

First Record of the Genus *Pseudohelice* Sakai, Türkay & Yang, 2006 from India and Description of a New Pseudocryptic Species (Crustacea: Brachyura: Varunidae)

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A new pseudocryptic species of the varunid crab genus *Pseudohelice* Sakai, Türkay & Yang, 2006, is described from India based on morphological and molecular evidence. The new species was collected from higher intertidal zones in the Vellar River estuary, Tamil Nadu, southeastern India, in a habitat composed of muddy and sandy sediment. *Pseudohelice annamalai* n. sp. is similar to *P. subquadrata* (Dana, 1851) and *P. latreillii* (H. Milne Edwards, 1837) in general form, but can be distinguished from the congeners by the characters of the infraorbital ridges, male first gonopod, and female vulvae. In addition, the mitochondrial cytochrome oxidase subunit I sequences also support the new species. The occurrence of *Pseudohelice* from India links the distribution gap between the western Indian Ocean and western Pacific Ocean. The new species provides additional evidence for the geographic isolation of the eastern Indian Ocean for some marine organisms.

Key words: Mitochondrial cytochrome oxidase subunit I, Morphology, *Pseudohelice annamalai*, Taxonomy, Tamil Nadu.

BACKGROUND

Species of the genus *Pseudohelice* Sakai, Türkay & Yang, 2006 (family Varunidae) are small-sized and distributed in subtropical and tropical regions of the Indo-West Pacific (Sakai et al. 2006). To date only two species have been confirmed within this genus. *Pseudohelice subquadrata* (Dana, 1851) was considered widely distributed in Indo-West Pacific (Sakai et al. 2006), but a recent study showed the range of this species to be from the eastern margin of the Indian Ocean to French Polynesia. *Pseudohelice latreillii*

(H. Milne Edwards, 1837) is distributed in eastern Africa, western Indian Ocean (WIO) (Hsu et al. 2022a). Based on the distributional range of the two species of *Pseudohelice* (Hsu et al. 2022a: fig. 4), species of this genus seems not distributed around the Indian subcontinent.

In the region of the Vellar River estuary, Tamil Nadu, southeastern India, 17 species of crabs have been recorded, including sesarmids and fiddler crabs (*Austruca annulipes* (A. Milne-Edwards, 1873) and *A. variegata* (Heller, 1862)) as the dominant species in mangroves (Khan et al. 2005; Fredrick and

Ravichandran 2013; Shih et al. 2019a). In recent surveys in this region, specimens of the genus *Pseudohelice* were collected, representing the first record of this genus from India. Using detailed comparison of the morphological characters with the other two congeners, and with support from analysis of mitochondrial cytochrome oxidase subunit I (*COI*), the specimens are confirmed to be a different species and the new species described herein.

MATERIALS AND METHODS

Specimens were collected from high intertidal areas in the Vellar River estuary, Parangipettai, Tamil Nadu, southeastern India, with sediments composed of mud and sand (Fig. 3). The mangroves in the habitats were artificially planted and covered an area of about 5 ha along the northern bank of the Vellar River, with two distinct zones including *Rhizophora* spp. toward the estuary and *Avicennia* spp. toward land in the intertidal area of estuary (Fig. 3).

The examined specimens were deposited into the reference collections of the crustacean research laboratory, Centre of Advanced Study in Marine Biology, Annamalai University (CASAU). However, the holotype of the new species will be moved to the national repository of the Zoological Survey of India (ZSI), Kolkata; the Museo Zoologico dell'Università di Firenze, Italy (MZUF); Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOO); the Queensland Museum, Brisbane, Australia (QM); and the Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore (ZRC).

Morphological characters were illustrated with the aid of a drawing tube attached to a stereomicroscope. The morphological characters and terminology follow those of Sakai et al. (2006), Guinot et al. (2013), and Davie et al. (2015). The abbreviation G1 is used for male first gonopod. Measurements are of the maximum carapace width (CW), and carapace length (CL) in millimeters.

Genomic DNA was isolated from the muscle tissue of legs or gills using the GeneMark tissue and cell genomic DNA purification kit (Taichung, Taiwan). A portion of the *COI* gene was amplified with PCR using the primers LCO1490 (5'-GGTCAACAAAT CATAAAGATATTGG-3'), HCO2198 (5'-TAAACT TCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994), LCOB (5'-CAAAYCATAAAGAYATYGG-3'), HCOex (5'-GCTCATACTACAAATCCTAAA-3'), HCOex2 (5'-GCTCANACTACAAATCCTAA-3') and

HCOex3 (5'-GCTCANACTACRAATCCTA-3') (Shih et al. 2022b). PCR conditions for the above primers were denaturation for 50 s at 94°C, annealing for 70 s at 45–47°C, and extension for 60 s at 72°C (40 cycles), followed by another extension for 10 min at 72°C. Sequences were obtained by automated sequencing (Applied Biosystems 3730) after verification with the complementary strand. Sequences of different haplotypes have been deposited into GenBank, with other sequences published in Shih and Suzuki (2008), Shih et al. (2020), and Hsu et al. (2022a) (accession numbers given in Table 1). *Parahelice daviei* (Sakai, Türkay & Yang, 2006), *Par. pilimana* (A. Milne-Edwards, 1873), and *Par. pilosa* (Sakai, Türkay & Yang, 2006) were selected as outgroups following Hsu et al. (2022a).

The best-fitting model for sequence evolution was determined by jModeltest (vers. 2.1.4; Darriba et al. 2012), selected by the Bayesian information criterion (BIC). The best model obtained was HKY+I+G, which was subsequently used for Bayesian inference (BI) and maximum likelihood (ML) analyses. The BI analysis was performed with MrBayes (ver. 3.2.3, Ronquist et al. 2012). Phylogenetic analyses were run with four chains for 10 million generations and four independent runs, with trees sampled every 1,000 generations. The convergence of chains was determined by the average standard deviation of split frequency values below the recommended 0.01 (Ronquist et al. 2020), and the first 2,650 trees were accordingly discarded as “burnin”. A ML analysis was conducted in MEGA (vers. 11.0, Tamura et al. 2021) with the HKY+I+G model and 2,000 replicates and the options of the Nearest-Neighbor-Interchange (NNI) in the ML heuristic method, NJ/BioNJ in the initial tree for ML, and moderate in the branch swap filter. A maximum parsimony (MP) consensus tree was also constructed using MEGA, with 2,000 bootstrap reiterations of a simple heuristic search, TBR branch-swapping (tree bisection-reconnection) (100 random-addition sequence replications; max no. of trees to retain = 10,000). Basepair (bp) differences and pairwise estimates of Kimura 2-parameter (K2P) distances (Kimura 1980) for genetic diversities between specimens were calculated with MEGA.

RESULTS

TAXONOMY

Family Varunidae H. Milne Edwards, 1853
Subfamily Cyclograpsinae H. Milne Edwards,
1853
Genus *Pseudohelice* Sakai, Türkay & Yang, 2006

***Pseudohelice annamalai* n. sp.**

(Figs. 1, 2, 4)

urn:lsid:zoobank.org:act:A5E686EC-C98A-48CF-81DF-47F1EBB3DF32

Material examined: Holotype: 1 male (17.3 × 14.6 mm) (CASAU CR-1011), Vellar River estuary, Tamil Nadu, India, coll. M. Prema and S. Ravichandran, 28 Feb.–5 Mar. 2022. Paratypes (same locality as holotype): 1 male (18.9 × 15.2 mm) (CASAU CR-1013), 21 Sep. 2020; 2 males (20.1 × 16.8, 16.7 × 14.0 mm) (CASAU CR-1014), 17 Dec. 2021; 2 males (16.4 × 13.1, 15.2 × 13.9 mm) (CASAU CR-1015), 25 Feb.

2022; 2 males (18.7 × 15.4, 16.7 × 14.5 mm) (CASAU CR-1016), 2 Mar. 2022; 2 females (18.3 × 15.4, 17.7 × 14.2 mm) (CASAU CR-1017), 5 Jun. 2021; 2 females (17.5 × 16.0, 10.4 × 9.5 mm) (CASAU CR-1018), 3 Mar. 2022, coll. M. Prema; 3 males (18.2 × 15.3, 16.7 × 14.3, 14.3 × 11.9 mm) (NCHUZOOL 17048), coll. M. Prema and S. Ravichandran, 12 Dec. 2020; 5 males (16.6 × 14.4, 18.2 × 15.9, 16.6 × 14.2, 17.1 × 14.3, 17.1 × 14.4 mm), 2 females (14.3 × 12.5, 13.9 × 11.6 mm), 1 ovig. female (18.8 × 15.3 mm) (NCHUZOOL 17049), coll. M. Prema and S. Ravichandran, 28 Feb.–5 Mar. 2022; 2 males (16.9 × 14.2, 17.2 × 14.5 mm), 2 females

Table 1. Haplotypes of the cytochrome *c* oxidase subunit I (*COI*) gene of specimens of *Pseudohelice* species from the Indo-West Pacific and the outgroups of *Parahelice* species

Species	Locality	Catalogue no.	Sample size	Haplotype	Access. no. of <i>COI</i>
<i>Pseudohelice annamalai</i>	India: Vellar R., Tamil Nadu	NCHUZOOL 17048	1	PA1	ON971913
	India: Vellar R., Tamil Nadu	NCHUZOOL 17049	1	PA2	ON971914
	India: Vellar R., Tamil Nadu	NCHUZOOL 17048	2	PA3	ON971915, ON971916
	India: Vellar R., Tamil Nadu	NCHUZOOL 17049	2	PA3	ON971917, ON971918
	India: Vellar R., Tamil Nadu	CASAU CR-1011	1	PA3	ON971919
	India: Vellar R., Tamil Nadu	ZRC 2022.0189	2	PA3	ON971920, ON971921
	India: Vellar R., Tamil Nadu	NCHUZOOL 17049	1	PA4	ON971922
	India: Vellar R., Tamil Nadu	ZRC 2022.0189	1	PA5	ON971923
	India: Vellar R., Tamil Nadu	NCHUZOOL 17049	1	PA6	ON971924
	India: Vellar R., Tamil Nadu	NCHUZOOL 17049	2	PA7	ON971925, ON971926
	India: Vellar R., Tamil Nadu	ZRC 2022.0189	1	PA7	ON971927
	India: Vellar R., Tamil Nadu	NCHUZOOL 17049	1	PA8	ON971928
	<i>Pseudohelice subquadrata</i>	Japan: Miyako, Ryukyus	NCHUZOOL 13102	1	PS1
Taiwan: Gangnan, Hsinchu City		NCHUZOOL 15724	1	PS2	LC511075
Taiwan: Sinfeng, Hsinchu County		NCHUZOOL 15723	1	PS3	LC511076
Taiwan: Dingtouer Shoal, Tainan		NCHUZOOL 15731	1	PS4	LC511077
Taiwan: Yanshuei R., Tainan		NCHUZOOL 15726	1	PS5	LC511078
Taiwan: Wanlitong, Pingtung		NCHUZOOL 15733	2	PS6	LC511079
Taiwan: Dongsha Island		NCHUZOOL 15725	1	PS7	LC511080
Guam		ZRC 2013.1034	1	PS8	LC511081
Indonesia: Bali		NCHUZOOL 15722	1	PS2	LC511082
Indonesia: Bali		NCHUZOOL 15727	1	PS9	LC511083
Australia: SE Queensland		QM W19922	1	PS10	LC511084
Australia: SE Queensland		QM W19922	1	PS11	LC511085
<i>Pseudohelice latreillii</i>		Egypt: Nabq-El Arwashie, Sinai	MZUF 3790	1	PL1
	Kenya: Mida Creek	MZUF 4985	1	PL2	MZ400782
	Kenya: Mida Creek	MZUF 4993	1	PL2	MZ400783
	Kenya: Gazi	MZUF 4987	1	PL4	MZ400784
	Kenya: Mida Creek	NCHUZOOL	1	PL3	MZ400785
	Seychelles: Mahé, Port Launay	MZUF 2935	1	PL4	MZ400786
	Mauritius: Poste Lafayette	MZUF 4994	2	PL2	MZ400787, MZ400788
	Mauritius: Melville	MZUF 4997	1	PL2	MZ400789
	Mauritius: Rodrigues I.	MZUF 4991	1	PL2	MZ400790
	Mauritius: Rodrigues I.	MZUF 4999	1	PL2	MZ400791
outgroups					
<i>Parahelice daviei</i>	Taiwan: Gangkou R., Pingtung	NCHUZOOL 15717	1		LC511052
<i>Parahelice pilimana</i>	Taiwan: Baoli R., Pingtung	NCHUZOOL 15681	1		LC511064
<i>Parahelice pilosa</i>	Taiwan: Gangkou R., Pingtung	NCHUZOOL 15700	1		LC511071

(15.6 × 13.2, 18.1 × 15.4 mm) (ZRC 2022.0189), coll. M. Prema and S. Ravichandran, 28 Feb.–5 Mar. 2022.

Comparative material: *Pseudohelice subquadrata* and *P. latreillii* (see material examined in Hsu et al. 2022a).

Description: Carapace (Fig. 2A) quadrate, slightly broader than long, 1.08–1.15 times as broad as long; surface convex, irregularly punctated and finely granulated; mesogastric and protogastric regions deeply low with noticeable epigastric groove. Frontal margin slightly concave. Anterolateral margins with 3 teeth including larger orbital tooth, second tooth slightly narrower than preceding, last tooth very small, distinct. Infraorbital ridge (Fig. 2C–E) heteromorphic in both sexes; in male, mesial part with 4 or 5 rounded, smooth, less interspaced small tubercles, followed by several large, elongated and less convex tubercles; lateral part with 1 significantly largest, very convex and elliptical tubercle, followed by 1 less convex tubercle, and 2 or 3 large convex tubercles (Fig. 2C); in female form I,

mesial part with several (5 or 6) dense, small rounded tubercles, followed by several larger, less convex elongated tubercles; lateral part with well-spaced 3 or 4 elliptical, more convex, larger tubercles, ending with 1 or 2 small rounded tubercles (Fig. 2D); in female form II, mesial part with several (5 or 6) dense, small rounded tubercles, followed by well-spaced several elongated and less convex tubercles, lateral part with 1 significantly largest elongated tubercle, and 2–5 closely spaced, larger tubercles (Fig. 2E).

Chelipeds (Fig. 2B) usually unequal in adult male and equal in some adult male (similar size) and all female; palm bulky, inner palm meagerly granulated, outer surface smooth, line of short setae present at base of anterior margin of palm.

Ambulatory legs (Fig. 1A–D) slender, anterior margins of merus, carpus, and propodus covered with short setae, posterior margins with sparse short setae.

Male G1 (Fig. 2F–I) slender, tapering, slightly curved towards lateral end in distal part, chitinous

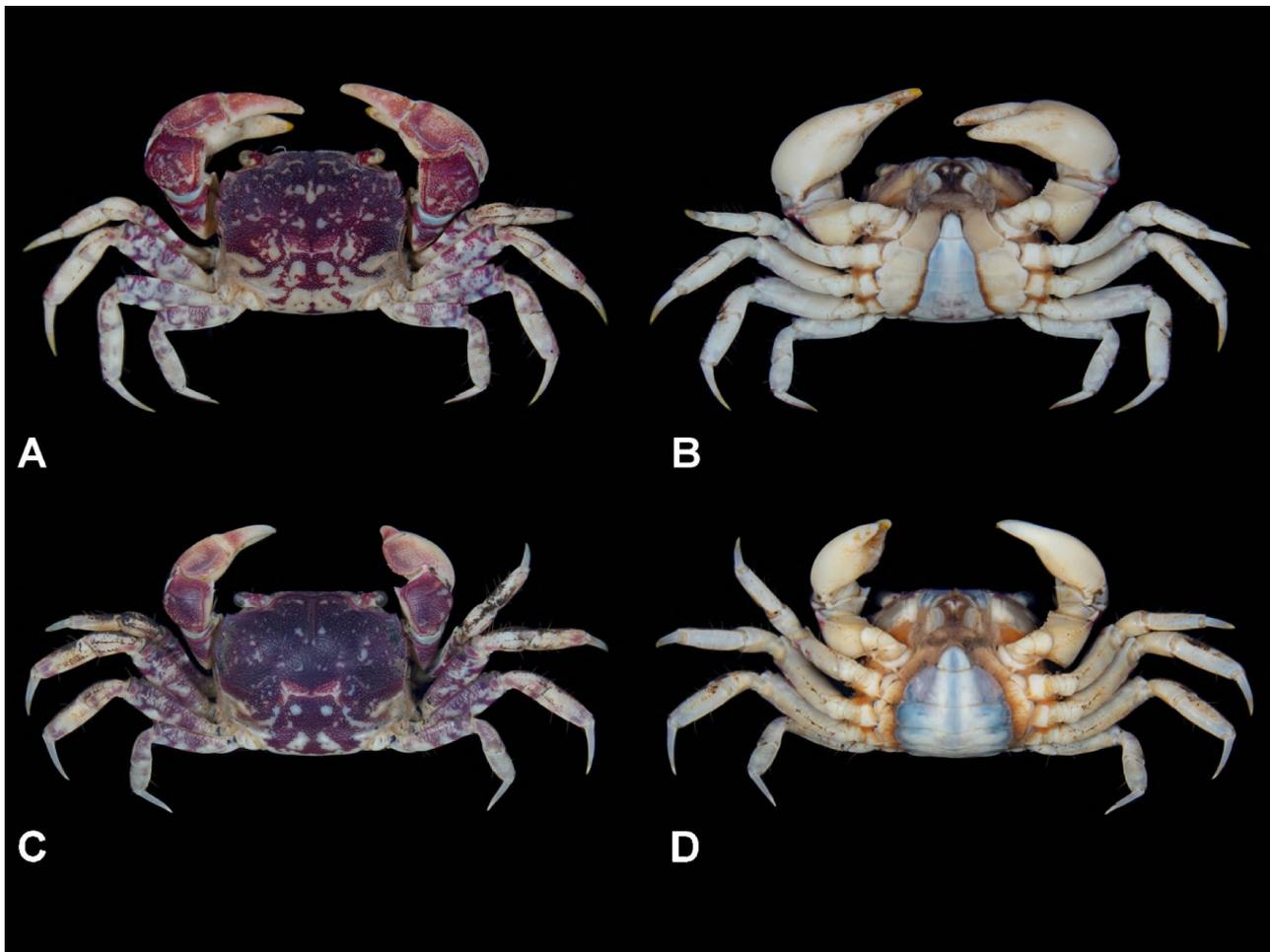


Fig. 1. *Pseudohelice annamalai* n. sp. with preserved coloration. A, dorsal view of male; B, ventral view of male; C, dorsal view of female; D, ventral view of female. A, B, holotype, CASAU CR-1011 (17.3 × 14.6 mm); C, D, NCHUZOOL 17049 (14.3 × 12.5 mm).

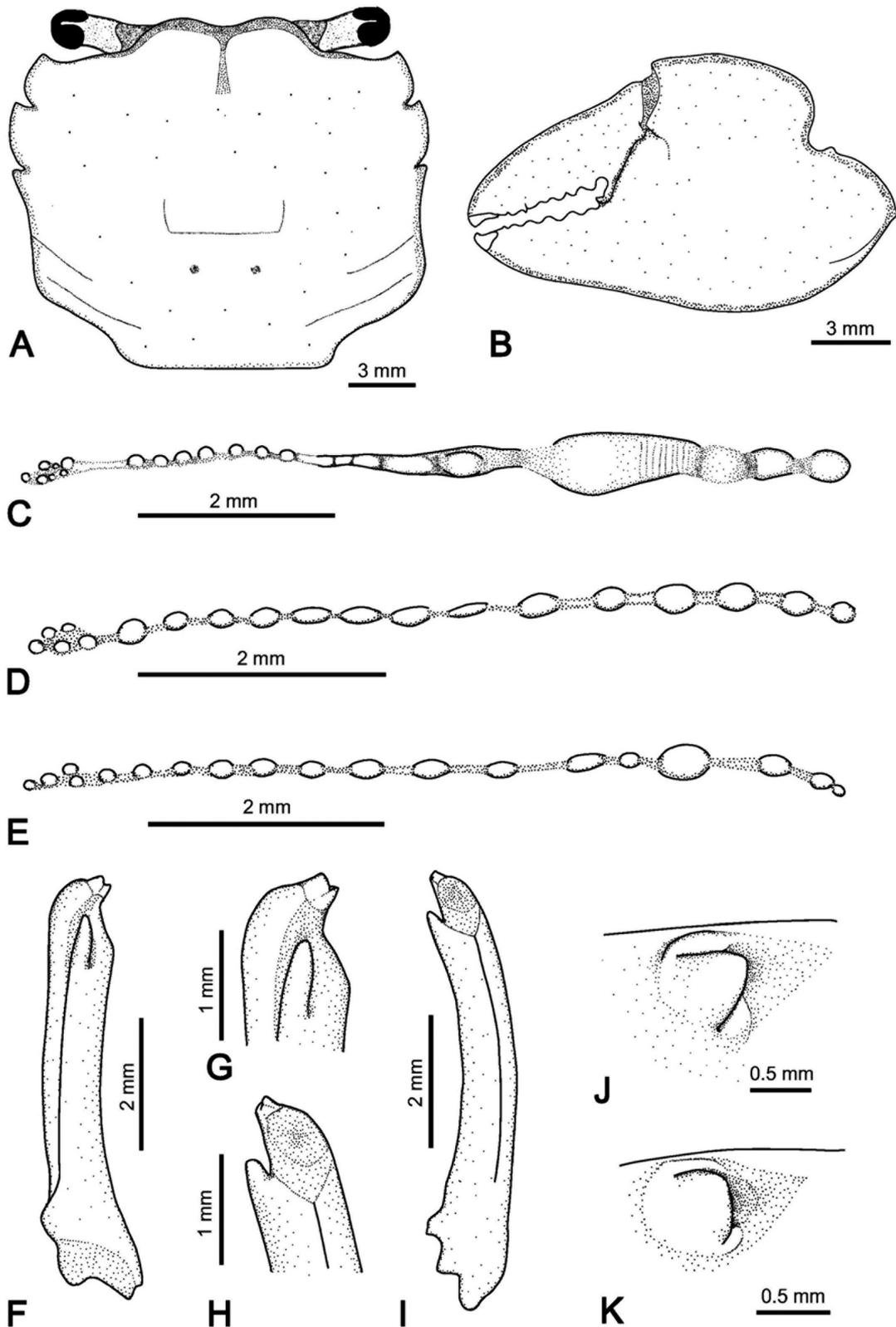


Fig. 2. *Pseudohelice annamalai* n. sp. A, carapace; B, outer view of male left cheliped; C, left male infraorbital ridge; D, E, left female infraorbital ridge (D, form I; E, form II); F, G, dorsal view of right G1; H, I, ventral view of right G1; J, K, right vulva (J, form I; K, form II). A, B, male, CASAU CR-1013 (18.9 × 15.2 mm); C, F–I, holotype male, CASAU CR 1011 (17.3 × 14.6 mm); D, female, NCHUZOOL 17049 (18.8 × 16.4 mm); E, J, female, ZRC 2022.0189 (18.1 × 15.4 mm); K, female, NCHUZOOL 17049 (13.9 × 11.6 mm).



Fig. 3. Habitats of *Pseudohelice annamalai* n. sp. A, higher intertidal zone of the Vellar River estuary, in front of the Centre of Advanced Study in Marine Biology, Tamil Nadu, southeastern India; B, artificial mangroves of *Rhizophora* sp. and *Avicennia* sp., in the margin of Vellar River.

endpiece shorter, wider and thicker, bilobed and rounded end; female vulvae (Fig. 2J, K) with a semicircular sternal vulvar cover; sunken on inner part.

Size: Largest male specimen is CW 20.1 mm (CASAU CR-1014); largest female is (ovigerous) CW 18.8 mm (NCHUZOO 17049).

Color in life: Varied from dark purple to dark gray, with irregular light brown, yellowish brown, or white patches on posterior carapace and some individuals on entire carapace as white dots or patches; some young individuals yellowish orange-brown. Chelipeds usually lighter brown (most of surface) and upper regions of palm lighter purple in adult male (Fig. 4A–G).

Ecological notes: In the southeastern coast of India, this species inhabits sand-muddy banks of mangroves (Fig. 3) and it is sympatric with *A. annulipes* (H. Milne Edwards, 1837). Some burrows (Fig. 4H) were located near the pneumatophores of *Avicennia* mangroves. Burrows have a depth of 25–30 cm and are branched, with larger-sized pellets around the burrow entrance.

Etymology: This species is named after Annamalai University, in honor of 100 years' service in education and research as a state university of India. In addition, the present specimens were collected from the intertidal areas in front of the Faculty of Marine Science, Research Centre (Centre of Advanced Study in Marine Biology, Annamalai University), Vellar River, Tamil Nadu. The name is used as a noun in apposition.

Distribution: Currently known only from the type locality, the Vellar River estuary, southeastern India.

Remarks: Morphologically, this new species is similar to *P. subquadrata* and *P. latreillii*, but can be distinguished by the infraorbital ridges, male G1s, and female vulvae. Both infraorbital ridges and male G1s (or female vulvae) should be compared to identify the species of *Pseudohelice*, because male infraorbital ridges and female vulvae are similar in *P. annamalai* and *P. subquadrata*, and male G1s and female infraorbital ridges are similar in *P. annamalai* and *P. latreillii* (Table 2).

Pseudohelice annamalai can be discerned from *P. subquadrata* by the infraorbital ridges in both sexes and male G1s. In *P. annamalai*, the lateral part of male infraorbital ridge has 1 less convex but obvious tubercle between the largest tubercle and 2 or 3 large convex tubercles (vs. this tubercle absent or not obvious in *P. subquadrata*) (Fig. 2C; Hsu et al. 2022a: fig. 1E); the lateral part of the female infraorbital ridge lacks the largest elongated tubercle (form I) or is present but smaller and less convex (form II) (vs. largest tubercle relatively larger and more convex in *P. subquadrata*) (Fig. 2D, E; Hsu et al. 2022a: fig. 1F). *Pseudohelice annamalai* has the G1 slender, the upper part tubular,

the chitinous endpiece with distal part shorter and thicker (vs. G1 stouter, upper part slightly flatter, chitinous endpiece with distal part relatively longer and thinner in *P. subquadrata*) (Fig. 2F–I; Hsu et al. 2022a: fig. 1G–J).

Pseudohelice annamalai can be differentiated from *P. latreillii* by the male infraorbital ridges and the female vulvae. In *P. annamalai*, the lateral part of male infraorbital ridge has 1 less convex but obvious tubercle between the largest elliptical tubercle and 2 or 3 large convex tubercles (vs. tubercle absent between the largest rounded tubercle and 2 or 3 large convex tubercles in *P. latreillii*) (Fig. 2C; Hsu et al. 2022a: fig. 3E). In females, the sternal vulvar cover is often longer (but shorter in some individuals) in *P. annamalai* (vs. shorter in *P. latreillii*) (Fig. 2J–K; Hsu et al. 2022a: fig. 1L, M).

Molecular analyses

We used 16 specimens from the Vellar River estuary for molecular study, with 8 haplotypes of *COI* (Table 1). The pairwise nucleotide divergences of K2P distances and bp differences among haplotypes of the species of *Pseudohelice* are shown in table 3. The intraspecific nucleotide divergences (and bp differences) of *P. subquadrata*, *P. annamalai* n. sp., and *P. latreillii* are $\leq 1.39\%$ (≤ 9 bp), $\leq 0.77\%$ (≤ 5 bp), and $\leq 1.86\%$ (≤ 12 bp), respectively. *Pseudohelice annamalai* has interspecific divergences $\geq 1.54\%$ (≥ 10 bp) with *P. subquadrata* and $\geq 3.45\%$ (≥ 22 bp) with *P. latreillii*.

The phylogenetic tree (Fig. 5) based on *COI* shows three clades within the genus *Pseudohelice*, corresponding to *P. subquadrata*, *P. annamalai*, and *P. latreillii*, although *P. annamalai* is not highly supported by BI and MP methods. The relationship between *P. subquadrata* and *P. annamalai* is closer, but the support values are not high in BI and MP methods.

DISCUSSION

Biogeographic implication

The occurrence of *Pseudohelice* from India links the distribution gap between the WIO and western Pacific Ocean. Currently the geographical distributions of the three species of *Pseudohelice* are different and do not overlap. From west to east, *P. latreillii* is distributed in the WIO (including the Red Sea, Arabian Sea, eastern Africa, Mauritius Island, and Rodrigues Island); *P. annamalai* is found from southeastern India (Vellar River estuary); and *P. subquadrata* is distributed widely from the eastern margin of the Indian Ocean (Koh Surin



Fig. 4. Color in life of *Pseudohelice annamalai* n. sp. (A–G) from higher intertidal zone of the Vellar River estuary, Tamil Nadu, India. A, B, dorsal view of male; C, D, frontal and dorsal views of female, respectively; E, F, dorsal and frontal views of an ovigerous female, respectively; G, male at the burrow entrance near mangrove pneumatophores; H, burrow in the habitat. A, CASAU CR-1012 (18.2 × 15.5 mm); B, CASAU CR-1016 (18.7 × 15.4 mm), C–F, specimens not collected; G, NCHUZOO 17049 (18.2 × 16.8 mm).

in western Thailand) to the eastern region of the West Pacific (French Polynesia) (Sakai et al. 2006; Hsu et al. 2022a: fig. 4; this study).

In addition to the endemic *P. latreillii*, other species of coastal crabs are known to be endemic in the WIO, e.g., *Metopograpsus cannicci* Innocenti, Schubart & Fratini, 2020 and *M. messor* (Forskål, 1775) (Grapsidae) (Fratini et al. 2018; Innocenti et al. 2020); *Neosarmatium africanum* Ragionieri, Fratini & Schubart, 2012 and *N. meinerti* (De Man, 1887) (Sesarmidae) (Ragionieri et al. 2009 2012); *Leptodius exaratus* (H. Milne Edwards, 1834) (Xanthidae) (Lee et al. 2013); seven species of fiddler crabs (Shih et al. 2022b); and several species or clades of ghost crabs (genus *Ocypode*) (Ocypodidae) (Sakai and Türkay 2013; Ma et al. 2018). Some marine barriers to larval dispersal in the WIO have been proposed, including the hydrochemical front found at approximately 10°S, the upwelling off the coast of Somalia and Arabia, and high salinity at the surface in the Arabian Sea (Ma et al. 2018). Several crab studies examined the

differentiation within the eastern Indian Ocean (EIO). Lai et al. (2010) reported that *Portunus reticulatus* (Herbst, 1799) distributed in the EIO is different genetically from *Por. pelagicus* (Linnaeus, 1758) from the western Pacific, although the Bay of Bengal may be a zone of hybridization of the two species. Shih et al. (2019a) confirmed two species of fiddler crabs in the EIO with *Austruca variegata* (Heller, 1862) in the Bay of Bengal and *A. bengali* (Crane, 1975) in the Andaman Sea, which differ genetically from *A. triangularis* (A. Milne-Edwards, 1873) in the western Pacific. Similarly, Lai et al. (2006) showed that *Calappa guerini* Brito-Capello, 1871 is only distributed in the Indian Ocean, which differ from *Calappa quadrimaculata* Takeda & Shikatani, 1990 from the western Pacific. It is suggested that some kinds of geographic isolation (e.g., ocean currents) in the EIO may promote speciation for some marine organisms.

Within the *Helice/Chasmagnathus* complex, species of *Parahelice* also have wide distributions, but less wide than species of *Pseudohelice* and the

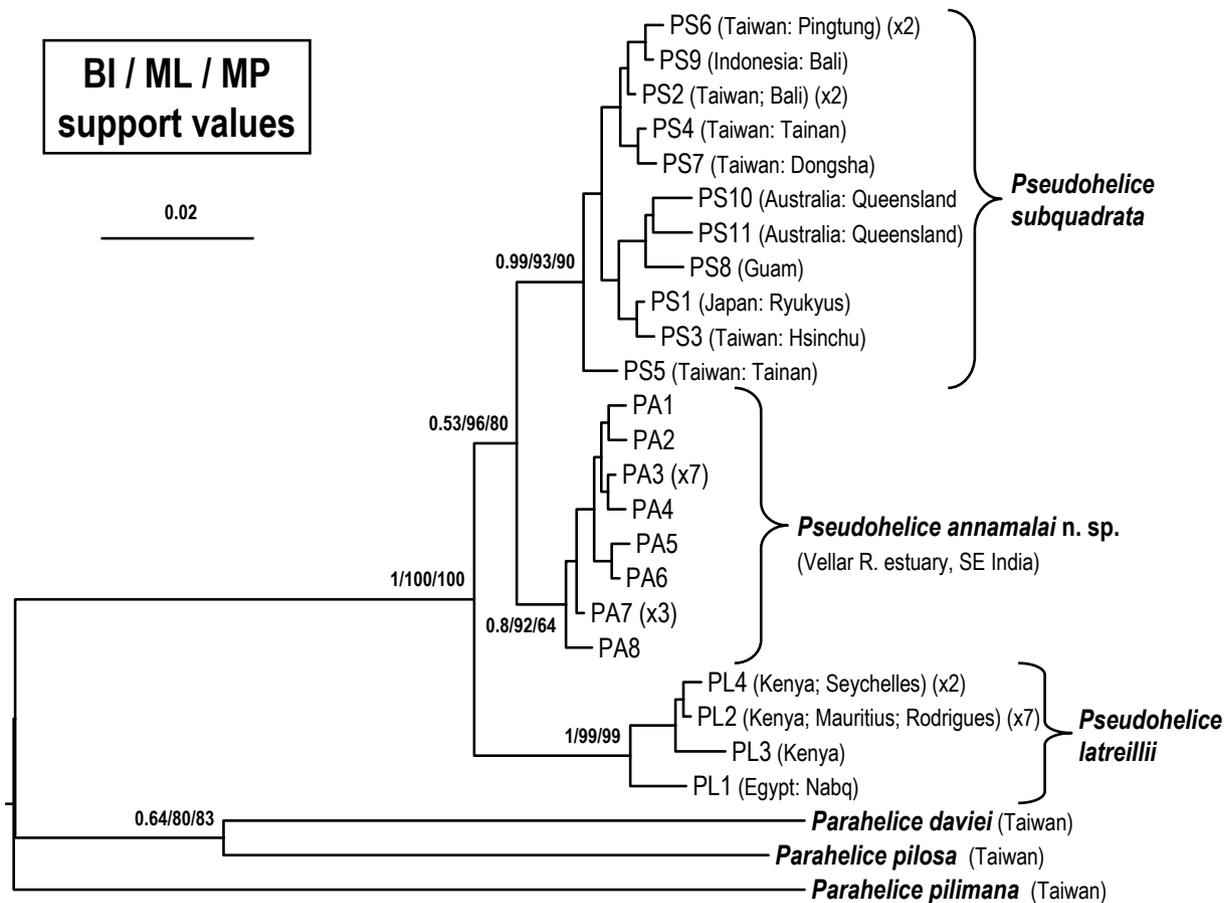


Fig. 5. A Bayesian inference (BI) tree for species of *Pseudohelice* and the outgroups, based on the cytochrome c oxidase subunit I (*COI*) gene. Probability values at the nodes represent support values for BI, maximum likelihood (ML) and maximum parsimony (MP). For haplotype names, see table 1.

ranges of some species overlap in the western Pacific (Sakai et al. 2006; Shih et al. 2020). On the contrary, distributions of species in the genera *Chasmagnathus*, *Helice*, and *Helicana* are relatively limited in East Asia (Korea, China, main islands of Japan, the Ryukyus, and Taiwan), and species of different genera might be sympatric, but the ranges of congeners rarely overlap (Sakai et al. 2006; Shih and Suzuki 2008; NK Ng et al. 2018).

Morphological and molecular comparison

The infraorbital ridge (= suborbital crest) is the structure below the suborbital edge on the suborbital region of the carapace (Sakai et al. 2006). Previous studies have assumed that the infraorbital ridge is probably used for sound production when rubbed by the horny crest in the anterior part of the upper surface of the cheliped merus, and in acoustic communication

Table 2. Comparison of characters of males and females between three species of *Pseudohelice*

Character	<i>P. subquadrata</i>	<i>P. annamalai</i> n. sp.	<i>P. latreillii</i>
Male infraorbital ridge	mesial part with several small tubercles, followed by several large, elongated and less convex tubercles; lateral part with 1 significantly largest, very convex and elliptical tubercle, and 2–5 large convex tubercles (Hsu et al. 2022a: fig. 1E)	mesial part with small, rounded tubercles, followed by several large, elongated and less convex tubercles; lateral part with 1 significantly one largest, very convex and elliptical tubercle, and 1 less convex but obvious tubercle between largest tubercle and 2 or 3 large convex tubercles (Fig. 2C)	mesial part with several small, rounded tubercles, followed by several large, elongated and less convex tubercles; lateral part with 1 largest, very convex and rounded tubercle, and 2 larger rounded and convex tubercles (Hsu et al. 2022a: fig. 3E)
G1	stouter and blunter, upper part slightly flatter; chitinous structure at top relatively smaller and thinner, tip pointed (Hsu et al. 2022a: fig. 1G–J)	slender, tapering, slightly curved towards lateral end in distal part, chitinous endpiece shorter, wider and thicker, bilobed and rounded end (Fig. 2F–I)	more slender, upper part tubular; chitinous structure at top relatively wider, shorter and thicker, tip blunter (Hsu et al. 2022a: fig. 3H–K)
Female infraorbital ridge	mesial part with several dense, small tubercles, followed by several closely spaced, larger tubercles, lateral part with 1 largest elongated tubercle and 2–5 larger convex tubercles (Hsu et al. 2022a: fig. 1F)	form I: mesial part dense, several small tubercles, followed by several larger elongated tubercles; lateral part with well-spaced 3 or 4 elliptical, more convex, larger tubercles, ending with 1 or 2 small rounded tubercles (Fig. 2D) form II: mesial part with several dense, small rounded tubercles, followed by well-spaced several elongated and less convex tubercles; lateral part with 1 significantly largest elongated tubercle and 2–5 closely spaced, larger tubercles (Fig. 2E)	form I: mesial part with several tubercles, followed by several well-spaced, isomorphically rounded tubercles in lateral part (Hsu et al. 2022a: fig. 3F) form II: mesial part with several well-spaced and larger tubercles, followed by several well-spaced, small tubercles, lateral part with 1 largest and 2–5 larger convex tubercles (Hsu et al. 2022a: fig. 3G)
Vulva	longer semicircular sternal vulvar cover Hsu et al. 2022a: fig. 1K	longer (common) (Fig. 2J) or shorter (rare) (Fig. 2K) semicircular sternal vulvar cover; sunken on inner part	shorter semicircular sternal vulvar cover (Hsu et al. 2022a: fig. 3L, M)

Table 3. Matrix of percentage of pairwise nucleotide divergences with Kimura 2-parameter (K2P) distances and number of basepair (bp) differences based on the cytochrome *c* oxidase subunit I (*COI*) gene within and between three species of *Pseudohelice*. In the right half, lower-left values are K2P distances and upper-right ones are bp differences. Range of values is given in parentheses

	Intraspecific		Interspecific		
	Nucleotide divergence	bp difference	<i>P. subquadrata</i>	<i>P. annamalai</i>	<i>P. latreillii</i>
<i>P. subquadrata</i>	0.77 (0–1.39)	5.05 (0–9)		13.48 (10–17)	25.15 (21–32)
<i>P. annamalai</i>	0.21 (0–0.77)	1.38 (0–5)	2.09 (1.54–2.65)		23.39 (22–26)
<i>P. latreillii</i>	0.39 (0–1.86)	2.51 (0–12)	3.96 (3.28–5.09)	3.67 (3.45–4.1)	

(Sakai et al. 2006; Guinot et al. 2018; Sal Moyano et al. 2019). Similar sound-producing behaviors have been reported in *Neohelice granulata*, such as rubbing the chelipedal merus against the pterygostomial region of the carapace (Sal Moyano et al. 2019); and in *Gecarcinus quadratus* (Gecarcinidae), such as the friction of the plectrum on chelipedal merus against the subhepatic region of the carapace (Abele et al. 1973). However, sound-producing behavior has not been reported in species of *Pseudohelice*. Similar structures of the infraorbital ridges and plectrum on the chelipedal merus have been reported in other grapsoid crabs (e.g., *Leptograpsus*, *Discoplax*, and *Epigrapsus*) (Guinot et al. 2018).

In some groups of varunids (e.g., the *Helice/Chasmagnathus* complex and *Metaplax*), the infraorbital ridges are useful morphological characters to distinguish closely related species, whereas in other groups of varunids, the infraorbital ridges are usually variable and sexually dimorphic in other groups. For example, the number of infraorbital granules is variable and overlapped among species of *Metaplax* (Shih et al. 2019b) and *Helice* (Sakai et al. 2006; NK Ng et al. 2018), as well as among the females of *Parahelice* and *Pseudohelice* species (Shih et al. 2020; Hsu et al. 2022a).

By using molecular evidence (e.g., the barcoding marker *COI*), it is possible to confirm the degree of morphological variation of some species, e.g., species of *Helice* (NK Ng et al. 2018), *Parahelice* (Shih et al. 2020), *Pseudohelice* (Hsu et al. 2022a; this study), *Metaplax* (Shih et al. 2019b), and *Ptychognathus* (Hsu and Shih 2020; Hsu et al. 2022b), but several studies also failed to distinguish *Helice formosensis* Rathbun, 1931, *H. latimera* Parisi, 1918, and *H. tientsinensis* Rathbun, 1931 genetically (see NK Ng et al. 2018). In our study, the morphological differences in *P. annamalai* are supported by *COI* sequences (Table 3, Fig. 5).

Although the minimum interspecific distances of *COI* among the three congeners are not high (1.54–3.45%) (Table 3), compared with other varunids (at least about 3% of the interspecific distances; see Hsu et al. 2022a), the monophyly of *P. annamalai* is more or less supported by *COI* (Fig. 5) and further support may be eventually discovered from other markers with high resolution. For example, by using the control region marker, two lineages were revealed in *Episesarma versicolor* (Tweedie, 1940) in western Thailand (Supmee et al. 2012) and *Tubuca arcuata* (De Haan, 1835) in East Asia and Vietnam (Shih et al. 2022a). Using this control region marker also supported the separation between *Paraleptuca crassipes* (White, 1847) and *P. boninensis* (Shih, Komai & Liu, 2013) (Shih et al. 2013).

CONCLUSIONS

In this study, *Pseudohelice annamalai* was established as a new species from southeastern India similar to the congeners *P. subquadrata* and *P. latreillii*, but can be distinguished by a suite of morphological characters which are also supported by the molecular evidence of *COI*. Biogeographically, the occurrence of this new species links the distribution gap between the WIO and western Pacific Ocean. From west to east, *P. latreillii* is distributed in the WIO; *P. annamalai* is found in the Bay of Bengal; and *P. subquadrata* is distributed widely from the eastern margin of the Indian Ocean to the eastern region of the West Pacific.

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