

Molecular and Morphological Investigations of Shovel-Nosed Lobsters *Thenus* spp. (Crustacea: Decapoda: Scyllaridae) in Thailand

Apinan lamsuwansuk¹, Jessada Denduangboripant^{1,*}, and Peter J.F. Davie²

¹Department of Biology, Faculty of Science, Chulalongkorn Univ., Phayathai Road, Phatumwan, Bangkok 10330 Thailand ²Queensland Museum, PO Box 3300, South Brisbane, Queensland 4101, Australia

(Accepted September 7, 2011)

Apinan lamsuwansuk, Jessada Denduangboripant, and Peter J.F. Davie (2012) Molecular and morphological investigations of shovel-nosed lobsters Thenus spp. (Crustacea: Decapoda: Scyllaridae) in Thailand. Zoological Studies 51(1): 108-117. Shovel-nosed lobsters (Thenus spp.) (or kang-kradan in Thai) are the basis of an increasingly important fishery in Thailand and other tropical Indo-West Pacific countries. In the past, only a single species was recognized, Thenus orientalis. However, a recent taxonomic revision, using both morphological and DNA sequence analyses, established that at least 3 species occur in Thai waters. The present work was designed to test the results of that earlier study as applied to the Thai fishery, and extend it by using a broader sampling regime. Thirty adult Thenus specimens were sampled from 3 provinces: Chonburi (on the eastern Gulf of Thailand), Phetchaburi (on the western Gulf of Thailand), and Phuket (on the Andaman Sea). Genomic DNA was extracted from pereiopods, and the mitochondrial cytochrome c oxidase subunit 1 (COI) gene was amplified and sequenced. A 403-base pair nucleotide data matrix was used to derive phylogenetic trees using maximum parsimony. The molecular phylogeny clearly separated the Thai specimens into 3 clades: 13 individuals of T. indicus, 7 of T. orientalis, and 10 of T. unimaculatus. The recently proposed morphological criteria were largely effective in separating Thenus species; however some of the morphometric ratios given in the previous paper need to be adjusted. New local Thai names are proposed: kang-kradan thammada (common shovel-nosed lobster) for T. indicus, kang-kradan kha-lai (spotted-leg shovel-nosed lobster) for T. orientalis, and kang-kradan kha-muang (purple-leg shovel-nosed lobster) for T. unimaculatus. It is evident that there is some ecological separation of the different species, and we hope this increased knowledge can be used to help establish sustainable management of their exploitation in Thailand. http://zoolstud.sinica.edu.tw/Journals/51.1/108.pdf

Key words: Cytochrome c oxidase subunit I, Molecular phylogenetics, Shovel-nosed lobster, Thailand, Thenus.

Thenus (Leach, 1815) is a genus of marine scyllarid lobster found in tropical and subtropical waters in the Indo-West Pacific region, and is becoming increasing commercially exploited (Department of Fisheries 1997, FAO 2010). Common names across the region include shovelnosed lobsters, slipper lobsters, flathead lobsters, and in Australia, Moreton Bay bugs. The common names are mostly derived from the peculiar shape of the broadly flattened cephalothorax. Generic characteristics were well described and illustrated

in several recent works (Holthuis 1991, Burton and Davie 2007). The length of the adult body ranges 12-25 cm.

Shovel-nosed lobsters are bottom dwellers and prefer sand and mud habitats at 10-50 m deep (Uraiwan 1977, Jones 2007, FAO 2010). They are widely distributed in Asia and Australia, and *Thenus* is the only genus in the family Scyllaridae to be significantly commercially exploited; and indeed, it is the main component of many trawl fisheries (Jones 1993, Burton and Davie 2007). Moreover,

*To whom correspondence and reprint requests should be addressed. Tel: 66-22185378. Fax: 66-2-2185386. E-mail:jessada.d@chula.ac.th

they are exported to European countries as frozen meat (Naiyanetr 1963, Uraiwan 1977, Sungthong 1979). Species of a related, typically deeper-water genus, *Ibacus*, also form small fisheries in some parts of the world (Holthuis 1991).

In Thailand, there is increasing demand for shovel-nosed lobsters (kang-kradan, in Thai), because they are considered less common and very good tasting. In 2003, whole-shelled shovelnosed lobsters were sold for only 140-240 baht (US\$5-8)/kg, but the current price (in 2011) is 400-600 baht (US\$13-20)/kg, 2.5 times higher than the past, and the supply cannot keep up with the everincreasing demand. Also, with decreasing stocks of marine shrimp due to overexploitation, fishermen are turning to the Thenus fishery to increase their economic returns. Unfortunately, research into the mariculture of shovel-nosed lobsters in Thailand has not yet been successful, and the fishery is thus still entirely based on wild-caught animals. Thus, there are significant concerns that natural populations of Thai shovel-nosed lobsters could become severely overexploited within a relatively short period of time.

Most earlier taxonomic studies of Thenus in Thailand (Naiyanetr 2007) recognized only 1 species: T. orientalis (Lund, 1793). However, Burton and Davie (2007) recently revised the genus using a concordance approach involving 3 techniques: morphometrics/morphology, starch gel isozyme electrophoresis, and mitochondrial (mt) DNA sequencing of 16S and cytochrome c oxidase subunit 1 (COI) genes. The COI gene has successfully been used for barcoding of several crustaceans such as freshwater shrimp (Liu et al. 2007) and mud crab (Shih and Suzuki 2008). They concluded that the genus contained 5 species: T. indicus Leach, 1815, T. orientalis (Lund, 1793), T. australiensis Burton and Davie, 2007, T. unimaculatus Burton and Davie, 2007, and T. parindicus Burton and Davie, 2007. Of these, T. indicus, T. orientalis, and T. unimaculatus were recorded from Thailand. However, they also stated that, "Despite significant genetic divergence, several sympatric species are morphologically similar and identification can be difficult." Because of the increasing exploitation of Thenus in Thailand, we undertook the present study to try and repeat some aspects of the work of Burton and Davie (2007) to ensure that we would be able to identify the local species, and also to try and better understand the population ecology and distribution of the species in Thai waters (since Burton and Davie (2007) examined

only a limited number of samples from a few locations in Thailand). We thus reexamined the external morphology, morphometric ratios, and COI sequences of 30 *Thenus* specimens sampled from both the Andaman Sea and Gulf of Thailand. We hope the information gained will be of use in establishing a sustainable fishery for *Thenus* species in Thai waters.

MATERIALS AND METHODS

In Apr., May, and Oct. 2010, 30 adult specimens of live or frozen shovel-nosed lobsters were randomly collected from 3 provinces in Thailand: 10 samples from Sri Racha District, Chonburi Province (on the eastern Gulf of Thailand), 10 from Cha-am District, Phetchaburi Province (on the western Gulf of Thailand), and 10 from Muang District, Phuket Province (on the Andaman Sea) (Fig. 1). All specimens were photographed to document the live color patterns, and labeled as Chon01-10 for Chonburi, Phet01-10 for Phetchaburi, and Phuk01-10 for Phuket samples. They were immediately preserved in 95% ethanol before later examination in the laboratory.

All 30 Thenus samples were identified

Fig. 1. Sample collecting sites in 3 provinces of Thailand: Phuket (star), Phetchaburi (circle), and Chonburi (triangle). The map was taken from http://www.nationsonline.org/oneworld/map/thailand map2.htm.



following Burton and Davie's (2007) taxonomic key to species. Important characters were: 1) the presence or absence of spots or blotches on the pereiopods, 2) the presence or absence of a spine on the merus of the 3rd maxilliped, and 3) the nature of the dentition on the ischium of the 3rd maxilliped (Fig. 2A). Width and length measurements of various parts of the body and legs were made, and morphological ratios were calculated following Burton and Davie (2007) (Fig. 2B-D, Table 1). These ratios were then also used as a further aid to discriminate species according to Burton and Davie's (2007) criteria (Table 2).

Genomic DNA was extracted from pereiopod tissue using a QIAamp DNA Mini Kit (QIAgen,

Hilden, Germany) using the animal tissue protocol supplied with the extraction kit. An approximately 700-base-pair (bp) fragment of the mitochondrial COI gene was amplified by a polymerase chain reaction (PCR) using Folmer's primers (Folmer et al. 1994): CO1-1490 (forward primer, 5'-GGTCA ACAAATCATAAAGATATTGG-3') and CO1-2198 (reverse primer, 5'-TAAACTTCAGGGTGACCAA AAAATCA-3'). PCR amplification was conducted on a 50-µl reaction volume containing 1x PCR optimized buffer, 0.24 mM of mixed dNTP, 2 units of Dynazyme *Taq* polymerase (Finnzyme, Vantaa, Finland), and 0.5 µM of each forward and reverse primer. The PCR conditions were modified from Folmer et al. (1994) as follows: 5 min at 95°C for

(A) (B)



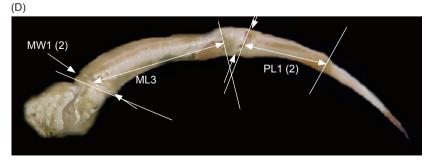


Fig. 2. Morphological characteristics and measurements used in this study. (A) Third maxilliped with spine (s) on the merus (m) and dentition (d) on the ischium (i); (B) carapace; (C) pereiopods; (D) 6th abdominal segment and telson. For details of other abbreviations see table 2.

pre-running, then 35 cycles of 60 s at 95°C for denaturation, 60 s at 49-52°C for annealing, and 90 s at 72°C for extension, followed by 5 min at 72°C for a final extension. Each PCR product was mixed with a loading dye (0.14% bromophenol blue) and electrophoresed in a 1.8% agarose gel stained with ethidium bromide at 80 V for 45 min. The gel was visualized under ultraviolet light.

The PCR products were purified using a QIAquick PCR purification kit (QIAgen) before being sent to an automated cycle-sequencing service (Macrogen, Seoul, South Korea). All of these COI sequences were submitted to GenBank with accession numbers JN165716-28 for *T. indicus*, JN165729-35 for *T. orientalis*, and JN165736-45 for *T. unimaculatus*. The COI sequences of our *Thenus* specimens

were compared to other Thenus sequences retrieved from the GenBank nucleotide database (T. parindicus GenBank no. HM015421, T. australiensis GenBank accession no. HM015433, T. orientalis no. HM015440, T. indicus no. HM015445, and T. unimaculatus no. HM015449) and aligned using the ClustalX vers. 2.0 program (Larkin et al. 2007). Ibacus peronii (GenBank accession no. HM015458) was added as an outgroup taxon. PAUP* vers. 4.10b (Swofford 2002) was used to reconstruct the phylogenetic trees (using maximum parsimony (MP), a heuristic search with random stepwise addition, TBR branch swapping, and steepest descent options). Bootstrap support for the clades was determined using PAUP* with 1000 pseudoreplicates.

Table 1. Abbreviations and their meanings for morphological measurements in this study, following Burton and Davie (2007)

Abbreviations	Morphological measurements							
Carapace (dorsally measured) (Fig. 2B)								
CL	Length of carapace from base of antennal plate sinus to posterior margin of carapace on dorsal side							
CW	Width of widest section: width of carapace with callipercaliper arms sitting on left and right postorbital spines							
Pereiopods 1, 2 and 3 (Fig. 2C)								
PL1(2)	Length of the 1st (and 2nd) propodus: anterior internal protrusion to posterior spine							
PW1	Width of the 1st propodus: widest posterior dimension at right angles to propodus length							
ML3	Length of the 3rd merus: anterior spine to posterior spine							
MW1(2)	Width of the 1st (and 2nd) merus: widest posterior dimension at right angles to merus length							
Abdomen (dorsally measured) (Fig. 2D)							
TL	Length of telson: from mid-anterior margin to posterior margin of the calcified region							
TW	Width of telson: from left to right latero-posterior spine							

Table 2. Morphological measurement ratios used to distinguish *Thenus* species following Burton and Davie (2007)

Morphological ratios	Suggested species						
	T. indicus	T. orientalis	T. unimaculatus				
CW/CL		-	> 1.29				
ML3/CL	> 0.45	-	-				
MW1/CL	< 0.07	-	-				
MW2/CL	-	< 0.079	-				
PL1/CL	-	-	< 0.23				
PL2/CL	-	-	> 0.39				
PW1/PL1	-	-	> 0.35				
TL/TW	-	> 0.31	-				

RESULTS

The morphological examination suggested that *T. indicus* and *T. orientalis* specimens (Fig. 3A, D, respectively) possessed numerous dark brown spots on the carapace, whereas *T. unimaculatus* typically had purple spotting (Fig. 3G). The carapace of *T. orientalis* also had pink spots and pink blotches near the orbits. The pereiopods of *T. orientalis* were all distinctively spotted (Fig. 3F), while *T. unimaculatus* had a purple blotch on the inner face of the merus of 1 or more pereiopods (Fig. 3I). *Thenus indicus* lacked any spots or blotches on the pereiopods.

The COI sequence data from the 30 Thai Thenus specimens were combined with 5 existing Thenus species in GenBank resulting in a 403-bp alignment. The PAUP* analysis produced 32 most parsimonious trees with a tree length of 210 steps (Fig. 4 shows an example tree). The semistrict consensus phylogenetic tree (Fig. 5) shows that the present Thai samples were all grouped into one of 3 major species-clades previously reported from Thai waters (Burton and Davie 2007). Clade A included all Thenus samples from Chonburi (Chon01-10) and 3 specimens from Phetchaburi (Phet01, -07, and -08), and all were strongly supported (100% bootstrap support) as T. indicus (reference GenBank sequence). Clade B strongly supported (98% bootstrap support) all other specimens from Phetchaburi Province (Phet02-06, -09, and -10) as being T. orientalis. Finally, clade C included all Thenus samples from Phuket (Phuk01-10) as T. unimaculatus (with 97% bootstrap support).

The morphometric ratios of all 30 Thenus shovel-nosed lobsters (according to Burton and Davie 2007) were calculated (Table 2) and used to identify the Thenus species. The results are presented in table 3. We found that around 1/2 of all Thenus samples (16 of 30) failed to be unambiguously identified on the basis of the calculated ratios, and only 10 samples (Chon01, -03, -05, -09, -10, Phet01, -03, -07, -08, and Phuk01) correctly matched the name given following the morphological and genetic identifications. Some samples (such as Chon02 T. indicus) presented with ratios that could place it in 2 separate species according the ratios given by Burton and Davie (2007), e.g., for Chon02 the ML3 to CL ratio was 0.51 (> 0.45) correctly indicating it to be T. indicus), but its MW2 to CL ratio was 0.077 (< 0.079) which also placed it as T. orientalis. This identification problem also occurred with

samples Phet04, -06, -10, and Phuk08, as for each of these cases, the identification based on the morphometric ratios differed from that based on simple morphological characteristics such as the color pattern. In total, only 33% (10 of 30) of all *Thenus* samples were unambiguously identified using the morphometric ratio technique.

DISCUSSION

We found that morphological identification of all samples using the key provided by Burton and Davie (2007) gave the same results as the COI analysis, thus proving the basic usefulness of the key for this region (Table 3). From this finding, morphological examinations combined with molecular phylogenetic analysis could identify at least 3 species of Thenus in Thailand, i.e., T. orientalis supposedly living only on the west coast of the Gulf of Thailand, T. indicus on both eastern and western sides of the Gulf, and T. unimaculatus distributed only in the Andaman Sea with the highest endemism among the 3 species. Our suggestion on the simple recognition of T. orientalis and T. unimaculatus as 2 additional Thenus species in Thailand is to look for the specific appearance of small brown spots on the pereiopods of T. orientalis and purple-blotched pereiopods of T. unimaculatus. Furthermore, we propose a new local Thai name for T. indicus of kang-kradan thammada (common shovel-nosed lobster), kang-kradan kha-lai (spotted-leg shovelnosed lobster) for T. orientalis, and kang-kradan kha-muang (purple-blotched leg shovel-nosed lobster) for T. unimaculatus.

In Burton and Davie's study (2007), their morphometric analysis gave strong, unambiguous results, and showed all species groups to be 100% discriminated for specimens for which complete datasets were available. Given a large dataset for analysis, it is clear that a canonical analysis can discriminate any given individual, even though this is not a practical technique for rapid field or laboratory identification. Unfortunately, they also stated that there is a large degree of overlap between species when any specific ratios were considered on their own. This means that simple ratios are of limited usefulness for uniquely identifying a particular species from all others. When all 5 species were compared, the ratio of the 1st pereiopod propodus width vs. length showed the best discrimination of means, but also showed overlapping ratios between some individuals of



Fig. 3. External morphological characteristics of 3 *Thenus* species of Thailand. (A-C) Specimen Phet08 as representative of *T. indicus*. (A) Dorsal view; (B) ventral view; (C) non-spotted pereiopods. (D-F) Specimen Phet06 as representative of *T. orientalis*. (D) Dorsal view; (E) ventral view; (F) brown spots and dots on pereiopods. (G-I) Specimen Phuk02 as representative of *T. unimaculatus*. (G) Dorsal view; (H) ventral view; (I) purple blotches on pereiopods.

all species. They did however suggest that the ratios may be of some use for identifying some individuals if they were above or below certain threshold values for that species.

Such a suggestion was confirmed in our present study. We followed Burton and Davie (2007) in calculating morphometric ratios for all 30 Thai *Thenus* shovel-nosed lobsters and found a problem of using these ratios for discriminating species in the absence of color. However, with some changes in the values, the success of identification improved. For example, the ML3/CL ratio should be adjusted to be > 0.48 (up from 0.45) for *T. indicus*, and the PW1/PL1 ratio should be changed to be > 0.33 (from 0.35) to more-accurately distinguish *T. unimaculatus*. It is likely that greater numbers of specimens may be needed

to more-finely calibrate these ratios for maximum usefulness.

According to the phylogenetic tree in figure 4, several samples within clade A (*T. indicus*) show marked separation from the main group (Chon01, Phet01, and Chon08), and this perhaps indicates some populational level genetic divergence that warrants further investigation. In addition, even though there was a strong cluster (100% bootstrap support) for clade B (*T. orientalis*), samples Phet03 and Phet04 had a 4-6-bp difference from the main grouping (see branch lengths in Fig. 4).

Biogeographically, it appears from the results so far that *T. orientalis* may be more abundant on the western side of the upper Gulf of Thailand with all of the present samples being confined to Phetchaburi Province. However, Burton and Davie

2

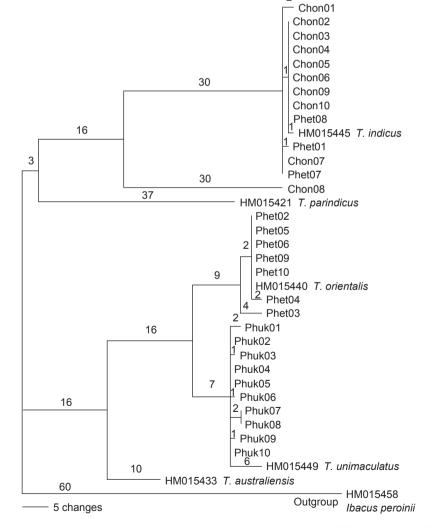


Fig. 4. One of 32 most-parsimonious trees based on the cytochrome c oxidase subunit 1 (COI) sequence of *Thenus* spp. (with a tree length of 210, a consistency index of 0.810, a retention index of 0.952, and a rescaled consistency index of 0.771).

(2007) previously reported 1 sample of this species from Pattaya, in Chonburi Province (eastern side). So it is evident that *T. orientalis* is also present on the eastern side of the Gulf, as one might expect given that its broader distribution is as far as Taiwan.

T. indicus occurs on both the eastern and western sides of the upper Gulf of Thailand (Chonburi and Phetchaburi Provinces, respectively), and the strong clustering of *T. indicus* specimens (Chon01-10 with Phet01, -07, and -08) suggests that there is gene flow between populations on both sides of the gulf. The oceanic circulation patterns in the upper Gulf of Thailand would likely widely distribute the planktonic phyllosoma larvae throughout the region. Currents move in a counterclockwise direction under the influence of the northeasterly monsoon (Nov.

to Jan.) and clockwise and counterclockwise under the influence of the southwesterly monsoon (May to Aug.) (Buranapratheprat 2008). Broader population-level studies, perhaps using microsatellites, are needed to properly investigate if there are any discrete populations elsewhere in Thai waters.

Interestingly, *T. indicus* was also recorded as far west as Pakistan and India (Burton and Davie 2007). However, while its distribution should therefore encompass the Andaman Sea coast of southern Thailand, no samples have so far been collected there. All samples from this area consisted only of *T. unimaculatus*. *T. unimaculatus*, although more-widely distributed in the Indian Ocean, is still only known from the Andaman Sea coast (Phuket Province), and has not been found in the Gulf of Thailand. This may

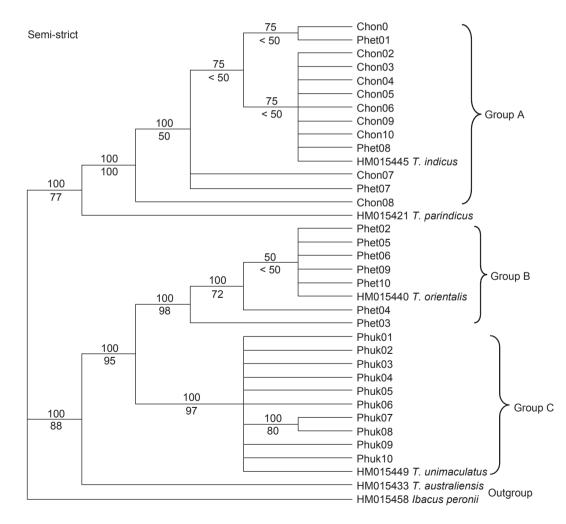


Fig. 5. Semi-strict consensus tree of the 32 most-parsimonious trees based on cytochrome oxidase subunit 1 (COI) sequences of *Thenus* spp. Numbers above the branches indicate percentages of nodes recovered in the most-parsimonious trees. Numbers below the branches show bootstrap support based on 1000 pseudoreplicates.

be the result of the deep-sea fishing fleet which trawls in the area dominated by *T. unimaculatus*, with *T. indicus* perhaps occurring in more-inshore shallower waters and thus not being taken. Further intensive sampling is needed to resolve this anomaly.

The regional geographic distribution patterns of *Thenus* species are likely to be influenced by the availability of habitat suitable for each species. For example, the preferred habitat of *T. parindicus* (referred to as the 'mud bug' in Australia) is shallow muddy inshore waters to 20 m deep, while *T. australiensis* (the sand bug) inhabits deeper, rockier areas (Jones 1993 2007). Similarly in Thailand, *T. indicus* appears to prefer shallower water than *T. orientalis*. Local fish sellers in Phetchaburi Province we interviewed reported that *T. indicus* is normally captured live by small local fishing boats in areas close to the coast. However, *T. orientalis* is usually caught by large commercial ships working in the open sea, and they must be frozen before being sent back to port. More-detailed studies of the ecological preferences of all 3 Thai species, *T. indicus*, *T. orientalis*, and *T. unimaculatus*, are urgently needed in order to enable effective conservation and management strategies to control their future exploitation.

Table 3. Morphological measurement ratios calculated from 30 live or frozen *Thenus* samples in this study. Bold numbers indicate in-range ratios which identify a species (see the criterion in Table 2). The specific names were suggested by morphological measurement ratios compared to those by morphological characteristics. Abbreviations of the specific names are: ind for *T. indicus*, ori for *T. orientalis*, and uni for *T. unimaculatus*

	Morphological measurement ratio									
Sample name	CW1/CL	ML3/CL	MW1/CL	MW2/CL	PL1/CL	PL2/CL	PW1/PL1	TL/TW	Species identified by measurement ratios	Species identified by morphological characteristics
Chon01 (live)	1.29	0.46	_ b	0.088	_ b	0.27	_ b	0.27	ind	ind
Chon02 (live)	1.29	0.51	0.09	0.077	0.28	_ b	0.19	0.25	ori, ind	ind
Chon03 (live)	1.24	0.47	0.10	0.083	0.27	0.33	0.27	0.34	ori, ind	ind
Chon04 (live)	1.29	0.45	0.10	0.083	0.27	0.35	0.30	0.25	ind	ind
Chon05 (live)	1.23	0.48	0.10	0.083	0.26	0.34	0.27	0.33	ori, ind	ind
Chon06 (live)	1.27	0.49	0.09	0.078	0.25	0.34	0.28	0.29	ori, ind	ind
Chon07 (live)	1.43	0.54	0.10	0.088	0.28	0.51	0.28	0.27	uni, ind	ind
Chon08 (live)	1.20	0.47	0.09	0.087	0.25	0.36	0.29	0.25	ind	ind
Chon09 (live)	1.24	0.47	0.10	0.084	0.26	0.35	0.30	0.29	ind	ind
Chon10 (live)	1.20	0.49	0.27	0.081	0.27	_ b	0.27	0.30	ind	ind
Phet01 (frozen)	1.25	0.56	0.35	0.080	0.29	0.36	0.21	0.26	ind	ind
Phet02 (frozen)	1.24	0.48	0.10	0.093	0.27	0.33	0.30	0.34	ori, ind	ori
Phet03 (frozen)	1.24	0.44	0.10	0.091	0.24	0.31	0.34	0.33	ori	ori
Phet04 (frozen)	1.22	0.46	0.41	0.089	0.26	0.31	0.30	0.29	ind	ori
Phet05 (frozen)	1.21	0.46	0.09	0.084	0.26	0.35	0.31	0.32	ori, ind	ori
Phet06 (frozen)	1.21	0.45	0.09	0.085	0.26	0.32	0.33	0.29	ind	ori
Phet07 (frozen)	1.28	0.53	0.09	0.088	0.27	0.32	0.26	0.29	ind	ind
Phet08 (frozen)	1.27	0.52	0.10	0.080	0.29	0.38	0.27	0.27	ind	ind
Phet09 (frozen)	1.22	0.44	0.09	0.080	0.25	0.31	0.30	0.28	_ a	ori
Phet10 (frozen)	1.24	0.46	0.09	0.086	0.26	0.32	0.29	0.31	ind	ori
Phuk01 frozen)	1.34	0.43	0.13	0.110	0.28	0.35	0.34	0.28	uni	uni
Phuk02 frozen)	1.27	0.38	0.12	0.115	0.24	0.33	0.38	0.34	ori, uni	uni
Phuk03 frozen)	1.27	0.40	0.12	0.099	0.25	0.30	0.36	0.37	ori, uni	uni
Phuk04 frozen)	1.22	0.38	0.11	0.100	0.24	0.31	0.35	0.35	ori, uni	uni
Phuk05 frozen)	1.23	0.38	0.11	0.097	0.23	0.29	0.42	0.36	ori, uni	uni
Phuk06 frozen)	1.21	0.36	0.10	0.098	0.23	0.30	0.38	0.32	ori, uni	uni
Phuk07 frozen)	1.26	0.38	0.11	0.097	0.24	0.30	0.37	0.36	ori, uni	uni
Phuk08 frozen)	1.23	0.40	0.12	0.100	0.27	0.32	0.33	0.34	ori	uni
Phuk09 frozen)	1.20	0.35	0.12	0.109	0.22	0.29	0.41	0.32	ori, uni	uni
Phuk10 frozen)	1.24	0.36	0.11	0.097	0.25	0.31	0.37	0.36	ori, uni	uni

^aPhet09 specimen was not identifiable to species by the measurement ratio. ^bSome ratios could not be calculated because of the loss of the 1st and 2nd pereiopods.

Acknowledgments: This project was financially supported by the A1B1-MICO (TRF) Research Fund and the Research Program on Conservation and Utilization of Biodiversity and the Center of Excellence in Biodiversity, Faculty of Science, Chulalongkorn Univ., the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, and the 90th Anniversary of Chulalongkorn Univ. Fund (Ratchadaphiseksomphot Endowment Fund). Special thanks go to Prof. Emeritus Phaibul Naiyanetr of Chulalongkorn Univ. for his suggestions on sample collection and taxonomy.

REFERENCES

- Buranapratheprat A. 2008. Circulation in the upper gulf of Thailand: a review. Burapha. Sci. J. **13(Supplement 1)**: 75-83.
- Burton TE, PJF Davie. 2007. A revision of the shovel-nosed lobsters of the genus *Thenus* (Crustacea: Decapoda: Scyllaridae), with descriptions of three new species. Zootaxa **1429:** 1-38.
- Department of Fisheries. 1997. Fish and other aquatic animals of Thailand. 3rd ed. Bangkok: Kurusapha. (in Thai)
- FAO. 2010. Fishery statistical collections: global production. Food and Agriculture Organization (FAO) of the UN.
- Folmer O, M Black, W Hoeh, R Lutz, R Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. **3:** 294-297.
- Holthuis LB. 1991. FAO species catalogue vol. 13: marine lobsters of the world. An annotated and illustrated catalogue of species of interest to fisheries known to date.

FAO Fisheries Synopsis 125(Supplement 13): 292.

- Jones CM. 1993. Population structure of *Thenus orientalis* and *T. indicus* (Decapoda: Scyllaridae) in northeastern Australia. Mar. Ecol. Progr. Ser. 97(Supplement 2): 143-155.
- Jones CM. 2007. Biology and fishery of the bay lobster, *Thenus* spp. *In* KL Lavalli, E Spanier, eds. The biology and fisheries of the slipper lobster. Crustacean issues Vol. 17. Boca Raton, FL: CRC Press, pp. 325-358.
- Larkin MA, G Blackshields, NP Brown, R Chenna, PA McGettigan, H McWilliam et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948.
- Liu MY, YX Cai, CS Tzeng. 2007. Molecular systematics of the freshwater prawn genus *Macrobrachium* Bate, 1868 (Crustacea: Decapoda: Palaemonidae) inferred from mtDNA sequences, with emphasis on East Asian species. Zool. Stud. **46**: 272-289.
- Naiyanetr P. 1963. Scyllarid lobsters and their phyllosoma larvae in the Gulf of Thailand. MSc thesis, Department of Biology, Faculty of Science, Chulalongkorn Univ., Bangkok, Thailand.
- Naiyanetr P. 2007. Checklist of crustaceans fauna in Thailand (Decapoda, Stomatopoda, Anostraca, Myodopoca and Isopoda). 2nd ed. Bangkok, Thailand: Office of Natural Resources and Environmental Policy and Planning, 196 pp.
- Shih HT, H Suzuki. 2008. Taxonomy, phylogeny, and biogeography of the endemic mudflat crab *Helicel* chasmagnathus complex (Crustacea: Brachyura: Varunidae) from East Asia. Zool. Stud. 47: 114-125.
- Sungthong S. 1979. Annual report. Tagging experiment of sand lobsters rearing in aquarium. Bangkok, Thailand: Department of Fisheries. (in Thai with English summary)
- Swofford DL. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Vers. 4. Sunderland, MA: Sinauer Associates.
- Uraiwan S. 1977. Annual report. Biological study of *Thenus* orientalis in the Gulf of Thailand. Bangkok, Thailand: Department of Fisheries. (in Thai with English summary)