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Research Article

Morphological and Genetic Characterisation of Seahorse Species (Syngnathidae: *Hippocampus* spp.) in the Waters of Sabah, Malaysia.

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ABSTRACT

Seahorse are teleosts belonging to the genus *Hippocampus* which consists of 57 species. Among the 57 species, only 12 species exist in Malaysian waters and 11 species in the coastal waters of Sabah. These records and checklists pre-date 2015, and no known studies or field surveys have been conducted in Sabah since then. In this study, a field survey was conducted in selected areas of Sabah waters to document the species composition through morphological and genetic identification. Out of the 11 species that were recorded previously, only two species (*Hippocampus barbouri and Hippocampus comes*) were found and identified using taxonomic keys. CO1 mitochondrial gene was used for genetic identification and phylogenetic tree reconstruction of Maximum Likelihood (ML). The dataset comprises sequences of 11 species from Malaysian waters (excluding *H. satomiae*). The genetic distances, i.e., p-distances, for *H. barbouri* and *H. comes* were recorded to be less than 1% inter-species and more than 10% intra-species, which confirmed the distinct species genetically. Furthermore, findings highlight the urgency of implementing conservation strategies to protect the remaining populations, in light of limitations of this study.

Keywords: Syngnathids; Barbour's seahorse; tiger-tail seahorse; morphology; phylogeny; Borneo.

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INTRODUCTION

Seahorses, the members of the genus *Hippocampus* are a group of captivating marine fishes belonging to the family Syngnathidae. This genus consists of 57 species, all of which form a diverse group. A total of 12 species in the Hippocampinae family namely, *Hippocampus barbouri, Hippocampus bargibanti, Hippocampus comes, Hippocampus denise, Hippocampus histrix, Hippocampus kelloggi, Hippocampus kuda, Hippocampus satomiae, Hippocampus spinosissimus, Hippocampus mohnikei, Hippocampus pontohi* and *Hippocampus trimaculatus* were recorded in Malaysia. Eleven species were found in Sabah but the presence of *H. mohnikei* has not been confirmed. The *Hippocampus* species has a body that is encased in a ring like rigid plates where the body is maintained in a vertical posture with the head bent towards the front forming a 90° sharp angle (Kuiter, 2000). Some general characteristics alone are not enough to identify them to species level but small details such as number of rings, height of coronet, and sharpness of spines are one of morphological characteristics that distinguish one species from another. The rings are divided into two: trunk rings which are the uppermost rings seen from the dorsal view to the ring immediately above the anal fin, and tail rings which are counted from the ring just below the anal fin to the ring before the tip of the (Wilson et al., 2001). Beside the morphological traits, seahorses also have a unique reproductive character, where males carry the fertilized eggs in a specialised brood pouch until the young are ready to be born (Stölting & Wilson, 2007). Seahorses inhabit subtropical and tropical shallow coastal waters, including threatened habitats, such as seagrass beds, coral reefs, mangroves, and river mouths (Lourie et al., 2004). They exhibit a range of vibrant colours and intricate patterns, which not only add to their allure but also serve as camouflage against predators (Wallis, 2004). Seahorses play a vital regulatory role in the marine ecosystem as both prey and predator. As predators, they control populations of small crustaceans and plankton. This in turn helps maintain balance in the food web by regulating the abundance of these organisms (Trewhella & Hatcher, 2017). Simultaneously, as prey, they provide sustenance for larger marine species, creating a critical link in the trophic chain (Trewhella & Hatcher, 2017). The presence of seahorses can therefore serve as an indicator of the overall health and integrity of these valuable marine ecosystems (Delunardo et al., 2015).

Despite their enchanting appearance and ecological importance, seahorses face significant threats. Habitat destruction due to coastal development, pollution, and destructive fishing practices have severely impacted their populations (Lim et al., 2011). Additionally, seahorses are often caught for the aquarium trade and traditional medicine, further endangering their numbers (Lourie et al., 1999). The combination of these factors makes seahorses vulnerable to population decline, highlighting the need for conservation efforts. The seahorse records and checklist data pre-dates to 2015, with Lim et al. (2011) conducting a study in 2011 on the diversity, habitats, and conservation threats of syngnathid fishes in Malaysia, while in 2015, Shapawi et al. (2015) explored the species and size composition of seahorses in the coastal waters and local markets of Kota Kinabalu, Sabah, Malaysia. As mentioned by Chen et al. (2021), there has been a lack of recent comprehensive surveys of seahorse populations in Sabah waters.

This study therefore aims to fill critical knowledge gaps by incorporating both morphological and genetic identification techniques to accurately document the *Hippocampus* species diversity in Sabah waters. Additionally, as previous research has primarily focused more on Peninsular Malaysia species (Lim et al., 2011; Ng et al., 2024), this study provides valuable data that further builds our understanding of seahorses in Sabah. By combining traditional and modern approaches, this study not only enriches the knowledge of seahorse taxonomy but also sets the stage to support future biodiversity management and conservation initiatives in Sabah.

MATERIALS AND METHODS

Fig. 1 shows the sampling sites that were covered in the present study. A total of 21 sampling stations were selected to carry out this field survey guided by occurrence information from past literature (Lim et al., 2011; Shapawi et al., 2015; Chen et al., 2021). These locations were chosen and prioritised, as this maximises the likelihood of encountering *Hippocampus* species by considering habitats and environmental conditions favourable for seahorses. In Table 1, detailed information on the sampling stations is recorded. The samples were collected using several non-destructive techniques during sampling to minimize the impact to habitat destructions: (i) SCUBA diving for seahorse surveys based on information from literature, dive centres, and local communities; (ii) purchasing samples from local fishermen, who provided confirmation that the samples were collected from the region with an estimated known location; and iii) using scoop net and dip nets to obtain seahorse species. On-site photographs of the specimens were taken prior to preservation to facilitate accurate species identification as the colouration of fish specimen often fades or becomes discoloured after preservation in ethanol (Carter, 2003). All collected samples were submerged in 99% undenatured ethanol solution after dipping them into chilled freshwater for DNA barcoding.

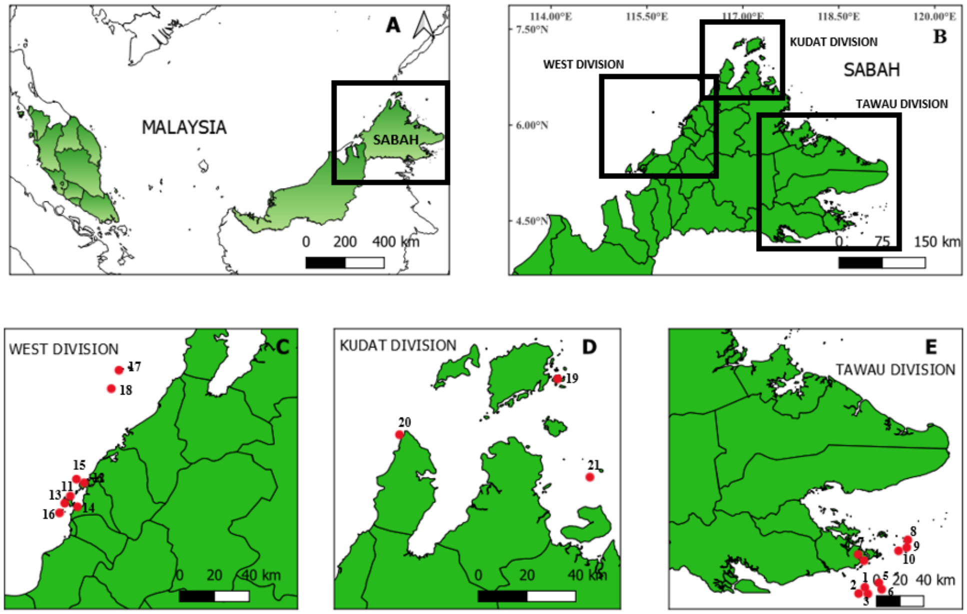

Figure 1: Sampling sites of seahorses in Sabah, Malaysia (1–21). **A**. Map of Malaysia with Sabah marked out. **B**. Map of Sabah with divisions marked out. **C**. Sampling locations in the West Division. **D.** Map outline of Kudat Division **E.** Map outline of Tawau Division. Sampling sites are plotted in red dots on three respective divisions.

Procedures were carried out according to ethical standards of Researcher’s Guidelines on Code of Practice for the Care and Use of Animal for Scientific Purposes (JHEUMS) and deposited in the collection of the Borneo Marine Research Institute (IPMB).

For morphological identification, meristic and morphometric measurements were measured up to the nearest centimetre (cm) in the laboratory (Lourie et al., 2004). The sex of collected specimens were distinguished by the presence of the brood pouch, which would indicate that the specimen was a male (Shapawi et al., 2015). The identification of seahorses was carried out using published taxonomic keys (Lourie et al., 1999; Kuiter, 2000; Lourie et al., 2004). Morphological traits such as trunk rings, tail rings, and spines are key taxonomic features for distinguishing seahorse species, as they vary among species (Lourie et al., 1999; Kuiter, 2000; Lourie et al., 2004).

For DNA extraction, approximately 5–10 mg of tissue from the specimen's tail was cut and inserted into a 1.5 ml microcentrifuge tube. The extraction was performed using the Toyobo MagExtractor™ Genome (NPK–101). The mitochondrial cytochrome c oxidase subunit 1 (CO1) gene (universal primer pair FishF1: 5’- TCA ACC AAC CAC AAA GAC ATT GGC AC -3’; FishR1: 5’- TAG ACT TCT GGG TGG CCA AAG AAT CA -3’), was used as a genetic marker for seahorses and pipefishes for the present study (Zhu et al., 2013; Chao et al., 2014; Chaiphongpachara et al., 2022). Gene amplification was performed using the protocol of Taq Polymerase (Vivantis PL1204, Malaysia) with 25µl reaction containing 13.3µl sterile distilled water, 2.5µl ViBuffer A (10x), 2µl dNTPs Mix (2.5 mM each), 1µl MgCl2 (50 mM), 2µl of each primer (10µM), 0.2µl Taq polymerase (5 u/µl) and 2µl DNA template. Thermal cycling was performed with initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 30 seconds, followed by 30 cycles annealing at 54°C for 30 seconds, and elongation at 72°C for 30 seconds, with an additional extension step of 7 min at at 72°C. Amplicons were visualized on a 1.8% agarose gel after the electrophoresis. Purification of amplicons was carried out using Monarch® Genomic DNA Purification Kit, and sequencing was done using Sanger Sequencing (Apical Scientific Sdn. Bhd). All obtained sequences were deposited in GenBank (Accession no. PP859224–PP859234).

The obtained nucleotide sequences were used to construct a phylogenetic tree based on Maximum Likelihood (ML). The COI dataset for the present study included only 11 sequences out of 12 recorded seahorse species found in Malaysian waters (except for *Hippocampus satomiae* that was unavailable in GenBank). *Corythoichthys haematopterus* (messmate pipefish) was used as an outgroup. ClustalX was used for multiple sequence alignment as proposed in (Thompson et al., 2003) and (Ng et al., 2022, 2023). The Bayesian Information Criterion (BIC) in jModelTest v.2.1.10 (Darriba et al., 2012) identified the Tamura-Nei model as optimal for the present dataset. The Maximum Likelihood tree was generated with 500 bootstraps, and intra- and inter-species p-distances were determined using MEGA-X (Kumar et al., 2018) and (Ng et al., 2022, 2023).

Table 1: List of sampling stations along with their respective coordinates, habitat type of that station, number of species count, and type of *Hippocampus* spp, and the sex of each obtained species.

Station	Division	Name of Location	Habitat Type	Count	Species	Sex	Survey Date
1	Tawau Division	Mabul House Reef	Reef	—	—	—	06 September 2023
2		Mabul Paradise 2	Reef	—	—	—	
3		Mabul Paradise 1	Reef	—	—	—	
4		Tampi-Tampi Island	Sandy Area	2	*Hippocampus comes*	1 male 1 female	
5		Kapalai House Reef 1	Reef	—	—	—	
6		Kapalai House Reef 2	Reef	—	—	—	07 September 2023
7		Semporna mangrove	Mangrove	—	—	—	
8		Mataking Island	Sandy - Reef	—	—	—	08 September 2023
9		Timba- Timba Island	Reef	—	—	—	
10		Pandanan Island	Sandy area	—	—	—	
11	West Division	ODEC, UMS	Sandy Area	1	*Hippocampus comes*	1 male	17 April 2023
12		Mengakabong	Mangrove	—	—	—	
13		Pulau Sepanggar	Sandy Area	—	—	—	
14		Tentera Laut Diraja Malaysia (Seagrass Patch)	Seagrass	2	*Hippocampus barbouri*	2 female	22 March 2023
15		Kibagu Island	Sandy - Seagrass patch	1	*Hippocampus barbouri*	1 female	22 March 2023
16		Pulau Gaya	Sandy Area	2 1	*Hippocampus barbouri* *Hippocampus comes*	1 male 1 female 1 female	08 June 2022
17	Kudat Division	Pulau Mantanani	Sandy - Reef	—	—	—	05 November 2022
18		Pulau Pandan	Sandy - Reef	—	—	—	
19		Pulau Sibogo	Reef	—	—	—	
20		Simpang Mengayau	Reef	—	—	—	23 July 2022
21		Pulau Tigabu	Reef	—	—	—	

Note: '—' = No individual that belongs to the genus *Hippocampus* was found in that specific location.

RESULTS

Two species of seahorses were collected from six of the 21 surveyed stations around Sabah, i.e., *Hippocampus barbouri* (# IPMB- I 01.00185 — IPMB- I 01.00190) and *H. comes* (# IPMB- I 01.00191, IPMB- I 12.00314, and IPMB- I 12.00315) (see Tables 1 and 2). The results of this study highlight key findings on morphological and genetic information of *H. barbouri* and *H. comes* from Sabah waters. Significant variations in physical traits and colouration were observed between species alongside information on the habitat. Phylogenetic analysis based on mitochondrial DNA (CO1), further clarifies the relationship between these two species and among other species in the same genus.

Systematics

Family **SYNGNATHIDAE** Bonaparte, 1831

Genus ***Hippocampus*** Rafinesque, 1810

Hippocampus barbouri Jordan & Richardson, 1908
(Figs. 2–3)

Figure 2: Female specimen of *Hippocampus barbouri* (IPMB-I 01.00185) collected from Pulau Gaya, Sabah, Malaysia.

Material examined: IPMB-I 01.00185, female, total length 6.7cm, location Pulau Gaya, habitat type sandy area, depth 1–4m, collection date 08 June 2022; IPMB-I 01.00187, male, total length 7.8cm, location Pulau Gaya, habitat type sandy area, depth 1–4m, collection date 08 June 2022; IPMB-I 01.00190, female, total length 7.9cm, location Kibagu Island, habitat type sandy-seagrass patch, depth 1–4m, collection date 22 March 2023; IPMB-I 01.00188, male, total length 10.5cm, location Tentera Laut Diraja Malaysia habitat type seagrass patch, seagrass area, depth 3m, collection date 22 March 2023; IPMB-I 01.001889, male, total length 5.4cm, location Tentera Laut Diraja Malaysia seagrass patch, habitat type seagrass area, depth 3m, collection date 22 March 2023.

Description

11 trunk rings; 33 tail rings were recorded for the observed specimens; two cheek spines and 1 sharp eye spine. Dorsal fin rays 17; pectoral fin rays 15; distinct raised high coronet (5 sharp spines); 2 + 1 rings supporting the dorsal fin; zebra- striped snout. The anal fin is absent; well-developed spine throughout the body; first dorsal trunk spine much longer than others and curved backwards; spines of different lengths in a regular series (e.g., long, short, long, short respectively). Snout appears narrow and loses its colour once submerged in ethanol, giving it a translucent look; double spines below eye; and body often covered with black spots. Fine lines radiate from the eye. Supraorbital spines are prominent, simple and acute. The nasal spine appears sharp.

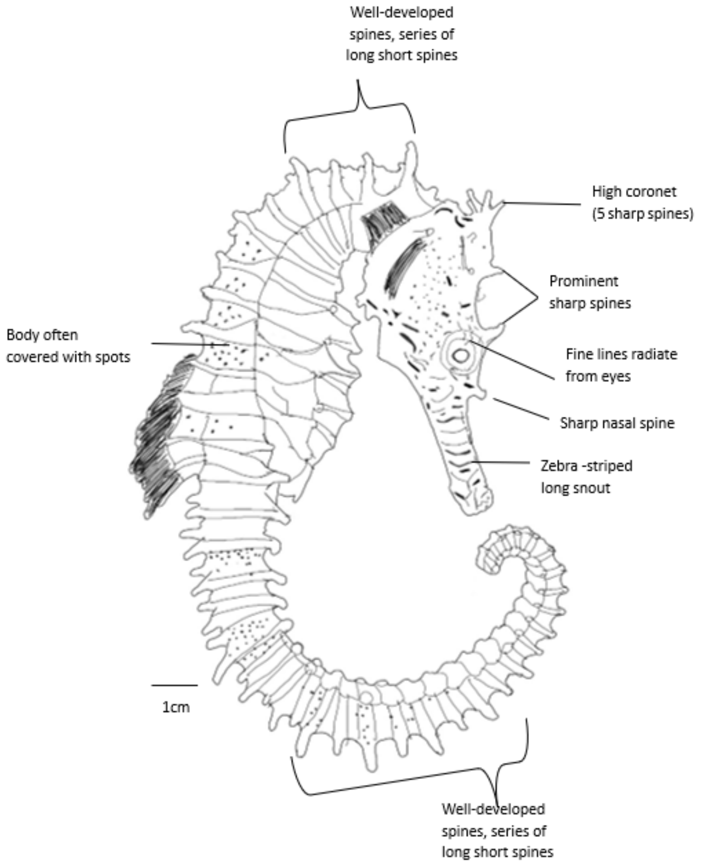

Figure 3: A detailed sketch of a female specimen of *Hippocampus barbouri* (IPMB-I 01.00185) collected from Pulau Gaya, Sabah, Malaysia. Scale bar, 1cm

Measurements: The mean total length (TL) of *H. barbouri* is 7.66 ± 3.60 (Standard deviation, SD) while mean snout length is 0.88 ± 0.28 (SD) for the collected samples. As for comparison, the highest recorded total length of *H. barbouri* to date is 15cm while length of snout falls in the range of 2.0–2.6 (Lourie et al., 1999). Additional meristic and morphometrics data are recorded in (Table 2).

Colour in life: Overall *H.barbouri* has an opaque colour ranging between white, pale yellow and pale brown (Fig. 2). Reddish, brown spots are present throughout the body. Dusky brown lines are present on body. Fine lines radiating from eye and stripes on the snouts range in colour shades of pale brown to dark brown. The tip of the spines is white in colour. However, after submerging in 99% undenatured ethanol, the specimen loses its colouration and became pale. Stripes on the snout became more visible. Black dots throughout the body became much more visible after being preserved for a period of time (Fig. 2).

Habitat: All individuals of *H. barbouri* for this field survey were collected in shallow waters, to a maximum depth of 5m. They were found in shallow seagrass patches parallel with previous reports (Lourie et al., 2005; Unsworth, 2021). Generally, the maximum reported depth for *H. barbouri* is at 10m (Kuiter, 2000). *Hippocampus barbouri* were often found clinging on to shallow seagrass beds using their prehensile tails; a common behaviour that has also been observed elsewhere (Lourie et al., 2016).

***Hippocampus comes* Cantor, 1849**

(Figs. 4–5)

Figure 4: Male specimen of *Hippocampus comes* (IPMB-I 12.00314), collected from Tampi-Tampi Island, Sabah, Malaysia.

Material examined: IPMB-I 12.00314, male, total length 15.1cm, location Tampi-Tampi Island, habitat type sandy area, depth 6m, collection date 6 September 2023**;** IPMB-I 12.00315, female, total length 15.1cm, location Tampi- Tampi Island, habitat type sandy area, depth 6m, collection date 06 September 2023; IPMB-I 01.00186, female, total length 7.3cm, location Pulau Gaya, habitat type sandy area, depth 1–4m, collection date 08 June 2022; IPMB-I 01.00191, male, total length 14.2cm, location ODEC, UMS, habitat type sandy area, depth 5m, collection date 17 April 2023.

Description

12 trunk rings; 32 tail rings were recorded for the observed specimens; two cheek spines and two eye spines. Dorsal fin rays 16; pectoral fin rays 18; 2 + 1 rings supporting the dorsal fin. This species possesses striped tail; small anal fin; pectorals appear to be shorter than dorsal, and anal fin is the shortest of all. All the spines of the salient angles are surmounted by oval or appear knob-like and blunt. A single spine appears to be present in between the head and the eyes. The coronet appears to be lower than the other species with five distinct rounded and knob-like spines. Possess a long and slender snout, and sometimes striped. The nose spine is sharp; double cheek spines and double spines below the eye. The body is heptagonal and its vertical diameter slightly exceeds the length of the snout to the nostrils. The tail is quadrangular, which is tapering into a point. The anus is situated nearly in the middle of the total length, opposite the posterior third of the dorsal.

Measurement: The mean total length (TL) of *H. comes* is 12.2 ± 5.51 (SD) while mean snout length is 1.23 ± 0.42 (SD) for the collected samples. As for comparison, the highest recorded total length of *H. comes* is 18.7cm (Project Seahorse, 2022) while length of snout falls in the range of 0.9 – 1.5 (Shapawi et al., 2015). while length of snout falls in the range of 0.9 – 1.5 (Shapawi et al., 2015). Number of pectoral fin rays and number of dorsal fin rays recorded are (16-18) and (17-19) respectively. Additional meristic and morphometrics data are recorded in (Table 2).

Colour in life: Overall, *H. comes* appears in hues of yellow and black, sometimes alternating; striped tail (although this may not be visible in dark specimens); molted or blotched pattern on body; may have fine white lines radiating from eye and rarely striped snout (Fig. 4).

Habitat: Individuals of *H. comes* were collected in shallow waters, to a maximum depth of 6m. They are typically found in a depth less than 10m and maximum reported depth of 20m (Kuiter, 2000). *Hippocampus comes* were found clinging on to artificial structures under the jetty using its prehensile tail. Past literature also recorded that *H. comes* inhabiting sandy areas clinging on to floating sargassum (Perante et al., 2002).

The morphological differences between *H. barbouri* and *H. comes* are central to distinguishing these closely related species, in particular, the individuals in this study showed key differences in characters, including in the snout, spine and colouration (see Table 3).

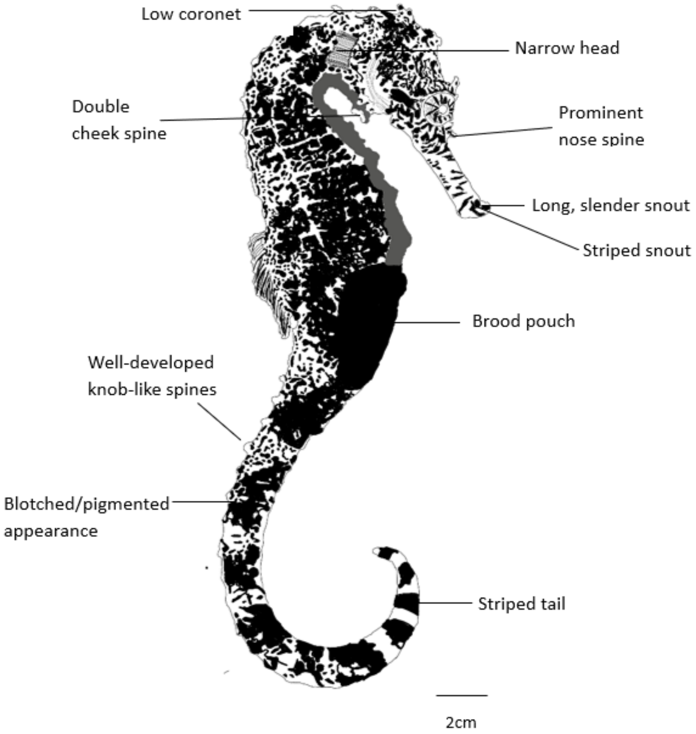

Figure 5: A detailed sketch a male specimen of *Hippocampus comes* (IPMB-I 12.00314), collected from Tampi-Tampi Island, Sabah, Malaysia. Scale bar, 2cm.

Molecular characterization: The Maximum Likelihood tree of COI (Fig. 6) consisting of 11 species that are found to exist in Malaysia including *H. barbouri* (observed species) and *H. comes* (observed species) was constructed. Overall, the ML tree of COI comprised one major clade as shown in Fig. 6. The observed specimen *H. barbouri* and *H. comes* in this study grouped together with the reference sequences of *H. barbouri* and *H. comes* with strong bootstrap value of 100% and 99%. *H. barbouri* grouped as sister species with *H. comes* in one sub clade with strong bootstrap value of 87%. The p-distance within *H. barbouri* is less than 1% while the distance of *H. barbouri* with molecularly closely related (*H. comes*) is less than 10% and morphologically closely related (*H. histrix*) is more than 10%. As for *H. comes*, the p-distance within *H. comes* is less than 1% while the distance with molecularly closely related (*H. barbouri*) is less than 10% and morphologically closely related (*H. kuda*) is 10%. Accordingly, based on comparison to published sequences of *Hippocampus*, the collected specimens in this study are confirmed as *H. barbouri* and *H. comes*, respectively.

Table 2: Meristic and morphometric characters examined in *Hippocampus* spp. seahorses collected from Sabah, Malaysia.

Species	***Hippocampus barbouri***						***Hippocampus comes***		
Accession number	IPMB-I 01.00188	IPMB-I 01.00189	IPMB-I 01.00187	IPMB-I 01.00185	IPMB-I 01.00190	IPMB-I 01.00186	IPMB-I 01.00191	IPMB-I 12.00314	IPMB-I 12.00315
Location	Tentera Laut Diraja Malaysia (Port Seagrass patch)		Gaya Island		Kibagu Island	Gaya Island	ODEC, UMS	Tampi- Tampi Island	
Morphometrics (cm)									
Total length	10.5	5.4	7.8	6.7	7.9	7.3	14.2	15.1	15.1
Height of coronet	0.5	0.5	0.4	0.5	0.3	0.1	0.1-0.2	0.2	0.1
Head length	2.8	2.5	2.9	2.8	2.3	2.7	2.7	3.1	2.9
Snout length	0.9	0.8	1.1	0.9	0.7	0.9	1.1	1.5	1.4
Eye diameter	0.5	0.4	0.5	0.5	0.4	0.5	0.5	0.5	0.4
Tail length	7.1	6.9	5.7	5.5	6.7	6.8	8.5	9.2	8.8
Snout depth	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2
Pectoral fin base length	0.5	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.4
Dorsal fin base length	1.2	1.2	1.3	1.2	1.1	1.2	1.3	1.3	1.2
Meristic									
Dorsal fin soft rays	19	18	19	17	14	16	17	16	17
Pectoral fin rays	17	14	16	15	17	16	16	18	19
Trunk rings	12	11	11	11	11	11	11	12	12
Tail rings	35	31	33	33	32	35	30	32	35
No. rings supporting dorsal fin	2 trunk ring and 1 tail ring	2 trunk ring and 1 tail ring	2 trunk ring and 1 tail ring	2 trunk ring and 1 tail ring	2 trunk ring and 1 tail ring	2 trunk ring and 1 tail ring	2 trunk ring and 1 tail ring	2 trunk ring and 1 tail ring	2 trunk ring and 1 tail ring

Note: IPMB-1 = Code for Borneo Marine Research Institute Ichthyological collection; 01–12 = Collection locality code; .000* = Specimen collection number collected from the collection locality code.

Table 3: Comparative morphological traits of *Hippocampus barbouri* and *Hippocampus comes* in Sabah, Malaysia.

Key Traits	***Hippocampus barbouri*** **(Barbour's Seahorse)**	***Hippocampus comes*** **(Tiger tail Seahorse)**
Height of Coronet	High	Low
Spines	Sharper and more prominent	Knob-like and rounded
Snout	Long, narrow and striped snout	Long, slender and rarely striped
Cheek spines	Double cheek spine	Double cheek spine
Body rings	More distinct, with sharper segments (long, short, long short spine series)	Fewer spines and more blunt trunk rings
Tail	No stripes	Striped/blotched/ pigmented appearance
Colouration	Pale to yellow or brownish shade	Dark brown to black shade

Table 4: Pairwise p-distances of *Hippocampus barbouri and H. comes* with other closely related species.

Species	***H. barbouri***	***H. comes***	***H. kuda***	***H. kellogi***	***H. spinossisimus***	***H. pontohi***	***H. denise***	***H. trimaculatus***	***H. bargibanti***	***H. histrix***	***H. mohnikei***	**Outgroup**
H. barbouri	< 0.01	0.06 - 0.07	0.11 - 0.12	0.10 - 0.11	0.11 - 0.12	0.16 - 0.17	0.16 - 0.17	0.11 - 0.12	0.16 - 0.17	0.09 - 0.10	0.11- 0.12	0.24 - 0.25
H. comes	0.06 - 0.07	< 0.01	0.11 - 0.12	0.11 - 0.12	0.11 - 0.12	0.14 - 0.15	0.15 - 0.16	< 0.10	< 0.16	0.07- 0.08	0.11- 0.12	0.24 - 0.25

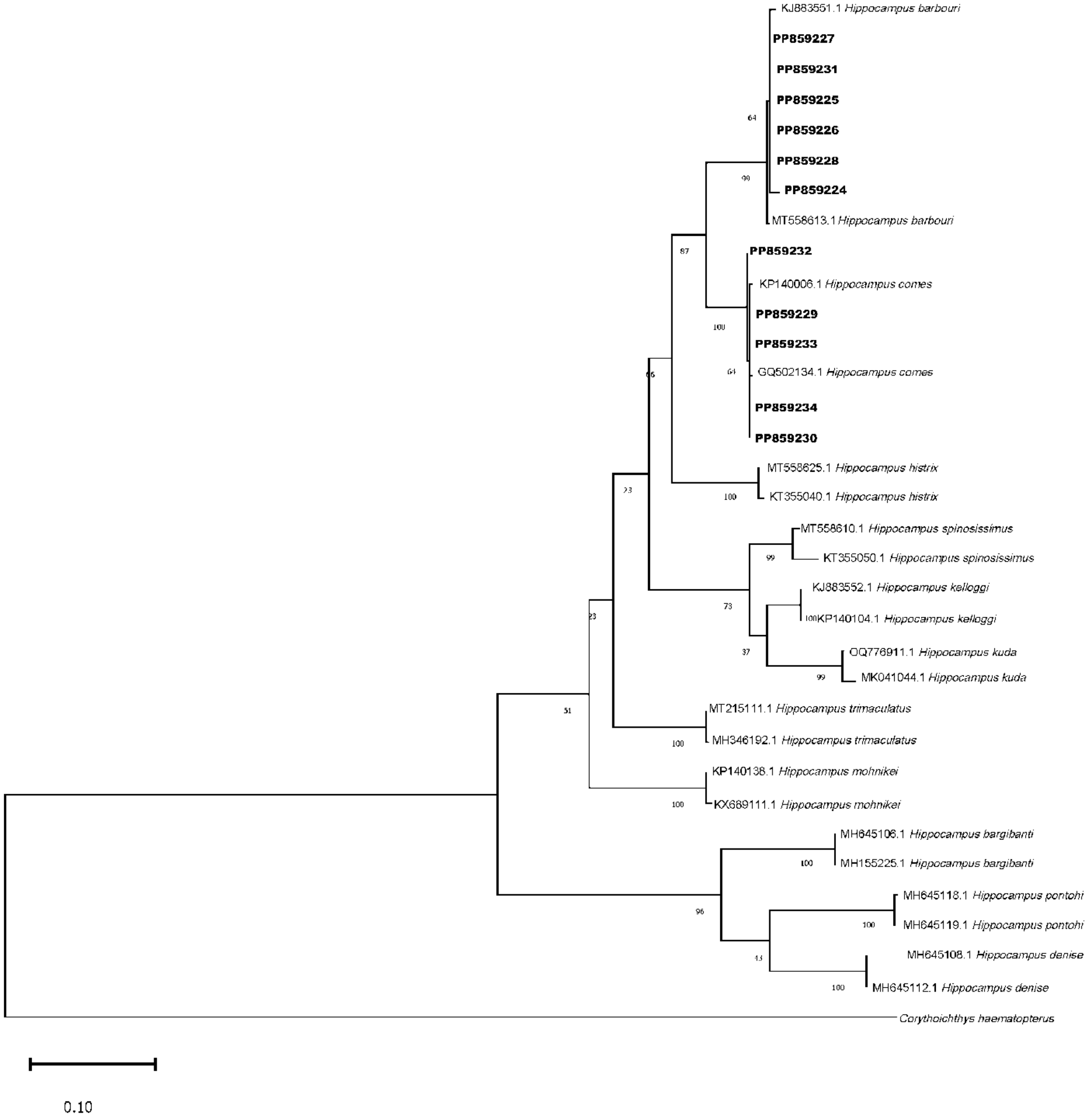

Figure 6: Maximum Likelihood (ML) tree inferred from the partial mitochondrial COI sequences of 11 species of *Hippocampus*, with *Corythoichthys haematopterus* as an outgroup. Values of the nodes correspond to bootstrap values; only values > 50% are shown. Samples from this study are highlighted in bold (**PP859224**: IPMB-I 01.00188; **PP859225**: IPMB-I 01.00187; **PP859226 & PP859231**: IPMB-I 01.00190; **PP859227**: IPMB-I 01.00189; **PP859228**: IPMB-I 01.00185; **PP859229 & PP859230**: IPMB-I 01.00191; **PP859232**: IPMB-I 12.00314; **PP859233**: IPMB-I 12.00315; **PP859234**: IPMB-I 01.00186)

DISCUSSION

Morphological observations

The morphological examination of the observed specimens of *H. barbouri* and *H. comes* revealed insights on the size variations within and between species. Among the collected specimens of *H. barbouri*, males were observed to be shorter in total length compared to females from the same sites. Although the current finding contradicted with (Faleiro & Narciso, 2011) and (Shapawi et al., 2015) in every seahorse species, further investigation is required for confirmation as only a few samples were obtained throughout our study in comparison to the previous studies that had a more robust sample size. However, in this study, apart from the key characteristics of each species, the most notable morphological difference observed is that *H. comes* is longer in total length compared to *H. barbouri*.

As for the colouration, among the collected specimens, the body colour of each individual varied from pale yellow to brown. This could be due to the environment they inhabit. During the field surveys, most of the *H. barbouri* were collected from sandy–seagrass patches. In order to camouflage itself to the sand flats, it turns to a yellowish-brown colour, which can account for the variation of colours in the collected specimens (Curtis & Vincent, 2005). Besides that, the *H. comes* individual collected during the field survey at Tampi-Tampi Island had skin discolouration compared to the individual that was collected from Gaya Island and ODEC, UMS. This could be due to the camouflage effects that all syngnathids possess. Syngnathids mimic vegetation in colour, shape and behaviour (Curtis & Vincent, 2005) which likely reduces their visibility to both predators and prey. The dark colours of *H. comes* collected could be due to camouflaging to the same colours as artificial structures.

Phylogenetic analysis

Based on the results of phylogenetic analysis of the present study, *H. barbouri* is a sister species to *H. comes* and *H. histrix*. Morphologically, *H. barbouri* is often misidentified with *H. histrix* but is distinguished by the length of the snout, number of fin rays, sharpness of spines, and number of cheek spines (Kuiter, 2000). Eventhough *H. barbouri* and *H. histrix* may look similar t morphologically, the molecular evidence indicates that it is more genetically related to *H. comes*. The overlapping distribution and shared habitats between *H. comes* and *H. barbouri* may have facilitated more genetic exchange and closer evolutionary ties between these two species compared to the more geographically distant *H. histrix* (Knowles, 2009). A study by Nurilmala et al. (2019), that investigated a different marker, 16S, also found a close genetic relationship between *H. comes* and *H. barbouri*. Other global studies with larger datasets and more genetic markers have resulted in robust conclusions (Thangaraj & Lipton, 2011; Zhang et al., 2014). It is possible that estimation of genetic similarity in smaller datasets and using only single genetic markers, may lead to underestimation and affect the assessments of species relationships, which is a limitation of this study and should be addressed in future research.

Taxonomic implications on genetic aspects

Morphologically, *H. barbouri* and *H. comes* are clearly different between each other. Although morphological identification can be sufficient for identifying seahorse species in Malaysia with the available taxonomic keys, the potential presence of cryptic species means that relying solely on external features may not always be reliable (Aylesworth et al., 2017; Woodall et al., 2018).

However, in Malaysia, seahorse surveys and monitoring programmes have traditionally relied on morphological identification techniques (Choo & Liew, 2003; Lim et al., 2011; Shapawi et al., 2015). This suggests the possibility of overlooking any presence of cryptic seahorses. Therefore, in our study, we incorporated molecular analyses as a crucial step to enhance the accuracy of species identification and address potential challenges in distinguishing morphologically similar seahorse species. Although our results did not uncover any cryptic species, the integration of molecular data has expanded our understanding of seahorse diversity and provided a valuable addition to traditional taxonomic classifications based on morphology alone.

Limitations and future directions

The outcomes of this study provide significant updates to the biodiversity records of *Hippocampus* species in Sabah, Malaysia. Out of 11 species historically reported in this region, only *H. barbouri* and *H. comes* were recorded based on morphology and molecular methods during this survey, which are in line with recent standard practice of using integrative methods to minimise misidentification in seahorses (Casey et al., 2004; Sanaye et al., 2020) Ongoing anthropogenic activities in Sabah such as excessive fishing pressure, fish bombing, and coastal development in the surveyed areas can potentially be destructive to seahorse populations (Wood & Ng, 2016). Although at a small scale, this study provides updated distribution and molecular data that may help refine species classifications, document range extensions, and highlight genetic linkages in Southeast Asian populations, which in turn is important for supporting conservation strategies.

One possible reason that this study recorded only 2 out of the 11 species previously recorded by (Lim et al., 2011) may have been because of the different sampling methods used, in particular, this study did not use trawl fishing, which has been banned and strictly enforced since 2013 (Nuruddin & Isa, 2013). In addition, the study may have overlooked significant populations of seahorses due to limited sampling, which focused on specific locations as indicated by previous studies (Lim et al., 2011; Shapawi et al., 2015). The reliance on visual surveys and manual capture techniques could introduce observer bias, as less conspicuous or cryptically coloured seahorses may have been overlooked (Brauwer et al., 2020).

Future research may include expanding the scope of surveys to include under-represented habitats (Hao et al., 2025), using multi-locus genetic analyses on larger sample sizes and broader geographical representation to strengthen the robustness of conclusions (Panithanarak, 2020), application of environmental DNA for more comprehensive surveys (Thomsen & Willerslev, 2014), promoting carefully designed aquaculture efforts to reduce the pressure on wild populations (Kumaravel et al., 2012), ensuring long-term monitoring and improved understanding of their population genetics. Regular monitoring of *Hippocampus* populations across a wider range of habitats in under-sampled regions (Cohen et al., 2017) should be established and addressing population decline should be prioritised to safeguard potential loss of seahorse diversity in Sabah waters and to ensure long term sustainability of seahorse biodiversity in the region.

CONCLUSIONS

This study has successfully identified and characterized two seahorse species, *H. barbouri* and *H. comes* in Sabah waters through both morphological and genetic analyses. Detailed morphological descriptions and genetic characterization using CO1 gene sequences provide a comprehensive taxonomic foundation for these species in the region. The phylogenetic analysis revealed a close genetic relationship between *H. barbouri* and *H. comes*, despite some morphological differences, highlighting the importance of integrating both morphological and molecular approaches in seahorse taxonomy and identification. The findings may not accurately represent a comprehensive updated database for the seahorse checklist in Sabah due to the limitations of this study. However, this study can be a reference to future researchers to seek alternative ways to monitor the status of seahorse population by using non-destructive methods such as environmental DNA (eDNA).

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DECLARATIONS

Research permits. This study was conducted with the approval of the Sabah Biodiversity Council Access License Ref. - JKM/MBS.1000-2/2 JLD.16 (39).
Ethical approval/statement. This study was conducted with the approval from Animal Ethics Committee UMS (Ref no: AEC0008/2023).
Generative AI use. We declare that generative AI was not used in this study nor in the writing of this article.

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