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SHORT PAPER

Genetic Evidence and Morphometry for Shovel Nosed Lobster, *Thenus Unimaculatus* from Andaman and Nicobar Islands, India

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Abstract

Since Burton & Davie revised the monotypic genus Thenus, the genus is represented by two species in Mainland India, *Thenus unimaculatus* (Burton and Davie, 2007) and *Thenus indicus* (Lund, 1793). In this study, we are reporting the occurrence of T. unimaculatus for the first time from Andaman and Nicobar Islands. We have investigated the species based on morphological characters and morphometric ratios following original description of T. unimaculatus. All the morphological characters and most of the morphometric ratios were found to coincide with the previous description. Mitochondrial COI gene sequencing also supports the landings of T. unimaculatus in these Islands.

Keywords: Morphology, ratios, lobster, mtDNA, COI gene.

Introduction

Lobsters fetch high price in domestic and international markets. *Thenus* is the only genus in 7 scyllarid genera of lobsters that is economically significant (Jones, 1990). *Thenus* Leach, 1815 in the family Scyllaridae Latreille, 1825 was considered to be a monotypic genus with only one species, *Thenus orientalis* (Lund, 1793) (Holthuis, 1985, 1991; Chan, 1998). The genus *Thenus* was revised and is now known to contain five species (Burton and Davie. 2007) *viz*. *Thenus indicus* Leach, 1815, *Thenus orientalis* (Lund, 1793), *Thenus australiensis* Burton & Davie, 2007, *Thenus unimaculatus* Burton and Davie, 2007 and *Thenus parindicus* Burton and Davie, 2007. Burton & Davie (2007) described *T. unimaculatus* based on the morphological and mitochondrial DNA sequence analysis. This species is currently known to be distributed in locations in the Indo-West Pacific Oceans near Thailand, the United Arab Emirates and Mozambique (Burton and Davie 2007), Thailand (Iamsuwansuk *et al*., 2012; Wongruenpibool and Denduangboripant, 2013) and India (Jeena *et al*., 2011).

The estimated total lobster landings of India during 2014-15 was 1568 t. Of the 30 species of lobsters belonging to 5 families occurring in India, 10 species belonging to 3 families (Palinuridae, Scyllaridae and Nephropidae) have been reported from Andaman and Nicobar Islands (Kumar *et al*., 2010) which is a Union territory of India enjoying the status of an archipelago with over 550 islands, islets and rocky outcrops in the Bay of Bengal, lying between 6°45' N and 13°41' N lat and between 92°12' E and 93°57' E long. With a land area of only 8293 sq. km, it has a total coastline of 1912 km which is about one-fourth of the total coastline of India. Lobsters are mainly used for catering the needs of tourists or shipped to mainland in live/frozen form. During 2013-14, 3.4 tons of lobsters were exported to mainland India (Fisheries Department, A&N Administration *pers comm*). Shovel nosed lobster belonging to the genus *Thenus* is one among the crustacean resources landed in multiday demersal trawlers operating in Andaman and Nicobar Islands. The genus *Thenus* is represented by two species in mainland India, viz., *T. unimaculatus* (Radhakrishnan *et al*., 2013) and *T. indicus* (Jeena, 2013). The species of shovel nosed lobster belonging to family Scyllaridae reported from the islands to date is *T. orientalis* (Shanmughan and Kathirvel, 1983). In the present study, *T. unimaculatus* is reported for the first time from Andaman and Nicobar Islands and investigations were carried out on this species based on the integrated taxonomic approach combining morphometry with mitochondrial COI gene sequencing to derive further confirmation of the occurrence.

Material and Methods

Shovel nosed lobsters were collected from trawl landings at $11^{0}39.25^{`}$ N and $92^{0}$ $43.30^{`}$ E, Port Blair (Figure 1) in Andaman and Nicobar Islands from November 2014 to April 2015. Samples were collected twice a month and a total of 64 samples in the size range of 45 mm to 85 mm carapace length were analysed for measurements. Species identification was done according to Burton and Davie (2007) for the identification of *Thenus* species following morphological characters along with morphometry (Table 1). All the morphometric measurements were taken using digital vernier calipers (Mitutoyo 500-197-30 AOS Digimatic Caliper). Muscle and pereiopods of representative samples (five numbers) preserved in 95% ethanol were used for DNA extraction.

Genomic DNA was extracted following the standard phenol chloroform method (Sambrook and Russell. 2001). Amplification of partial sequences COI genes was accomplished using primer set LCO1490 (GGTCAACAAATCATAAAGATATTGG)/ HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer *et al*., 1994). PCR reactions for gene amplifications were carried out in BIORAD T100 ™ thermal cycler (Biorad, USA). All the reactions were performed in 25µl reactions containing 2.5 µl 10x assay buffer, 1.5 µl $MgCl_2$ (1.5µM), 0.5µl of 10µM of each primer, 0.5 µl, 10µM dNTPs, 1 U Taq DNA polymerase (Sigma Aldrich, USA) and 1µl of 50-100 ng template DNA. The PCR cycling profiles were as follows: 4min at 94°C for initial denaturation, 30 cycles of denaturation for 30s at 94°C, 30s annealing at 42 °C, 45s extension at 72°C, and a final extension for 7 min at 72°C. The PCR products were visualized on 1.5% agarose gels. All samples were sequenced and the raw DNA sequences were edited using BioEdit sequence alignment editor version 7.2.5 (Hall, 1999). The sequences were compared to the GenBank database using the NCBI BLAST server. The software MEGA version 6 (Tamura *et al*., 2013)

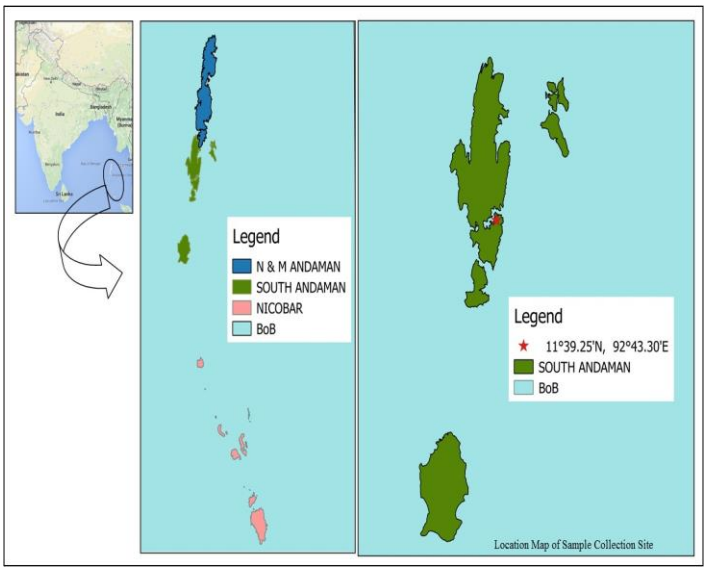

Figure 1. Location map of sample collection site.

Table 1. List of measurements used for determining morphometric ratios

CL	Length of carapace
CW	Width of carapace at widest section
A1L	Length of antenna 1
A1W	Width of antenna 1
A2L	Articulation notch on posterior margin of antenna 2
A2W	Width of antenna 2
PL1;PL2	Length of propodus on the first and second pereopod respectively
PW1	Width of propodus on the first pereopod
ML3	Length of merus on the third pereopod
MW1, MW2	Width of merus on the first and second pereopod respectively
TL	Length of telson
TW	Width of telson

These measurements were used for finding out the morphometric ratios according to Burton and Davie, 2007

was employed for calculating the sequence divergence values.

Results

In the present study, all the 64 samples of shovel nose lobsters collected from trawl landings were identified as *Thenus unimaculatus* based on the purple blotches on carapace and the inner face of one or more pereiopods (Figure 2), spine on merus of third maxilliped, dentition on ishium of third maxilliped (Figure 3) and setae on the propodus of second pereopod. Based on our findings, a paratype specimen [ZSI/ANRC-12469] was deposited with the Andaman and Nicobar regional centre of Zoological Survey of India, Port Blair. The morphometric ratios of *T. unimaculatus* collected from Andaman and Nicobar Islands were compared with the earlier reports and are presented in Table 2. The morphometric ratios of TL/TW, CW/CL, MW2/CL, A1L/A1W, A2L/A2W, A2L/CL, PL1/CL, PL2/CL, PW1/PL1, MW1/CL and ML3/CL were in the range of 0.23-0.33, 1.17-1.34, 0.076-0.112, 0.59-0.77, 0.55-0.67, 0.25-0.37, 0.22-0.27, 0.24-0.34, 0.21-0.38, 0.088-0.127 and 0.34-0.43 respectively based on our study. The values of five morphometric ratios (A2L/CL, PL1/CL, PL2/CL, PW1/PL1, MW1/CL and ML3/CL) were within the range observed for the species (Burton and Davie. 2007). The morphometric ratios of TL/TW, CW/CL, MW2/CL, A1L/A1W and A2L/A2W showed 16, 1, 6,

Figure 2. Dorsal and Ventral view of *Thenus unimaculatus.*

Figure 3. Third maxilliped of *Thenus unimaculatus* with spine on merus and dentition on ischium.

2 and 3 outlier measures respectively which differed from the results of Burton and Davie (2007). Linear regression between measurements of these morphometric values were described by linear regression equation, y = mx+c with their corresponding r-square values after removal of outlier values (Figure 4a-4e). The sequences were submitted to GenBank (www.ncbi.nlm.nih.gov) [GenBank: KT362350, KT362351, KT362352, KT362353 and KT362354]. The COI gene sequences (628bp) when compared with GenBank submissions of the species showed high similarity with samples from Andaman Sea of Thailand [GenBank: JN 165736- JN 165745]. K2P genetic distance of 1.2% was observed with *T. unimaculatus* from India [GenBank: JQ 229927-JQ 229938].

Discussion

All the 64 samples of shovel nose lobsters collected from multiday trawlers at regular time interval were identified as *T. unimaculatus* following the morphological characters as described by Burton and Davie (2007). The morphometric analysis by Burton and Davie (2007) gave strong, unambiguous results, and showed all species groups to be 100% discriminated for specimens with complete datasets (Iamsuwansuk *et al*., 2012). About 96% of measurements in this study fell under the values described by Burton and Davie (2007). We also found 23%, 1.6%, 9.4%, 3.1% and 4.7% outlier values in the individual ratios of TL/TW, CW/CL, MW2/CL, A1L/A1W and A2L/A2W respectively. Iamsuwansuk *et al*. (2012) during their morphological investigations on *Thenus* spp. also presented values of morphometric ratios of *T. unimaculatus* different from that of Burton and Davie (2007). But the morphometric ratios employed by them in their study for identification of *T. unimaculatus* had a lot of differences from the present study further more from

Table 2. Comparison of morphometric ratios of *Thenus unimaculatus* from Andaman & Nicobar Islands with earlier reports

	Present study		Earlier reports	
Morphometric ratios	Mean±sd	Range	Burton and Davie. 2007	Iamsuwansuk *et al*., 2012
Length of telson / Width of telson (TL/TW)	0.273±0.025	0.23-0.33	≤ 0.29	
Width of carapace / Length of carapace (CW/CL)	1.226±0.029	1.17-1.34	1.18-1.4	> 1.29
Width of merus on the second pereopod / Length of carapace (MW2/CL)	0.093±0.008	0.076-0.112	≥ 0.083	
Length of antenna1 / Width of antenna 1 (A1L/AW1)	0.654±0.03	0.59-0.77	≤ 0.7	
Articulation notch on posterior margin of antenna 2 / Width of antenna 2 (A2L/A2W)	0.595±0.022	0.55-0.67	≤ 0.63	
Articulation notch on posterior margin of antenna 2 / Length of carapace (A2L/CL)	0.318±0.016	0.25-0.37	≤ 0.37	
Length of propodus on the first pereopod / Length of carapace (PL1/CL)	0.249±0.011	0.22-0.27	≥ 0.19	< 0.23
Length of propodus on the second pereopod /Length of carapace (PL2/CL)	0.307±0.016	0.24-0.34	≤ 0.45	> 0.39
Width of propodus on the first pereopod / Length of propodus on the first pereopod (MW1/PL1)	0.29±0.027	0.21-0.38	≤ 0.42	> 0.35
Width of merus on the first pereopod / Length of carapace (MW1/CL)	0.109±0.009	0.088-0.127	≥ 0.088	
Length of merus on the third pereopod / Length of carapace (ML3/CL)	0.382±0.015	0.34-0.43	≤ 0.44	

*Morphometric ratios of *Thenus unimaculatus* from Andaman & Nicobar Islands were compared with the similar ratios of the same species from the published reports of Burton and Davie (2007) and Iamsuwansuk et al., 2012

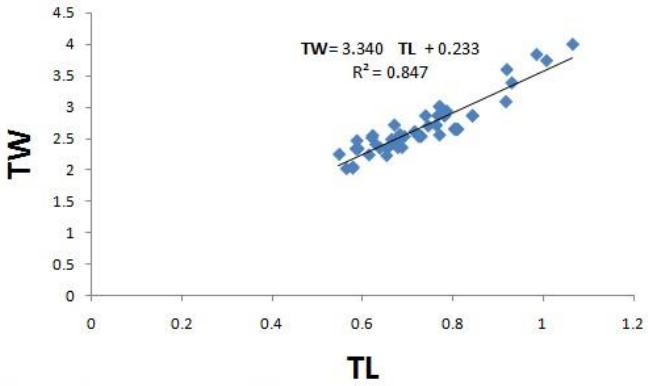

Figure 4a. Scatter plot of TL and TW.

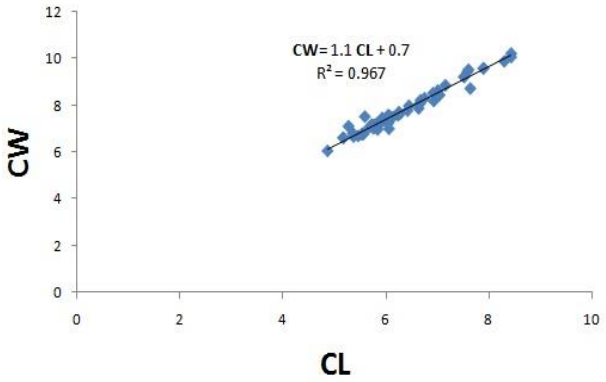

Figure 4b. Scatter plot of CL and CW

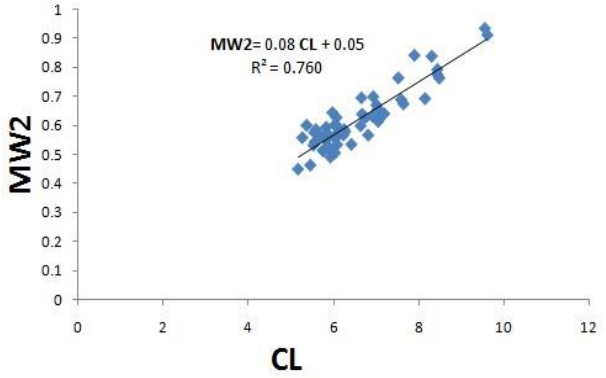

Figure 4c. Scatter plot of CL and MW2.

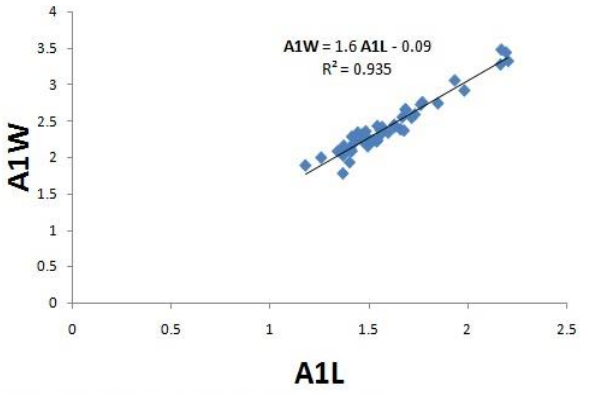

Figure 4d. Scatter plot of A1L and A1W.

Burton and Davie (2007). Altogether only 3 values of morphometric ratios of this study established relation with the values of Iamsuwansuk *et al*. (2012). Nevertheless we found the morphometric ratios of A2L/CL, PL1/CL, PL2/CL, PW1/PL1, MW1/CL and ML3/CL were within the ratios of Burton and Davie (2007). In our study, the maximum ratio for TL/TW was found to be 0.33 which suggest that the value of this ratio need to be increased. During the period of our study, we did not come across any samples of *T. orientalis* and all samples were having the morphological and morphometric characters similar to *T. unimaculatus.* Further the genus *Thenus* was monotypic with only one species at the time of reporting *T. orientalis* by Shanmughan and Kathirvel (1983) from Andaman and Nicobar Islands. This points undoubtedly that the species of *Thenus* dominantly occurring in seas near Andaman and Nicobar Islands is *T. unimaculatus*. The present work also supports the fact that species of *Thenus* commonly occurring along Indian coast is *T. unimaculatus* (Jeena *et al*., 2011). COI gene is widely employed in molecular studies in *T. unimaculatus* (Burton and Davie, 2007; Palero *et* al., 2008; Jeena *et*

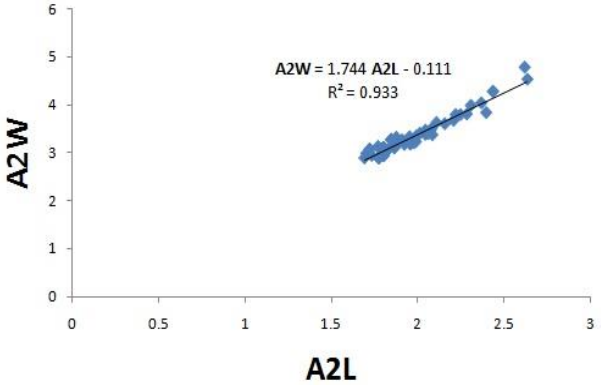

Figure 4e. Scatter plot of A2L and A2W.

al., 2011; Iamsuwansuk *et al*., 2012; Wongruenpibool and Denduangboripant, 2013; Jeena *et al*., 2016a; Jeena *et al*., 2016b). DNA barcoding using the COI gene confirmed the species identity and supported the occurrence of *T. unimaculatus* in this region. The close similarity to GenBank submissions from Andaman sea of Thailand might be due to the circulation patterns of sea currents occurring in this region.

The presence of identical morphological characters and 96% similar morphometric values similar to previous workers and mitochondrial sequencing supports the incidence of *T. unimaculatus* in Andaman and Islands. Based on our investigations using both morphometric and genetic tool, we conclude that the shovel nosed lobsters landed in Andaman and Nicobar Islands is *T. unimaculatus*. This paper describes *T. unimaculatus* from Andaman and Nicobar Islands genetically, morphologically and morphometrically and can facilitate in the future studies for delineating different *Thenus* spp besides helping in population genetic studies between mainland India and Andaman and Nicobar Islands.

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