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REVİEW

Unveiling Biodiversity: A Critical Review of Morpho-molecular Identification in Crabs

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Abstract

Crabs are a diverse and ecologically important group of crustaceans found in both marine and freshwater habitats. Accurate species identification is essential for understanding biodiversity, ecological balance, and conservation management. Traditional morphological taxonomy, based on traits such as carapace shape, cheliped structure, and setal pattern, remains the foundation of classification but is often limited by intraspecific variation, phenotypic plasticity, and cryptic species. To overcome these limitations, this review integrates morphological and molecular approaches to assess their combined effectiveness in crab identification. A systematic review of 117 peer-reviewed studies published between 2000 and 2025 was conducted following PRISMA guidelines. Molecular tools, including DNA barcoding using COI and 16S rRNA genes, ITS-1, and RAPD markers, provide higher accuracy and reveal cryptic diversity. The integration of morphological and molecular data enhances taxonomic resolution, confirms species boundaries, and enables detection of invasive and new taxa. Despite these advances, challenges persist due to incomplete genetic databases, lack of standardized protocols, and limited access to affordable sequencing technologies. This review highlights the need for harmonized methodologies and global collaboration to improve crab taxonomy. A holistic approach combining ecological, behavioral, and genetic data is essential for effective biodiversity assessment and conservation.

Introduction

Crabs, belonging to the infraorder *Brachyura*, represent one of the most diverse and ecologically significant groups of decapod crustaceans, with approximately 700 genera and 7,000 species distributed across 98 families (Kaestner, 1970; Ng, 1988; Peter et al., 2008; Tsang et al., 2014). Their remarkable adaptability has allowed them to thrive in an astonishing array of environments, including abyssal seas, coral reefs, rocky shores, tropical rainforests, volcanic vents, and even arid deserts (Davie, 2021). This ecological versatility, coupled with their complex behaviors and unique physiological traits, underscores the importance of crabs in marine and terrestrial ecosystems. Although crabs are ecologically and biologically diverse, their accurate identification at the species level remains a persistent challenge. Traditional taxonomy, which relies primarily on morphological and morphometric traits such as carapace shape, cheliped morphology, and body proportions, often struggles to distinguish closely related or cryptic species (Klinbunga et al., 2000; Fazhan et al., 2017b; Waiho et al., 2018).

Additionally, body size variations further distinguish these species, with *S. serrata* being the largest and *S. olivacea* the smallest and most robust due to their adaptation to low-salinity intertidal habitats (Fazhan et al., 2017a). Morphological and morphometric analysis has long been the cornerstone of crab taxonomy and species identification. Farmers, crabbers, and researchers alike rely on physical traits such as carapace shape, cheliped morphology, and body proportions to differentiate species. However, while these traits are useful, they can sometimes be ambiguous due to overlapping features or environmentally induced variations (Waiho, Fazhan & Ikhwanuddin, 2016b). For example, closely related species within the genus *Scylla* exhibit subtle morphological differences that require detailed and precise measurements to achieve accurate identification (Ikhwanuddin et al., 2011; Fazhan et al., 2017b). Many brachyuran species exhibit high morphological similarity, particularly among closely related taxa and juvenile individuals, making traditional morphological approaches unreliable in some cases (Schubart et al., 2001; Yamasaki et al., 2011). Additionally, crabs play a critical role in marine bioinvasions, necessitating precise species identification to assess and manage their impact on estuarine and coastal ecosystems (Brockerhoff & McLay, 2011). To address these challenges, molecular tools have proven invaluable in crab identification and biodiversity assessment. DNA barcoding techniques, which analyze a standardized region of the mitochondrial cytochrome c oxidase I (COI) gene, have proven highly effective in distinguishing closely related species. Additionally, these techniques facilitate the identification of larval, juvenile, and adult stages of crabs by providing genetic confirmation of species identity, even when morphological differences obscure taxonomic classification (Hebert et al., 2003; Schindel & Miller, 2005). This approach has been successfully applied to a wide range of animal taxa, including fish, birds, mollusks, and crustaceans, revealing a clear barcoding gap between intraspecific and interspecific genetic divergence (Davison et al., 2009; Feng et al., 2011). Furthermore, molecular analyses incorporating additional genetic markers, such as mitochondrial 16S rRNA and nuclear genes, have enhanced our ability to resolve taxonomic ambiguities in crabs (Markert et al., 2014; Yamasaki et al., 2011).

The objective of this critical review is to compile and synthesize current knowledge on morpho-molecular identification techniques in crabs, emphasizing their applications in biodiversity assessment, taxonomy, and related biological fields. This review aims to enhance understanding of crab diversity and resolve taxonomic ambiguities by integrating traditional morphology with molecular approaches and evaluating key genetic markers. Species-level identification remains hindered by morphological variability, cryptic taxa, and inconsistent molecular references. This review bridges these gaps through a crab-specific framework linking diagnostic traits to marker selection, sampling, analysis, and harmonized protocols. Unlike earlier works that isolate morphology or barcoding or treat decapods broadly, this review provides a PRISMA-guided synthesis (to Jan 2025), a traits-to-markers crosswalk and a phylogenetic scaffold. we aim to highlight the vast, still-unexplored biodiversity of crabs and encourage standardized molecular protocols for accurate identification, conservation, and sustainable utilization of these ecologically and economically important organisms.

Methods

Literature Search Strategy

This systematic review was conducted following the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020)* guidelines. A comprehensive literature search was performed across five databases: Scopus, Web of Science, PubMed, ScienceDirect, and Google Scholar, covering publications up to January 2025. The search strategy combined Boolean operators, wildcards, and controlled vocabulary (where available) to ensure thorough retrieval of studies on crab morpho-molecular identification. The primary search string used was:

("crab" OR "Brachyura" OR "crustacean") AND
("molecular identification" OR "DNA barcoding" OR
"genetic marker" OR "phylogenetic analysis") AND
("morphology" OR "morphometric" OR "taxonomy")

This core query was adapted for each database to align with specific indexing rules. Filters were applied to include only *peer-reviewed articles* and *English-language publications*. Reference lists of selected papers were manually screened to identify additional relevant studies (backward and forward citation searching). All searches were conducted initially in August 2024 and last updated on January 15, 2025. The search results were exported to EndNote for duplicate removal, after which titles and abstracts were screened.

Study Selection and Eligibility Criteria

The inclusion and exclusion process adhered to predefined criteria to ensure methodological consistency. Eligible studies were required to: Conduct morphological identification of crab species; Employ molecular markers (e.g., COI, 16S rRNA, ITS, or nuclear genes) for species identification; Focus on taxonomy, phylogenetic relationships, or biodiversity assessment; Be published in peer-reviewed journals or well-documented preprints with full methodological details. Studies were excluded if they: Focused solely on ecological, physiological, or aquaculture aspects without taxonomic analysis; Lacked molecular or morphological

data; Were review papers, conference abstracts, or theses without full data access.

Two independent reviewers (Author A and Author B) screened all titles and abstracts. Discrepancies were resolved through discussion, and unresolved cases were adjudicated by a third expert reviewer (Author C). Inter-reviewer agreement was quantified using Cohen's kappa (κ= 0.87), indicating high reliability.

Screening and PRISMA Flow Diagram

A total of 757 records were identified across databases and reference lists. After removing 86 duplicates, 671 records were screened. Of these, 289 full-text articles were assessed for eligibility, and 154 were excluded for not meeting inclusion criteria (e.g., lacking molecular data, incomplete methods, or non-peer-reviewed sources). Finally, 117 studies were included in the qualitative synthesis. The full selection process is summarized in Figure 1 (PRISMA Flow Diagram), following PRISMA 2020 standards with four main phases: *Identification, Screening, Eligibility,* and *Inclusion.*

Data Extraction and Management

Data extraction was performed using a structured Excel template. For each included study, the following data were collected: Author(s), year, and study location; Crab species examined and taxonomic group; Morphological methods and diagnostic traits used; Molecular markers and primer sequences; Sequencing platforms and analytical tools; Key taxonomic findings and limitations. When necessary, corresponding authors were contacted to obtain missing or unclear methodological details. All extracted data were cross-verified by two reviewers for consistency.

Quality Assessment

The quality of the included studies was assessed using a modified version of the Newcastle-Ottawa Scale for observational studies and experimental research (Luchini et al., 2017). Studies were evaluated on five dimensions:

(1) clarity of morphological description,

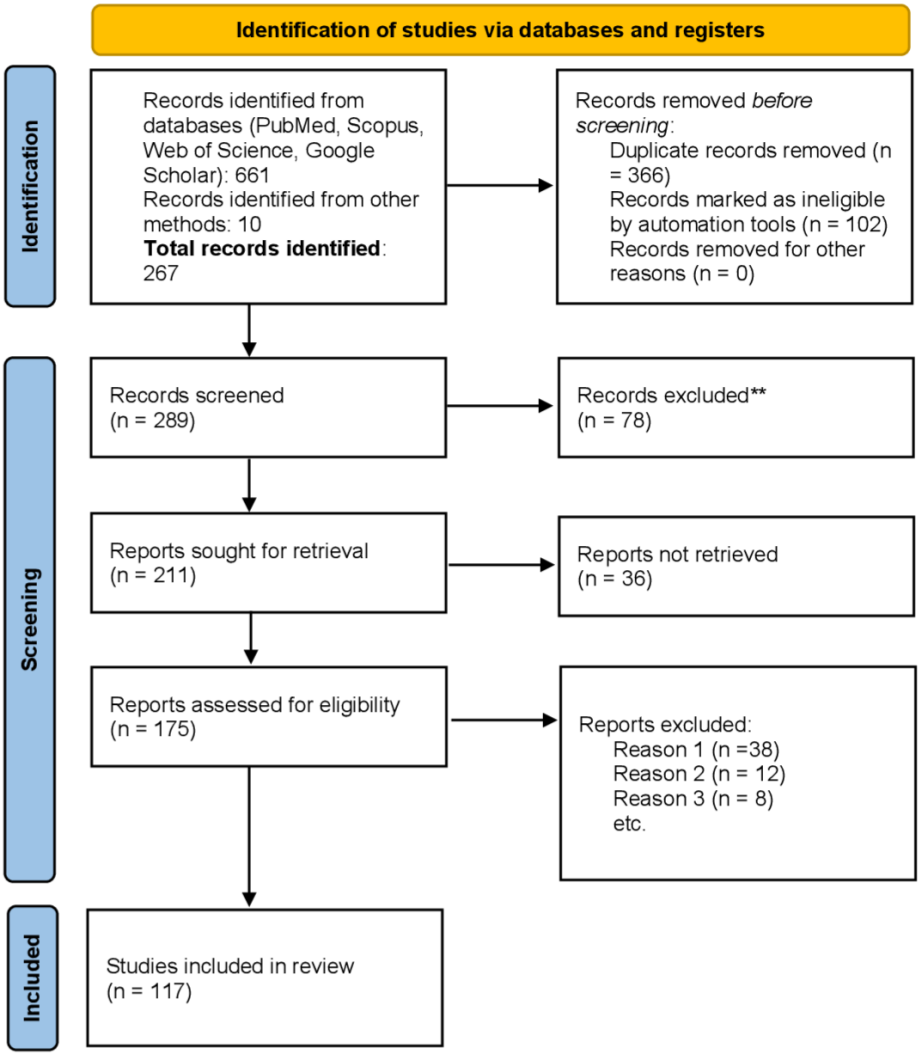

Figure 1. Screening literature using Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).

(2) appropriateness of molecular markers,
(3) accuracy of taxonomic classification,
(4) sample size and design adequacy, and
(5) statistical robustness.

Each criterion was rated on a 3-point scale (0-2), and studies scoring below 6/10 were subjected to sensitivity analysis or excluded.

Data Synthesis and Analysis

Due to heterogeneity among methodologies, statistical meta-analysis was not feasible. Instead, a comprehensive descriptive and qualitative synthesis was undertaken using data from 117 reviewed studies. Frequencies of molecular markers (COI, 16S rRNA, ITS-1, NaK, 18S rDNA, RAPD/RFLP, and NGS/eDNA), taxonomic groups, and geographic distributions were compiled in Microsoft Excel and analyzed in RStudio (v4.5.1). Bar charts, pie charts, and temporal trend plots illustrated marker usage patterns, while a heat map highlighted associations between marker type and research purpose (e.g., species identification, phylogeny, or hybrid detection). Results revealed a dominance of mitochondrial genes (COI, 16S) and a gradual rise in genomic tools. Biases, knowledge gaps, and methodological inconsistencies were critically evaluated to identify research needs in crab morpho-molecular taxonomy.

Morphological Identification

Morphological identification has traditionally formed the cornerstone of crab taxonomy, relying on diagnostic traits such as carapace structure, cheliped morphology, and coloration patterns (Keenan et al., 1998; Ng, 1998). While these features remain fundamental, their reliability and reproducibility have been increasingly questioned because of phenotypic plasticity, sexual dimorphism, and observer bias (Waiho et al., 2016; Fazhan et al., 2017). Therefore, a critical comparison of both classical and modern morphometric methods is essential to strengthen taxonomic precision.

Classical Morphological Approaches

Traditional taxonomy has historically depended on linear morphometrics and qualitative traits such as the number of anterolateral spines, frontal-lobe shape, and cheliped proportions (Stephenson, 1972; Serène, 1984). These features are often measured from standard anatomical landmarks of the carapace and appendages (Figure 2; Farrag, 2022), although they can vary with environmental and ontogenetic conditions, leading to

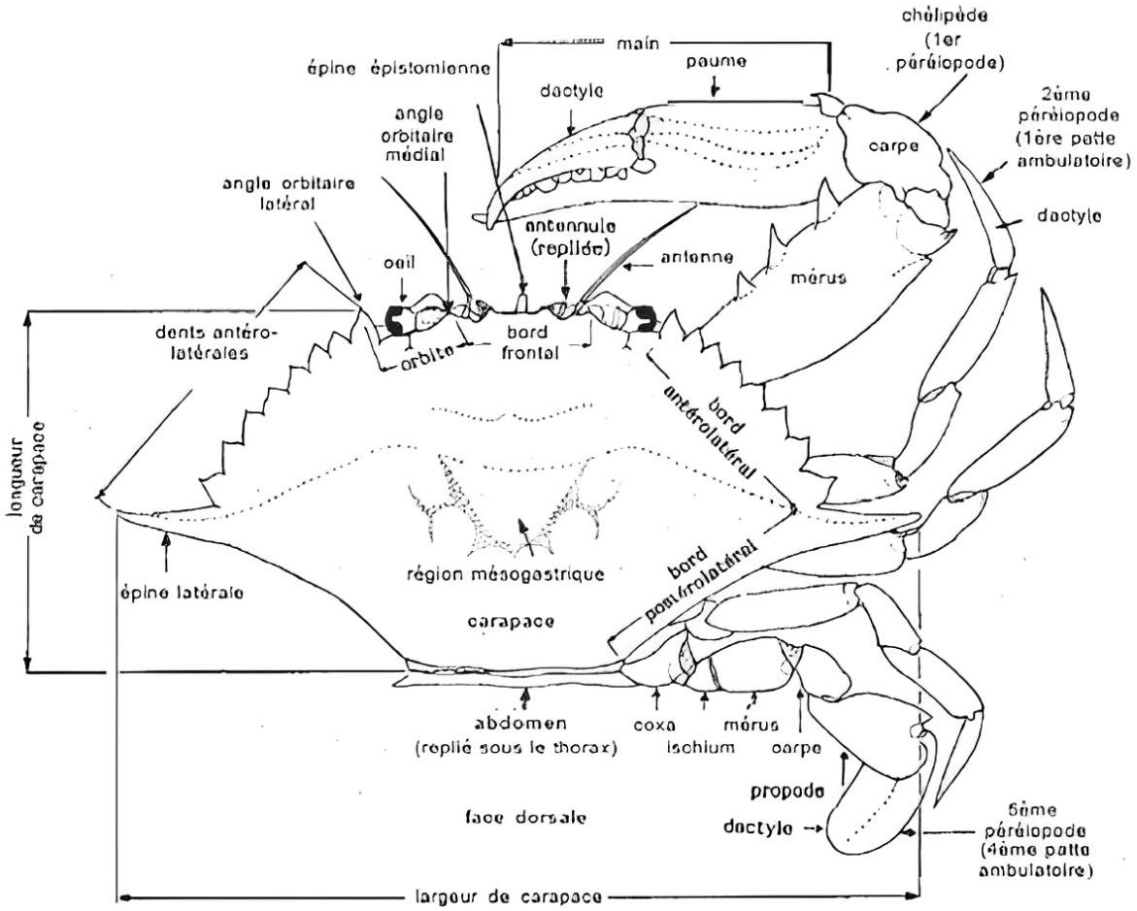

Figure 2. General Morphometric measurement and description of the common form of crabs (Farrag, 2022).

intraspecific overlap (Ikhwanuddin et al., 2011; Naim et al., 2020). *Measurement inconsistency is another limitation:* caliper-based readings and subjective interpretation can introduce inter-observer error (Reiter et al., 2025). Vermeiren et al., (2021) reported calibration protocols or error margins, making reproducibility difficult. To improve reliability, researchers are urged to employ replicate measurements, calibration against reference specimens, and inter-observer precision tests.

Modern Morphometric and Image-Based Techniques

Recent decades have seen the rise of geometric morphometrics and image-analysis-based tools that capture the geometry of structures through landmark coordinates instead of simple distances (Grinang et al., 2019; Xu et al., 2023). Luo, (2024) reported that software such as tpsDig, SHAPE, and MorphoJ allow quantitative assessment of carapace shape variation via principal-component and canonical-variates analyses, providing statistical measures of shape differentiation. Beyond geometric morphometrics, machine-learning image classifiers and deep-learning convolutional neural networks (CNNs) have shown promise in automating species recognition from photographs of carapaces or chelipeds (Malik et al., 2024). These computational tools can minimize human bias and scale to large ecological datasets, supporting citizen-science biodiversity monitoring.

Integrative Reliability, Applications, and Future Directions

Morphological and molecular comparisons have revealed varying degrees of congruence. In *Scylla* and *Charybdis*, morphological identifications differ from molecular results in up to 30% of cases (Barua et al., 2021; Markert et al., 2014), often due to hybridization or environmental effects. Conversely, geometric morphometric datasets show greater alignment with DNA-based species boundaries (Sultana et al., 2022), suggesting that statistically defined shape variables more accurately reflect genetic divergence. Representative examples of the morphological and morphometric techniques used across crab taxa, together with their diagnostic challenges which are summarized in Table 1. This comparative overview underscores how diverse morphological approaches, from scanning electron microscopy to geometric and molecular analyses, are increasingly combined to resolve species boundaries, especially in taxa exhibiting cryptic diversity. To ensure reliability in future morphological studies, a comprehensive framework should include:

- Standardized and error-quantified measurement protocols;
- Molecular barcoding (COI, 16S rRNA) for validation; and
- Cross-validation of morphological clusters with genetic lineages.

Finally, developing digital image repositories and morpho-molecular databases that link shape descriptors to barcode data will preserve morphological evidence as a quantitative and verifiable pillar of modern integrative taxonomy.

Molecular Identification

Molecular identification has transformed crab taxonomy by supplementing traditional morphological approaches with precise genetic tools. Over the past two decades, molecular markers have been extensively applied to resolve taxonomic ambiguities, detect cryptic diversity, and validate morphologically similar taxa. Instead of focusing on laboratory steps, this section synthesizes how these methods have been used across studies, highlighting marker preferences, success rates, and remaining limitations.

Patterns in Molecular Approaches

Across the 117 reviewed studies, mitochondrial genes overwhelmingly dominate crab molecular identification efforts. The COI gene is the most frequently applied marker, appearing in roughly 65% of studies due to its universality and high interspecific divergence. COI barcoding effectively distinguished *S. serrata*, *S. tranquebarica*, and *S. olivacea* (Ma et al., 2012; Mandal et al., 2021) and remains the “gold standard” for *Decapoda*. The 16S rRNA gene follows in popularity (≈ 40% of studies), often paired with COI to strengthen phylogenetic resolution (Markert et al., 2014). Nuclear markers such as ITS-1, NaK, and 18S rDNA appear in about 20% of publications, typically for resolving recent divergences or hybridization events (Imai et al., 2004; Mandal et al., 2014b). Although less common, microsatellites, NGS, and eDNA are emerging in population-level and environmental studies (Li et al., 2021). Collectively, these findings indicate a gradual shift from single-gene barcoding toward multilocus and high-throughput frameworks (Figure 3A-B).

Methodological Variations and Tissue Sources

As summarized in Table 2, DNA extraction and tissue selection practices varied considerably among studies. Muscle tissue from chelae or ambulatory legs was most frequently used, followed by hepatopancreas and gill samples. About 70% of studies employed either the phenol-chloroform or Proteinase K methods, achieving comparable DNA purity to commercial kits such as Qiagen DNeasy®. Extraction success depended more on tissue preservation (ethanol vs. frozen) than on extraction method type. RNA-based studies, such as *Uca*

Table 1. Morphological Identification Techniques for Different Crab Species

Species	Morphological Traits	Techniques and Tools	Taxonomic Challenges	Reference
Scylla spp.	Frontal lobe spine shapes (distinct among species)	Molecular markers (internal transcribed spacer 1, 16S rDNA), Fourier transformation, SHAPE software, Resemble.js	Difficulty in species identification in early developmental stages due to soft tissue extraction limitations	(Cruz-Abeledo et al., 2018; PARVIN et al., 2018)
Scylla species from the Philippines	Geometric patterns in swimming and walking legs, asymmetry in chelipeds, continuity in frontal lobe spines	Morphological species identification, Morphometric techniques, Molecular techniques (ITS-1 fragments)	High intraspecific variation, Continuity of diagnostic features, Misidentification due to morphological ambiguities	(Vincecruz-Abeledo & Lagman, 2018).
Brachyuran crabs (general)	Zoeal morphology, detailed setation of mouthparts, larval stages. Adaptations to mid-water habitat, family and sub-family level features in zoeae. Variability in zoeal groupings at higher taxonomic levels (e.g., family, sub-family). Proposed revision of Brachyura systematics in relation to zoeal features	Morphological analysis, setation study. Comparative analysis of larval features. Phylogenetic analysis Zooplankton collection, species identification	Past failure to categorize zoeae corresponding to adult groupings. Convergence in adult characters; failure to recognize ecological adaptations. Potential need for radical rearrangement based on zoeal features. Groups based on evolution grades rather than phylogenetic lines	(Rice, 1980)
Grapsus albolineatus, Ozius truncates, Uca (Galasimus) tetragonon	Shape, color, and size; triangular abdomen (female), tapered triangle abdomen (male)	Morphological examination, field observation	Difficulty in differentiating between closely related species based on morphology alone	(Rustikasari et al., 2021)
River Crab(*Potamonautes sidneyi, Potamonautes unispinus*)	Presence of epibranchial teeth, gonopod 1 shape, 4 diagnostic loci	Electrophoretic analysis (11 enzyme systems)	Morphological similarity with other river crabs, requiring genetic analysis to differentiate, Distinguishing between two distinct forms of river crabs in South Africa	(Stewart & Cook, 1998)
Blue crab (*Callinectes sapidus*)	Aesthetasc tuft divided into mesial and lateral halves; presence of aesthetascs and new sensilla	Scanning Electron Microscopy, Behavioral Tests	Differentiating between sensilla types; functional confirmation of sensilla	(Gleeson, 1982).
Charybdis japonica	Diagnostic traits include carapace shape and features, chela structure, and other intertidal adaptations.	Mitochondrial DNA sequencing (cytochrome oxidase 1 gene), morphological analysis.	Overlap in traits with native and non-native species; identifying juvenile or cryptic forms.	(Smith et al., 2003)
Eriocheir sinensis	Carapace shape changes over time; relative distortion score highlights differences after "bathing" culture.	Geometric morphometrics; 35 established landmarks; stepwise discriminant analysis; visualization tools.	Morphological convergence incomplete after one month; distinguishing "bathed" crabs from original populations.	(Xu et al., 2023)
Hemigrapsus penicillatus	Smaller setal patches on male chelae; dark spots on cephalothorax, abdomen, mandibles, and limbs.	Starch gel electrophoresis, morphological analysis, visual pigmentation inspection.	Morphological similarity with sibling species; distinguishing females when pigmentation fades.	(Takano et al., 1997; Mingkid et al., 2006; Asakura & Watanabe, 2005; Markert et al., 2014)
Hemigrapsus takanoi	Larger setal patches on male chelae; absence of dark spots; distinct first pleopod morphology in males.	Field surveys, microscopic examination, morphometric measurements, habitat mapping.	Intermediate traits in overlapping zones; males with conflicting pigmentation and morphological features.	(Mingkid et al., 2006; Asakura & Watanabe, 2005; Markert et al., 2014)
Pagurus spp.	Variation in shell occupancy, chelae size and shape, and carapace morphology across species.	Phylogenetic analysis using mitochondrial (16S, COI) and nuclear markers; sequence concatenation.	Resolving taxonomic positions of newly described species and unassigned morpho-groups.	(Sultana et al., 2022)
Charybdis (Charybdis) hellerii	Carapace dentation, cheliped shape, and color patterns distinctive for identification.	Morphological description, COI gene sequencing (DNA barcoding).	Overlap in morphological traits with closely related species; reliance on genetic confirmation.	(Abbas et al., 2016)
Portunus (Portunus) pelagicus	Broad carapace, distinctive blue coloration in males, and pereiopod shape variations.	Morphological identification and mitochondrial COI gene analysis.	Phenotypic plasticity due to environmental factors; distinguishing regional populations.	(Abbas et al., 2016; Hidayani et al., 2018)
Liocarcinus corrugatus	Corrugated carapace with defined ridges; first record in the Egyptian Red Sea.	Morphological description, mitochondrial COI sequencing for confirmation.	First report in this region; previously known in other oceans, requiring broader comparisons.	(Abbas et al., 2016)
Atergatis roseus	Smooth carapace, distinctive rose-like coloration, and robust chelipeds.	Morphological traits supported by DNA barcoding using COI gene sequencing.	Challenges in distinguishing juveniles from other Atergatis species; habitat-based phenotypic variations.	(Abbas et al., 2016)

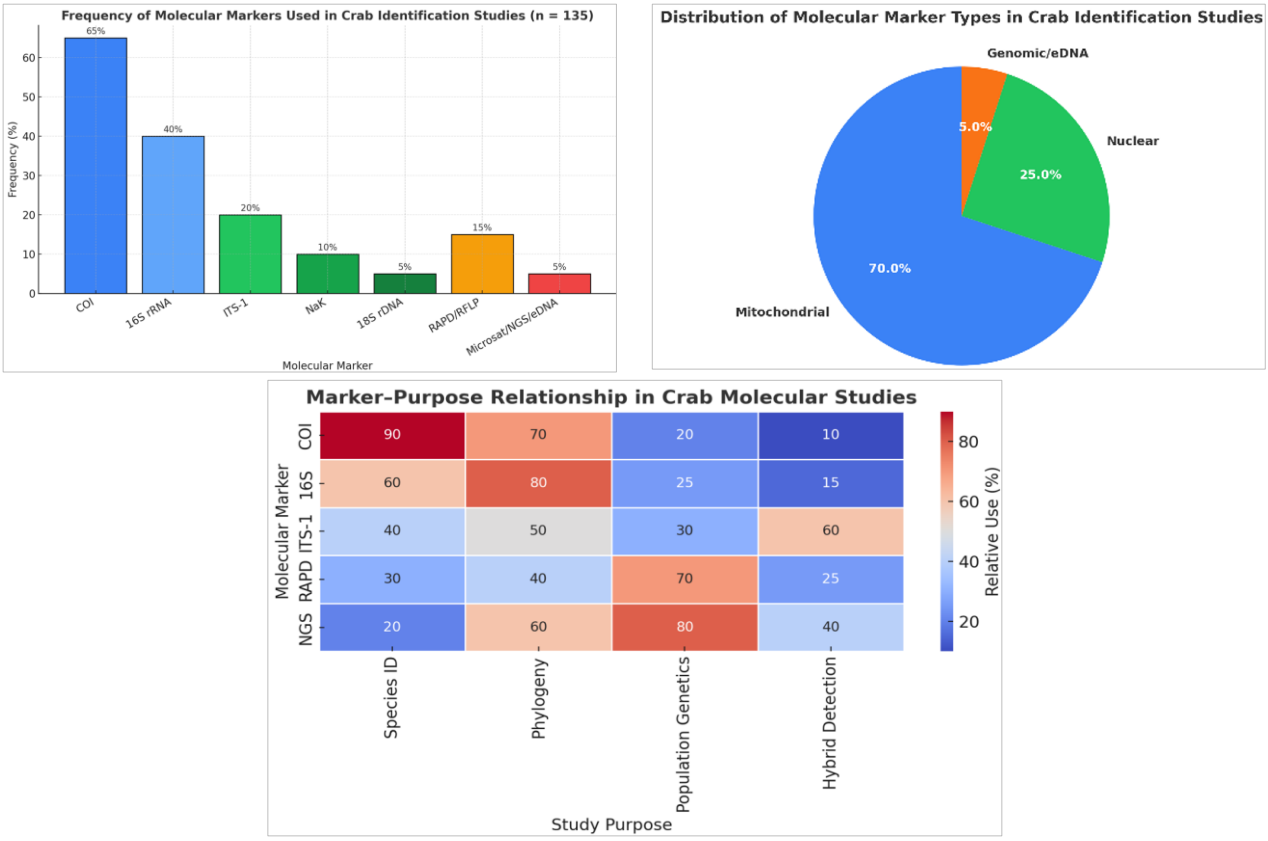

Figure 3. Molecular identification trends in crab studies (n = 117): (A) Frequency of molecular markers used, showing COI dominance; (B) Proportional distribution of mitochondrial, nuclear, and genomic/eDNA markers; and (C) Heatmap linking marker types with research objectives such as species identification, phylogeny, and biodiversity monitoring.

Table 2. DNA Extraction Methods and Quality Assessment in Crab Species

Species Name	Tissue Used	DNA Extraction Method	DNA Quality Check	Fluorescent Dye	Final DNA Concentration	Reference
Scylla spp.	Alcohol-preserved muscle tissue (~25 mg)	Proteinase K and phenol–chloroform - Isoamyl alcohol method	Electrophoresis on 0.7% agarose gel	Non-mutagenic (EZ Vision In-Gel Solution, Amresco, USA; 0.1 µl/ml)	Diluted to 75 ng/µl with sterile double-distilled water	(Mandal et al., 2014; Mandal et al., 2021)
U. pugilator	Eyes of male fiddler crabs (n = 5)	TRIzol reagent (manufacturer's protocol, Gibco BRL, Gaithersburg, MD, USA)	Synthesized cDNA from RNA; PCR amplification	No fluorescent dye used for RNA extraction	cDNA synthesized from ~1 µg of RNA, PCR amplification used for opsin gene sequences	(Rajkumar et al., 2010)
Uca forcipata and *Uca triangularis*	Genomic DNA from fiddler crab tissue (collected from Surabaya, Indonesia)	DNA kit extraction (Molecular Physiology Laboratory PKNU, Busan, Korea)	Molecular identification via BLASTN (99% certainty)	No fluorescent dye used during extraction	Not specified, used for BLASTN analysis	(Andriyono et al., 2019)
Charybdis japonica	Crab muscle tissue (22 individuals)	Proteinase K-based method	PCR-based with five ONT primers, electrophoresis in 1.4% agarose gel	Ethidium bromide (EtBr) for gel electrophoresis	5 ng of template DNA per PCR reaction	(Yoon, 2022)
Potamon spp	Muscle tissue from ambulatory leg (<2 mm)	DNeasy® Blood & Tissue Kit (QIAGEN); purified using BioSprint 15	PCR amplification with LCO1-1490/HCO1-2198 primers; confirmed via electrophoresis	-	Eluted in 200 µl AE buffer; 1–2 µl template DNA used in PCR	(Rezaei et al., 2022)

pugilator opsin gene amplification (Rajkumar et al., 2010), remain uncommon, reflecting the dominance of DNA-level taxonomy. These methodological insights emphasize that while protocols differ, most yield reliable material for amplification and sequencing when coupled with rigorous quality checks and contamination control (see Table 2).

Primer Design and Marker Optimization

Table 3 details the range of primers applied across taxa. Universal COI primers designed by Folmer et al. (1994) were used in nearly half of the studies, while species-specific primers (e.g., *Scy-F/Scy-R* from Ma et al., 2012) increased diagnostic precision for *Scylla* species. ITS-1 primers (Chu et al., 2001) proved particularly valuable for detecting hybridization and intra-genus variation, whereas mitochondrial 16S primers (Imai et al., 2004) supported phylogenetic reconstructions. The prevalence of COI-based primer sets highlights an ongoing trade-off between universality and specificity: universal primers facilitate cross-study comparison, but taxon-specific primers offer higher resolution for closely related or regionally endemic crabs (see Table 3).

Success Rates and Comparative Insights

Success rates in species identification correlate strongly with marker choice and DNA quality. Studies using COI and 16S achieved over 90% accuracy, whereas RAPD and RFLP markers often fell below 70%. Ma et al. (2012) and Naim et al. (2020) reported clear barcode gaps among *Scylla* species, but hybrid populations occasionally blurred boundaries. Similarly, research on *Hemigrapsus* and *Charybdis* (Markert et al., 2014; Abbas et al., 2016) demonstrated that COI outperformed ITS-1 and RFLP in resolving species complexes. Geographic patterns were also evident: Indo-Pacific species exhibited higher COI divergence than Atlantic or temperate taxa, reflecting regional evolutionary rates (Figure 3C).

Data Reliability, Databases, and Analytical Tools

Approximately 80% of the studies relied on Sanger sequencing, valued for cost-effectiveness and accuracy in single-specimen work. The recent transition to NGS and metabarcoding platforms (Li et al., 2021) enables large-scale biodiversity monitoring. Nearly all studies compared sequences against GenBank or BOLD, yet about 25% of entries lacked voucher data or metadata, leading to potential misidentifications (Meiklejohn et al., 2019; Ari & Arikan, 2016). Analytical tools such as MEGA, RAxML, and BEAST were most frequently used for tree construction, reflecting a shift toward Bayesian and maximum-likelihood frameworks (see Table 4).

Limitations and Future Directions

Persistent limitations include primer bias, incomplete reference databases, and contamination from degraded tissues. DNA degradation, especially in preserved museum material, continues to hinder amplification (Zimmermann et al., 2008). Hybridization and incomplete lineage sorting can also produce discordant morphological and molecular signals. Recent developments such as multilocus barcoding, cross-marker validation, and integration with ecological metadata are beginning to mitigate these issues. Future crab biodiversity research should prioritize standardized marker sets, regional reference databases, and

Table 3. Details of Primers Used in PCR Amplification

Primer Name	Sequence (5' → 3')	Target Gene	Additional Notes	Reference
L-SP-1–3′	ATTTAGCTGCGGTCTTCATC	ITS-1 Region of ribosomal DNA	Used for amplifying ITS-1 region.	(Chu et al., 2001; Imai et al., 2004)
H-SP-1–5′138	CACACCGCCCGTCGCTACTA	ITS-1 Region of ribosomal DNA	-	(Chu et al., 2001; Imai et al., 2004)
Scy-F	GATACSCGAGCTTAYTTTACATC	COI (Cytochrome Oxidase Subunit I)	Designed for species-specific PCR.	(Ma et al., 2012)
Scy-R	TAGGATTAAGRGAYAAACCTGTAAA	(COI)	Produces a 325 bp product for all species.	(Ma et al., 2012)
ScyS-R	AATAAATCCTAAAGCCCATAATATA	COI (Species-specific for *S. serrata*)	Amplifies a 138 bp product when paired with Scy-F.	(Ma et al., 2012)
ScyO-R	GTGTCATGTAGGATAATATCGATG	COI (Species-specific for *S. olivacea*)	Amplifies a 212 bp product when paired with Scy-F.	(Ma et al., 2012)
ScyP-R	AACATAGTGGAAATGGGCTACG	Species-specific fragment	-	(Mandal et al., 2014a)
LCO1490	GGTCAACAAATCATAAAGATATTGG	(COI)	Universal primer for DNA barcoding; amplifies a 709 bp fragment.	(Folmer et al., 1994)
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	(COI)	-	(Folmer et al., 1994)
mtd10	TTGATTTTTTGGTCATCCAGAAGT	(COI)	Heavy strand primer; used for insects and *Scylla* species; amplifies a 597 bp fragment.	(Roehrdanz , 1993)
C/N2769	TTAAGTCCTAGAAAATGTTGRGGGA	(COI)	Light strand primer; used for insects and *Scylla* species.	(Gopurenko et al., 1999)

quantitative performance analyses comparing marker reliability and success rates.

Overall, molecular identification in crabs has evolved from simple COI barcoding to complex, multi-gene, and genomic frameworks. While COI remains the benchmark, integrating mitochondrial (COI, 16S) and nuclear (ITS-1, NaK) markers yields the highest taxonomic confidence. Properly curated datasets such as those outlined in Tables 2-4 are fundamental for sustaining accurate, reproducible, and globally comparable molecular taxonomy. Continued refinement of databases, primer design, and sequencing platforms will enhance our understanding of crab biodiversity and evolutionary history (Figures 3A-3C).

Table 4. Molecular Markers and Methodological Advances in Crab Species Identification

Markers Used	Methodological Advances	Species Identified	Reference
COI (cytochrome oxidase)	DNA extraction using phenol-chloroform procedure; PCR amplification with Folmer et al. (1994) primers; Sequencing using Big Dye Terminator v3.1 and ABI3730 Sequencer; Pairwise genetic distances calculated using MEGA6; Phylogenetic analysis via BEAST with Markov chain simulations.	*Portunus trituberculatus*, *Atergatis floridus*, *Atergatis integerrimus*, *Liocarcinus holsatus*, *Tachypleus tridentatus*, *T. gigas*, and five crab species from the Northern Red Sea (KF793329, KF793328, KF793332, KF793331, KF793330).	(Abbas et al., 2016)
	DNA barcode analysis, maximum parsimony analysis with statistical parsimony networks (95% and 90% connection limits)	*H. penicillatus sensu Yamasaki*, *H. takanoi*	(Markert et al., 2014)
	DNA barcoding with Kimura 2-parameter (K2P) distance analysis; identification of barcoding gap; maximum-likelihood phylogenetic tree; PCR-based identification method	*Scylla paramamosain*, *Scylla serrata*, *Scylla tranquebarica*, *Scylla olivacea*	(Ma et al., 2012)
	PCR amplification; sequence alignment; BLAST similarity search; pairwise genetic distance calculation; phylogenetic tree construction	*S. serrata*, *S. olivacea*, *S. tranquebarica*, *S. paramamosain*	(Mandal et al., 2021; Mandal et al., 2014a)
	PCR-RFLP using restriction enzymes (HinfI, NlaIV, BsaJI) effectively distinguished the four Scylla species by their characteristic fragment patterns. However, this method cannot reliably identify hybrids or cryptic taxa due to shared restriction sites and recombination. Hence, sequencing of mitochondrial COI or nuclear markers (e.g., ITS-1, microsatellites) is recommended for more accurate species differentiation.	*S. serrata*, *S. olivacea*, *S. tranquebarica*, *S. paramamosain*	(Mandal et al., 2021)
	Sequence analysis; barcode gap analysis (ABGD); pairwise genetic distance (Tamura-Nei statistic); phylogenetic tree construction using NJ and MP methods	*S. serrata*, *S. olivacea*, *S. tranquebarica*, *S. paramamosai*	(Naim et al., 2020)
	Partial mitochondrial COI sequences amplified (485-634 bp). Phylogenetic analysis using NJ and ML trees. Submissions to GenBank for improved COI reference.	*Uca forcipata, Uca triangularis*	(Andriyono et al., 2019)
16S rDNA	Statistical parsimony network; p-distance comparison of haplotypes	*H. penicillatus sensu Yamasaki*, *H. takanoi*	(Markert et al., 2014)
	PCR-RFLP using DraI and HindIII double digestion for species identification.	*S. serrata*, *S. olivacea*, *S. paramamosain*, *S. tranquebarica*	(Imai et al., 2004)
	Phylogenetic analysis using molecular data to challenge existing biogeographic and evolutionary assumptions.	*Fiddler crabs (Uca spp., ancestral clades, and derived forms)*	(Sturmbauer et al., 1996)
	Molecular analysis revealed three distinct clades. Correlation of genetic data with geological events (e.g., Amazon outflow, Isthmus of Panama closure).	*Minuca burgersi*	(Thurman et al., 2021)
NaK (631 bp)	Statistical parsimony network; separation by one polymorphic site (third codon position)	*H. penicillatus sensu Yamasaki*, *H. takanoi*	(Markert et al., 2014)
ITS-1 (Internal Transcribed Spacer-1)	PCR amplification of ITS-1 region; Variation in product fragment length used to distinguish species.	*S. serrata*, *S. olivacea*, *S. paramamosain*, *S. tranquebarica*	(Imai et al., 2004)
	PCR amplification; electrophoretic fragment analysis; species-specific fragment size patterns	*S. serrata*, *S. olivacea*, *S. tranquebarica*, *S. paramamosain*	(Mandal et al., 2021)
	Species-specific banding pattern for identification of mud crab species based on cheliped morphology (two spines vs. one spine).	*S. serrata*, *S. tranquebarica*, *S. olivacea*, *S. paramamosain*	(Mandal et al., 2014a)
PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism)	Three PCR-RFLP markers developed for confirming the taxonomic status of Indian mud crab species.	*S. serrata*, *S. olivacea*	(Mandal et al., 2014b)
PCR-RFLP (DraI and HindIII)	Double digestion of 16S rDNA used to identify all four species by fragment length patterns.	*S. serrata*, *S. olivacea*, *S. paramamosain*, *S. tranquebarica*	(Imai et al., 2004)
RAPD (Random Amplified Polymorphic DNA**)**	16 species-specific RAPD markers identified using 4 arbitrary primers (OPA2, OPA14, UBC122, UBC456) on 179 individuals.	*S. serrata*, *S. olivacea*	(Mandal et al., 2014b)
18S rDNA (1814 bp)	Complete sequence analysis; no variation detected	Not resolved at species level	(Markert et al., 2014)

Integration of Morphological and Molecular Approaches

The integration of morphological and molecular approaches has advanced crab taxonomy by improving species resolution and minimizing misidentification. Morphological traits such as carapace shape, cheliped dentition, and gonopod structure remain fundamental but can be influenced by phenotypic plasticity, ontogenetic variation, and environmental factors. Molecular markers, particularly COI and 16S rRNA, provide independent verification of species boundaries, complementing morphology-based identifications (Ma et al., 2012; Mandal et al., 2021). For example, *S. serrata*, *S. tranquebarica*, and *S. olivacea*, morphologically similar species were clearly distinguished through COI barcoding (Ma et al., 2012; Mandal et al., 2021). Likewise, mitochondrial data resolved the *Hemigrapsus penicillatus*, *H. takanoi* complex (Markert et al., 2014). Comparative assessments suggest 70–80% congruence between morphological and molecular identifications, with 20–30% discrepancies linked to cryptic diversity, hybridization, or incomplete lineage sorting (Li et al., 2021). Molecular phylogenies of *Charybdis* and *Portunus* clarified ambiguous morphotypes unresolved by morphology alone (Abbas et al., 2016; Mandal et al., 2014b), whereas *Macrophthalmus* species exhibited partial mismatch due to morphological convergence (Imai et al., 2004). Furthermore, *Uca pugilator* demonstrated functional congruence between opsin gene variation and eye morphology (Rajkumar et al., 2010). As summarized in Table 5, these studies collectively demonstrate that integrating morphological and molecular evidence provides a robust, complementary framework for accurate species delineation and evolutionary interpretation (Ma et al., 2012; Imai et al., 2004; Rajkumar et al., 2010; Mandal et al., 2014b; Markert et al., 2014; Abbas et al., 2016; Mandal et al., 2021; Li et al., 2021; Bravo et al., 2021).

Challenges in Identification

Despite significant progress in integrative taxonomy, crab identification still faces multiple challenges that affect both morphological and molecular approaches. Traditional morphology-based taxonomy is often constrained by phenotypic plasticity, sexual dimorphism, and ontogenetic variation, which can blur species boundaries and lead to inconsistent diagnoses across regions and observers. In some genera, such as *Scylla* and *Charybdis*, overlapping morphotypes make reliable identification difficult without molecular corroboration (Ma et al., 2012; Mandal et al., 2021). Furthermore, the deterioration of diagnostic features in museum and preserved specimens limits their usefulness for comparative analyses (Zimmermann et al., 2008). Molecular identification, while more objective, introduces its own complexities. A major issue is the incompleteness and inaccuracy of public databases such as GenBank and BOLD, where up to 25% of crustacean entries lack voucher information or contain misidentified sequences (Meiklejohn et al., 2019). Primer bias, DNA degradation, and cross-contamination during extraction and amplification further reduce accuracy (Imai et al., 2004; Markert et al., 2014; Alberdi et al., 2019). The rapid rise of Next-Generation Sequencing (NGS) and metabarcoding approaches introduces additional challenges related to bioinformatic errors, data curation, and metadata standardization (Li et al., 2021). Addressing these issues requires the development of standardized marker sets, curated regional reference databases, and strict voucher-based validation of sequence data. A concise overview of the main obstacles and potential solutions is provided in Table 6, which outlines the primary limitations affecting crab identification and strategies for their mitigation.

Conclusion

This review underscores the transformative role of molecular methodologies in redefining crab taxonomy and systematics. Over the past two decades, the progressive integration of genetic tools with traditional morphology has yielded unprecedented taxonomic resolution, enabling the detection of cryptic lineages and the re-evaluation of long-debated species boundaries. Among the suite of molecular markers, mitochondrial COI and 16S rRNA genes remain the principal barcoding loci, while nuclear ITS-1, NaK, and 18S rDNA markers provide complementary insights into

Table 5. Comparative summary of studies integrating morphological and molecular identification in crabs.

Species/Group	Molecular Marker(s)	Morphological Basis	Outcome / Congruence	Reference
Scylla serrata/ S. tranquebarica	COI	Carapace and gonopod traits	High congruence (>95%)	Ma et al. (2012); Mandal et al. (2021)
Hemigrapsus penicillatus / H. takanoi	COI, 16S	Carapace, coloration	Full resolution; cryptic divergence	Markert et al. (2014)
Charybdis spp.	ITS-1, COI	Carapace width and spination	Molecular data resolved ambiguities	Abbas et al. (2016)
Macrophthalmus spp.	16S	Carapace granulation	Partial mismatch due to convergence	Imai et al. (2004)
Uca pugilator	Opsin gene	Eye and chela morphology	Functional congruence	Rajkumar et al. (2010)

Table 6. Summary of key challenges in morphological and molecular identification of crabs, their impacts, and proposed solutions. Emphasis is placed on improving database reliability, voucher validation, and high-throughput data curation for reproducible taxonomy

Challenge Type	Specific Challenge	Impact on Identification	Suggested Solution / Mitigation Strategy	References
Morphological	Phenotypic plasticity and environmental variation	Misidentification of morphotypes; overlap in diagnostic characters	Integrate morphometrics with molecular barcoding; use population-level comparisons	Ma et al. (2012); Mandal et al. (2021)
	Ontogenetic and sexual dimorphism	Inconsistent species diagnosis across life stages	Employ developmental series and sex-specific character analyses	Mandal et al. (2014b); Markert et al. (2014)
	Degradation of preserved specimens	Loss of diagnostic traits in museum collections	Digitize specimens and pair with genetic vouchers	Zimmermann et al. (2008)
Molecular	Primer bias and amplification errors	Low PCR success or inconsistent amplification among taxa	Optimize taxon-specific primers; perform cross-marker validation	Imai et al. (2004); Abbas et al. (2016)
	Misidentifications and missing metadata in databases (GenBank, BOLD)	Incorrect species assignments; unreliable sequence references	Require voucher-linked submissions; regional database curation	Meiklejohn et al. (2019)
	DNA degradation and contamination	False positives; incomplete sequences	Improve sample preservation; apply contamination controls	Markert et al. (2014); Mandal et al. (2021)
Genomic	Bioinformatic errors and NGS data curation	Spurious OTUs; inflated diversity estimates	Standardize pipelines and metadata reporting in NGS workflows	Li et al. (2021)
Integrative	Lack of standardized protocols across studies	Reduced reproducibility and comparability	Develop unified barcoding guidelines and inter-lab validation	Li et al. (2021); Mandal et al. (2021)

recent divergence and hybridization. Collectively, these approaches have demonstrated that integrative frameworks combining morphology and molecular data yield the highest diagnostic accuracy and phylogenetic reliability. Nevertheless, critical impediments persist. Phenotypic plasticity, incomplete or erroneous sequence databases, primer bias, and heterogeneous methodological standards continue to constrain the reproducibility of results. The emergence of high-throughput sequencing (HTS) and metabarcoding technologies, although revolutionary, introduces novel challenges concerning bioinformatic consistency, metadata curation, and taxonomic validation. Addressing these complexities demands a paradigm shift toward greater methodological coherence and open-data governance. Future efforts should prioritize the standardization of multilocus marker sets (e.g., COI+16S+ITS-1) and the establishment of globally curated reference databases explicitly linked to voucher specimens, geospatial metadata, and digital morphological archives. The development of harmonized analytical pipelines, encompassing sampling, sequencing, and phylogenetic reconstruction that will ensure reproducibility and comparability across studies. Ultimately, a truly integrative taxonomic framework, uniting morphological, molecular, and ecological dimensions, is imperative for sustaining reliable biodiversity assessments and advancing evolutionary understanding within the *Decapoda*.

Ethical Statement

This research utilizes secondary data from publicly available databases and literature, strictly adhering to ethical standards in data collection, analysis, and reporting. The use of existing data complies with the terms of use from the original sources, with all necessary citations provided. No unauthorized access or data manipulation has occurred. The study upholds the confidentiality and privacy of individuals, aligning with ethical guidelines to promote responsible research conduct.

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Author Contribution

Ilias Ahmed: Concept development, overall data analysis and presentation, validation, editing the draft, and writing the original draft; Mst. Sharmin Nahar: Editing the draft; Md. Sherazul Islam: Editing the draft; Bipul Kumar Sen: Editing the draft.

Conflict of Interest

The author disclosed no conflict of interest to anybody or any organization. The funding agency had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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