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1. Crustacean Fisheries Division, Central Marine Fisheries Research Institute, Ernakulam North, Cochin, Kerala, India 2. Department of Biosciences, Mangalore University, Mangalagangothri, Karnataka State, India Notes on *Plesionika alcocki* (Anderson, 1896) and *Plesionika narval* (Fabricius, 1787) from the southern coast of India

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### Abstract

The present study provides the taxonomic information on two deepsea caridean shrimps *Plesionika alcocki* and *Plesionika narval. P. alcocki* is the new record from the southeast coast of India (fishing off Bay of Bengal, 8°47′40″N, 78°09′37″E) obtained in the depth of 200-300 m. The species *P. narval* obtained from Kalamuku fish landing centre (fishing off Arabian Sea, 9°59′02.91″N 76°14′33.14″E) from deepsea bottom trawlers between 250-300 m depth. In addition to this the phylogenetic tree of 16S and COI mitochondrial genes of the Indian *Plesionika* was provided.

Keywords: Plesionika alcocki, deepsea shrimps, COI, 16S, India

## 1. Introduction

Plesionika Bate, 1888 [1] represent a major group under the family Pandalidae with 93 species worldwide distributed in the tropical and temperate oceans [2]. Most of the species under the genus feed on both pelagic and benthic resources. The genera Plesionika contributes majorly to the commercial landing of deepsea shrimps from the southern coast of India. From the Indian waters, 15 species under the genera *Plesionika* are recorded, namely *P. alcocki*, *P.* bifurca, P. ensis, P. martia, P. ocellus, P. quasigrandis, P. unidens, P. sindoi, P. williamsi, P. adensameri and P. longicuda, P. narval, P. reflexa, P. semilaevis, P. persica [3-6]. The species P. alcocki Anderson, 1896 [7] which is widely distributed in the southwest coast off Arabian Sea and southeast Andaman Island off Bay of Bengal at the depth between 496 – 1093 m [8-10]. Here we report the first record along the Tamil Nadu coast off Bay of Bengal in the lower depth range between 200 - 300 m and on the species P. narval [11] a short technical note was recorded from southern India [4]. In particular, targeted deepsea shrimp fishing started in the early 1990s in the Indian EEZ. Considering the tropical importance of sustainability in deepsea fisheries, the present study gathers detailed taxonomic information on pandalid shrimp resources of India with special importance on P. alcocki and P. narval together with molecular barcoding data.

## 2. Materials and Methods

The samples were collected from deepsea bottom trawlers with a mesh size of 20-26 mm in its cod end operated along southern coast of India at depths of 200-350 m. The specimens were obtained from two major fish landing centers along the southern coast of India during 2014-2015. The species *P. alcocki* from the southeast coast was obtained during the period of January 2015 while from the southwest coast the species *P. narval* was obtained in the month of April 2014. The specimens were preserved in 95% ethanol and provided the voucher number and deposited at Central Marine Fisheries Research Institute (CMFRI), Cochin, India. Partial sequences of mitochondrial genes (COI & 16S rDNA) were amplified using universal primers [12, 13]. PCR purified products were sequence by dideoxy chain termination method [14] using ABI Prism 3770 automated sequences from Scigenom, India. For phylogenetic analysis, the Maximum-Likelihood (ML) method was used for individual gene sequences to compare the similarity between tree topology and MEGA 7.0 was used to select the best-fit model for individual and combined data.

Corresponding Author: Rekha Devi Chakraborty Crustacean Fisheries Division, Central Marine Fisheries Research Institute, Ernakulam North, Cochin, Kerala, India General time-reversible model with a gamma distribution and invariable sites (GTR+G+I) (COI), and Tamura–Nei model with a gamma distribution and invariable sites (TrN+G+I) (16S) were used to generate ML gene trees with 1000 bootstrap replicates [15, 16]. The morphological identification keys, mainly followed the descriptions by [8, 17-19, 9] for the present work.

### 3. Results

In the present study two species viz., *Plesionika alcocki* (Anderson, 1896) are first time reported from the coastal waters of Tamil Nadu, India and *Plesionika narval* (Fabricius, 1787) with detailed taxonomic and distribution notes from the southwest coast of India. Along with the phylogenetic tree of 16S and COI mitochondrial genes of the Indian *Plesionika* was provided.

## 4. Systematic accounts

## 4.1 Plesionika alcocki (Anderson, 1896) - Fig 1

*Pandalus alcocki* — Anderson, 1896:92 [type-locality: Laccadive Sea off Malabar Coast of India; 9°34'57"N 75°36'30"E; 743 meters].

*Pandalus (Plesionika) alcocki* — Alcock and McArdle, 1901, pi. 52: figs. 2, 4.

*Plesionika alcocki*: De Man, 1920: 105; Balss, 1925: 278, fig. 48; Mohamed and Suseelan, 1973; Chace, 1985: 55; Hayashi, 1986: 127, 270, fig. 81; Kensley, Tranter & Griffin, 1987: 313; Dave, 2002: 347; Fransen, 2006: 62-65, fig.17.

**Material examined:** India, Tamil Nadu, Tuticorin fishing port, fishing off Bay of Bengal, 8°47′40″N, 78°09′37″E, 200-300 m, Jan 2015, 3 males CL 22-25 mm, (CMFRI).

**Description:** Rostrum armed dorsally with 4-6 teeth, ventrally armed with 8-23 teeth becoming obscure anteriorly; orbital margin rather regularly concave; abdomen without posteromesial tooth or median dorsal carinae on 3<sup>rd</sup> somite, 4<sup>th</sup> somite pleuron rounded, without marginal denticle, 5<sup>th</sup> somite pleuron rather sharply acute posteroventrally; telson with 4 pairs of dorsolateral spinules, including a pair adjacent to lateral pair of posterior spines; stylocerite rather narrowly acute; antennal scale long as wide, distolateral tooth not reaching level of distal margin of blade; third maxilliped with epipod; pereiopods with epipods on 4 anterior pairs, 3<sup>rd</sup> pair overreaching antennal scale by lengths of dactylus, propodus, and 1/3 of carpus, dactyl about ½ as long as propodus, none of the pereiopods extremely slender or thread like.

Coloration: Deep red to pale orange

**Distribution:** Western Indian Ocean: East Africa, Gulf of Aden, Maldives, Bay of Bengal (BOB) and Andaman Sea, Indonesia, Japan and Philippines; at depths between 287 and 1170 m [8, 20, 17, 2]. The present specimens were obtained from the southeast coast, off the BOB, depths of 200-300 m from India.

**Remarks:** The present specimen shows similarity with the identification key proposed to this species <sup>[8, 17]</sup>, particularly with the following diagnostic characters: Rostrum armed dorsally with 4-6 teeth, ventrally armed with 8-23 teeth becoming obscure anteriorly; abdomen without posteromesial tooth or median dorsal carinae on 3<sup>rd</sup> somite, 4<sup>th</sup> somite pleuron rounded, without marginal denticle, 5<sup>th</sup> somite pleuron rather sharply acute posteroventrally; telson with 4

pairs of dorsolateral spinules. In the present study, three male specimens were obtained at a depth of 200-300 m, while in the earlier records from Indian water it has been reported at higher depths of 496-1093 m.

**Genetic distance:** COI (accession number: KX530799, KX530800) and 16S (accession number: KX364188) sequences of *P. alcocki* were submitted to GenBank. The sequence lengths are 618 and 464 bp for cytochrome c oxidase I (COI) and 16s rDNA genes, respectively. The intraspecies genetic divergence between the present specimen and other sequences retrieved from NCBI was recorded for COI (KP759494 & JX681738: 23% & 24%) and 16S (KX364188 & KX364189: 10% & 11%).

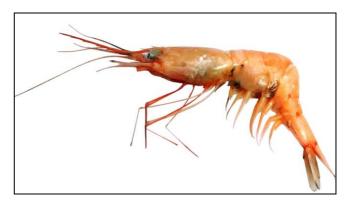


Fig 1: Plesionika alcocki, location: Tuticorin, southeast coast, off Bay of Bengal, India, depth 200-300 m

## 4.2 Plesionika narval (Fabricius, 1787) – Fig 2 (a-k)

Astacus narval Fabricius, 1787: 331 (type locality: probably Nice, Mediterranean).

*Parapandalus serratifrons*: De Man, 1920: 146, Pl. 12, fig. 34a, c, Pl. 13, fig. 34, 34b, d, e (non Borradaile, 1900).

Parapandalus spinipes: Kubo, 1965: 611, fig. 958 (non Bate, 1888).

Parapandalus narval: Crosnier and Forest, 1973: 221, fig. 69a; Crosnier, 1976: 235, fig. 4b.

*Plesionika serratifrons*: Chace, 1985: 121, figs. 55, 56; Hayashi, 1986: 139, 274, fig. 89 (non Borradaile, 1899).

*Plesionika narval*: Lemaitre and Gore, 1988: 385, figs. 3k-m, 4; Chan and Crosnier, 1991: 443, figs. 12a-c, 13a, 14a-c, 15a-e, 34-36; Miyake, 1998: 61, Pl. 21, fig. 1; Li and Komai, 2003: 265; Li, 2006a: 370; Li and Davie, 2006: 161.

**Material examined:** India, Kerala, Kalamukku fishing port, City of Kerala, fishing off 9°59'02.91"N 76°14'33.14"E, 250-300 m, 4<sup>th</sup> April 2014, 14 males CL 10-15 mm, 7 ovigerous females CL 13-14 mm, 5 non ovigerous females CL 13-15. Voucher specimen (ED.2.4.3.4) was deposited in Marine Biodiversity Referral Museum of Central Marine Fisheries Research Institute, Cochin, India.

**Description:** Body size is usually small. The morphological analysis of the specimens was performed using conventional methods, revealed the absence of denticle on the 4<sup>th</sup> abdominal pleura; rostrum is 1.7-1.8 times as long as carapace and the dorsal margin armed with 48-61 teeth over the entire length, including 5-6 teeth on carapace posterior to the level of orbital margin, ventral margin armed with 33-38 teeth, posterior 10 ventral teeth corresponding to 15 dorsal teeth. Eye diameter 0.1-0.3 times of carapace, cornea broader than the eyestalk, antennular peduncle with stylocerite acute barely

overreaching 1st antennular segment, antennal scale 4.7-5.3 times as long as wide with distolateral tooth overreaching rounded blade. Antennal spine stronger while pterygostomian spine was found to be weak. Abdomen with 3<sup>rd</sup> somite rounded posteriorly unarmed, without median dorsal carinae, pleura of 3<sup>rd</sup> and 4<sup>th</sup> somite rounded while that of 5<sup>th</sup> is acute. Sixth abdominal somite is 2.2-2.6 times as long as maximum height. Telson is 1.1 times as long as 6th abdominal somite and armed with 3 pairs of dorsolateral spinules and two pairs of longer lateral spines were found at its posterior end. Maxilliped III with well-developed epipod, overreaching antennal scale and its penultimate segment (9 mm) is almost 1.8 times than terminal segment (5 mm), terminal and penultimate segments combined 1.1-1.3 times as long as carapace (12 mm) and the ultimate segment is equal to carapace length. Pereiopods without epipods extremely slender and thread like.

Coloration: Body and appendages generally reddish, body with 1 subdorsal and 2 lateral red coloured longitudinal stripe extending along almost entire body length from anterior carapace to posterior abdomen on both sides, subdorsal stripe running from upper orbital margin and fading on abdominal somite IV and V, 1 lateral stripe running from antennal spine to tailfin another running from pterygostomian spine to uropod, slightly faded than subdorsal stripe, rostrum red, margins and teeth white; antennular flagellum white, basal portion of antennal flagellum, anterior appendages and basal parts of posterior appendages light red to white; pereiopods red distally and pink proximally, uropods translucent with orange-red margins; eggs pale blue.

**Distribution:** Indo-West Pacific from Madagascar to French Polynesia, Japan, Korea, Mediterranean, East Atlantic from Gibraltar to Cape Verde Islands, South Atlantic, Red Sea, and northward to Japan, at depths of 35-400 m. Appeared in stray numbers among the deepsea shrimp landed in Kerala, India.

**Remarks:** *Plesionika narval* is recorded for the first time from India (off the Arabian coast). Overall 26 specimens were segregated from the deepsea shrimp discards which include 14 male and 12 female were obtained. Total length, carapace length, rostral length, weight in male and female ranged from 54-73 mm, 10-15 mm, 15-24 mm, 0.6-1.2 g and 65-78 mm, 13-18 mm, 17-25 mm, 0.8-1.5 g, respectively. Of the total 12 female specimens recorded 5 were non-berried and the rest were ovigerous. The fecundity in *P. narval* ranged from 162 to 698 (TL: 70-78 mm, CL: 13-14 mm and weight: 1.1-1.5 g) with an average width of the egg was found to be 0.2 mm. The eggs were spherical in shape with bluish green colour in the freshly acquired berry. The present specimens agree well with the diagnosis [19].

**Genetic distance:** COI (accession number: KP398863, KP398864) and 16S (accession number: KM057378, KM047390, KM047389, KP398866) sequences of *P. narval* were submitted to GenBank. The intraspecies genetic divergence between the present specimen and other sequences retrieved from NCBI varied for COI (KJ670310, JQ305934, JN412729, JQ305933: 15% - 16%) and 16S (KJ670313, JN412691 & MK470812: 8% - 16%) while the intraspecies genetic distance for the Indian *P. narval* was negligible the COI (0%) and 8 – 13% in 16S.

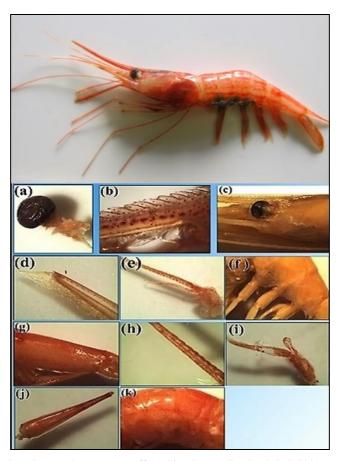


Fig 2: Plesionika narval, location: Kalamuku, southwest coast, off Arabian Sea, India, depth 250-300 m, (a) Eye tubercle; (b) Rostral teeth; (c) Antennal spine; (d) Stylocerite (tip); (e) Third maxilliped (exopod); (f) Posterior ventral abdomen (III, IV, V); (g) Abdomen (VI); (h) Second pereiopod (left) carpels; (i) First pleopod (right); (j) Telson; (k) Presence of bands in pleuron.

### 5. Molecular results

## 5.1 Cytochrome Oxidase subunit I (COI) Taxon diversity of the southern coast of India

Overall 29 molecular sequences of COI for five species of genera *Plesionika* were presented, the identified species are *Plesionika quasigrandis*, *Plesionika narval*, *Plesionika semilaevis*, *Plesionika alcocki* and *Plesionika reflexa* (Table 1). The amplified sequence length varied from 665 bp in *P. reflexa* to 652 bp in *P. narval*. The overall mean distance of individuals among the genera was estimated as 13%. The maximum interspecific K2P distance was 33% between *P. semilaevis* and *P. alcocki* and minimum was 21% divergence between *P. reflexa* and *P. semilaevis*. The minimum and maximum intraspecies genetic distance (0%) was observed between the *Plesionika* species. Moreover, the recently

misidentified *Plesionika quasigrandis* from the Indian water <sup>[22]</sup> (n=21) were collected from a wide geographic area and described off Kerala coast; Cochin and Kollam (Arabian Sea) and off Tamil Nadu coast; Tuticorin (Bay of Bengal). Between the southwest and southeast coast the genetic distance was 0% within the intraspecies. The other species *Plesionika narval, Plesionika reflexa* and *Plesionika semilaevis* (n=2, each) obtained off Kerala and *Plesionika alcocki* (n=2) which was obtained from off Tamil Nadu coast were studied for genetic analysis. The phylogenetic tree was also constructed based on ML which shows that *P. quasigrandis, P. reflexa* and *P. semilaevis* conquer well in one clade while *P. narval* and *P. alcocki* formed a separate cluster distinctly away from the major cluster.

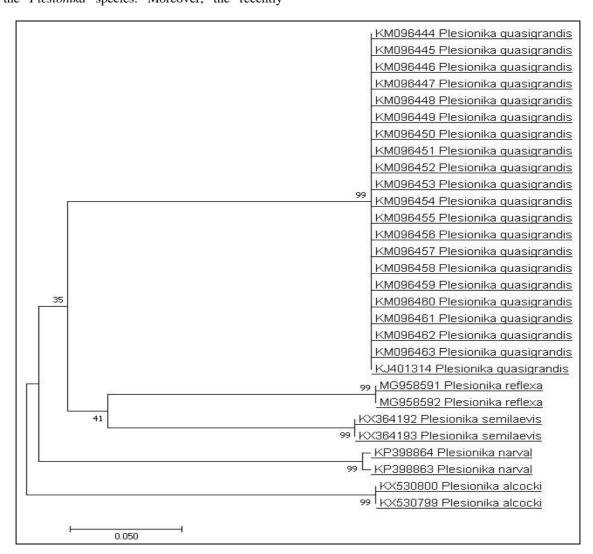


Fig 3: Phylogenetic tree of the genera *Plesionika* from the southern coast of Indian using Maximum-Likelihood with 1000 bootstraps under the best fitting model GTR+G+I inferred from DNA Sequences of mitochondrial gene COI

### 5.2 16S rRNA analysis

The genus *Plesionika* with five species are presented, namely *Plesionika quasigrandis* (n=23), *Plesionika narval* (n=2), *Plesionika alcocki* (n=2), *Plesionika reflexa* (n=4) and *Plesionika semilaevis* (n=2). In total 33 molecular sequences of these five species are obtained and the maximum length varied from 644 bp in *Plesionika reflexa* to a minimum of 484 bp in *Plesionika narval*. The overall mean distance of individuals among the genera was estimated as 0.11 (11%). The maximum interspecific K2P distance was 0.32 (32%) between *Plesionika narval* and *Plesionika semilaevis* and

minimum was 0.15 (15%) between *Plesionika reflexa* and *Plesionika semilaevis*. The intraspecific genetic distance between various *Plesionika* species varied from 0-1% (*P. quasigrandis* (0%), *P. narval* (1%), *P. reflexa* (0%), *P. semilaevis* (0%) and *P. alcocki* (1%)). The phylogenetic tree was constructed using ML with 1000 bootstraps, showed the grouping of all the five species of genera *Plesionika* in one cluster with three sister lineages. There was a distinct separation of *P. alcocki* from remaining four species of the genera *Plesionika* (*P. quasigrandis*, *P. narval*, *P. semilaevis* and *P. reflexa*).

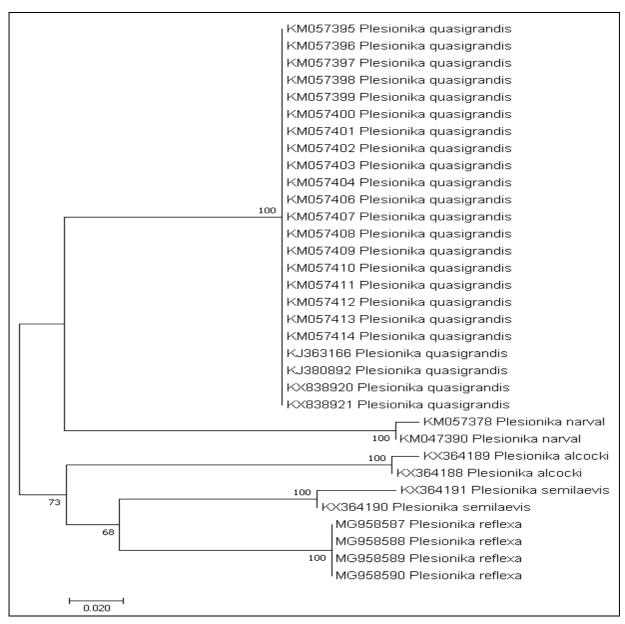


Fig 4: Phylogenetic tree of the genus *Plesionika* deep sea caridean shrimp species from the southern coast of India using Maximum-likelihood with 1000 bootstraps under the best fitting model GTR+G+I inferred from DNA Sequences of mitochondrial gene 16S

Table 1: Species, sampling location, and GenBank accession numbers of the genera Plesionika along southern coast of India

Sl no	Species	Collected location	COI	16S rRNA
1	Plesionika quasigrandis	Sakthikulangara	KM096444	KM057395
		Sakthikulangara	KM096445	KM057396
		Sakthikulangara	KM096446	KM057397
		Sakthikulangara	KM096447	KM057398
		Sakthikulangara	KM096448	KM057399
		Sakthikulangara	KM096449	KM057400
		Sakthikulangara	KM096450	KM057401
		Sakthikulangara	KM096451	KM057402
		Sakthikulangara	KM096452	KM057403
		Sakthikulangara	KM096453	KM057404
		Sakthikulangara	KM096454	KM057405
		Sakthikulangara	KM096455	KM057406
		Sakthikulangara	KM096456	KM057407
		Sakthikulangara	KM096457	KM057408
		Sakthikulangara	KM096458	KM057409
		Sakthikulangara	KM096459	KM057410
		Kalamuku	KM096460	KM057411
		Kalamuku	KM096461	KM057412
		Kalamuku	KM096462	KM057413
		Kalamuku	KM096463	KM057414

		Kalamuku	KJ401314	KJ363166
		Kalamuku	KF938650	KJ380892
		Tuticorin	KX838917	KX838920
		Tuticorin	KX838918	KX838921
2	Plesionika narval	Kalamuku	KP398864	KM057378
		Kalamuku	KP398863	KP398866
		Kalamuku	-	KM047390
		Kalamuku	-	KM047389
3	Plesionika semilaevis	Kalamuku	KX364192	KX364190
		Kalamuku	KX364193	KX364191
4	Plesionika alcocki	Tuticorin	KX530799	KX364188
		Tuticorin	KX530800	-
5	Plesionika reflexa	Sakthikulangara	MG958591	MG958587
	-	Sakthikulangara	MG958592	MG958588
		Sakthikulangara	-	MG958589
		Sakthikulangara	-	MG958590

### 6. Discussion

Shrimps of the infraorder Caridea from India majorly reported from RIMS survey during 1885-1900 [23, 24]. The studies that have concentrated in detail on the classification and evolutionary history within the Caridea have relied entirely on morphological characters. Here we report the base molecular data on deep sea shrimp genera Plesionika sp diversity using COI and 16S rRNA data from the southern part of India. The phylogenetic tree resulting from two mitochondrial genes (COI and 16S) were similar and strongly reveal that genera Plesionika from Indian water is divided into five species (P. quasigrandis, P. narval, P. semilaevis, P. alcocki and P. reflexa) formed in one cluster group with well supported bootstrap values. P. quasigrandis is the dominant species among the deep sea shrimps landed in southern coast of India and the molecular data of *P. quasigrandis* compared with its type locality in the Philippines, showed 5.8-8.4% COI sequence divergence between the Indian and Philippines [4]. P. quasigrandis which has been earlier misidentified as P. spinipes in Indian water [22] through morphological identification was confirmed with molecular data and morphological identification for the first time from India. The Plesionika martia [25] was considered to be an important catch in deep sea fishery in India [26] and the specimens collected from three deep sea fish landing centres along the southern coast of India (AS: Kalamuku and Sakthikulangara and BOB: Tuticorin) actually represents P. semilaevis without exception and the record of P. semilaevis from the southwest coast of India was confirmed [5] with a morphological description. Based on the molecular data of COI and 16S, P. semilaevis and P. martia sequences retrieved from NCBI GenBank which is compared with present Indian P. semilaevis sequences. The obtained sequence from the NCBI of P. semilaevis for 16S (Accession no: KP725640) and COI (KX364192 and KX364193), revealed less genetic distance of 16.3% in comparison with COI (5.6 -17.8%). However, with P. martia 16S (KP725638 and JN412688) exhibited 16.8-18.6% which was found to be lesser than COI (26.1%: JN412728). The COI sequence divergence higher than 3% has been generally considered to form a different species in crustaceans. The species P. reflexa from the present study revealed the genetic comparisons of the present Indian specimens (1 males, 1 ovig. female, CMFRI ED.2.4.3.8, NTOU M02081; GenBank nos. MG729438-729440) with topotypic material of P. ensis (GenBank no. MG729442, versus type locality Barbados [27]) and *P. reflexa* (from the Philippines: eastern Luzon, AURORA stn CP2695, NTOU M02080, GenBank no. MG729441, versus type locality S.E.

Luzon) showed that there is high divergence in the barcoding gene COI amongst the specimens from different localities (9.3%, 10.3–10.7%, 14.2–14.5% divergences between material from Lesser Antilles/Philippines, Philippines/India and Lesser Antilles/India, respectively). However, there are only 0.0-0.3% genetic divergence amongst the Indian material. The high genetic difference of the Indian from topotypic material of both P. ensis and P. reflexa, and the reduction or absence of epipods at the pereiopod III and IV may urge the separation of the Indian form as another species (Chan et al., 2018). The species P. narval obtained from southwest coast of India was characterised based on its morphology was found closely related with the species, P. grandis and the phylogenetic tree, pair wise genetic distance of 16S and COI genes with that of closely related species was constructed using maximum likelihood method. The level of interspecies divergence among other species of the genus was retrieved from the NCBI database for 16S and COI of P. narval (genetic distance: 8.7 to 13.1%) with P. grandis (GQ131900) and P. ensis (AY612883) using COI sequences P. narval (19.1-20.2%) was closely related with P. spinipes (JX681792) and P. williamsi (JX681801). Another species P. alcocki which was obtained from the southeast coast of India showed lesser intraspecies genetic distance for both COI and 16S (0%) between the individuals of the species and phylogenetic tree has been constructed based on maximum likelihood showed high bootstrap values which confirms the occurrence of P. alcocki along the Indian coast.

## 7. Acknowledgement

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