

Integrative Taxonomic Identification of *Panulirus versicolor* (Latreille, 1804) Through Morphological Analysis and COI Barcoding

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ABSTRACT

The present study aimed to refine the taxonomy of Panulirus versicolor from Kanyakumari District, Tamil Nadu, by integrating morphological and molecular approaches. Specimens were collected from local fish landing centers and identified morphologically using 15 diagnostic traits recorded in a coding matrix. DNA barcoding was performed by amplifying and sequencing the mitochondrial COI gene, followed by phylogenetic analysis using Neighbor-Joining, Bayesian Inference, and BEAST methods. The morphological traits confirmed species identity, while molecular analysis revealed a low genetic divergence of 0.03 and a genetic distance of 0.71% from closely related species. Phylogenetic trees consistently clustered P. versicolor into a monophyletic clade along with sequences from China, Palau, and Sri Lanka. The study confirms that combining morphological data with COI based barcoding is effective for accurate species identification and understanding genetic relationships in spiny lobsters.

Keywords: Crustaceans, lobsters, *Panulirus versicolor*, Morpho-taxonomy, DNA barcoding, BOLD systems.

Mathematics Subject Classification: 62P12

Journal of Economic Literature (JEL) Classification: Q57

1. INTRODUCTION

Lobster fisheries in India rank fifth globally, with major contributions from the south-east and north-west coasts, the latter accounting for nearly three-quarters of the nation's total lobster landings (Vijayanand et al. 2007; Radhakrishnan & Manisseri, 2003; Radhakrishnan, 1995; Kagwade et al. 1991). Among the diverse species, the spiny lobster *Panulirus versicolor* (Latreille, 1804) of the family Palinuridae is ecologically important and commercially valuable. This species inhabits coral reefs across the tropical Indo-Pacific, including the coastal waters of Kanyakumari, and plays a vital role in supporting local fisheries (Holthuis, 1991).

Traditionally, morphological identification of animals (Singh, 2017; Linnaeus, 1758) and plants (Katuuk et al. 2018) is crucial for understanding biodiversity, conservation, and ecological relationships by recognizing species based on structural characteristics. In lobsters, species identification has been based on morphological features such as carapace shape, supination, and antennal structure (Iamsuwansuk et al. 2012; Holthuis, 1985). While these traits aid classification, they are often affected by intraspecific variation, ontogenetic changes, or environmental influences, leading to potential misidentification or underestimation of biodiversity (Abramson & Wells, 2018; Williams, 1986). To overcome these limitations, molecular tools have gained recognition in crustacean taxonomy, especially through the use of genetic markers such as 16S rRNA, 28S rDNA, and cytochrome c oxidase subunit I (COI), which offer greater tenacity at the species level (Hettiarachchi et al. 2022; Patwardhan et al. 2014; Ratnasingham & Hebert; 2007).

Despite the success of molecular approaches in resolving taxonomic uncertainties in many crustacean groups (Nishita 2024; Raj et al. 2023; Silva et al. 2010; Palero et al. 2009), there is a significant gap in molecular characterization of lobster species from underexplored coastal regions like Kanyakumari. Previous studies have either relied solely on morphological characters or molecular tools in isolation, without integrating both approaches for a comprehensive analysis. The present study addresses this gap by employing an integrated morpho-molecular approach for the identification and corroboration of *P. versicolor* from Kanyakumari District. Morphological traits were quantified using a diagnostic coding matrix, while molecular data were derived from COI gene sequencing. Phylogenetic relationships were inferred using advanced computational tools including Neighbour-Joining, Bayesian Inference, and BEAST. This combined approach enhances the reliability of species identification and contributes to a clearer understanding of genetic relationships within *P. versicolor*.

2. MATERIALS AND METHODS

Live specimens of *P. versicolor* were collected from the major lobster landing centers, Chinnamuttom (8.097752°N, 77.565699°E) and Kadiapattinam (8.134921°N, 77.303753°E), Kanyakumari district, Tamil Nadu (Fig. 1).

2.1. Morphological identification

The morphological study of the lobster followed the terminology and measurements specified by Radhakrishnan (2013), Holthius (1991) and Austin (1986). Species identification was conducted by examining various morphological features, such as body colouration, spines on the antennular plate, and the presence of transverse grooves on the abdominal segments. The matrix and morphological coding are mentioned in the table 1.

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	Character	Code
Carapace	Carapace shape	Sub cylindrical (0); Rounded (1); Flattened (2); Dorsoventrally flattened (3)
	Carapace spine	Absent (0); Present (1)
	Frontal horn	Absent (0); Present (1)
	Supraorbital spine	Absent (0); Present (1)
	Post-orbital spine	Absent (0); Present (1)
	Cervical groove	Absent (0); Present (1)
Antenna	Antennal spine	Absent (0); Present (1)
	Antennular plate	Absent (0); Present (1)
	Antennular plate (spine detail)	Absent (0); One pair of equal principal spines (1); Two pairs of sub-equal principal spines (2)
	Antennular plate (spine detail)	Absent (0); One pair of equal principal spines (1); Two pairs of sub-equal principal spines (2)
	Second antenna	Long, flagellate (0); Stout, flagellate (1); Short & broad (2); Broadly flattened & expanded (3)
	Antennular flagella	Absent (0); Longer than peduncle (1); Shorter than peduncle (2)
Abdominal Segment	Abdominal segment 2–5	Without transverse groove (0); With transverse groove (1); Incomplete transverse groove (2)
	Telson	Uncalcified & flexible distally (0); Calcified distally (1); Weakly calcified distally (2)

Tail	Uropod exopod diaeresis	Absent (0); Present (1)
	Uropod endopod	Reduced (0); Equal to exopod (1)

A coding matrix with 15 morphological keys (4 multivariate, 9 bivariate) was created in Mesquite 2.75 (Maddison & Maddison, 2011), based on data from 13 taxa. As outlined by Saitou and Nei (1987), a phylogenetic tree was constructed using the Neighbour-Joining (NJ) method in the PAUP software. The tree's reliability was evaluated using the Skewedness test (Hillis, 1991), analyzing 1,000,000 random tree topologies, and the G1 value (Sokal & Rohlf, 1981) was recorded to measure tree length distribution skewness.

2.2. Molecular taxonomy

DNA was extracted from the vas deferens utilizing the methodology established by Sambrook and Russell (2001), employing the NucleoSpin tissue kit (Macherey-Nagel). The tissue was broken down using T1 buffer and proteinase K, incubated at 56°C, and treated with RNase to remove RNA. After adding B3 buffer and incubating at 70°C and to precipitate the DNA, ethanol was added. The sample was then passed through a NucleoSpin column, washed, and the purified DNA was collected for further analysis. PCR amplification was performed using a GeneAmp thermal cycler (Applied Biosystems) with the Big Dye Terminator v3.1 cycle sequencing kit. The reaction mixture included Phire PCR buffer, dNTPs, DNA, Phire HotStart II polymerase, BSA, DMSO, Betaine, and COI specific primers. The amplified products were visualized using a 1.2% agarose gel, and the quality of the sequences was evaluated with Sequence Scanner Software version 1.0 (Applied Biosystems). The sequences were subsequently edited utilizing Geneious Pro version 5.1 (Drummond et al. 2010).

The COI sequences were comparison-aligned with those from fifteen known spiny lobster species utilizing MEGA version 11 (Tamura et al., 2021) alongside BioEdit version 7.2.5 (Hall, 1999). A phylogenetic tree based on Maximum Likelihood (ML) was generated in MEGA version 7 (Kumar et al., 2016), while genetic distances were determined using the Kimura 2-Parameter model. Further, an ML phylogram with bootstrap support was created using IQ-TREE on XSEDE 8.1.12 via the Prescience Gateway (Miller et al. 2010), using 1000 ultrafast bootstrap replicates (UFBoot). The tree was visualized in FigTree v1.4.4 (Rambaut & Drummond, 2018). The best model of sequence evolution for the COI gene was selected using TPM2+F+I based on the Bayesian Information Criterion (BIC), replacing several other substitution models as per Lecocq et al. (2013). Bayesian Inference (BI) was conducted utilizing BEAUTi v1.10.4 and BEAST v1.10.4, executing a Markov Chain Monte Carlo (MCMC) run for 10 million generations with tree sampling occurring every 1000 generations. The resulting phylogenetic trees were illustrated using FigTree.

The COI partial gene sequences of *P. versicolor* were submitted to GenBank and the Barcode of Life Data System (BOLD), which uses the COI gene for species identification and comparison. The barcode phylogram generated through BOLD grouped sequences based on genetic similarity (Austerlitz et al. 2009; Ratnasingham & Hebert, 2007; Hebert et al. 2003).

3. RESULTS

3.1. Systematics

Order: Decapoda Latreille, 1802

Infraorder: (Achelata Scholtz & Richter, 1995)

Family: *Palinuridae* Latreille, 1802

Species: *Panulirus* sp. (Fig 2. A & B)

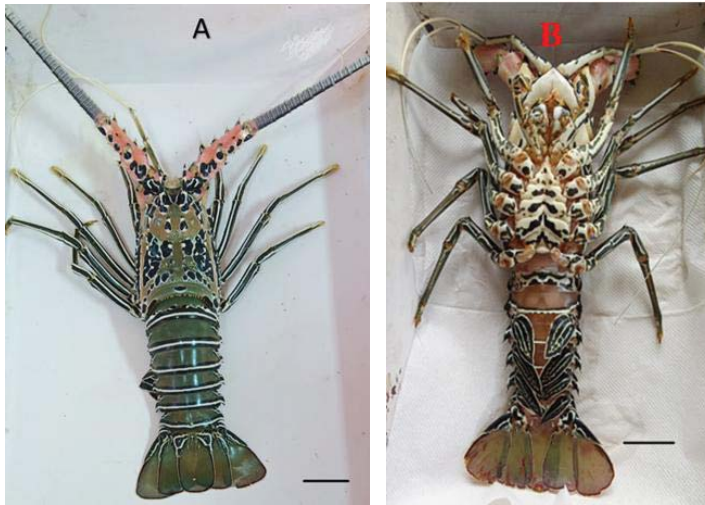


Figure 2. *P. versicolor* collected from Chinnamuttom, Kanyakumari District, Tamil Nadu, India, showing the colour of a living specimen. A, whole animal dorsal view; B, whole animal ventral view. Scale bars = 5 cm (A), 5 cm (B).

3.2. Material examined

Holotype: Adult male (36.6 mm x 11.7 mm) (Museum Number: HCZLO2), Kadiapattinam (8.134921°N; 77.303753°E) and Chinnamuttom (8.097752°N; 77.565699°E) Kanyakumari District, Tamil Nadu, India, 8.2731°N; 77.1607°E coll., Shyla Suganthi A, and Tyni Joice Raj T G, 10 Dec 2019.

3.3. Description

The carapace is subcylindrical, slightly shorter than the abdomen, and covered in sharp spines that point forward. Large spines (horns) are located above the eyes, pointing anterodorsally, with smaller spines towards the back. There are two rows of four gastric spines, and a distinct cervical groove, with other areas marked by shallow grooves. The segments between the cardiac, branchial, and intestinal regions are barely divided. The abdomen is smooth, with shallow, incomplete grooves on somites (S2–S5) that are interrupted in the middle, especially on somites (S3–S4). The telson is sub-rectangular, flexible at the end, and lacks diaeresis. The antennular plate has two pairs of similar spines, with the outer and inner spines on the antennal peduncle being different in length. The distal antennal segment has a large spine on its ventral surface and a dorsal spine. The third maxilliped lacks an exopod but has

a crista dentate, while the second maxilliped has an exopod. The first pereopod (P1) is normal and not inflated, with an oblique propodus, and the fifth pereopod (P5) has a coxal spine.

Based on the character status summary in table 2, three characters are parsimony-uninformative, and twelve are parsimony-informative characters. 10,00,000 random trees were generated from each data set. The skewness of the tree length frequency distribution is G1 values ($g1 = -0.239433$, $p < 0.02$) (Fig 3). The NJ tree confirms that *P. versicolor* from Chinnamuttom clusters monophyletically with other *P. versicolor* populations from the Indian and Indo-Pacific coasts, showing minimal genetic divergence (0.03) (Fig 4).

Table 2: Morpho-taxonomic phylogenetic tree analysis.

Species / Location	Character states
<i>Panulirus versicolor</i> (Chinnamuttom)	011111112010010
<i>P. versicolor</i> (Palabuharatu Bay)	011111112010010
<i>P. versicolor</i> (Indian coast)	011111112010010
<i>P. versicolor</i> (Indian coast)	011111112010010
<i>P. versicolor</i> (West Indian coast)	011111112010010
<i>P. versicolor</i> (Kenya)	111111112010010
<i>P. meripurpuratus</i>	011111112012010
<i>P. polyphagus</i> (Mayalibit Bay)	111111111010010
<i>P. ornatus</i> (Kenya)	111111112010010
<i>P. homarus</i> (Kenya)	111111112011010
<i>P. longipes longipes</i> (Indonesia)	111111111111011
<i>Ibacus brucei</i> (Japan)	300000200000000
<i>Homarus americanus</i>	000001100100101



Figure 3. Skewed frequency distribution of 1000000 random trees for 13 taxa and characters of *P. versicolor*, all unordered and unscaled.

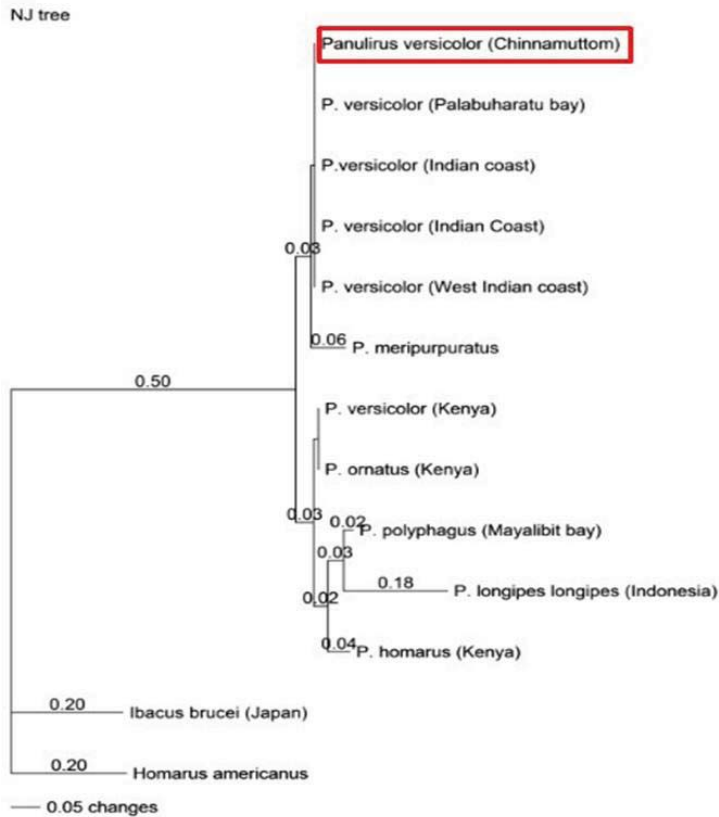


Figure 4. *P. versicolor* relationship supported by Neighbour-Joining phylogram using morphological characters: The tree length – 21, consistency index – 0.857, retention index – 0.813, rescaled consistency index – 0.696, homoplasy index – 0.143.

3.4. Molecular phylogenetic analysis

The partial COI gene sequence of *P. versicolor*, with a length of 675 base pairs, was successfully isolated and submitted to GenBank (Accession No. MN273739) (Fig 5). Following alignment, phylogenetic analysis using the ML method revealed that *P. versicolor* formed a monophyletic clade with a strong bootstrap support value of 86%, confirming its taxonomic validity (Fig 6). BI further supported this topology, with posterior probabilities ranging from 0.5 to 1 across most nodes. The TPM2+F+I model was selected as the best-fit evolutionary model based on the BIC. The BI search consisted of four independent MCMC runs, each conducted for 10 million generations, with samples taken every 1,000 generations and the first 1 million generations discarded as burn-in. (Fig.7). Pairwise alignment and distance analysis revealed that *P. versicolor* from Chinnamuttom showed 99.53%, 99.51%, and 99.47% similarity with sequences from Palau Island, Sri Lanka, and Indonesia, respectively. The closest genetic relationships were with *P. versicolor* MW041243 and MG062681 (both from Indonesia), showing the smallest pairwise distance of 0.70 (Tables 3 & 4). Barcode analysis through the BOLD system confirmed the monophyletic grouping of the Chinnamuttom specimen with *P.*

versicolor from other geographical locations, supporting its distinct but closely related genetic identity within the species complex (Table 5 & Fig.8).

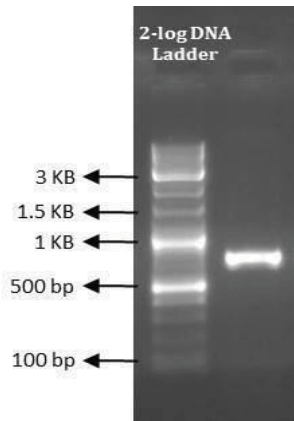


Figure 5. Electropherogram of PCR COI gene of *P. versicolor*.

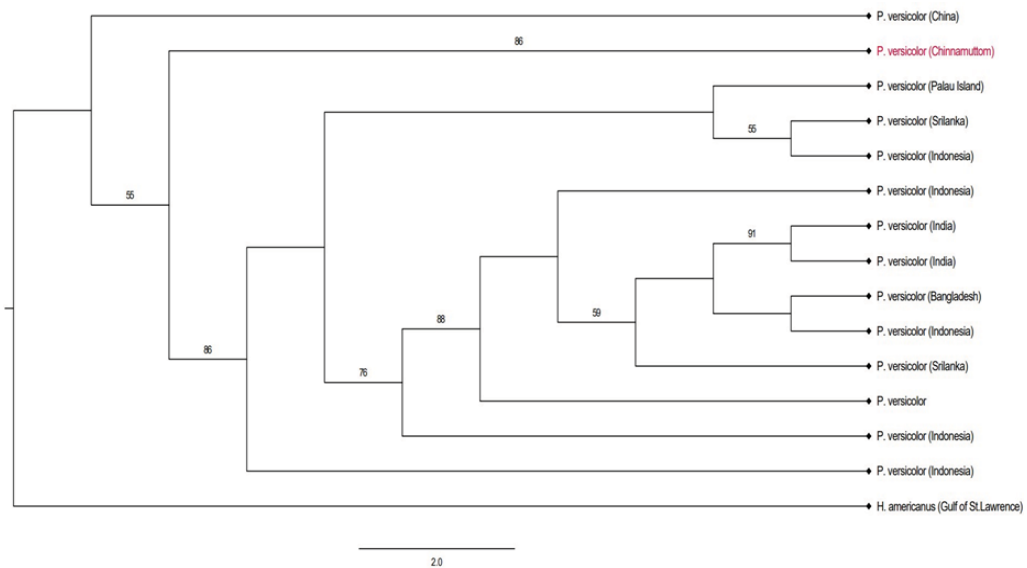
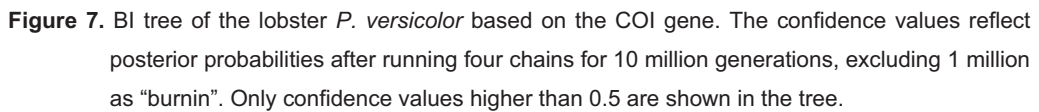


Figure 6. Maximum Likelihood phylogeny of the Spiny lobster *P. versicolor* based on the COI gene, showing a distinct clade, and the numbers at the branches show bootstrap support. Maximum Log-likelihood of -2340.8942, the unconstrained log-likelihood -3128.1862, total tree length 2.2056, the sum of internal branch length 0.0080.

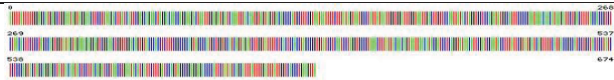


S. No	Species	Per. Identity	Accession
1	<i>Paratuberculosis</i>	100.00%	MM276730

Table 4: Estimation of Evolutionary Divergence between the species pairs (pair-wise distance).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>P. versicolor</i> MN273739														
<i>P. versicolor</i> AF339472	0.73													
<i>P. versicolor</i> KF548586	0.73	0.00												
<i>P. versicolor</i> MW041243	0.70	0.70	0.70											
<i>P. versicolor</i> MW041249	0.73	0.69	0.69	0.71										
<i>P. versicolor</i> MW041248	0.73	0.69	0.69	0.70	0.01									
<i>P. versicolor</i> JQ229882	0.77	0.77	0.77	0.78	0.77	0.77								
<i>P. versicolor</i> MT749755	0.74	0.75	0.75	0.75	0.77	0.77	0.75							
<i>P. versicolor</i> JN591366	0.71	0.75	0.75	0.78	0.75	0.74	0.76	0.69						
<i>P. versicolor</i> KF548584	0.73	0.01	0.01	0.70	0.69	0.69	0.77	0.75	0.75					
<i>P. versicolor</i> MW832712	0.71	0.75	0.75	0.79	0.77	0.77	0.73	0.71	0.69	0.75				
<i>P. versicolor</i> MW041250	0.82	0.78	0.78	0.75	0.77	0.76	0.68	0.78	0.75	0.78	0.77			
<i>P. versicolor</i> MG062681	0.70	0.74	0.74	0.78	0.76	0.76	0.76	0.71	0.68	0.75	0.69	0.74		
<i>P. versicolor</i> KX275386	0.73	0.73	0.73	0.68	0.70	0.70	0.78	0.75	0.76	0.73	0.77	0.77	0.73	
<i>H. americanus</i> FJ581693	0.75	0.77	0.77	0.77	0.78	0.78	0.75	0.78	0.77	0.77	0.78	0.70	0.77	0.79

Table 5: The Barcode of the partial COI gene of *P. versicolor*.

S. No	Name of the species	Barcode	BIN
1.	<i>P. versicolor</i>		AAD3352

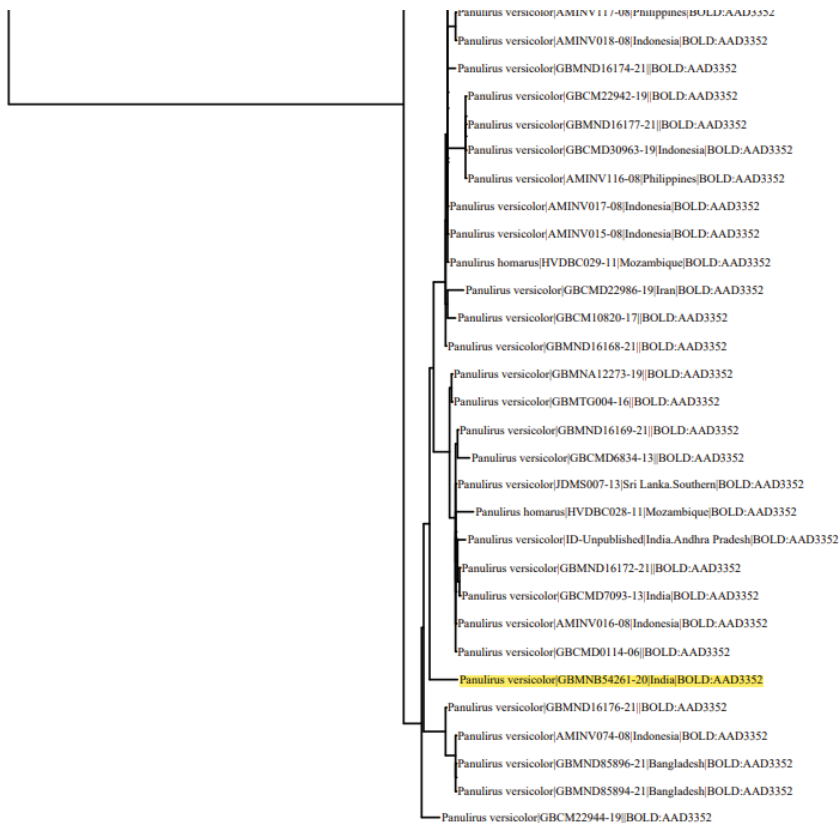


Figure 8. COI-based barcode phylogram of *P. versicolor*, including the Kanyakumari specimen highlighted with yellow.

4. DISCUSSION AND CONCLUSION

The integrative analysis in the present study confirmed the identification of *P. versicolor* from Kanyakumari by combining morphological features, molecular data, and DNA barcoding. While earlier studies have generally employed either morphological or molecular tools independently, this study is among the first to apply all three methods in tandem for this species along the southern Indian coast. The observed morphological characteristics corresponded with the recognized diagnostic features outlined by De Grave et al. (2009) and Holthuis (1991). The diagnostic matrix included 15 characters, of which 12 were parsimony-informative (Fitch, 1971), reinforcing the morphological diagnosis and enabling comparative evaluation with closely related taxa.

Molecular analysis of the mitochondrial COI gene grouped the Kanyakumari specimen with *P. versicolor* sequences from Indonesia, Palau, and Sri Lanka. The alignment between morphological and molecular results illustrates the strength of an integrated approach, especially in data-deficient regions. ML analysis confirmed a monophyletic grouping of *P. versicolor*, supported by a bootstrap value of 86% (Felsenstein, 1985). BI applied using the TPM2+F+I model yielded high posterior probabilities,

contributing strong support for the evolutionary relationships among the sampled individuals (Huelsenbeck & Ronquist, 2001; Yang, 1994). The application of MCMC-based methods (Larget & Simon, 1999) further refined the inferred phylogenetic structure.

The NJ tree also grouped the Kanyakumari specimen with those from Sri Lanka and Indonesia, and the low genetic distances (Saitou & Nei, 1987) indicate close affinity and potential gene flow within the Indo-Pacific region. DNA barcoding through the BOLD system validated these results by clustering the specimens within a global *P. versicolor* clade. The use of BOLD not only enhances taxonomic precision but also integrates genetic data with geographic and ecological metadata (Ratnasingham & Hebert, 2007; Hebert et al. 2003).

To conclude, this study highlights the effectiveness of an integrated morpho-molecular approach for the accurate identification of *Panulirus versicolor* from the Kanyakumari coast. Morphological examination using 15 diagnostic traits, supported by COI-based DNA barcoding and phylogenetic analysis, confirmed species identity with high confidence. The consistent clustering of *P. versicolor* with sequences from China, Palau, and Sri Lanka in all phylogenetic trees indicates strong genetic coherence across its distribution. The BOLD system's application further emphasizes how important easily accessible genomic databases are for biodiversity research and species authentication. Overall, this integrative approach proves to be a reliable tool for resolving taxonomic ambiguities and enhancing our understanding of spiny lobster genetics.

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6. REFERENCES

- Abramson, C.I., Wells, H., 2018, An inconvenient truth: Some neglected issues in invertebrate learning, *Perspect. Behav. Sci.* **41**, 395-416. <https://doi.org/10.1007/s40614-018-00178-8>
- Austerlitz, F., David, O., Schaeffer, B., Bleakley, K., Olteanu, M., Leblois, R., ... & Laredo, C., 2009, DNA barcode analysis: a comparison of phylogenetic and statistical classification methods, *BMC Bioinform.* **10**, 1-13. <https://doi.org/10.1186/1471-2105-10-S14-S10>
- Linnaeus, C., 1758, *Systema Naturae per Regna Tria Naturae* 10, Stockholm: Laurentius Salvius.
- Darriba, D., Taboada, G.L., Doallo, R., & Posada, D., 2012, jModelTest 2: more models, new heuristics and parallel computing, *Nat. Methods.* **9**, 772-772. <https://doi.org/10.1038/nmeth.2109>
- De Grave, S., Pentcheff, N.D., Ahyong, S.T., Chan, T.Y., Crandall, K.A., Dworschak, P.C., ... & Wetzer, R., 2009, A classification of living and fossil genera of decapod crustaceans, *Raffles. B. Zool.* **21**, 1-109. <http://hdl.handle.net/10088/8358>
- Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., Wilson, A., 2010, Inspirational Software for Biologists, Geneious. <http://www.geneious.com>
- Felsenstein, J., 1985, Confidence limits on phylogenies: an approach using the bootstrap, *Evol.* **39**, 783-791. <https://doi.org/10.2307/2408678>
- Fitch, W.M., 1971, Toward defining the course of evolution: minimum change for a specific tree topology, *Syst. Biol.* **20**, 406-416. <https://doi.org/10.2307/2412116>
- Hall, T.A., 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucleic Acids Symp. Ser.* **41**, 95-98.
- Hebert, P.D., Cywinska, A., Ball, S.L., & de Waard, J.R., 2003, Biological identifications through DNA barcodes, *Proc. Biol. Sci.* **270**, 313-321. <https://doi.org/10.1098/rspb.2002.2218>
- Hettiarachchi, S.A., Hyeon, J.Y., Mahardini, A., Kim, H.S., Byun, J.H., Kim, H.J., ... & Hur, S.P., 2022, DNA barcoding and morphological identification of spiny lobsters in South Korean waters: a new record of *Panulirus longipes* and *Panulirus homarus homarus*, *PeerJ.* **10**, e12744. <https://doi.org/10.7717/peerj.12744>
- Hillis, D.M., 1991, Discriminating between phylogenetic signal and random noise in DNA sequences, *Phylogenetic analysis of DNA sequences.* 278-294. <https://doi.org/10.1093/oso/9780195066982.003.0013>
- Holthuis, L., 1985, A revision of the family Scyllaridae (Crustacea Decapoda Macrura). I. subfamily Ibacinae, *Zool. Verh.* **218**, 1-130. <https://repository.naturalis.nl/pub/317894/ZV1985218001.pdf>
- Holthuis, L.B., 1991, Marine lobsters of the world. An annotated and illustrated catalogue of species of interest to fisheries known to date, *FAO Fish. Synop.* **13**, 1-292. <https://www.fao.org/4/t0411e/t0411e00.htm>
- Huelsenbeck, J.P., Ronquist, F., 2001, MRBAYES: Bayesian inference of phylogenetic trees, *Bioinformatics.* **17**, 754-755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Iamsuwansuk, A., Denduangboripant, J., Davie, P.J., 2012, Molecular and morphological investigations of shovel-nosed lobsters *Thenus* spp. (Crustacea: Decapoda: Scyllaridae) in Thailand, *Zool. Stud.* **51**, 108-117. <https://zoolstud.sinica.edu.tw/Journals/51.1/108.pdf>
- Kagwade, P.V., Manickaraja, M., Deshmukh, V.D., Rajamani, M., Radhakrishnan, E.V., Suresh, V., ... & Rao, G.S., 1991, Magnitude of lobster resources of India, *J. Mar. Biol. Assoc. India.* **33**, 150-158. <http://eprints.cmfri.org.in/id/eprint/832>

- Katuuk, V., Wagey, B.T., Mantiri, D., Gerung, G., & Bucol, L.A., 2018, Variation in morphological parameters of the seagrass *Thalassia hemprichii* (Ehrenberg) Ascherson) across substrate types and locations in Northern Sulawesi, Indonesia, *Int. J. Ecol. Dev.* **33**, 64-72. <http://www.ceser.in/ceserp/index.php/ijed/article/view/5602>
- Kumar, S., Stecher, G., Tamura, K., 2016, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, *Mol. Biol. Evol.* **33**, 1870-1874. <https://doi.org/10.1093/molbev/msw054>
- Larget, B., Simon, D.L., 1999, Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees, *Mol. Biol. Evol.* **16**, 750-759.
- Latreille, P.A., 1802, Histoire naturelle des crustacés et des insects, *Orthoptera*. **3**, 267-284. <https://www.biodiversitylibrary.org/item/80064#page/7/mode/1up>
- Latreille, P.A., 1804, Des langoustes du Muséum national d'Histoire naturelle, *Ann. Hist.-Nat. Mus. Natl. Hung.* **3**, 388-395. <https://www.biodiversitylibrary.org/item/93709#page/2/mode/1up>
- Lecocq, T., Vereecken, N.J., Michez, D., Dellicour, S., Lhomme, P., Valterova, I., ... & Rasmont, P., 2013, Patterns of genetic and reproductive traits differentiation in mainland vs. Corsican populations of bumblebees, *PLoS ONE*. **8**, e65642. <https://doi.org/10.1371/journal.pone.0065642>
- Maddison, W.P., Maddison, D.R., 2011, Mesquite: A Modular System for Evolutionary Analysis, Version 2.75. <https://www.mesquiteproject.org/>
- Nishita, L., Anilkumar, G., 2024, Revisiting the phylogenetic status of a field crab (Decapoda, Brachyura) from Chelari, Malappuram District (Kerala, India): an integrative approach through morphological and molecular taxonomy, *Crustaceana*. **97**, 21-53. <http://dx.doi.org/10.1163/15685403-bja10356>
- Palero, F., Crandall, K.A., Abelló, P., Macpherson, E., Pascual, M., 2009, Phylogenetic relationships between spiny, slipper and coral lobsters (Crustacea, Decapoda, Achelata), *Mol. Phyl. Evol.* **50**, 152-162. <https://doi.org/10.1016/j.ympev.2008.10.003>
- Patwardhan, A., Ray, S., Roy, A., 2014, Molecular markers in phylogenetic studies-a review, *J. Phylogenet. Evol. Biol.* **2**, 131. <http://dx.doi.org/10.4172/2329-9002.1000131>
- Radhakrishnan, E.V., 2013, Taxonomy and identification of commercially important crustaceans of India, Crustacean Fisheries Division, Central Marine Fisheries Research Institute. 137-156.
- Radhakrishnan, E.V., Manisseri, M.K., 2003, Lobsters. In: Status of exploited marine fishery resources of India, CMFRI. 195-202.
- Raj, T.G., Suganthi, A.S., Raj, T.G., Anilkumar, G., 2023, Morphological and molecular identification of the freshwater crab, *Barytelphusa cunicularis* from Kanniyakumari, India, *J. Environ. Biol.* **6**. <http://doi.org/10.22438/jeb/44/6/5088>
- Ratnasingham, S., Hebert, P.D., 2007, BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>), *Mol. Ecol. Notes*. **7**, 355-64.
- Saitou, N., Nei, M., 1987, The neighbour-joining method: a new method for reconstructing phylogenetic trees, *Mol. Biol. Evol.* **4**, 406-425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Sambrook, J., Maccallum, P., Russell, D., 2001, Molecular Cloning: A Laboratory Manual 3, Cold Spring Harbor Press. ISBN 0-87969-577-3.
- Scholtz, G., Richter, S., 1995, Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca), *Zool. J. Linn. Soc.* **113**, 289-328. <https://doi.org/10.1111/j.1096-3642.1995.tb00936.x>
- Singh, S.P., Groeneveld, J.C., Al-Marzouqi, A., Willows-Munro, S., 2017, A molecular phylogeny of the spiny lobster *Panulirus homarus* highlights a separately evolving lineage from the Southwest Indian Ocean. *PeerJ*. **5**: e3356. [DOI 10.7717/peerj.3356](https://doi.org/10.7717/peerj.3356)

Sokal, R.R., Rohlf, F.J., 1981, Biometry 2. W. H. Freeman and Company.

Tamura, K., Stecher, G., Kumar, S., 2021, MEGA 11: Molecular Evolutionary Genetics Analysis Version 11, *Mol. Biol. Evol.* **38**, 3022-3027. <https://doi.org/10.1093/molbev/msab120>

Vijayanand, P., Murugan, A., Saravanakumar, K., Khan, S.A., Rajagopal, S., 2007, Assessment of lobster resources along Kanyakumari (South East Coast of India), *J. Fish. Aquat. Sci.* **2**, 387-394. <http://dx.doi.org/10.3923/jfas.2007.387.394>

Williams, A.B., 1986, Lobsters-identification, world distribution, and US trade, *Mar. Fish. Rev.* **48**, 1–36. https://marine-lobsters.linnaeus.naturalis.nl/linnaeus_ng/app/views/literature2/reference.php?id=19805

Williams, A.B., 1986, The Diversity of the Crustacean Lobsters. *Marine Biology*. **93**, 475–483.

Yang, Z., 1994, Estimating the pattern of nucleotide substitution, *J. Mol. Evol.* **39**, 105-111. <https://doi.org/10.1007/bf00178256>