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Bargibant's pygmy seahorse *Hippocampus bargibanti* in Taiwan Bank: hidden biodiversity of Taiwan Strait

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Abstract

During a scientific expedition in the Taiwan Bank in the southern Taiwan Strait in 2021, five pygmy seahorses, measuring between 18.1 mm and 28.3 mm in standard length, were inadvertently collected along with *Muricella* sea fans. Morphometric measurements of these pygmy seahorse specimens coincided with the previous data on *H. bargibanti*, but they had a proportionally greater head depth and snout length. Genetically, DNA marker analysis of partial mitochondrial COI sequences confirmed that our specimens belonged to the same lineage as *H. bargibanti*, with a p-distance of 0.54% among populations. This discovery enhances our knowledge of the limited biodiversity in the Taiwan Bank, which includes a complex geomorphology of both reef banks and sandy substrates, and highlights the importance of strict laws prohibiting fishing with inadequate gear, invasive or illegal fishing, and sand dredging to protect unexplored coral reef biodiversity in the area.

Key words: biogeography, conservation, coral reef, new record, sand dredging

Introduction

The pygmy seahorses belong to the genus *Hippocampus* of the family Syngnathidae. Molecular evidence from 32 *Hippocampus* species suggests that the genus originated in the Indo-Pacific biome, with the pygmy seahorse being an ancient divergence of this group from the main clade of seahorses (Teske *et al.* 2004). A global revision on the taxonomy and biogeography of the genus, along with some new discoveries, revealed that seven species of pygmy seahorses, *H. bargibanti*, *H. colemani*, *H. denise*, *H. japapigu*, *H. nalu*, *H. pontohi*, and *H. satomiae*, have been confirmed as valid based on morphological and genetic data (Whitley 1970; Kuiter 2003; Lourie & Randall 2003; Lourie & Kuiter 2008; Gomon & Kuiter 2009; Lourie *et al.* 2016; Short *et al.* 2018, 2020). Most pygmy seahorse species are widely distributed in the Coral Triangle region in the western Pacific Ocean, except for *H. nalu*, which is endemic to South Africa in the Indian Ocean (Short *et al.* 2020). With five reported species, Taiwan is a global hotspot for pygmy seahorse biodiversity (Heard *et al.* 2019).

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All species of pygmy seahorses are small, with sizes ranging from 11 mm (*H. satomiae*) to 27 mm (*H. colemani*) in maximum recorded height/length (Lourie *et al.* 2016; Koning & Hoeksema 2021). They are known for their complex homochromy, or ability to mimic the colors of their hosts such as sea fans, colonial hydrozoans, bryozoans, seagrasses, and algae, providing an effective camouflage and making it difficult for observers to spot them in their habitats (Lourie & Kuiter 2008; Reijnen *et al.* 2011; Lourie *et al.* 2016). Therefore, apart from studies of new discoveries and taxonomic examinations, information on their biology and ecology in the wild is relatively scarce. Smith *et al.* (2012) studied the extreme habitat specialization and population structure of two gorgonian-associated pygmy seahorses, *H. bargibanti* and *H. denise*, in southeast Sulawesi, Indonesia. Shepherd *et al.* (2017) described the mating, birth, larval development, and settlement of a pair of *H. bargibanti* collected from the Philippines and reared at the Steinhart Aquarium of the California Academy of Sciences. A review of the diversity of seahorse species in the international aquarium trade revealed that information on most pygmy seahorses was either not evaluated or data deficient in the IUCN Red List categories (Koning & Hoeksema 2021). This indicates that data obtained from any individual collected in the wild will be beneficial to the understanding of the natural history and biogeography of pygmy seahorses.

On August 13, 2021, five pygmy seahorses were inadvertently collected along with *Muricella* sea fans (FIGURE 1) by beam trawling during a scientific expedition onboard R/V *Fishery Researcher II* in the Taiwan Bank. This area is a shoal in the southern Taiwan Strait that measures <40 m in depth. To our knowledge, this is the first time pygmy seahorse specimens have been recorded and officially archived from the waters around Taiwan. Previous reports have only included images from citizen science projects shared on social media (after Heard *et al.* 2019). In this study, we identify the pygmy seahorses found in the Taiwan Bank both morphologically and genetically, provide preliminary insights into the population genetics and the basic biology of the species in the western Pacific Ocean, and indicate the immediate threats to the coral reef biodiversity in the Taiwan Bank.



FIGURE 1. *Muricella* sea fans and pygmy seahorse *Hippocampus bargibanti* collected from Taiwan Bank in the southern Taiwan Strait. (A) samples of the collected sea fans; (B) branches and polyps of the sea fan; and (C) morphology and fresh color of the pygmy seahorse on board.

Materials and Methods

A combined scientific expedition aboard R/V *Fishery Researcher II* from August 11 to 17 in 2021, conducted a demersal fauna survey and carried out marine environmental and ecological research in the Taiwan Bank area (22°48'N; 118°50'E) of the Taiwan Strait. Pygmy seahorses and inhabiting sea fans were inadvertently collected by a French-type beam trawl (2.5-m span with a wooden beam and forerun tickler chain [length 4.5 m, weight 10 kg] and a pair of iron side-skis; Tsai *et al.* 2009) at a depth of approximately 25 m. Trawling was disrupted due to the wooden beam breaking, which was caused by the complex topography of the reef banks.

The morphometric measurement and sex determination of the pygmy seahorse were conducted according to

Lourie *et al.* (1999) and Lourie and Randall (2003). The standard length (SL) of a seahorse is defined as the sum of the head length, trunk length, and tail length, with measurement of the trunk and tail lengths following the curve of the body. The number of rings on the trunk and tail were determined from radiographs captured with exposure settings of 40 kV and 120 μ A using a Thermo Kevex X-ray Source Control Interface 5.5.9 and SCap 1.1.5 for capturing X-ray images (Thermo Scientific, USA).

Species	Origin	NCBI accession no.	Archive record no.
Pygmy seahorses			
Present study	Taiwan: Taiwan Bank	-	NMMB-P035690
		PQ140446	NMMB-P040318
		PQ140447	NMMB-P040319
		PQ140448	NMMB-P040320
		PQ140449	NMMB-P040321
H. bargibanti	Philippines: Sulu Sea	KY066105	HH-1011
	Indonesia: West Papua, Kaimana, Selat Iris	MH155225	I.47832-002
	Indonesia	MH645106	I.47832-002
		MH645107	I.47858-002
H. denise	Papua New Guinea: Arafura Sea	KY066106	HH-1014
	Indonesia: Raja Ampat, Wayilbatan	MH155224	I.47855-001
	Indonesia	MH645108	I.47851-001
		MH645109	I.47851-001
		MH645110	I.47855-002
		MH645111	I.47855-003
		MH645112	I.47855-003
		MH645113	I.47855-003
		MH645114	I.47857-001
		MH645115	HH-1111
		MH645116	I.48031-001
H. japapigu	Japan: Hachijo-jima	MH142380	KAUM:I:111770
H. pontohi	Indonesia	MH645129	I.47960-003
		MH645134	I.47834-006
		MH645136	I.47834-005
Seahorses			
H. abdominalis	New Zealand	HQ322642	-
H. comes	China	MK287967	MR1818
H. fuscus	Chinese herbal markets	MT558617	-
H. histrix	China	KP140007	SCSMBC001727
H. patagonicus	Brazil	KF725695	-
H. trimaculatus	Philippines: Bohol, Nasingin Island	GQ502173	RM2023

TABLE 1. *Hippocampus* species, origin, NCBI accession number, and archive record number of the specimen for the partial cytochrome c oxidase subunit I gene used in this study.

Genomic DNA of the pygmy seahorses and also the sea fan coral they inhabit were extracted from muscle tissue and coral branches, respectively. Due to the deficiency of muscle tissue from pygmy seahorses, the Illumina Novaseq X Plus platform was used for next-generation sequencing to obtain cytochrome c oxidase subunit I (COI) sequences for both pygmy seahorses and sea fan coral. The 150bp paired-end short reads were mapped to the mitogenome of *Hippocampus bargibanti* (NCBI accession no. NC065492) by Geneious V11 software (Auckland,

New Zealand) to produce complete COI sequences. In addition, the partial COI gene sequence of six congeneric species of seahorses (*H. abdominalis*, *H. comes*, *H. fuscus*, *H. histrix*, *H. patagonicus*, and *H. trimaculatus*) and pygmy seahorses (four *H. bargibanti*, 11 *H. denise*, one *H. japapigu*, and three *H. pontohi*) available from the National Center for Biotechnical Information (NCBI) GenBank were also included for the phylogenetic analyses (TABLE 1).

Phylogenetic trees were reconstructed using both Bayesian and maximum likelihood (ML) methods. The Bayesian method was applied using the program MrBayes 3.2.7 (Huelsenbeck & Ronquist 2001) with 1 million steps in a Monte Carlo Markov Chain (MCMC) simulation. The effective sample sizes (ESS) of parameters sampled from the MCMC were 1,517 (acceptable ESS is >200). Trees were sampled every 200 generations, with the initial 25% being discarded as burn-in to ensure that the Average Standard Deviation of Split Frequencies (ASDSF) is <0.01. Additionally, the convergence diagnostic PSRF (Potential Scale Reduction Factor) should approach 1 as runs converge (Gelman & Rubin 1992). The ML method was applied using the program MEGA11 (Tamura *et al.* 2021) with 1,000 bootstraps. Models with the lowest BIC scores (Bayesian Information Criterion) were considered to describe the substitution pattern. The best substitution model evaluated from MEGA11 with invariable sites (TN93+G+I, I=0.56, G=0.70, Ts/Tv ratio=6.93) was selected for constructing ML tree, while GTR+G+I was selected for constructing a Bayesian tree with both phylogenetic trees using *Hippocampus patagonicus* (GenBank accession numbers: KF725695), *H. fuscus* (MT558617), *H. histrix* (KP140007), *H. comes* (MK287967), *H. trimaculatus* (GQ502173), and *H. abdominalis* (HQ322642) as the outgroups. The p-distance of 654 bp of the COI gene between species and among the population was also determined using MEGA 11 (Kumar *et al.* 2018).

The pygmy seahorse specimens were archived as NMMB-P035690 and P040318–P040321 in the Pisces collection of the National Museum of Marine Biology and Aquarium. The DNA sequences that were obtained have been registered in GenBank (under the accession numbers PQ140446–PQ140449). The COI sequence of the sea fan coral in this study was also blast to the GenBank for species identification.

Results

In this study, five pygmy seahorses and the sea fan they inhabited were collected. The pygmy seahorses were yellow to orange in color, which matched the similar color morph of the collected sea fan, demonstrating their excellent camouflage (FIGURE 1). The sea fan was subjected to DNA barcoding (Accession no. PQ150045) and blasted to NCBI, suggesting a 99% identity to *Muricella* sp. ANT77 (OL616247).

Two male (22.6–24.5 mm in SL) and three female (18.1–28.3 mm in SL) pygmy seahorse specimens were morphologically examined. The snout was short with a bulbous tip. The body was fleshy with a rounded knob-like coronet and irregular bulbous tubercles (FIGURE 2). The pygmy seahorses had 12 trunk rings, 30–32 tail rings, 9–11 pectoral fin rays, 13–14 dorsal fin rays, and no anal fin rays. The number of rings and proportional measurements of the specimen in our study were similar to the data on *H. bargibanti* provided by Lourie and Randall (2003), although the head shape slightly differed, with our specimen having a proportionally greater head depth and snout length (TABLE 2).

	Present study		Lourie and Randall	
	Male	Female	Total	(2003)
Number of fish	2	3	5	8
Standard length (SL, mm)	23.53±1.34	23.20 ± 5.08	23.3±3.66	$24.4{\pm}1.7$
Count				
Trunk ring (TrR)	12	12	12	12
Tail rings (TaR)	31	30-32	30–32	31-32
Pectoral fin ray (PF)	9	10-11	9–10	10
Dorsal fin ray (DF)	13–14	13–14	13–14	14
Anal fin ray (AF)	0	0	0	0

TABLE 2. Counts and morphometric measurements (expressed as percentages of standard length, head length, or snout length as indicated) of *Hippocampus bargibanti* discovered by the present study and Lourie and Randall (2003). Values are ranges or means \pm standard deviations.

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TABLE 2. (Continued)

	Present study			Lourie and Randall
	Male	Female	Total	(2003)
Proportional measurement				
Trunk length (TrL):SL	26.95±2.33	25.00 ± 4.69	25.78±3.67	28±1.61
Tail length (TaL):SL	57.25±1.77	59.07±4.17	58.34±3.23	56±1.29
Head length (HL):SL	15.80±0.57	15.93 ± 0.86	15.88 ± 0.68	17±1.44
Head depth (HD):HL	75.85±2.62	79.70±1.01	78.16±2.58	66±3.28
Snout length (SnL):HL	30.35±0.49	28.23 ± 0.38	29.08 ± 1.22	22±1.83
Snout depth (SnD):SnL	104.85 ± 6.86	106.67 ± 4.89	$105.94{\pm}4.97$	100±20.23
Orbital diameter (OD):HL	20.75±1.06	21.87 ± 2.08	$21.42{\pm}1.68$	18 ± 3.10
Post-orbital length (PO):HL	53.25±0.49	53.93 ± 3.79	53.66±2.72	57±4.06
Coronet height (CH):HL	65.05 ± 6.43	$70.90{\pm}12.18$	68.56±9.74	57±6.26
Trunk depth anterior to the dorsal	12.95 ± 0.78	11.37 ± 0.38	$12.00{\pm}0.99$	13±1.61
fin base, between the 9th and				
10th trunk rings (TD9):SL				
Length of dorsal fin base (DL):SL	9.40±1.84	9.20±0.66	9.28±1.04	8±0.74
Length of pectoral fin base (PL):SL	3.05 ± 0.07	3.20±0.95	3.14 ± 0.68	3±0.52



FIGURE 2. The five pygmy seahorse *Hippocampus bargibanti* collected from Taiwan Bank in the southern Taiwan Strait. (A) preserved and (B) X-ray photos.



FIGURE 3. (A) Bayesian and **(B)** maximum-likelihood (ML) phylogenetic tree of the 654-bp cytochrome c oxidase subunit I gene for *Hippocampus* pygmy seahorse species, with their relatives as outgroup species. Values above the branches of the ML tree are bootstrapping values.

In addition, morphological identification was genetically supported through the available DNA marker analysis of the partial mitochondrial COI sequences of congeners from NCBI GenBank. According to the constructed maximum-likelihood and Bayesian phylogenetic trees, the lineage of the pygmy seahorse was different from that of its relatives (FIGURE 3). The genetic relationship suggested that our specimens were in the same lineage with a

p-distance of 0.54% among populations of *H. bargibanti*. The p-distance between species and among populations of pygmy seahorses was 9.37%–13.77% and 0.31%–0.54%, respectively (TABLE 3), indicating our specimens belong to *H. bargibanti*.

11	1 150 5 1 7	0 1	5	
	H. bargibanti	H. denise	H. japapigu	H. pontohi
H. bargibanti	(0.0054)			
H. denise	0.1050	(0.0042)		
H. japapigu	0.1377	0.1053	(-)	
H. pontohi	0.1129	0.0937	0.1162	(0.0031)

TABLE 3. p-Distances of the partial cytochrome c oxidase subunit I gene sequences between and within (in parentheses) known *Hippocampus* pygmy seahorse species, including the sequence obtained in this study.

Discussion

Identification of pygmy seahorse species is not always an easy task. For instance, due to color variations and lack of distinct differences from a single specimen, *H. severnsi* Lourie & Kuiter 2008 and *H. waleananus* Gomon & Kuiter 2009 were considered as synonyms of *H. pontohi* Lourie & Kuiter 2008 and *H. satomiae* Lourie & Kuiter 2008, respectively (Lourie *et al.* 2016). However, in the case of *H. bargibanti*, its unique characteristics such as irregular bulbous tubercles scattered over its body and tail made it immediately identifiable, despite slight variations in head shape. Further analysis based on the construction of phylogenetic trees indicated a distinct phylogenetic split between the lineage of the four pygmy seahorses and other six seahorses. This coincided with the result from one species of pygmy seahorse (*H. bargibanti*) and other 31 congenerics (Teske *et al.* 2004). These findings also support the speculation on the designation of pygmy seahorses as a separate genus (Lourie *et al.* 2016).

Based on keyword searches and underwater photographs from social media and citizen scientists, H. bargibanti has been the most frequently recorded pygmy seahorse species since its first sighting in southern Taiwan in 2004 (Heard et al. 2019). It is widely distributed among main diving locations, including sightings in 2011 and 2016 off the northeastern coast of Chaojing Park, Keelung in northern Taiwan (FIGURE 4). The first Taiwanese specimen described herein was discovered in the Taiwan Bank area of the southern Taiwan Strait. This offshore continental location is distinct from other coastal coral reef sites around Taiwan. The Taiwan Bank area is around 13,000 km² with a water depth of less than 40 m, averaging 20–25 m and the shallowest point is 8.6 m. The hydrology of the Taiwan Bank is significantly influenced by the South China Sea Warm Current and Kuroshio Branch Current. It is one of the major upwelling areas in the Taiwan Strait (Lan et al. 2009) and the traditional fishing ground for the residents of the Penghu Islands in Taiwan (Lu et al. 1986; Weng et al. 2021). The seabed in this region comprises hard reef banks and soft sandy substrates, which have been adversely affected by the destructive rockhopper trawling and sand extraction, respectively, carried out by China (Jeng 2019; Lee 2021). Hippocampus bargibanti is an extreme habitat specialist exclusively found on gorgonian Muricella spp. (Gomon 1997; Baine et al. 2008; Reijnen et al. 2011; Smith et al. 2012; this study). Habitat loss and degradation could directly impact the distribution of the host gorgonians and subsequently reduce the population size of the associated pygmy seahorses. Our finding highlights the habitat complexity of the Taiwan Bank and the importance of enforcing strict laws to protect unexplored biodiversity of coral reef in this area through the prohibition of fishing with inadequate fishing gear as well as invasive and illegal fishing and sand dredging.

Conclusions

A combined morphological and genetic identification has confirmed the occurrence of the pygmy seahorse *H. bargibanti*, in the Taiwan Bank in the southern Taiwan Strait. Immediate threats to the unexplored coral reef biodiversity in the Taiwan Bank need to be urgently addressed and prohibited.



FIGURE 4. Reported discoveries of *Hippocampus bargibanti* near Taiwan: Open circles indicate previously reported locations, and the solid circle indicates the new discovery. Topography and water depth were given in meters.

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