JFMSE, 28(4), pp. 1159~1170, 2016. www.ksfme.or.kr
수산해양교육연구, 제28권 제4호, 통권82호, 2016. http://dx.doi.org/10.13000/JFMSE.2016.28.4.1159

Taxonomical Reexamination and Distribution of Sea horses in the Southern Sea of South Korea

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한국 남해연안에 서식하는 해마류의 분포와 분류학적 재검토

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Abstract

This study examines the distribution and taxonomy of sea horses in South Korea, specifically sea horses that were caught in the Southern Sea, centering on Yeosu, South Korea. Specimen collection methods The samples were collected by set net, skimming nets, dragnets, and landing nets on a boat, as well as scuba diving. A total of 128 sea horses belonging to three species were collected. To investigate the exact distribution pattern, the catch per unit effort and the population density per 1,000 ㎡ were calculated for each site in the Southern Sea. The result shows the highest catch by set nets was 29 sea horses a day in Site B at Dolsan-eup, Port Impo, and the highest catch by scuba diving was 8 sea horses a day in Site B at Gijang-gun, Busan. The highest population density was 61.2 sea horses in the coast of Gijang-gun, Busan. Genetic information analysis and morphological analysis were performed for determination of species. As a result, four *Hippocampus trimaculatus*, (flat-faced sea horses), 45 *Hippocampus coronatus* (Crowned sea horses), and 79 *Hippocampus mohnikei* (Japanese sea horses) were distinguished.

Key words : Sea horse, Crowned sea horse, Flat-faced sea horse, Japanese sea horse, Yeosu sea horse

Ⅰ. Introduction

The sea horse is a marine fish belonging to the order Syngnathiformes and the family Syngnathidae. It features curled tails with no tail fin, a right angle between the head and the body, and a high pectoral fin, which easily distinguishes it from other types of fish (Fritzsche, 1980). Their main habitats are seaweed and eelgrass beds, and the subtropical species are mainly found in coral reefs (Foster & Vincent,2004). One characteristic of sea horses is that the female deposits eggs in the male's brood pouch and the male carries the eggs until they are fully developed. This reproduction pattern is unique among the bony fishes. Other unique features include fecundity throughout the year, eating food by inhaling, and body color change (Koh et al., 2004). Based on their coastal settlement

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※ This study was conducted jointly with the Hanwha Marine Biology Research Center as part of the Functional Improvement and Recovery of Marine Ecosystem Habitats Project 2014 sponsored by the Ministry of Maritime Affairs and Fisheries.

characteristic with a limited swimming area, sea horses have been reclassified of late into 32 species in 7 habitats categories using their morphological and genetic traits (12 and 16S mtDNA) (Lourie et al., 1999b). The life span of sea horses is one year or longer for the dwarf sea horse *H. zosterae* (Strawn, 1958) and up to five years for the cape sea horse *H. capensis* (Lockyear etal., 1997). Their life span is mainly influenced by predation and diseases but varies widely by type and researcher (Jordan &Gilbert, 1982).

Sea horses historically have had high commercial values, and have been used for various purposes. "Donguibogam" (Principles and Practices of Eastern Medicine) claims that the sea horse helps refresh the kidney and replenish one's energy. In clinical settings, they are used as an excitatory tonic and have also been found to be effective to treat senility and mental breakdown. In China and Southeast Asian countries, sea horses have a high pharmaceutical value and are known to reduce asthma and cholesterol as well as prevent arteriosclerosis. Furthermore, due to their unique shapes, sea horses are being exhibited in aquariums for ornamentation. Consequently, the population of sea horses is rapidly decreasing (Joung, 2008), and some species are facing the risk of extinction in Southeast Asia (Vincent, 1996; Lourie et al.,1999b). Pertinently, *Red List in 2002,* published by the International Union for Conservation of Nature and Natural Resources (IUCN), classified one of 32 species of sea horses as endangered, 20as vulnerable, and 11 as data-deficient (IUCN, 2002). As a result, the catching and commercial trade of wild sea horses have been restricted (CITES, 2002).

In South Korea, sea horses are rarely used commercially; however, their coastal habitats are being threatened nonetheless due to reclamation projects, the proliferation of industrial complexes, the construction of large housing complexes, and the inflow of contaminated water from various industrial activities (Choi, 2006). It seems that there is an urgent need for investigating the distribution and taxonomy of sea horses inhabiting the Southern Sea of South Korea in order to keep track of the sea horse population in South Korea. Therefore, this study was conducted to obtain basic data for sea horses in the Southern Sea of South Korea as a basis for a series of forthcoming research. Through morphological and genetic analyses, this study strives to understand the domestic distribution of South Korean sea horses that have been designated as protected animals and arerapidly decreasing in number around the world.

Ⅱ. Materials and Methods

To examine the distribution pattern of sea horses, various methods for specimen collection were used in ten collection sites. To ensure safety in transporting and to reduce travel time and distance, the investigation was centralized to the shores near Yeosu, where the researchers' facility (Hanwha Aquaplanet, Yeosu) is located. Eight sites for specimen collection were selected, including Port Impo in Dolsan-eup; Samseom Village in Anpo-ri, Hwayang-myeon; Nam-myeon, Ando-ri; Gijang-gun, Busan; and Sangju Beach in Namhae-do. The selected locations, their GPS coordinates, and satellite images are provided in <Table 1>.

Specimen collection methods were determined by depth of water and the environment of the sea floor at the collection sites. To minimize the death of specimen during transport, the sea horses were transported to the research lab on the day of

<Table 1> Specimen collection methods and the site environment

Environment	Pictures	Sites
Water depth 1-2 m, no bedrocks, even floor		Site H
Water depth 2-10 m, many bedrocks, seaweed bed		Site C Site D Site E Site F Site J
Water depth 1-2 m, many bedrocks, coastal area		Site C Site H
Water depth 20-30 m, wide area		Site A Site B

collection. For the sea horses that had been caught from sites that were far away, portable aeration devices and Styrofoam boxes were used. The collection methods selected based on the environment of the sites are presented in <Table 2>.

For the collection of sea horses, set nets were used at sites A and B. sea horses were captured using set nets 10 times from July to November.

Skimming nets and dragnets were used in the relatively shallow coastal areas, and scuba diving was used in the seaweed beds of other areas that were suspected to be habitats of sea horses. All specimen collection, except for collection using set nets, was carried out by the researchers.

After pressure sand filter circulation, the sea horses were placed in two water tanks in the authors' company premises for temporary storage. To create conditions similar to their natural habitats, natural seawater was supplied for 24 hours at 2 ℓ/min after sand filtering. Furthermore, the water tank was divided internally for each sea horse, and various structures were installed inside the water tank for the stable feeding of the sea horses, which are vulnerable to currents. The material and thickness of the structures were selected to allow the sea horses to wrap their tails around the structures, and artificial aquatic plants and 5×5 mm meshes were used for the structures. Furthermore, Kajima and sea staghorns were collected from the collection sites and placed inside the water tank. For feeding, a mixture of 24 g of one-day-refrigerated adult brine shrimp (*Artemia sp.*) and hatched *Artemia* Nauplius were fed to the sea horses, and any leftover was removed daily. To ensure effective removal of leftovers, no substrates were used in the tank.

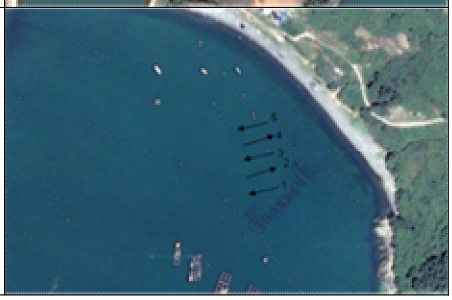
For definite classification, 11 sea horses belonging to three species were sent to the Fish Laboratory of the Marine Biology Department of Pukyong National University. The captured sea horses were classified as Morphological Analysis and Genetic Analysis.

At first, The isotype of Morphological Analysis that was used for the comparison was based on the literature presented by Lourie et al.(2004).

For the measurement criteria of each part, according to the method of Lourie et al.(1999b), the coronet height(CH) was defined as the part from the end of the crown center groove to the center of the cleithral ring; the head length (HL), from the mouth end to the center of the cleithral ring; the trunk length (TrL), from the center of the cleithral ring to the center of the last trunk ring; and the tail length (TaL), from the center of the last trunk ring to the tail end. The standard length(SL) was determined by adding up HL, TrL, and TaL (Choi, 2006).

Next, Genetic Analysis is used to the mitochondria DNA 16s rRNA region.

<Table 2> GPS coordinates of the collection sites and the collection methods

	Collection sites	GPS coordinates		Satellite images
	Collection methods	Latitude	Longitude	
Site A	Port Impo in Dolsan-eup, Yeosu, location A	34°34'44.1"N	127°48'23.8"E	
	Set net			
Site B	Impo Port, Dolsan-eup, Yeosu, location B	34°34'55.2"N	127°47'42.0"E	
	Set net			
Site C	Anpo-ri, Hwayang-myeon, Yeosu	34°38'11.31"N	127°38'27.12"E	
	Scuba diving, skimming net			
Site D	Nam-myeon, Ando-ri, Yeosu	34°28'49.77"N	127°48'32.28"E	
	Scuba diving			

Site E	Sangju beach, Namhae-do	34°42'55.55"N	127°59'30.08"E	
	Scuba diving			
Site F	Sinwol-dong Yachting Course, Yeosu	34°43'50.55"N	127°41'21.41"E	
	Scuba diving			
Site G	Jongpo Marine Park, Yeosu	34°44'12.88"N	127°44'33.01"E	
	Landing net			
Site H	Mosaguem Beach,Yeosu	34°47'45.46"N	127°45'25.86"E	
	Dragnet, skimming net			
Site I	Gijang-gun, Busan, location A	35°18'20.86"N	129°15'35.62"E	
	Scuba diving			
Site J	Gijang-gun, Busan, location B	35°19'13.02"N	129°16'3.90"E	
	Scuba diving			

Ⅲ. Results And Discussion

1. Results of Specimen Collection in the South Sea, South Korea

Sea horses were caught 20 times between July 8 and November 16, 2014 in the Southern Sea, South Korea, including Yeosu in Jeollanam-do area. A total of 128 sea horses belonging to three species were collected, consisting of 4 flat-faced sea horses (*Hippocampus trimaculatus*), 45 crowned sea horses (*Hippocampus coronatus*), and 79 Japanese sea horses (*Hippocampus mohnikei*). The Japanese sea horses and flat-faced sea horses were mostly caught with set nets, while the crowned sea horses were caught with scuba diving and skimming nets in the coasts. With regard to the water depth, *H. trimaculatus* (flat-faced sea horse) and *H. mohnikei* (Japanese sea horse) were caught in waters 30 m deep, while all *H. coronatus* (crowned sea horses) were caught in waters 1-6 m deep.

The numbers and species collected for each site are outlined in <Table 3>. [Fig. 1] shows a picture of a captured sea horse.

[Fig. 1] A captured sea horse

2. Population Density and Catch per Unit Effort (CPUE)

The population density per site was determined by the number of captured sea horses per 1,000 ㎡. The total area

Was determined by scuba divers, who estimated the range of vision to be about 2 ㎡, at which depth they could still see the seabed in the water. In the case of the set net, the extent of the net was estimated to be 5,000 ㎡. To examine the variations in appearance of the sea horses in each site the CPUE was represented by the number day-1 divided by the hauling count(Choi, 2006). One hauling count for scuba diving was determined to be the movement in a straight line until the end point in an area before reverse movement. The CPUE was determined for each area around the location where the sea horses were caught. The results are shown in <Table 4>. The highest CPUE for catching with set nets was 29 sea horses per day in Site B. The highest CPUE for catching with scuba diving was 8 sea horses per day in Site I.

The population density per 1,000 ㎡ of each area was estimated by the number of captured sea horses and the area. The results are outlined in <Table 4>. The lowest population density was found to be Site A, whereas the highest population density was 61.2 in Site I.

3. Morphological Analysis of the Captured sea horses

Sea horses have been known to mostly settle in coastal areas and to form a single pedigree group in the same habitat, with no movement(Vincent & Sadler, 1995), but a molecular biological study for

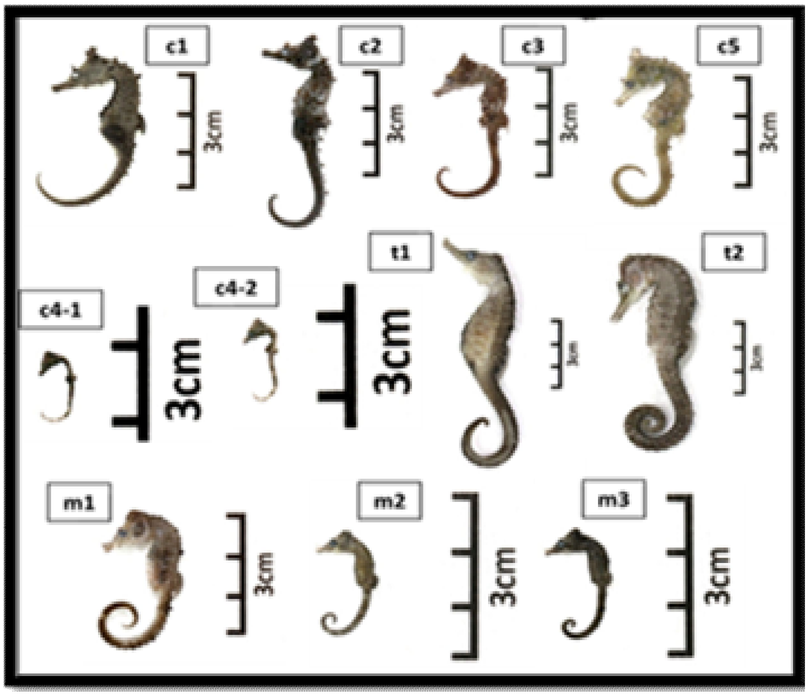
<Table 3> Collection data by location

date	Location	Species	No. of sea horses	Water depth (m)
2014/7/8	Site A	*H. trimaculatus*	1	30
2014/7/11	Site I	*H. coronatus*	6	6
2014/7/20	Site A	*H. trimaculatus*	1	30
2014/7/25	Site J	*H. coronatus*	24	3-6
2014/7/28	Site C	*H. coronatus*	8	6
2014/8/9	Site A	*H. trimaculatus*	2	30
2014/8/19-22	Site B	*H. mohnikei*	50	30
2014/8/24	Site B	*H. mohnikei*	29	30
2014/8/27	Site E	*H. coronatus*	3	6
2014/9/2	Site B	*H. coronatus*	1	30
2014/9/13	Site F	*H. coronatus*	1	6
2014/9/24	Site G	*H. coronatus*	1	6
2014/11/16	Site B	*H. coronatus*	1	30
Total			**128**	

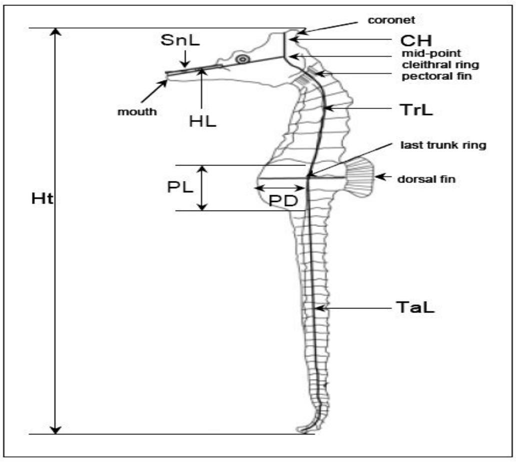
<Table 4> CPUE and Population Density

Location	Species	CPUE (sea horses/day)	Population density (per 1,000㎡)	Collection method
Site A	*H. trimaculatus*	2	0.1	Set net
Site B	*H. mohnikei*	29	2	
Site C	*H. coronatus*	5	32.4	Scuba diving, skimming net
Site E	*H. coronatus*	2	2.7	Scuba diving
Site F	*H. coronatus*	1	3.3	
Site G	*H. coronatus*	0.125	12.1	Landing net
Site I	*H. coronatus*	6	23.6	Scuba diving
Site J	*H. coronatus*	8	61.2	

classification by Lourie et al.(1999b) suggested that there is a need for a re-examination of the taxonomical standards due to the frequent generation of hybrids(Koh et al., 2004). Syngnathiformes fishes are easily distinguishable from other fishes because their larvae have external traits similar to those of mature fish. In particular, the fins and body rings have already grown close to the crown(Pham et al., 1998). For species classification, the number of dorsal fins and the number and shape of the body rings are essential traits(Kanou & Kohno, 2001, Okiyama, 1988). The criteria for the species judgment of the captured sea horses were based on literature showing the morphological characteristics of sea horses. Pictures of the species of the captured sea horses indicating their sizes and shapes are shown in [Fig. 2]. The morphological characteristics were distinguished based on the criteria defined by Lourie et al.(1999b). Reference pictures for measurement are shown in [Fig. 3].



[Fig. 2] Body length analysis of the captured sea horses



[Fig. 3] Measurements used in this study (CH, coronet height; HL, head length; Ht, height; PD, pouch depth; PL, pouch length; SL, standard length =HL+TrL+TaL. SnL, snout length; TaL, tail length; TrL, trunk length. From Lourie et al., 1999b and Lourie, 2003.)

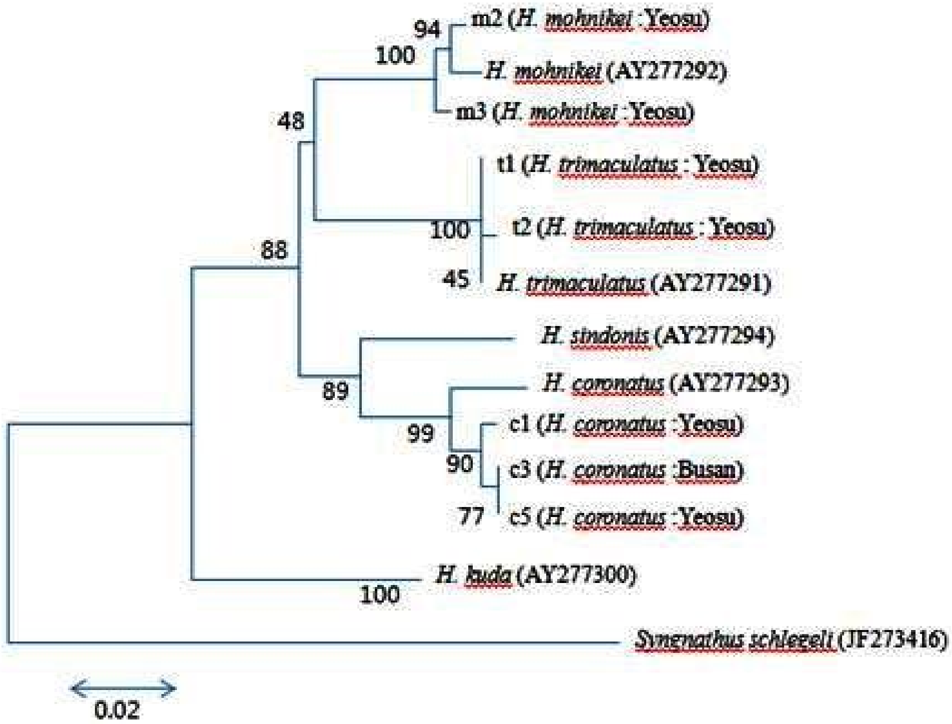
The results of the measurements are outlined in <Table 5> and the results of the comparison with the isotypes for species judgment are shown in <Table 6>. The data of the isotypes that were used for the comparison were based on the literature presented by Lourie et al.(2004).

<Table 5> Morphological measurements of the samples

Samples	Dorsal fin rays	Pectoral fin rays	Trunk rings	Tail rings	Coronet type	Height (mm)	Head length (mm)	Snout length (mm)
c1	12	11	10	34	Fluted, turned backtop	61.0	13.9	5.1
c2	13	12	10	36	Fluted, turned backtop	69.3	13.1	5.1
c3	13	12	10	37	Fluted, turned backtop	58.1	12.2	3.5
c4-1	12	11	10	36	Fluted, turned backtop	13.4	3.0	0.7
c4-2	13	12	10	37	Fluted, turned backtop	13.9	3.1	1.0
c5	14	12	10	38	Fluted, turned backtop	65.7	14.1	5.1
t1	22	18	11	41	Tiny points	121.5	24.7	11.1
t2	20	17	11	42	Tiny points	130.0	25.7	12.8
m1	16	11	11	39	Ridge-like	64.7	12.4	4.4
m2	16	11	11	40	Ridge-like	27.6	6.0	2.2
m3	17	13	11	37	Ridge-like	30.0	7.1	2.3

<Table 6> Comparison with isotypes (Lourie et al. (2004)

Samples	Item	Results	Lourie et al. (2004)	Judgment
C1-5	Dorsal fin rays	12-14	14	*H. coronatus*
	Pectoral fin rays	11-12	12	
	Trunk rings	10	10	
	Tail rings	34-38	38-40	
	Coronet shape	Fluted, turned backtop	Fluted, turned backtop	
	(Mouth length/head length)*100	2.3-3.9	2.3-2.5	
t1-2	Dorsal fin rays	20-22	18-22	*H. trimaculatus*
	Pectoral fin rays	17-18	16-19	
	Trunk rings	11	11	
	Tail rings	41-42	38-43	
	Coronet shape	Tiny points	Tiny points	
	(Mouth length/head length)*100	2.0-2.2	1.9-2.4	
M1-3	Dorsal fin rays	16-17	15-16	*H. mohnikei*
	Pectoral fin rays	11-13	12.14	
	Trunk rings	11	11	
	Tail rings	37-40	37-40	
	Coronet shape	Ridge-like	Ridge- or wedge-like	
	(Mouth length/head length)*100	2.8-3.1	2.8-3.9	



[Fig. 4] Genetic analysis results of the captured sea horses

As a result, the 11 captured sea horses were found to be 6 *H. coronatus,* 2 *H. trimaculatus,* and 3 *H. mohnikei.* In [Fig. 2], c1-5 are *H. coronatus.* Among these, c4-1 and c4-2 are larvae of the crowned sea horses that were born in August and September. Furthermore, t1-2 are H. trimaculatus and m1-3 are *H. mohnikei.*

4. Genetic Analysis Results of the Captured sea horses

Results of the analysis using the mitochondria DNA 16s rRNA region are shown in [Fig. 4].

The larvae of the crowned sea horses that were born in August and September were also sent for genetic analysis. The sample numbers of the crowned sea horse larvae are c4-1 and c4-2.

The genetic analysis results for the 11 sea horses revealed that they belonged to three species: c1-5 were *H. coronatus,* t1-2 were *H. trimaculatus*, and m1-3 were *H. mohnikei.*

Ⅳ. Conclusion

This study seeks to investigate the current status of sea horses in South Korea to help preservation efforts of sea horses, which are rapidly decreasing in number around the globe. To obtain data for sea horses in South Korea, sea horses were captured in the Southern Sea, and their distribution, morphology, and genetic characteristics were analyzed. sea horses were collected over 20 incidents in five months, between July 8 and

November 16, 2015, around Yeosu using set nets, skimming nets, dragnets, landing nets, and scuba diving. As a result, 128 sea horses belonging to three species were captured, consisting of 4 *Hippocampus trimaculatus*, 45 Hippocampus coronatus, and 79 *Hippocampus mohnikei*. In the deep seas of 20 m deep or deeper, mostly *H. trimaculatus* and *H. mohnikei* were captured, whereas *H. coronatus* were mostly captured in the shallow coasts. This suggests that the habitats of the *H. trimaculatus* and *H. mohnikei* are deeper than those of *H. coronatus*. Considering that three species were captured in the coasts of Yeosu, more intensive research in more diverse areas and water depths will be necessary based on the results of this study.

The distribution patterns of the sea horses captured in each area were examined. When the captured sea horses were counted by location, the highest number of sea horses were found to have been caught in sites A and B, where 85 sea horses belonging to three species were caught. For a more accurate examination of the distribution pattern, the population density and CPUE were calculated. The CPUE was analyzed by catching method because different environments required different catching methods. Consequently, the highest CPUE for catching with a set net was 29 sea horses a day in Site B. The highest CPUE for catching with scuba diving was 8 sea horses a day in Site J. In the case of the set net, the spaces of the meshes were not designed for sea horses, and it was practically impossible to adjust the mesh spaces just for catching sea horses. In the future, the nets must be selected in line with the target species for the investigation of the population density and catch. The population density per 1,000 ㎡ in each area was estimated from the captured sea horses and the study area size. As a result, Site J showed the highest population density (61.2).

The c1-5 were classified as *H. coronatus*, the t1-2 as *H. trimaculatus,* and the m1-3 as *H. mohnikei,* which coincided with the results of the molecular analysis. The morphological analysis revealed that *H. trimaculatus* and *H. mohnikei* meet the criteria presented in Lourie et al. (2004), but *H. coronatus* had 34-38 tail rings, and their mouth length: head length ratio was 2.3:3.9, which did not exactly match. Even though morphological classification has a clear definition of the reference points, the counters can commit errors because the sea horses are classified based on the eyes and hands of the counters as shown in Figure 3.

Even though sea horses are settling fishes, three species were captured in the sea near Yeosu, suggesting the diversity of the species. In the future, the characteristics of sea horses based on preferred habitats need to be explored in other coastal regions of South Korea according to the water environments. Such further study is hoped to help reveal the distribution patterns of sea horses in South Korea and their morphological characteristics.

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• Received : 01 July, 2016
• Revised : 29 July 2016
• Accepted : 10 August, 2016