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COMMUNICATION

VARYING COLOUR PATTERN, YET GENETICALLY SIMILAR: PEBBLE CRAB *SEULOCIA VITTATA* (STIMPSON, 1858) (BRACHYURA: LEUCOSIIDAE) FROM THE SOUTHEASTERN COAST OF INDIA

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Varying colour pattern, yet genetically similar: Pebble Crab *Seulocia vittata* (Stimpson, 1858) (Brachyura: Leucosiidae) from the southeastern coast of India

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Abstract: Five adult specimens of leucosiid crab *Seulocia vittata* (Stimpson, 1858) were recently collected off the coast of Palk Bay, southern India. Typical morphological examination revealed the presence of two colour patterns: grey and red. Interestingly, molecular analysis based on the barcoding gene cytochrome oxidase sub unit I (COI) revealed that both grey and red colour patterns in *S. vittata* showed 0% sequence divergence between the specimens. This indicates a situation of reverse cryptic behavior in this crab. Surprisingly, the evolutionary and ecological processes leading to the absence of genetic divergence and variation in morphology (colour pattern) in *S. vittata* complex remain to be addressed.

Keywords: Colouration in crab, DNA barcoding, leucosiid, Mandapam, molecular phylogeny, Tamil Nadu.

Tamil abstract: லுக்ோசிட் நண்டு *சிலோசியா விட்டாட்டா* (ஸ்டிம்ப்ஸன், 1858), பாக் விரிகுடாவின் கடற்கரையில் (5 மாதிரிகள்) சமீபத்தில் சேகரிக்கப்பட்டன. வழக்கமான உருவ பரிசோதனையின் மூலம் அதன் இரண்டு வண்ண வடிவங்கள்: சாம்பல் மற்றும் சிவப்பு நிறம் இருப்பது கண்டறியப்பட்டது. மேலும், மூலக்கூறு டிஎன்ஏ பார்ஓடிங் சைட்-டோக்ரோம் ஆக்சிடேஸ் சப்்யூனிட் 1 (COI) ஐ அடிப்படையாகக் கொண்ட பகுப்பாய்வு மேற்கொண்டபோது சாம்பல் மற்றும் சிவப்பு நிறங்கள் கொண்ட நண்டு வகையின் வேறுபாடு 0% ஆக இருந்தது. இந்த நிலைமை ரிவேர்ஸ் கிரிப்டிக் பிஓஹெவியர் ஐ குறிக்கின்றது. ஆச்சரியப்படும் விதமாக, *சிலோசியா விட்டாட்டா* வகை நண்டுகளில் மரபணு மாறுபாடு மற்றும் உருவவியல் (வண்ண முறை) மாறுபாடு இல்லாததற்கு, அதன் பரிணாம வளர்ச்சி மற்றும் சுற்றுச்சூழல் செயல்முறைகளே காரணமாக இருக்கக்கூடும் என்பது இன்னும் விவாதத்திற்குரிய ஒன்றாகும்.

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Author details: SANJEEVI PRAKASH is a marine biologist, specialized with the taxonomy of coral-reef dwelling decapod crustaceans. Currently, he is focusing on the integrative approach such as morphology and molecular taxonomy to understand the cryptic species diversity in the coral reef associated caridean shrimps of the world. AMIT KUMAR is a marine biologist. He has been using molecular markers to understand marine biodiversity. He is also interested in studying how marine organisms respond to predicted climate change stress conditions.

Author contribution: SP and AK designed and performed the study. SP identified the species using morphology. AK did the phylogeny. SP and AK interpreted the results and wrote the manuscript.

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INTRODUCTION

Pebble crab or leucosiid crab belonging to the family Leucosiidae (Samouelle, 1819) is rich in diversity (Ng et al. 2008; Galil & Ng 2015). It mostly inhabits the sandy and silty areas adjacent to seagrass beds, coral reef flats, as well as intertidal areas usually buried in the sand (Naderloo & Apel 2012; Ng & Komatsu 2016). Leucosiid crabs of India have a long history where the key to the Indian *Leucosia* was first provided by Alcock (1896) during an “Investigator” expedition. At present, 97 species belonging to 35 genera of the family Leucosiidae have been reported from India (Trivedi et al. 2018). While revisiting the Leucosiidae classification, Galil (2005) proposed a new genus *Seulocia* which differs from other genera in the shared characters such as third to sixth abdominal somites fused in males and the straight shaft in the first pleopod of males twisted once on its axis. So far, 11 species have been described in this genus (Ng et al. 2008; WoRMS 2019), of which six species *S. cristata* (Galil, 2005), *S. pubescens* (Miers, 1884), *S. pulchra* (Galil, 2005), *S. rhomboidalis* (De Haan, 1841), *S. truncata* (Alcock, 1896), and *S. vittata* (Stimpson, 1858) have been recorded in Indian waters (Trivedi & Vachhrajani 2017; Trivedi et al. 2018). All of these above records were mainly based on the morphological characteristics and lack of information on genetic relatedness among them.

DNA barcoding along with morphological examination has been considered as a useful tool for the validation of species (Madhavan et al. 2020). This method can effectively identify cryptic species due to differences in their genetic character (Bucklin et al. 2007). No such study has been reported for the genus *Seulocia*. Hence, in the present study along with the detailed morphological examination, we used mitochondrial cytochrome oxidase subunit I (COI) gene to validate the taxonomy of *S. vittata* from the southeastern coast of India.

MATERIALS AND METHODS

The fish landing centre at Mandapam in Ramnad District of Tamil Nadu, India is one of the major landing sites in the southeastern coast of India (9.286°N & 79.153°E). A total of five crab specimens were hand-picked from the freshly discarded by-catch of commercial trawlers at the fish landing during June–July 2019. Specimens were quickly cleaned to remove sediments and photographed (Cannon Powershot G16) in the

field to record fresh colouration. The specimens were preserved in 95% ethanol and brought to Sathyabama Marine Research Station, Rameswaram for further detailed examination.

Morphological examination

The specimens were examined by comparing key morphological features and photographs described by Galil (2005). Four specimens (3 male, 1 female) were in red colouration (LR) and 1 male was in typical bluish-grey colour (LG) as described by Galil (2005). The carapace length (cl in mm) was measured from the tip of the rostrum in the anterior region to the posterior border of the carapace. The carapace width (cw in mm) was measured from the lateral margins of the carapace. The specimens were then deposited in the national zoological collection of the Marine Biological Regional Centre (MBRC), Zoological Survey of India (ZSI), Chennai, Tamil Nadu, India.

Molecular identification

One representative each of red and grey coloured specimens was subjected to molecular identification. Total genomic DNA from the propodus/meri region of the major cheliped of the crab was extracted using OMEGA BIO-TEK E.Z.N.A. Blood & Tissue DNA Kit, USA following the manufacturer’s protocol. PCR amplification was done for the mitochondrial cytochrome oxidase subunit I (COI) gene using LCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAATCA-3′) primers (Folmer et al. 1994). Each PCR contained 12.5μL 2X PCR master mix (Ampliqon, Denmark), 2.5μL each of the two primers (10nM), and 2.5μL of template DNA (10–20 ng) and water to make a final volume of 25μL. PCR conditions were as follows: initial denaturation at 95°C for 10 min, 35 cycles of 95°C for 45 sec, 50°C for 45 sec, and 72°C for 45 min and final extension at 72°C for 10 min. PCR products were then visualized on 1% agarose and products with the high intensity band were sequenced with ABI Prism 3730 Genetic Analyzer based on BigDye Terminator Chemistry.

Chromatograms were visualized, edited, and contigs were prepared using consensus sequences from both the strands in the BioEdit (Hall 1999). Sequences obtained in the present study were deposited in NCBI GenBank. Sequences of COI from the present study were then compared with published COI sequences of related taxa from NCBI GenBank using BLASTn tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). COI sequences of species of the genus *Seulocia* and other

related genus belonging to the family Leucosiidae were downloaded and aligned in the web version of Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). The alignment consisted of two sequences obtained in the present study (MN786514, MN786515), two published sequences of the genus *Seulocia*: *S. vittata* (MH675982), *S. latirostrata* (MH675981) as ingroup terminals, as well as four sequences from other genera as outgroup terminal: *Leucosia rubripalma* (MH675986), *L. craniolaris* (MH675985), *Euclosiana scitula* (MH675980), and *E. crosnieri* (MH675978). The pairwise genetic distance between the species was determined by the Kimura 2-parameter method (Kimura 1980) using MEGA 7 (Kumar et al. 2016). The phylogenetic tree was constructed using maximum likelihood method based on the Tamura-Nei model in MEGA 7 (Kumar et al. 2016). Bootstrap test was conducted using 1,000 replications to get the best topology from a 75% majority rule consensus tree (Felsenstein 1985).

RESULTS

Systematic accounts

Order Decapoda (Latreille, 1802)
 Infraorder Brachyura (Latreille, 1802)
 Family Leucosiidae (Samouelle, 1819)
 Genus *Seulocia* (Galil, 2005)
Seulocia vittata (Stimpson, 1858), Image 1 & 2.

Restricted Synonymy

Cancer craniolaris; Herbst, 1783: 90, pl. 2, fig. 17.
Leucosia craniolaris; Fabricius, 1798: 350 (part); K. Sakai, 1999: 19, pl. 7E.
Leucosia vittata Stimpson, 1858: 159; Shen & Dai, 1964: 28, fig.; Chhappgar, 1968: 609; Chen & Sun, 2002: 436, fig. 197, pl. 16.8.
Leucosides craniolaris; Rathbun, 1910: 310 (part).
Leucosia sinica Shen & Chen, 1978: 80, pl. 2, figs 12, 13, text-fig. 5; Huang, 1994: 580; Chen & Sun, 2002: 440, fig. 199.

Materials examined: Grey colouration: MBRC/ZSI D1-609, 11.vii.2019, 1 male, (cl 22mm; cw 20mm), India, Tamil Nadu, Mandapam fish landing site (Palk Bay), 9.286°N & 79.153°E, depth 10–15 m, col. Prakash & Amit Kumar.

Red Colouration: MBRC/ZSI D1-610, 11.vii.2019, 1 male and 1 female, (cl 22 each; cw 19 and 20), same collection data as above.

MBRC/ZSI D1-610, 11.vii.2019, 2 males, (cl 19 and 22; cw 18 and 20), same collection data as above.

Short description

Carapace sparsely punctate anteriorly, anterior margin tridenticulate with median denticle slightly larger than the adjacent ones. Anterolateral margin with minute beaded lines. Margin of epibranchial angle of carapace finely milled, epimeral margin evenly milled throughout. Posterior margin of carapace slightly rounded in male specimens (Both Grey and Red) and rounded in female specimens (Red). Thoracic sinus deep, pterygostomian region anteriorly defined by scalloped, overhanging and oblique margin. Fused abdominal segment bearing granule medially in male, smooth without granules in females. Merus of the major cheliped periform, tubercles on the lateral margins, few tubercles (3–5) on the dorsal as well as ventral region. The upper margin of carpus and palm smooth and lower margins periform, movable finger with upper margin carinate (both grey and red). Meri of the remaining pereopods bearing beaded lines on the dorsal and ventral margin, carpi prominently carinate dorsally. Propodi of the pereopods carinate in dorsal and ventral margins, dactyli flat and non-carinate on the lateral margins.

Colouration – Grey (Male)

Carapace bluish-grey, with median dorsal reddish-brown band becoming broader posteriorly (Image 1A–C). Presence of two oblique bands of reddish-brown on each side diverging from the front. Major cheliped meri, carpi and pal with a combination of reddish-brown and bluish-grey band, distal region reddish, fixed and movable finger whitish anteriorly and reddish posteriorly. Meri, carpi, propodi, and dactyli of the remaining pereopods with a combination of reddish and white bands, tip of the dactyli brown to black. Abdominal region almost whitish (Image 1B). Terminal end of the maxillipeds dark bluish-grey (Image 1C).

Colouration – Red (Male and Female)

Anterior region of carapace bluish-grey, latter half of the carapace brick red to reddish-brown in colour in females (Image 2A–C). In males, carapace with bluish-grey extended to latter half, posterior region of carapace brick-red (Image 2D–F). Oblique bands are not visible on carapace (Image 2D). Dorsal and ventral region of the meri, carpi, and propodi of the major chelipeds brick-red to reddish colour, dactyli of the movable dark red with a whitish tip (Image 2A,B). Meri of the remaining pereopods brick-red in colour, carpi, propodi, and dactyli dark brown to black in colour. Abdomen brick red with big black patch at the centre in both females and males

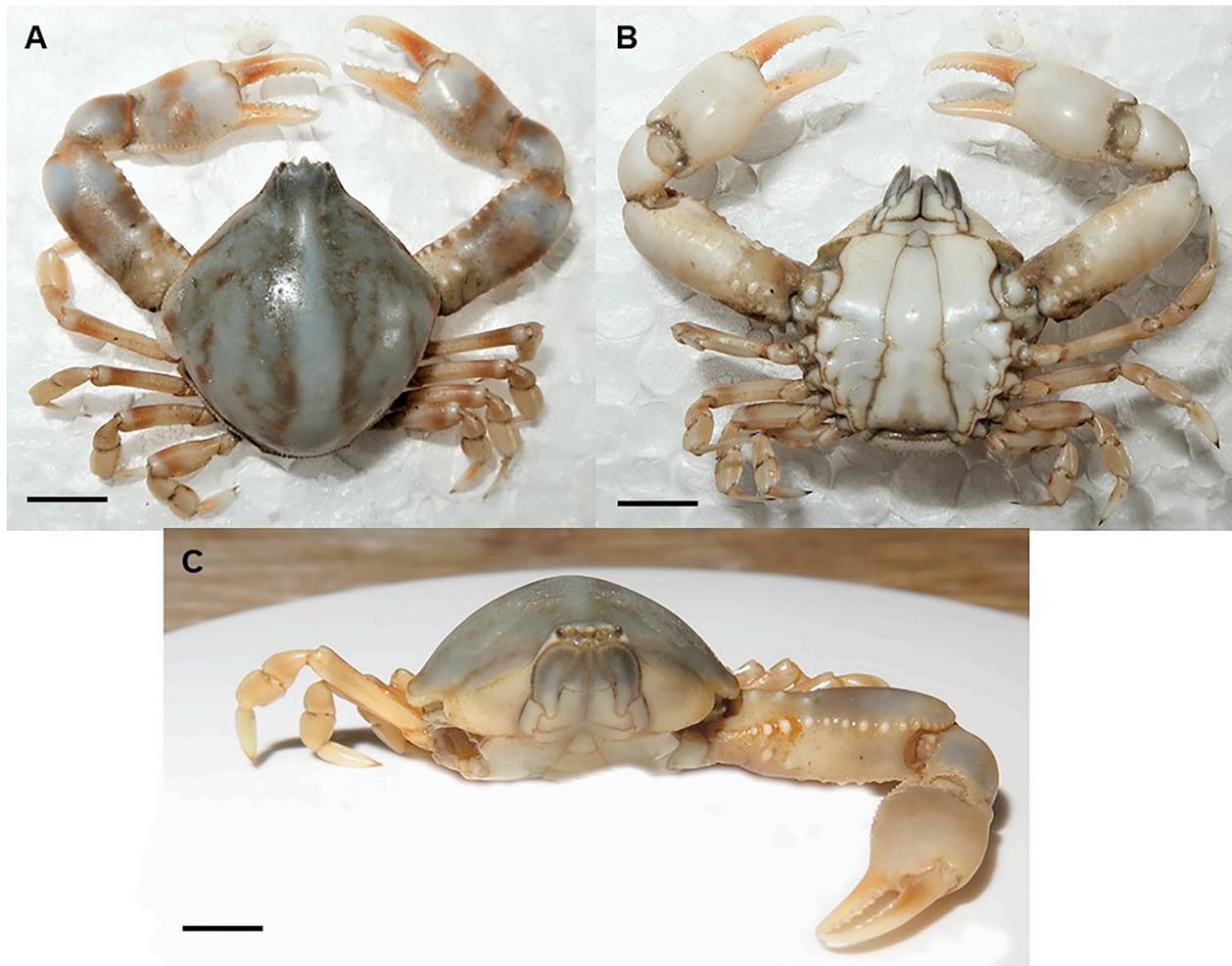


Image 1. Pebble crab *Seulocia vittata* (Stimpson, 1858) (Leucosiidae) male from Palk Bay with grey colour pattern. MBRC/ZSI D1-609. A—dorsal view | B—ventral view | C—frontal view of mouthparts. Scale bar: A–C = 6 mm. © Sanjeevi Prakash.

(Image 2B, 2E). Propodi and carpi of the major cheliped were black ventrally (Image 2B, 2E). Terminal end of the maxillipeds dark bluish-grey in both females and males (Image 2C, 2F).

DNA barcode and phylogenetic relationship

BLAST analysis revealed that the sequences for LG and LR exhibited 99.67% and 99.83% similarity with existing COI sequence of *Seulocia vittata* sequence in the NCBI GenBank. The phylogenetic analysis based on ML tree constructed using single mitochondrial gene fragment (COI – 653bp) resulted in tree topology that *S. vittata* (grey and red colour patterns) are closely related to *S. vittata* available in the GenBank (Figure 1). Out of 653 sites, 148 were parsimony informative sites. In the ML tree, all the *S. vittata* clustered together to form a monophyletic clade. In addition, *S. vittata* is sister to its congener comprising *Seulocia latirostrata* and other

related genera *Leucosia* and *Euclosiana* (Figure 1).

Furthermore, the calculated pairwise genetic distance of COI gene fragment using Kimura-2 parameter revealed that LR, LG, and published *S. vittata* in the GenBank have no genetic divergence. However, the genetic distance of 0.177 was calculated between *S. vittata* and *Seulocia latirostrata*, which is comparable to the genetic distance of more than 0.200 with other genera such as *Leucosia* and *Euclosiana* in the family Leucosiidae (Table 1).

DISCUSSION

A nomenclatural and taxonomical validation of the brachyuran crabs of the world includes over 6793 species belonging to 1,271 genera and 93 families (Ng et al. 2008). Interestingly, recent advances in the molecular techniques have received greater attention in

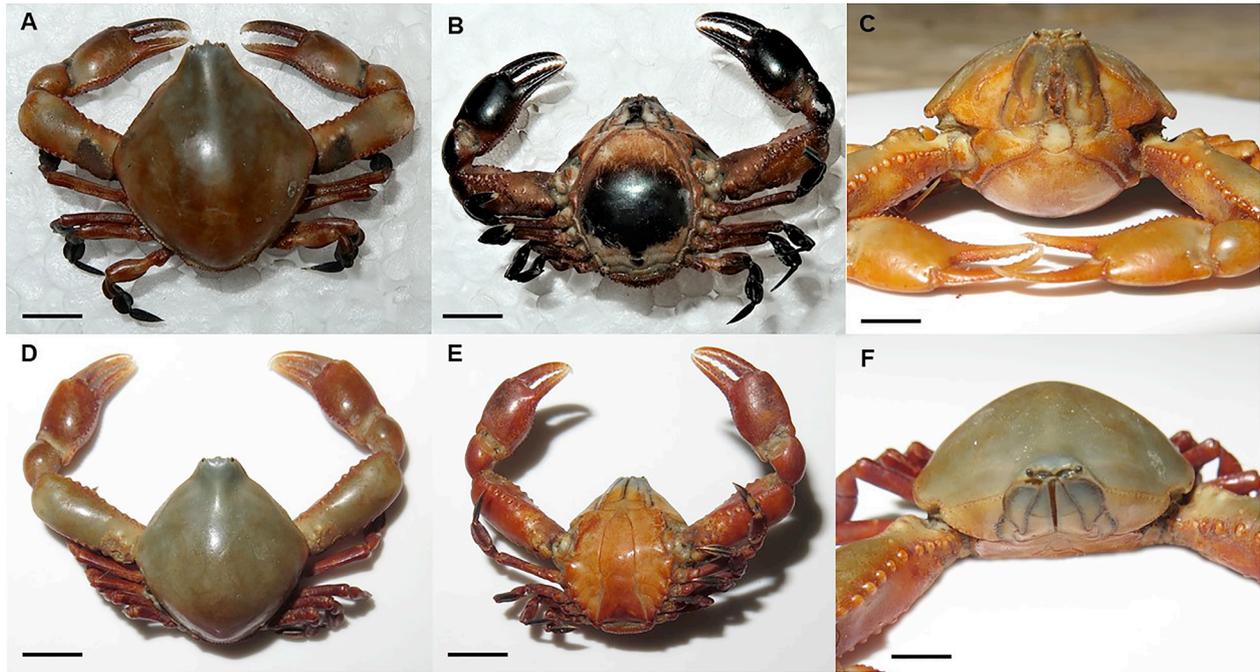


Image 2. Pebble crab *Seulocia vittata* (Stimpson, 1858) (Leucosiidae) females and males from Palk Bay – red colour pattern. MBRC/ZSI D1-610. A—Female, dorsal view | B—ventral view | C—frontal view of mouth parts | D—male, dorsal view | E—ventral view | F—frontal view of mouth parts. Scale bar: A–B = 6mm, C = 4mm; D–E = 5mm; F = 4mm. © Sanjeevi Prakash.

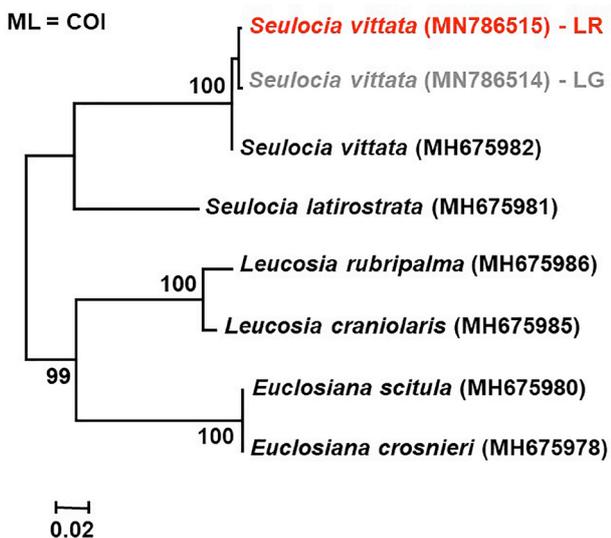


Figure 1. Maximum Likelihood phylogenetic tree of leucosiid crab *Seulocia vittata* (grey and red colouration) based on COI gene sequence data (653 bp, out of which 148 are parsimony informative sites). Numbers above or below the branches indicate bootstrap support based on ML. GenBank accession numbers are mentioned next to the species name in parentheses.

understanding the evolutionary perspectives of marine brachyuran crabs (Hultgren & Stachowicz 2008; Lai et al. 2013; Fratini et al. 2018; Mantelatto et al. 2018;

Chen et al. 2019). The integrative approach of both morphological and molecular analyses offers robust information not only on the taxonomic ambiguity of the species, including cryptic species (Baeza & Prakash 2019) but also in the monitoring of commercial crabs for seafood safety (Rath et al. 2018). In India, the molecular based study in brachyuran crabs was very limited (Vartak et al. 2015; Apreshgi et al. 2016; Ravichandran et al. 2017; Rath et al. 2018; Madhavan et al. 2020). In the present study, we performed the molecular phylogeny as well as identified the pairwise sequence divergence study of *Seulocia vittata* and its congeners based on the COI gene fragment. The ML tree suggests that *S. vittata* was sister to the *S. latirostrata* and other related species that are supported with high bootstrap values.

Seulocia vittata has a wide geographic distribution in the Indo-Pacific from Mauritius, India, Singapore, Malaysia, Thailand, Indonesia, China, and Philippines (Galil 2005). The present study represents a rediscovery of *S. vittata* in the south east coast of India after the original report of Alcock (1896) during the “investigator” expedition. Moreover, no information exists on its varying colour pattern. We observed that though the individuals of *S. vittata* differed in their colour pattern (grey and red variants), their genetic distance showed no variation between these two-colour forms. This

Table 1. Pairwise genetic distance calculated using Kimura 2-parameter based on COI gene fragment of *S. vittata* and other closely related taxa.

Species name	1	2	3	4	5	6	7
<i>Seulocia vittata</i> (MH675982)							
<i>Seulocia latirostrata</i> (MH675981)	0.177						
<i>Seulocia vittata</i> - LG (Present study)	0	0.177					
<i>Seulocia vittata</i> - LR (Present study)	0	0.177	0				
<i>Leucosia rubripalma</i> (MH675986)	0.214	0.212	0.214	0.214			
<i>Leucosia craniolaris</i> (MH675985)	0.202	0.196	0.202	0.202	0.026		
<i>Euclosiana scitula</i> (MH675980)	0.215	0.204	0.215	0.215	0.188	0.182	
<i>Euclosiana crosnieri</i> (MH675978)	0.215	0.204	0.215	0.215	0.188	0.182	0

indicates a situation of reverse cryptic behaviour in this crab. Surprisingly, the evolutionary and ecological processes leading to the absence of genetic divergence and variation in morphology (colour pattern) in *S. vittata* complex remain to be addressed. There could be several possible explanations: a) *S. vittata* possess the capacity to change colour and camouflage in nature as anti-predatory mechanisms (Stevens et al. 2014); b) the colour variation could be due to ecological adaptation to different depths and habitats such as reefs and open sand flats (Darnell 2012); c) morphological colour variation with low genetic structuring may indicate high dispersal capacities of *S. vittata* throughout the evolutionary history of this species. However, to validate the above hypotheses, extensive sampling efforts and detailed examinations at larger geographical scales are required.

Based on the outcome of this study, we recommend integrative taxonomic and phylogeographic approaches to demonstrate the extent and magnitude of species complexity in the leucosiid crabs. This goal needs to be prioritized as there is a recent increase in the trawl net operations in the south eastern coast of India that could lead to decline in the benthic biodiversity (Purohit 2017). This could cause profound implications in the conservation planning, stock assessment, biogeography, evolutionary as well as the natural history of leucosiids. Lastly, the species complexity in *S. vittata* will provide the opportunity to understand the important mechanisms of speciation among the leucosiid crabs.

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