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A New Record of *Panulirus homarus homarus* (*Panulirus* spp.) from Malaysia Waters with its Molecular Phylogeny

WEI-LING NG[1], CHENG-ANN CHEN*[1], SING-TUNG TENG[2], AUDREY DANING TUZAN[1],
MUHAMMED NAYEEM MULLUNGAL[3] & TIN-YAM CHAN[4]

[1]Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia; [2]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [3]Environmental Science Program, Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, P.O. Box: 2713, Qatar; [4]Institute of Marine Biology and Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan
*Corresponding author: chengann@ums.edu.my
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ABSTRACT

Spiny lobsters *Panulirus* spp. of the family Palinuridae Latreille, 1802 occupies the shallow tropical and subtropical waters (<100 m depth). These species are ubiquitous and abundant, yet knowledge of its range, biodiversity, and population abundance in Malaysia is limited. *Panulirus homarus* is listed as “Least Concern” along with other 11 species out of 21 spiny lobster species in the International Union for Conservation of Nature and Natural Resources (IUCN) red list in 2022. A total of five species were reported from Malaysia waters. Whilst, this study reports the sixth spiny lobster species, *Panulirus homarus homarus,* with morphology and molecular genetic information (Cytochrome oxidase I (COI) gene) provided. The p-distance within *P. h. homarus* was 0.2 – 0.6%, n = 12; between *P. h. homarus* with *P. h. rubellus* and *P. homarus* “Brown” was 6.4 – 6.8%, n = 12 and 0.8 – 3.3%, n = 12, respectively. Intra-species p-distance within *Panulirus* spp. was below 3%, while inter-species was above 10%. This validates the collected specimen as *P. h. homarus* and *P. h. rubellus* is the subspecies, but leaving *P. h.* “Brown” taxonomic status unclear. Thus, genetic information is important in solving many of these systematic and taxonomic problems, because often these species are difficult to distinguished based on morphological criteria. A key to all *Panulirus* spp. identified in Malaysia waters was constructed. This study updates the marine biodiversity list in Malaysia, where the acquisition of biological knowledge is vital to the protection of the world’s natural resources as well as to the sustainable management of spiny lobster fisheries.

Keywords: Molecular, *Panulirus* spp., Sabah, spiny lobster

INTRODUCTION

The term "lobsters" is typically used to denote the clawed lobsters belonging to the Family Nephropidae. However, it is important to note that there are other closely related species of lobsters, including the spiny/rock lobsters, slipper lobsters, reef lobsters, and blind lobsters. Spiny lobsters, scientifically classified as Decapoda: Achelata: Palinuridae, are a species of crustacean known for their substantial size, with body lengths reaching up to 60 cm. They are characterised by their extended lifespan, exceeding 10 years. Spiny lobsters are found in diverse environments and depths, as documented by Phillips (2008). They typically reside within the substrate, taking refuge in crevices within coral formations, holes, and the rocky seashore floor, as noted by Booth (2001) and Setyanto *et al.* (2019). According to Lipcius *et al.* (2001), the habitat of these lobsters spans from the coastal areas to the limit of the continental shelf. This range is observed in tropical and subtropical waters, with a depth of less than 100 metres, in both the northern and southern (Radhakrishnan *et al.*, 2019). They remain concealed during the day and are active to forage at night - nocturnal, with no fixed diet by eating on specific prey exclusively, but typically browse on sand regions for a variety of food items (Chan, 1998; Childress & Jury, 2013; Senevirathna *et al*., 2017; Setyanto *et al*., 2019). Moreover, the coexistence of many *Panulirus* species in the same geographical area is frequently observed worldwide, as a result of their shared ecological preferences such as rocky and reef habitats (Briones-Fourzán & Lozano-Álvarez, 2013).

The family Palinuridae comprises 54 extant species/subspecies arranged in 12 genera (Chan, 2010) which the most diverse genus is *Panulirus* White (1847), with 24 species/subspecies listed in World Registered Marine Species (WoRMS), most recently updated by Sammy (2021). In 2022, the International Union for Conservation of Nature and Natural Resources (IUCN) red list listed a total of 21 species of spiny lobster; however, nine species are presently classified as "Data Deficient" and the remaining 12 species are "Least Concern." *Panulirus homarus* is categorised as "Least Concern" on the IUCN red list, which was last accessed in December 2009, more than a decade ago. Moreover, evolutionary divergence has occurred within the species, as two subspecies, *P. h. homarus* and *P. h. rubellus*, have been identified (Lavery *et al*., 2014), whereas the population of *P. homarus* is unknown according to IUCN (2023).

Panulirus spp. are crucial components of marine ecosystems, serving as both prey and predator. However, due to a dearth of surveys, information on the distribution, biodiversity, and population abundance of spiny lobsters in Malaysia is limited. Ikhwanuddin *et al.* (2014) and Halim and Siow (2019) reported only two species (*P. polyphgus* and *P. ornatus*) in Peninsular Malaysia and Waiho *et al*., (2021) did a study on the length-weight relationship and size at morphometric maturity of *P. polyphagus* population from Johor Strait. Whilst, Biusing and Chio (2004) identified three species (*P. ornatus*, *P. versicolor*, and *P. longipes*) in Sabah. Chen and Fatihah (2018) reported that *P. ornatus* and *P. versicolor* are present in Labuan. In addition, the most recent discovery was made by Ng *et al.* (2022), who added *P. femoristriga* to the list. Thus, a total of five species have been documented in the Malaysian waters. The understanding on the fundamental knowledge of all the five *Panulirus* spp. that possess different physiology, spawning seasonality and different length of planktonic larval phase is crucial for better conservation and fisheries management efforts in Malaysia (Holthuis, 1991; Sachlikidis, 2010; Briones-Fourzán, 2014). Gaining insights into the availability of species in Malaysia waters is essential to interpreting the reasons for vulnerability of lobster resources and developing sustainable management systems in a rapidly changing marine environment. Therefore, this study describes the sixth species of spiny lobster, *P. h. homarus*, with morphological and molecular genetic data. Data obtained from the current study not only founded the baseline data on the distribution of spiny lobster biodiversity in Malaysian waters, yet contribute to the policy making in fisheries resource management that geared towards the 14th Sustainable Development Goals (SDGs) on life under the water.

MATERIALS AND METHODS

A specimen of *Panulirus homarus homarus* was caught by hand while scuba diving in the region of Kuala Penyu (N 5°32'32.73", E 115°32'5.999"), located in Sabah, Malaysia (Figure 1). The specimen was spotted at depth of approximately 8 metres, in rock crevices. Water turbidity during the dive was slightly high with visibility of 5 – 6 m. The specimen was initially identified at the sample site prior to being euthanized in a solution of ice-cold water. One pereopod was then removed, fixed in absolute ethanol (99%), and transferred to the laboratory for further analysis.

The specimen was subsequently sent to the laboratory at Universiti Malaysia Sabah for the purpose of capturing photographs of several anatomical features, including the sternum, lateral view of the abdomen, antennular plate, ventral view of the antenna peduncle, frontal horn and the entirety of the specimen. The measurements taken included carapace length (CL), carapace width (CW), total length (TL), and weight. The taxonomic keys supplied by Chan (1998) were employed for the morphological identification of the species. Subsequently, a modified key to the species in Malaysian waters was constructed. For genetic analysis, approximately 10 mg of preserved tissue was used for total genomic DNA extraction using Toyobo MagExtractor-Genome-Kit. Universal primer pair LCO1490 + HCO2198 (Folmer *et al*., 1994) were used in this study for the COI gene with sequence LCO1490: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and HCO2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' of a 710 bp fragment in *Panulirus* sp. lobster specimens. Gene amplification was performed using the protocol of Vivantis Taq DNA Polymerase (recombinant) kit: 25 µl reaction containing 13.3 µl sterile distilled water, 2.5 µl ViBuffer A (10x), 2 µl dNTPs Mix (2.5 mM each), 1 µl $MgCl_2$ (50 mM), 2 µl of each primer (10 µM), 0.2 Taq polymerase (5 u/µl) and 2 µl DNA template with thermal cycle performed with initial denaturation at 94 °C for 2 min, denaturation at 94 °C for 30 sec, followed by 30 cycles of annealing at 48 °C for 30 sec, and elongation at 72 °C for 30 sec, with an additional extension step of 7 min at 72 °C. Amplicons were visualized on a 1.8% agarose gel. Purification of the amplicons was performed using Monarch PCR & DNA Cleanup Kit prior to DNA sequencing (two ways) by 1st BASE DNA Sequencing Service using Sanger Sequencing. Then, the sequences obtained were assembled and clean before uploading to BLAST for preliminary identification of spiny lobster species.

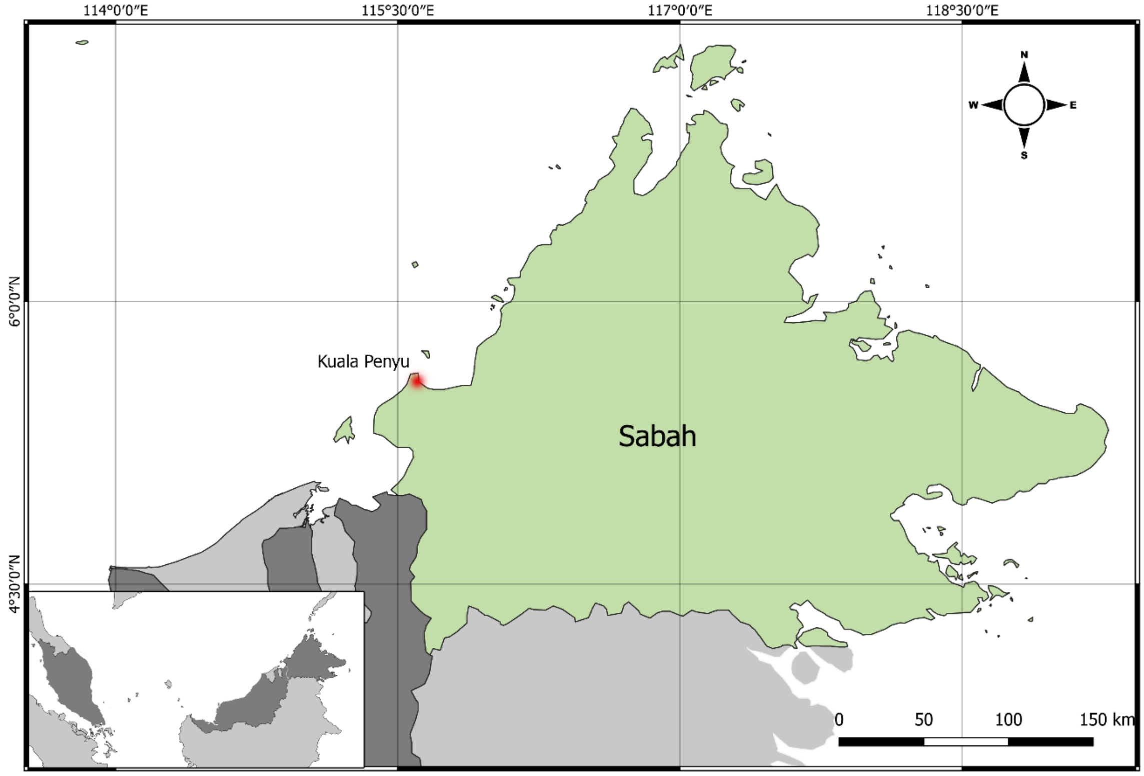

Figure 1. Sampling location in Kuala Penyu, Sabah, Malaysia

The nucleotide sequences obtained were also used to reconstruct the phylogeny, based on Maximum Likelihood (ML) (Ng *et al*., 2022). The dataset comprised of a total of 64 nucleotide sequences, including the *P. h. homarus* specimens collected in this study and the closest outgroup species, *Parribacus antarcticus* Lund, 1793 (Decapoda, Scyllaridae). The outgroup was selected, based on the molecular phylogenetic investigation of Palinuridae as described in Ravago and Juinio-Meñez (2003). The programme ClustalX (Thompson *et al*., 2002) was used to multiple align the sequences and the Akaike Information Criterion (AIC) of jModelTest v.2.1.10 was used to evaluate and select an optimal evolutionary model (Darriba *et al*., 2012). The ML tree was generated using 100 bootstraps using RaxML while intra- and inter-species p-distances were determine using MEGA-X (Kumar *et al*., 2018).

RESULTS

Panulirus homarus homarus (Linnaeus, 1758)

Material examined. One female: CL 73.56 mm, CW 59.18 mm, TL 228.00 mm, weight 355 g, December 2021, Kuala Penyu, Sabah, Malaysia (Figure 2).

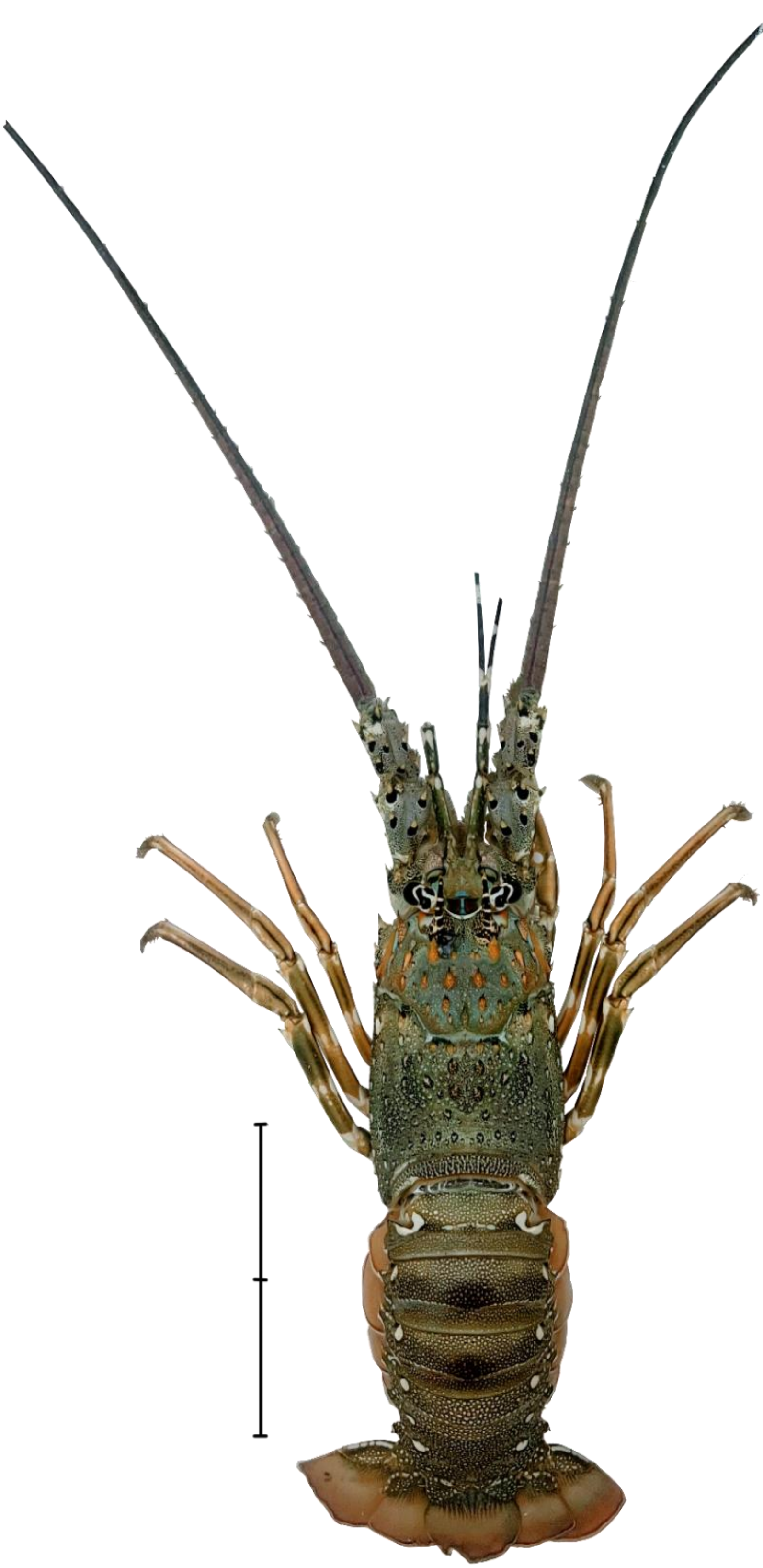

Figure 2. A female specimen of *Panulirus homarus homarus* was collected from Kuala Penyu, Sabah, Malaysia. Scale bar, 100 mm

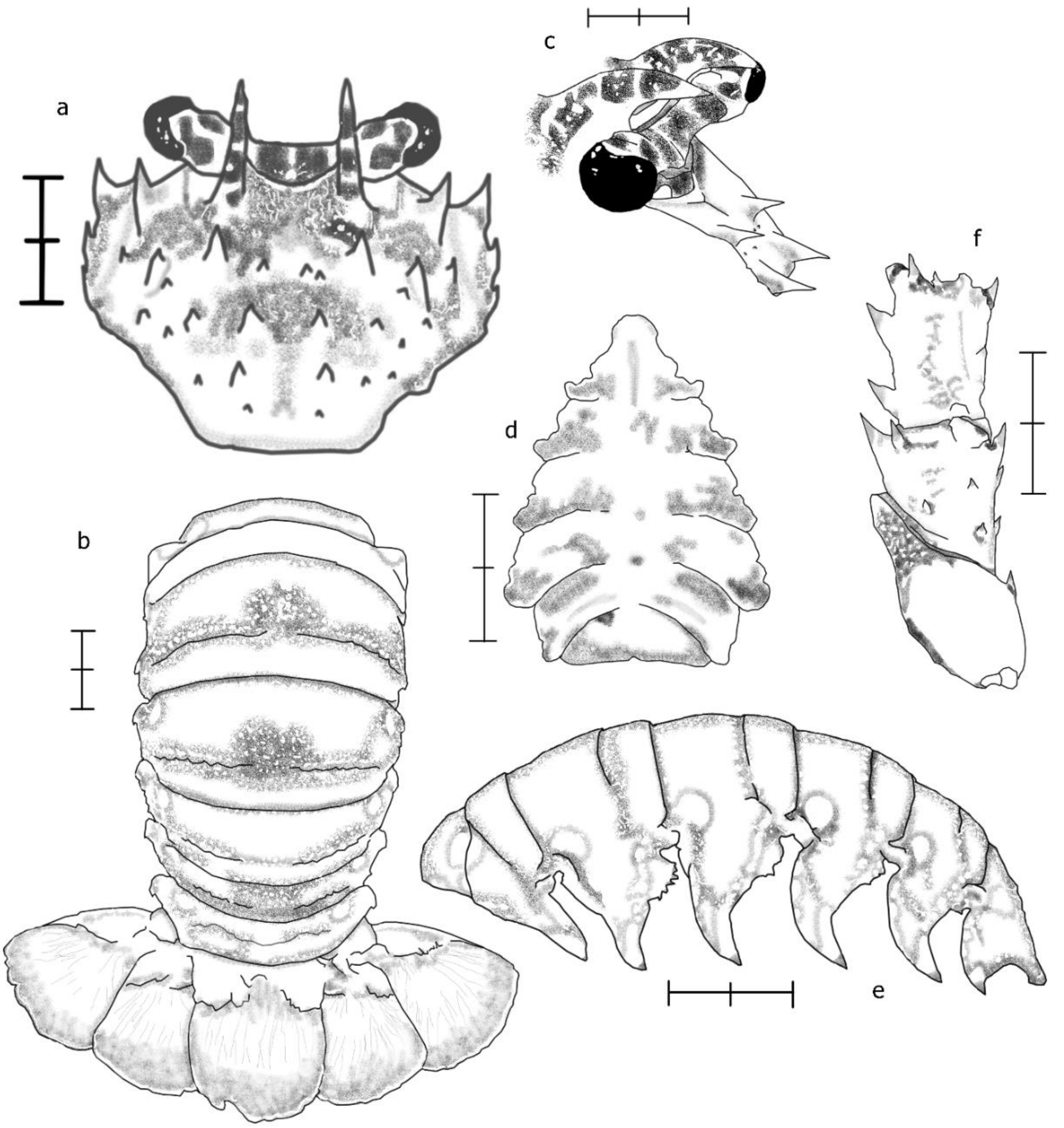

Figure 3. *Panulirus homarus homarus* (Linnaeus, 1758), Kuala Penyu, Sabah (Female: a. Anterior carapace; b. Abdomen, dorsal view; c. Antennular plate, dorsolateral; d. Thoracic sternum; e. Abdomen, lateral view; f. Antennal peduncle, ventral view. Scale bars, 20 mm)

Description. Carapace rounded and spiny; rostrum absent; anterior margin armed with four regularly spaced large spines other than frontal horns; spinules absent in between frontal horns (Figure 3a). Abdominal segments with a slightly crenate transverse groove, sometimes interrupted at the middle (Figure 3b and 3e). Antennular plate at bases of antennae bearing two pairs of well-separated principal spines (anterior pair slightly larger) and some spinules (Figure 3c). Thoracic sternum without protrusions (Figure 3d). Ventral surfaces of middle antennal segments with some scattered spinules (Figure 3f). Antennules with flagella longer than peduncle. First four pairs of legs without pincers. Posterior half of tail fan soft and flexible. Colour: body greenish to brownish. Eyes dark brown. Anterior carapace and region between eyestalks with bright orange and blue markings. Frontal horns banded with black and white. Antennular flagella alternated with brown and white bands. Legs blotched. Abdomen covered with tiny white spots. Pleopods red-brown with white margin. Table 1 shows the key to this species in Malaysian waters.

Table 1. Keys to *Panulirus homarus* subspecies and other *Panulirus* spp. identified in Malaysia Waters (Modified from Chan, 1998)

No.	Descriptions	Species
1a.	Abdomen provided with transverse grooves	→ 2
1b.	Abdomen without transverse grooves or only with broad sunken pubescent areas.	→ 4
2a.	Anterior margins of transverse grooves on abdomen crenulated, grooves incomplete or interrupted in the middle; antennular plate bearing 4 well-separated principal spines and some small spinules; regions between eyestalks with bright orange and blue markings; legs blotched;	
	(i) body dark green to olive green; small squamae	*P. h. homarus*
	(ii) body brick red; large squamae	*P. h. rubellus*
2b.	Transverse grooves on abdomen with straight anterior margins, not crenulated; antennular plate with 2 principal spines; anterior margin of third abdominal pleuron not spinous; transverse groove of second abdominal segment joining corresponding pleural groove	→ 3
3a.	Median area at anterior carapace behind frontal horns usually bearing a longitudinal row of 3 spines only; ventral surfaces of distal 2 antennal segments each with 2 large spines only; thoracic sternum with 2 strong submedian protrusions; antennules with outer flagella dark brown and inner flagella entirely whitish; antennal peduncle including stridulating pad pinkish; lateral carapace with 2 complete longitudinal white strips extending along the entire carapace; legs striped	*P. femoristriga*
3b.	Median area at anterior carapace behind frontal horns always bearing some smaller, irregular spines in addition to the regular row of 3 spines; ventral surfaces of distal 2 antennal segments each with 1 large spine (sometimes also with several other scattered spinules; thoracic sternum without strong submedian protrusions; antennules alternated with dark brown and white bands; antennular peduncle brown to purple and with stridulating pad bright blue; lateral carapace with 1 short (upper) and 1 long (lower) longitudinal white stripes; legs striped or spotted	*P. longipes longipes*
4a.	At least second and third abdominal segments with broad sunken pubescent areas; legs striped; antennular plate armed with 2 pairs of principal spines only; fourth to sixth abdominal segments smooth; body deep blue and green, abdomen with narrow transverse white bands, antennal and antennular flagella whitish	*P. versicolor*
4b.	Abdomen naked and smooth; legs blotched	→ 5
5a.	Antennular plate armed with two pairs of principal spines; body greenish and abdomen with broad transverse dark bands, legs and antennules conspicuously ringed with light yellow and black	*P. ornatus*
5b.	Antennular plate armed with one pair of principal spines; body pale green and abdomen with narrow transverse yellowish white bands	*P. polyphagus*

Molecular characterisation. The best model of the COI fragment dataset in this study estimated by jModelTest selected with corrected AIC was the GTR+I+G model (Generalised Time Reversible model with invariant sites and non-uniform evolutionary rates or gamma distribution). The analysis of all the data resulted in two monophyletic lineages, Clade I and Clade II (Figure 4). The collected *P. homarus* in this study is grouped together with *P. h. homarus* (KX275378, KX275379, KX275380) from Kenya, with a moderately strong bootstrap value of 61% as it is grouped together with its subspecies, *P. h. rubellus,* and a slight diverged *P. homarus* species, *P. homarus* “Brown”. The p-distance within *P. h. homarus* was 0.2 – 0.6%, n = 12; while the p-distance of *P. h. homarus* with *P. h. rubellus* and *P. homarus* “Brown” was 6.4 – 6.8%, n = 12 and 0.8 – 3.3%, n = 12 respectively (Table 2). Accordingly, the spiny lobster in this study is confirmed as *P. h. homarus.* For more information and records, a new sequence dataset is uploaded to the National Centre for Biotechnology Information (NCBI) GenBank, with the accession number: OR497507. The specimens have also been deposited in Borneo Marine Research Institute collection with voucher number: IPMB-Cr 05.00001.

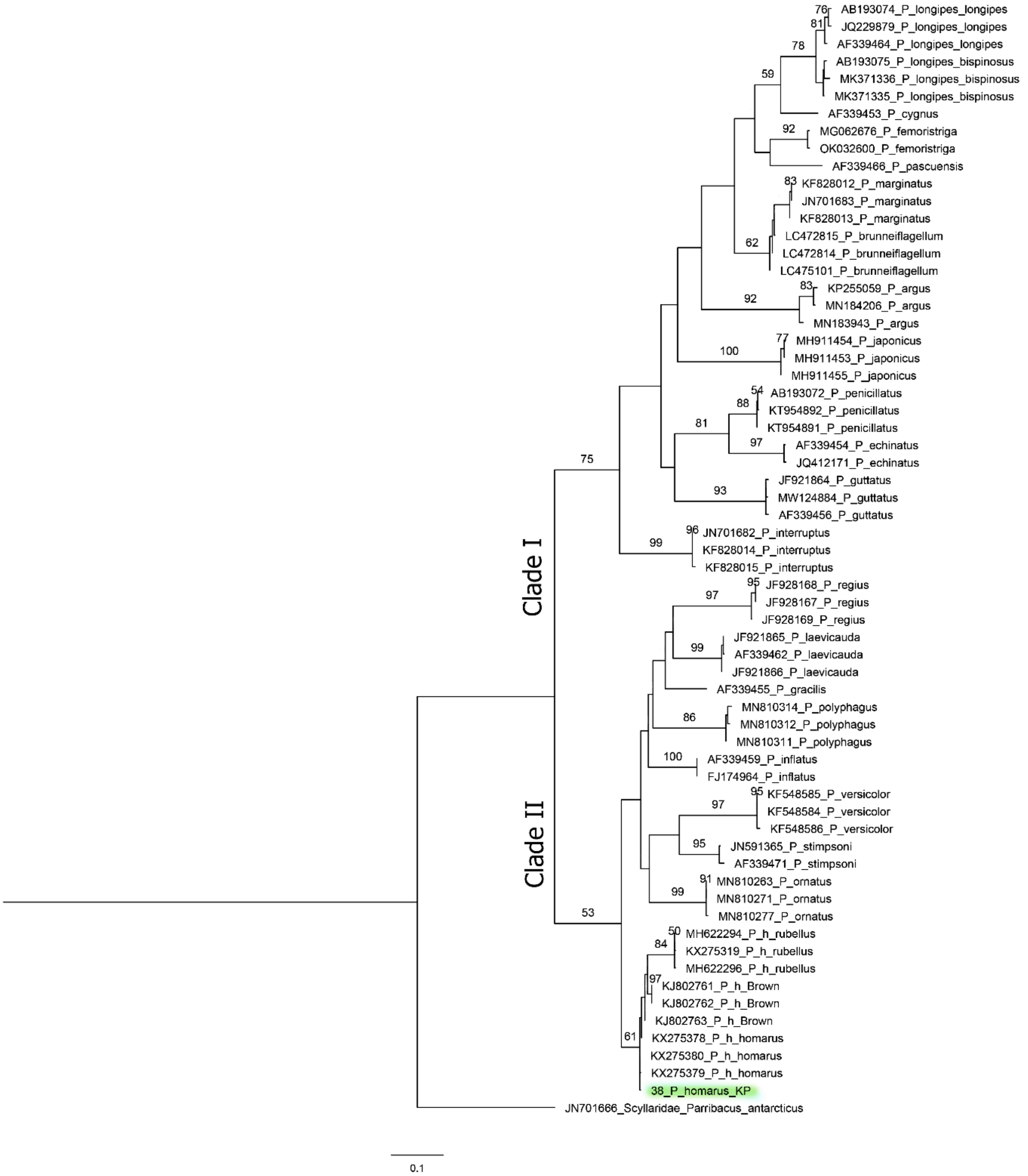

Figure 4. Maximum Likelihood (ML) tree inferred from the partial mitochondrial COI sequences of 64 species of *Panulirus*, with *Parribacus antarcticus* as outgroup. Specimen in this study is highlighted in green. Values of the nodes correspond to bootstrap values, only values >50 are shown

Table 2. Pairwise p-distance of the *Panulirus homarus homarus* and its closely related species. n, number of sequences

	P. h. homarus	*P. h. rubellus*	*P. h.* "Brown"
P. h. homarus	0.0022-0.0066	0.0639-0.0682	0.0088-0.0330
n	12	12	12

DISCUSSION

The collected specimen *Panulirus homarus homarus* was observed and documented in the region of Kuala Penyu, Sabah, Malaysia, through the utilisation of the scuba diving technique at an approximate depth of 8 metres sandy bottom with scattered rocks. This is inline with the observation by Holthuis (1991) where *P. h. homarus* is frequently observed inhabiting reef areas characterised by sandy substrates inside the surf zone, and occasionally in murky waters at depths ranging from 1 to 5 m. Despite wide distribution across the Indo-West Pacific region, spanning from the eastern coast of Africa to Japan, Australia, and the Marquesas Archipelago, it has not been reported in Malaysia waters. Two main reasons could possibly contributed to the presence of this species in Malaysia water previously: habitat preference in turbid water which makes it difficult to be spotted and high similarity in coloration between *P. h. homarus* (less contrast) and *P. ornatus* (brighter and more contrast) confuses local fishermen, as they typically classify these lobsters based on color.

Previous studies have suggested the presence of four subspecies, distinguished by variations in morphology such as the pattern of sculpturing on the abdomen and coloration (Berry, 1974; Holthuis, 1991; George, 2006). These subspecies have distinct geographic ranges, with the exception of *P. h. homarus*, which is found throughout South Africa and the Pacific region (Table 3).

Table 3. Morphololological difference between the subspecies of *Panulirus homarus*

Subspecies	Squamae	Color	Type locality; distribution	References
P. h. homarus	Small	Mainly green	Amboina, Indonesia; South Africa through Pacific	George (2006)
P. h. megasculpta	Large	Yellowish spots in abdomen, and yellowish line along margin s of tergites and pleura	South Yemen; Arab Sea region	Berry (1971, 1974)
P. h. rubellus	Large	Brick red	Eastern coast of South Africa; restricted to Southern Africa coast and Madagascar	Berry (1971, 1974)
P. h. “Brown”	Small	Brown	Marquesas Island; Marquesas Island	George (2006)

In a study conducted by Lavery *et al.* (2014), both mitochondrial DNA and nuclear DNA sequencing techniques were employed. The results of this investigation demonstrated that there is no discernible genetic distinction between *P. h. megasculpta* and *P. h. homarus*. In the aforementioned investigation, it was demonstrated that *P. h. rubellus* and *P. h.* 'Brown' are genetically separate lineages. However, *P. h.* 'Brown' exhibited a reduced degree of divergence compared to the lineage of the former species in relation to the remaining *P. homarus*. Consequently, the taxonomic classification of *P. h.* 'Brown' remains uncertain. According to Radhakrishnan *et al.* (2019), the present recognition of subspecies is limited to *P. h. homarus* and *P. h. rebellus* only. The findings of the present investigation demonstrate consistent outcomes, wherein the p-distance observed between *P. h. homarus* and *P. h. rubellus* exceeds 5%, hence supporting their classification as distinct subspecies. The current study's ML phylogenetic tree also demonstrated that the *P. h. homarus* specimen (38_P_homarus_KP) collected in this study forms a clade with other *P. h. homarus* specimens collected from Kenya by Singh *et al*. (2017), hence providing confirmation of the respective species.

In contrast, the intra-species p-distance within the *Panulirus* spp. in present study was found to be below 3% and reveals that *P. h.* "Brown" exhibits a p-distance ranging from 3% to 5%, which aligns with the findings of a prior study conducted by Lavery *et al*. (2014). Furthermore, it was also determined that there is no significant variation in morphology and colouring between *P. h. homarus* and *P. h.* "Brown" in their study. Hence, the observed divergence is inadequate to classify it either as a subspecies or as the same species as *P. h. homarus*. It is possible that in the future, the subspecies *P. h.* "Brown" may undergo speciation as a result of prolonged isolation and limited geographical range within the Marquesas Islands. According to previous research, the segregation of groups, such as the isolation caused by temperature fluctuations (referred to as chemical barriers) in conjunction with climatic cycles (referred to as physical barriers), can result in the isolation of populations (Tolley *et al.*, 2005). This isolation, over a period of time, may become substantial enough to justify a taxonomic reassessment and the recognition of distinct species (Gopal *et al.*, 2006; Groeneveld *et al.*, 2006).

In addition, it is worth noting that populations of species can undergo reproductive isolation due to prolonged allopatric separation or morphological and genetic divergence while they coexist in the same geographic area (Knowlton, 1993; Quenouille *et al.*, 2011). The survey undertaken by Berry (1974) investigated the distribution of *P. h homarus* and *P. h. rubellus* throughout the east coast of Africa. The findings indicated that *P. h homarus* was solely seen at the Northern Mozambique coast, and *P. h. rubellus* was exclusively found at the southeast Madagascar coast. However, it was noted that both subspecies were present along the southeast African coast. Lavery *et al*. (2014) proposed that the sympatric distribution of *P. homarus* is accompanied by genetic divergence in both mitochondrial and nuclear genes. This divergence indicates the presence of a biological reproductive barrier between the two forms, leading to a limited mixing of the gene pool. The occurrence of hybridization between *P. h. homarus* and *P. h. rubellus* was documented by Berry in 1974. However, the prevalence of these hybrid individuals decreased to a mere 0.1% in the same year. The study conducted by Lavery *et al*. (2014) identified a single heterozygous individual out of a total of 44 individuals sampled. Subsequently, the study conducted by Singh *et al*. (2017) did not find any evidence of hybridization but proposed a reassessment of the taxonomic classification of two species, suggesting that *P. h. rubellus* should be recognised as a distinct species, *Panulirus rubellus*. Present study showed distinct clustering of *P. h. homarus* and *P. h. rubellus* in the phylogenetic tree, thus, provides confirmation that the collected species is not a hybrid but rather a genuine *P. h. homarus*. However, the current study lacks sufficient evidence to substantiate the claim, mostly due to the restricted sampling site and the utilisation of phylogenetic tree sequences that were not derived from the most recent specimen. Consequently, additional information is necessary to accurately assess the current condition of this species.

CONCLUSION

The current investigation documented a novel occurrence of *P. h. homarus* in the spiny lobster database inside the maritime boundaries of Malaysia. The research on spiny lobster in Malaysia is still constrained, particularly with regards to the comprehensive understanding of its distribution. Additional research is necessary to ascertain the rarity of the species in Sabah, Malaysia, given the discovery of only one specimen. In order to ensure the sustainable exploitation and management of *P. h. homarus*, it is imperative to get a comprehensive understanding of its biological characteristics and behaviours. Given the significance of spiny lobsters as a focal species in the fishing industry, it is imperative to prioritise the acquisition of biological knowledge for the purpose of sustainable fisheries management. This endeavour is closely intertwined with the commitment to safeguard the Earth's natural resources, with a special emphasis on the preservation of marine life.

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