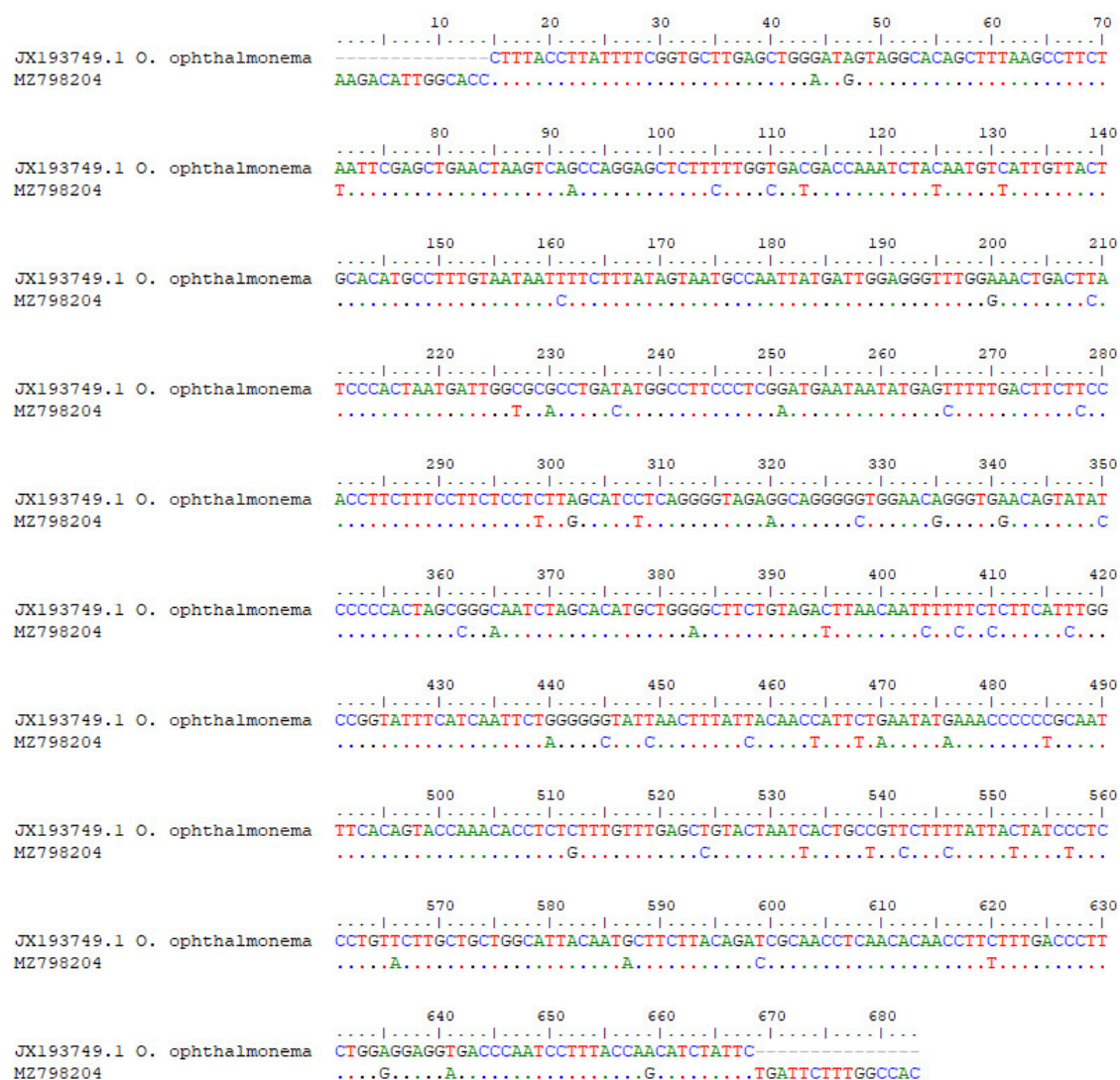


Figure 5. Results comparing nucleotide sequences of fish sample MZ798204 with similar sequences in Genbank

The detailed analysis of the remaining sequences shows that fish samples named MZ798206 and MZ798213 have 93.65% similarity with *O. ophthalmonema* (JN242585.1), in which there is a difference in 41 nucleotide positions. However, both samples differed in 40 similar positions, and 1 nucleotide position difference from the COI gene sequence of *O. ophthalmonema* was different. Fish samples MZ798205, MZ798214, MZ798216, MZ798217, MZ798208, MZ798210, MZ798209, MZ798207, MZ798211, MZ798212 and MZ798215 have 93.81% similarity with *O. ophthalmonema* (JN242585.1), with a difference in 40 nucleotide positions. In these samples, the MZ798207 pattern at the 348<sup>th</sup> different nucleotide position (T-G) is different from the rest (T-A). The most similarity to *Favonigobius gymnauchen* (KJ013043.1) was 99.84%, with only 1 nucleotide position could not be identified, and the similarity to *O. microlepis* (MK777350.1) was 99.67%, with 2 nucleotide positions difference, but only 495 nucleotide sizes were compared.

Results comparing gene distance and similarity between COI gene fragments of 14 samples of *O. ophthalmonema* are shown in table 8.

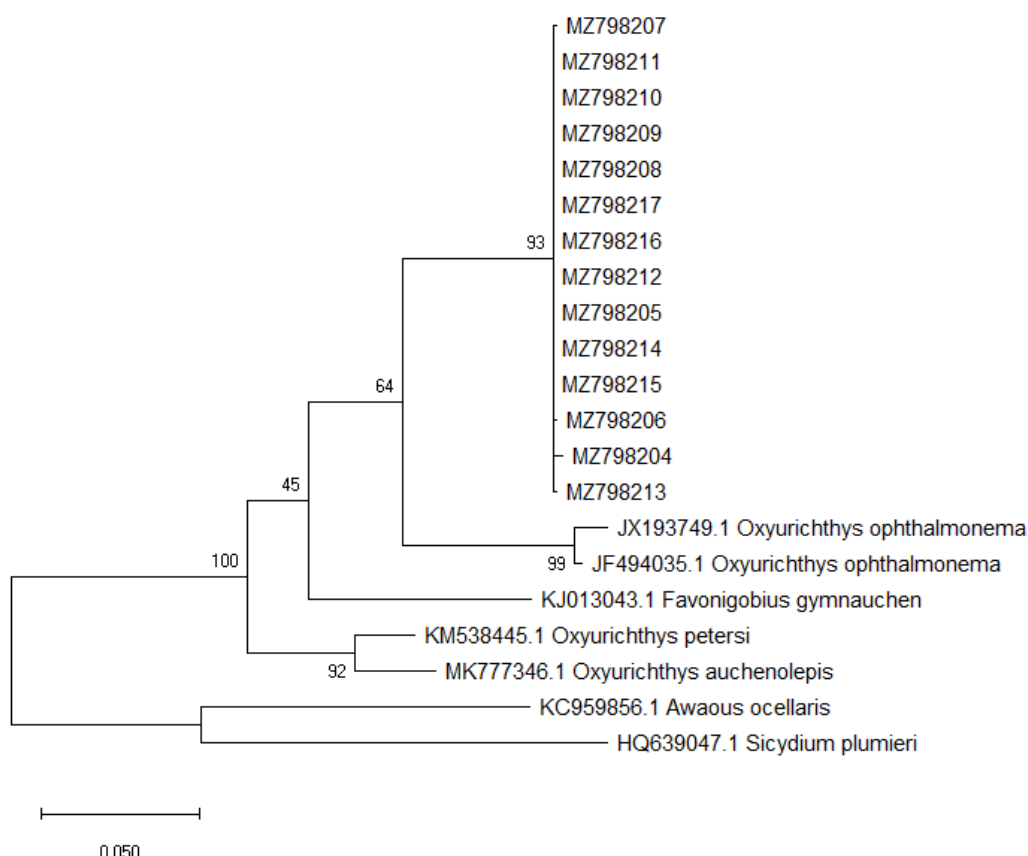
Table 8. The similarity of COI gene of 14 samples of *O. ophthalmonema* in central Vietnam

	MZ 798 204	MZ 798 216	MZ 798 217	MZ 798 208	MZ 798 206	MZ 798 209	MZ 798 210	MZ 798 207	MZ 798 211	MZ 798 212	MZ 798 205	MZ 798 213	MZ 798 214	MZ 798 215
MZ 798 204	*** **	0.0 089	0.0 089	0.0 029	0.0 044	0.0 029	0.0 029	0.0 044	0.0 029	0.0 029	0.0 029	0.0 044	0.0 029	0.0 029
MZ 798 216	0.9 91	*** **	0.0 000	0.0 059	0.0 074	0.0 059	0.0 059	0.0 074	0.0 059	0.0 059	0.0 059	0.0 074	0.0 059	0.0 059
MZ 798 217	0.9 91	1,0 00	*** **	0.0 059	0.0 074	0.0 059	0.0 059	0.0 074	0.0 059	0.0 059	0.0 059	0.0 074	0.0 059	0.0 059
MZ 798 208	0.9 97	0.9 94	0.9 94	*** **	0.0 015	0.0 000	0.0 000	0.0 015	0.0 000	0.0 000	0.0 000	0.0 015	0.0 000	0.0 000
MZ 798 206	0.9 95	0.9 92	0.9 92	0.9 98	*** **	0.0 015	0.0 015	0.0 029	0.0 015	0.0 015	0.0 015	0.0 029	0.0 015	0.0 015
MZ 798 209	0.9 97	0.9 94	0.9 94	1,0 00	0.9 98	*** **	0.0 000	0.0 015	0.0 000	0.0 000	0.0 000	0.0 015	0.0 000	0.0 000
MZ 798 210	0.9 97	0.9 94	0.9 94	1,0 00	0.9 98	1,0 00	*** **	0.0 015	0.0 000	0.0 000	0.0 000	0.0 015	0.0 000	0.0 000
MZ 798 207	0.9 95	0.9 92	0.9 92	0.9 98	0.9 97	0.9 98	0.9 98	*** **	0.0 015	0.0 015	0.0 015	0.0 029	0.0 015	0.0 015
MZ 798 211	0.9 97	0.9 94	0.9 94	1,0 00	0.9 98	1,0 00	1,0 00	0.9 98	*** **	0.0 000	0.0 000	0.0 015	0.0 000	0.0 000
MZ 798 212	0.9 97	0.9 94	0.9 94	1,0 00	0.9 98	1,0 00	1,0 00	0.9 98	1,0 00	*** **	0.0 000	0.0 015	0.0 000	0.0 000
MZ 798 205	0.9 97	0.9 94	0.9 94	1,0 00	0.9 98	1,0 00	1,0 00	0.9 98	1,0 00	1,0 00	*** **	0.0 015	0.0 000	0.0 000
MZ 798 213	0.9 95	0.9 92	0.9 92	0.9 98	0.9 97	0.9 98	0.9 98	0.9 97	0.9 98	0.9 98	0.9 98	*** **	0.0 015	0.0 015
MZ 798 214	0.9 97	0.9 94	0.9 94	1,0 00	0.9 98	1,0 00	1,0 00	0.9 98	1,0 00	1,0 00	1,0 00	0.9 98	*** **	0.0 000

MZ														
798	0.9	0.9	0.9	1,0	0.9	1,0	1,0	0.9	1,0	1,0	1,0	0.9	1,0	***
215	97	94	94	00	98	00	00	98	00	00	00	98	00	**

#### Genetic relationships of Goby populations distributed in central Vietnam

Development of a phylogenetic tree of 14 fish samples with 10 *COI* sequences of 5 species of the genus *Oxyurichthys* (sequences from Genbank) is shown in figure 6. The results indicated that all fish samples belong to the same clade as *O. ophthalmonema* with a bootstrap value of 64%.



**Figure 6. Relative relationship of Goby in central Vietnam with 5 species of genus *Oxyurichthys***

Through the results of sequence analysis and pedigree building of 14 fish samples identified as *O. ophthalmonema* species, the following results were obtained: the Goby samples in the same province or between the study areas have differences in the number of nucleotides and in the sequence of one or several nucleotides (MZ798204, MZ798206, MZ798207, MZ798215, MZ798213, MZ798216, MZ798217). However, the remaining fish samples in the study areas did not show any significant difference (MZ798205, MZ798214, MZ798209, MZ798210, MZ798208, MZ798211, MZ798212). The difference between the *COI* gene sequence of *O. ophthalmonema* (Genbank) and the fish samples is quite high. This difference was expressed in the positions of 40 to 43 nucleotide, the similarity is not high. This can be explained because the *COI* gene sequence region is prone to mutations, thus leading to errors in the pairing process as well as efficient use of primers in PCR.

In summary, when studying the morphological characteristics of all samples distributed in different areas in the Central region, the obtained results showed that there were differences in the morphology of the research subject in each analysis criterion. Although they were only small differences, they were also significant in specific features for identification. When studying the COI gene sequencing and analyzing the genetic tree, it was found that the Goby populations had genetic diversity showing differences in nucleotide sequences in the analyzed genes. However, there were still some differences in nucleotide sequences. The genetic results firmly confirmed the morphological taxonomic criteria. Therefore, for fish species whose morphology is still uncertain, gene sequencing is required to identify not only the COI gene but also many other genes in the fish's mitochondria. For 1 out of 15 samples collected from Nghe An, when analyzing genetics, the similarity with the bootstrap value was still 100%. However, for this area, it is necessary to collect more samples and conduct genetic analysis to get accurate data.

## CONCLUSION

Species *O. ophthalmonema* had an average size ranging from 67.70 - 190.20 mm, corresponding to a weight of 4.70 - 29.25g. It was found that there was a correlation between body length and body mass. Among 23 surveyed criteria, the ratio of criteria has significant difference ( $p < 0.05$ ) between fish populations distributed in 4 study areas as follows: Thua Thien Hue and Quang Tri are different in 11 criteria (48%), Thua Thien Hue and Quang Binh differ in 14 criteria (61%), Thua Thien Hue and Ha Tinh differ in 18 criteria (78%), Quang Tri and Quang Binh differ in 7 criteria (28%), Quang Tri and Ha Tinh differ in 13 criteria (57%), Quang Binh and Ha Tinh differ in 12 criteria (52%).

19 total DNA samples of Goby in 5 areas of central Vietnam were extracted, in which 14 samples were amplified and sequenced for COI gene region; the results obtained the sequences that had differences at different positions in comparison to the COI gene region of *O. ophthalmonema*. The phylogenetic tree construction for 14 fish samples with COI sequences of *O. ophthalmonema* and 5 species of the genus *Oxyurichthys* showed that the fish samples belonged to *O. ophthalmonema* species with a 64% bootstrap value. However, their genetic distances were far apart and there were also some differences between the researched fish samples.

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