

DNA barcoding of an amphidromous palaemonid shrimp recruiting to the Palu River, Central Sulawesi, Indonesia

Eka Rosyida
Fisheries and Marine Science
Department
Universitas Tadulako
Palu, Indonesia
ekarosyida16@gmail.com

Abigail Mary Moore
Graduate School
Universitas Hasanuddin
Makassar, Indonesia
abigail@pasca.unhas.ac.id

Novalina Serdiati
Fisheries and Marine Science
Department
Universitas Tadulako
Palu, Indonesia
novalimbongallo@gmail.com

Samliok Ndobe*
Fisheries and Marine Science
Department
Universitas Tadulako
Palu, Indonesia
samndobe@yahoo.com

Judistira Siddik
Fisheries and Marine Science
Department
Universitas Tadulako
Palu, Indonesia
yudistirasiddik@gmail.com

Abstract—Sulawesi is the largest island in the Wallacea biodiversity hotspot, but many aspects of this biodiversity are still poorly known, including diadromous fish and invertebrates. As part of a research program to identify the taxa migrating upstream in the Palu River estuary together with multispecies schools of anguillid glass eels, sampling during the August 2022 dark moon period yielded glass eels, other fish, mollusks, and crustaceans (shrimp and crabs). This study used molecular methods to identify the shrimp collected. DNA barcoding targeted a segment of the Cytochrome Oxidase I Mitochondrial DNA (COI mtDNA) molecular marker (Sanger sequencing of PCR product) and analysis was implemented in MEGA 11. The NCBI BLAST-n online routine was used to obtain homologous nucleotide accessions similar to the 612 bp Palu crustacean barcode. Matches with 100% identity submitted as *Macrobrachium australe* ranged from 91–100% coverage (Accessions MN526188–MN526195) while matches with 100% identity submitted as *M. indicum* had 85% coverage (Accessions KX866570–KX866577, KX866580–KX866582 and KX866585). Phylogenetic analysis (Maximum Likelihood) showed the Palu crustacean nested in the genus *Macrobrachium* consistent with identification based on external morphology, within an *M. australe* and *M. indicum* clade, deeply divided from the clade containing *M. rosenbergii*.

Keywords—*Macrobrachium australe*, *Macrobrachium indicum*, COI mitochondrial DNA, Palaemonidae, Wallacea

I. INTRODUCTION

Sulawesi is the largest island in the Wallacea biodiversity hotspot, but many aspects of its biodiversity are still poorly known, including riverine and diadromous taxa [1], [2]. The distributions and species composition of the valuable catadromous anguillid eels are still in need of research [3], while there are substantial gaps in knowledge regarding amphidromous species such as gobies of the Family Eleotridae and Gobiidae [2], [4], [5] and invertebrates including mollusks and crustacea [6].

Previous research in and around Sulawesi estuaries has revealed a wide range of diadromous taxa migrating upstream, mostly in larval stages hard to identify based on external morphology alone, e.g. [3], [7]. A research program has been initiated with the aim of identifying the anguillid eels and other taxa migrating upstream in the Palu River estuary in multispecies schools. The initial sampling of these

multispecies schools yielded glass eels, non-anguillid fish, gastropod mollusks, and crustaceans (shrimps and crabs).

DNA barcoding is a method that is increasingly used to identify specimens where classical taxonomy based on morphological traits is challenging or even impossible (e.g. due to the life-stage or condition of specimens, cryptic species, etc.) [8]–[11]. This molecular biology approach is based on the analysis of relatively conserved DNA sequences (molecular markers) that remain similar within taxa but differ between taxa, referred to as barcodes [11]–[13]. The most common barcoding region is the mitochondrial DNA cytochrome oxidase I (mtDNA COI or COI) gene fragment [11]. This marker has been tested in many taxa, including crustaceans [11], [14]–[16]. The purpose of this study was to apply the DNA barcoding molecular approach to identify the shrimp collected while migrating upstream in the Palu River estuary during the dark of the moon in August 2022.

II. METHODS

A. Study Site and Sample Collection

Sampling of taxa migrating upstream in the Palu River estuary (0.8856 S, 119.859 E) were collected during the August 2022 dark moon period using hand-held push-nets following [3]. A typical specimen of the shrimp collected was photographed (Figure 1) and a sample for genetic analysis preserved in 96% absolute ethanol. Preliminary identification based on external morphology referred to literature including [17]–[20] and on-line resources, e.g. [21].



Fig. 1. Shrimp collected migrating upstream in the Palu River estuary

B. DNA Barcoding and Phylogenetic Analysis

DNA extraction and PCR targeting the Cytochrome Oxidase I mitochondrial DNA (COI mtDNA) molecular marker were performed at Bionesia (Bali). DNA was isolated from approximately 10 g of shrimp tissue using a Blood & Tissue Kit (Qiagen) following the manufacturer's extraction protocol. PCR (Applied Biosystems™ 2720 Thermal Cycler) followed the BIONESIA laboratory protocol using the forward primer jgLCO (5'-TIT CIA CIA AYC AYA ARG AYA TTG-3') and reverse primer jgHCO (5'- TAI ACY TCI GGR TGI CCR AAR AA-3') [16]. The 25 µL PCR reaction comprised: 1 µL DNA template, 4 µL buffer (Applied Biosystems), 2.5 µL dNTPs (10 mM), 1.25 µL of each primer (10 mM), 2 µL MgCl₂ (25 mM), 0.125 µL AmpliTaq Gold™ enzyme (Applied Biosystems) and 14.5 µL ddH₂O. The PCR profile was: initial denaturation at 94 °C for 3 min; 38 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 60 s; final extension at 72 °C for 2 min. The PCR product was verified through electrophoresis on 1% Agarose gel with Nucleic Acid Gel Stain (GelRed®) staining (Figure 2) before Sanger dideoxy DNA sequencing at PT. Genetics Science Jakarta.

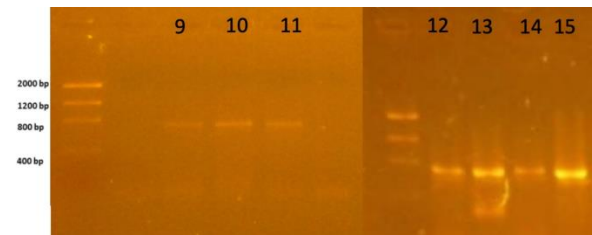


Fig. 2. PCR product electrophoresis: column 15 is the trace for the DNA extracted from a shrimp migrating upstream in the Palu River estuary

The forward and reverse Sanger sequencing trace files were cleaned, aligned and trimmed in MEGA 11 [22], producing a 612 bp consensus sequence. The NCBI BLAST-n tool was used to find similar homologous sequences (accessions) in the GenBank repository. Additional sequences were obtained by searching GenBank for COI accessions of specific *Macrobrachium* taxa. Sequences obtained were aligned using ClustalW and phylogenetic analyses conducted in MEGA 11 [22] using Neighbor-Join [23], [24] and Maximum Likelihood (Kimura 2-parameter model, 100 bootstrap replications [25]) methods with default parameters. Accession KX219190 of *Calin euria californica* was used as an outgroup. Resultant trees were exported as

Newick files for editing in the Interactive Tree of Life on-line tool [26], [27].

III. RESULTS AND DISCUSSION

A. Identification and DNA Barcoding

Based on morphological characters, the juvenile shrimp recruiting to the Palu River estuary was identified as belonging to the Family Palaemonidae and most likely the genus *Macrobrachium*. Out of the top 100 significant BLAST-n matches, 99 belonged to 9 named species in the genus *Macrobrachium*, the one exception being accession MZ560320 (deposited as *Alpheidae* sp.) collected from a study of invertebrates recruiting to an artificial refuge in a marine environment [28]. This accession likely represents a misidentification of an early life-stage stage, especially as members of the families Alpheidae and Palaemonidae can be found co-habiting in coastal waters, in particular in dead coral [29] which could provide similar habitat to the ARMS modules used to attract invertebrate settlement in [28].

The BLAST-n results returned 20 sequences having 100% identity of overlapping nucleotide positions with the 612 bp barcode of the shrimp collected in the Palu River estuary. These included eight *M. australe* accessions with 91-100% coverage (MN526188-MN526195) collected from Bali and Java, Indonesia [30]; 11 *M. indicum* accessions with 85% coverage (KX866570-KX866577, KX866580, KX866582, KX866585) from India [31], and one accession with 68% coverage (GU205043, unpublished) of unknown geographical origin. The five other *M. indicum* accessions (all from [31]) had identity values over 99%. *Macrobrachium* accessions from 56 named species comprised 837 of the top 1000 significant BLAST-n matches, including other *M. australe* accessions from sources other than [30], with identity values in the range 79.87-83.14%.

B. Phylogenetic analysis

Phylogenetic analyses using Maximum Likelihood and Neighbor-Join routines produced similar trees. Both methods placed the Palu shrimp closest to *M. australe* or *M. indicum* accessions, exact placement varying between iterations. The Maximum Likelihood tree in Figure 3 used the 20 closest GenBank accessions (with four additional *M. australe* accessions), while Figure 4 used the 98 closest accessions.

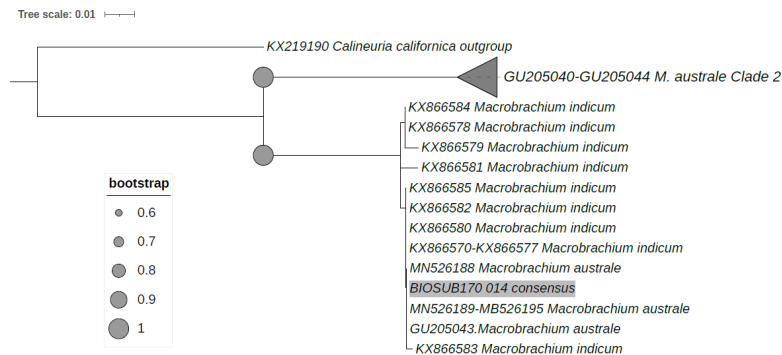


Fig. 3. Maximum Likelihood phylogenetic tree based on COI mtDNA barcodes of the shrimp collected migrating upstream in the Palu River estuary (grey highlight), 20 closest GenBank accessions of the genus *Macrobrachium* (BLAST-n, top 100 matches), outgroups *C. californica* and *M. australe* Clade 2.

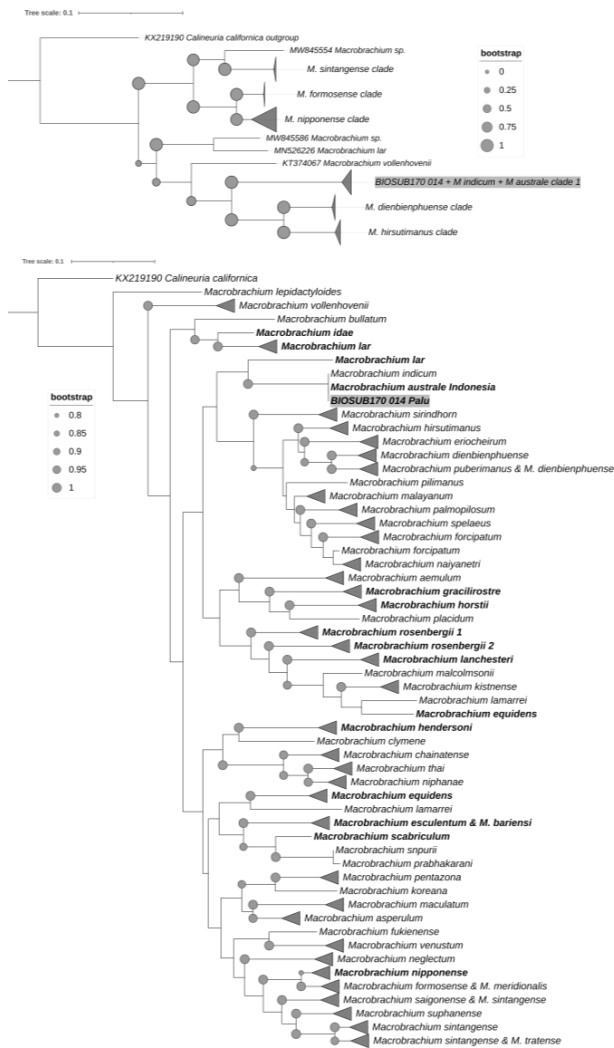


Fig. 4. Condensed Maximum Likelihood phylogenetic trees based on COI mtDNA sequences of the shrimp collected migrating upstream in the Palu River estuary (grey highlight) and with *C. californica* as outgroup: Above: 98 closest GenBank accessions; Below: 348 accessions of the genus *Macrobrachium* with taxa reported from Sulawesi in bold font.

C. Phylogenetic analysis

The results of the DNA barcoding analysis are consonant with visual observations of specimen morphology. Therefore, the juvenile shrimp recruiting to the Palu River can be identified as belonging to the genus *Macrobrachium* with a high level of confidence. However, the 100% identity of the DNA barcode sequence with accessions representing two nominal taxa (*M. australe* and *M. indicum*) as shown in Figure 3 means that assigning a species name to the Palu shrimp specimen is problematic, as these taxa are not currently considered synonymous [21], despite their apparent identity based on the accessions used to construct Figure 3. Furthermore, the status of *M. indicum* as a separate species has been supported by research using a COI mtDNA molecular marker [31].

The known distributions of these two species with barcode matches to the Palu River shrimp differ in both extent and location [21]. Each of these taxa presents issues which complicate making an identification with a high level of certainty based on the similarity of GenBank accessions

with the Palu Bay shrimp barcode. On the one hand, *M. australe* Guérin-Méneville, 1838 is considered one of the most widespread amphidromous shrimps and has been reported from Africa to the eastern Pacific [21], [32], including the Banggai Archipelago in eastern Central Sulawesi [20], a range clearly encompassing the Makassar Strait and therefore Palu Bay and Palu River. However, *M. australe* is thought to comprise several cryptic species [32], and phylogenetic analysis of all 18 GenBank COI accessions of this taxa comprised 3 clades, with the third clade deeply separated from the two clades in Figure 3. On the other hand, *M. indicum* Jayachandran & Joseph, 1986 was described from Kerala State, India [33] with a known distribution limited to the southern tip of India [21], [31], [33], a long way from Palu Bay on the Makassar Strait coast of Sulawesi. Despite the relatively recent description, the holotype was lost, and a neotype designated; the GenBank barcode accessions used in this study represent specimens collected and identified based on the original description [31]. The original justification of *M. indicum* as a new species was based on differences between six specimens collected in 1980 (holotype and five paratypes) and previously described congeneric species, with *M. australe* being the least different [33].

The original description of *M. australe* by Guérin-Méneville in 1838 as *Palaemon australis* was based on an unknown number of specimens from Tahiti in the Pacific [34] with a much more detailed description given in the report of the Sibolga Expedition by Holthuis in 1950 [35]. Numerous nominal taxa have been synonymized with *M. australe*, including *Leander lepidus* de Man, 1915; *M. danae* Heller, 1865; *Palaemon alphonsianus* Hoffman, 1874; *P. danae* Heller, 1865; *P. dispar* von Martens, 1868; *P. malliardi* Richters, 1880; *P. parvus* Hoffman, 1874; and *P. spec* Richters, 1880 [21].

Both studies describing *M. indicum* [31], [33] appear to have used [35] as a key reference. It is not made clear in [31], [33] whether or what *M. australe* material was examined for comparison, or whether the comparison was based solely on references. Therefore, given the concerns regarding the monophyly of *M. australe* [32], reflected in the structure of the tree in Figure 4, there is room for doubt regarding the identity of the *M. australe* material used (directly or through references) as a basis for establishing *M. indicum* as a new species.

The analysis underpinning the tree in Figure 4 was complicated by the variety of primer sets used by different studies, so that many *Macrobrachium* COI-mtDNA sequences (barcodes) have a limited overlap with each other, and/or with the COI mtDNA region amplified in this study. This meant that some *Macrobrachium* COI mtDNA accessions did not meet the criteria for inclusion in the analysis. Furthermore, several molecular phylogenetic studies on *Macrobrachium* and related taxa have used different molecular markers, including the 16S rRNA [36]–[38] and 18S rRNA markers [37], while GenBank accessions do not cover all currently recognized species within the genus. Therefore this tree does not represent a full range of the species within *Macrobrachium* or even those with molecular data available. Other *Macrobrachium* species reported from Sulawesi are shown in Table 1; however few of the reports are from studies including DNA barcoding.

TABLE I. MACROBRACHIUM TAXA REPORTED FROM SULAWESI

Macrobrachium Species	Reported Locations ^a in Published Literature					
	CS/MS	CS/BA	CS/LA	SS	NS	OT
<i>M. acanthurus</i>				[43]		
<i>M. australe</i> ^b	[44]	[20]				
<i>M. esculentum</i> ^b	[44]			[45] [41]		
<i>M. equidens</i> ^b		[20]		[43]		
<i>M. gracilirostre</i> ^b					[41]	
<i>M. hendersoni</i> ^b				[43]		
<i>M. horstii</i> ^b	[44]					
<i>M. idea</i> ^b				[46] [43]		
<i>M. lanchesteri</i> ^b			[39] [40]			
<i>M. lar</i> ^b	[44]	[20]			[41]	
<i>M. latidactylus</i>		[20]				
<i>M. nipponense</i> ^b						[42]
<i>M. placidulum</i>	[44]					
<i>M. rosenbergii</i> ^b				[47] [43]		
<i>M. scabriculum</i> ^b	[48]					
<i>M. weberi</i>				[43]		

^a. CS = Central Sulawesi; SS = South Sulawesi; NS = North Sulawesi; OT = other; MS = Makassar Strait; BA = Banggai Archipelago; LA = lakes.

^b. GenBank accessions available and included in Figure 4.

Despite the above-mentioned limitations, the top 1000 BLAST-n matches enabled the construction of Figure 4 showing *Macrobrachium* accessions submitted as 54 nominal species (out of the 279 species in [21]). The tree topology comprises several major clades, with some nominal species nested in more than one cluster or sub-clade

Among the 16 *Macrobrachium* species in Table 1, 12 had homologous COI mtDNA GenBank accessions. Of these taxa, only *M. lanchesteri* is thought to be an introduced and potentially invasive species, and a threat to endemic taxa in Lindu Lake [39] and Poso Lake [40]. Based on known distributions [21], the report of *M. acanthurus* (from North and South America) is likely a misidentification. Taxa with Sulawesian COI mtDNA barcode accessions included *M. esculentum* (accession FM958064 [41]) and *M. gracilirostre* (accession FM986613, [41]), while *M. nipponense* was detected through eDNA barcoding (16S rRNA marker) in coastal waters of Indonesian Fishing Management Areas (FMAs) around Sulawesi, including the Makassar Strait [42].

The tree configuration in Figure 4 reflects the considerable uncertainty reported by many other studies regarding the actual number and identity of species within the Palaemonidae as a family (e.g. [49], [50]) and *Macrobrachium* as a genus (e.g. [36], [50]–[52]). The growing evidence for cryptic species within the genus *Macrobrachium* also reinforces concerns over taxonomic uncertainty with regards to both recent and historical occurrence reports, as pointed out *inter alia* by [32].

At least some of the challenges in *Macrobrachium* taxonomy are likely related to the biology and ecology of species within this taxonomic group. These shrimps exhibit sexual dimorphism with key diagnostic features often only present or visible on adult male specimens [53]. Furthermore, there is evidence of considerable phenotypic

plasticity, especially under varying environmental conditions, leading to considerable intra-species diversity in morphological traits, with considerable overlap in traits between species [51].

With respect to reproductive patterns, the genus *Macrobrachium* comprises both primary freshwater and diadromous (amphidromous or catadromous) species [6], [54]–[57] where the pelagic phase enables dispersal between watersheds and islands. For example, the giant long-armed prawn *M. lar* has been shown to have common haplotypes spread over considerable distances [58], indicating relatively recent or current genetically significant connectivity, although another study found significant genetic differentiation between geographically separate populations of this species [37].

The Palu shrimp nested within an *M. australe* and *M. indicus* sub-clade, while *M. rosenbergii* (a widespread shrimp [21] reported from Sulawesi, e.g. [43], [47]) formed sub-clades in a different main clade. The *M. australe* accessions clustered with the Palu sequence and the *M. indicum* sequences in Figure 3 are from a population in Indonesia (Java and Bali) [30]. There do not seem to be any previously deposited Sulawesian accessions for *M. australe* or indeed most of the species reported from this island; however, many *Macrobrachium* accessions in GenBank lack information on specimen geographical origin. A study on the transport and stranding of marine debris [59] shows seasonal currents in the Indian Ocean and Indonesian waters that could enable dispersal patterns (e.g. through rafting with debris) accounting for a widespread taxon such as that apparently represented by this clade. However, to confirm that this clade does in fact represent a species-level taxon would require further study, ideally using multiple molecular markers as well as detailed examination of adult male specimens from each location/population.

The circumstances in which the Palu specimen was collected strongly indicate that it belongs to an amphidromous species or population, and therefore has a pelagic larval phase enabling dispersal between watersheds and islands. The presence of a species previously known only from India in Palu, Sulawesi in a river linked to the Makassar Strait cannot be ruled out. It is possible that *M. indicum* may not be an Indian endemic, and may have a wide distribution including watersheds around the Makassar Strait as well as Java and Bali.

Other recent studies have highlighted the need for further combined classical and molecular taxonomic work on crustaceans, including amphidromous shrimps of the Palaemonidae (e.g. [30]), and in particular the genus *Macrobrachium* (e.g. [32]). The use of multiple molecular markers, including nuclear DNA as well as mtDNA, may also help resolve some of the issues, as proposed by [51].

IV. CONCLUSION

The shrimp collected in the Palu River belongs to the genus *Macrobrachium*, based on external morphology and DNA Barcoding. However, despite the high confidence level of the BLAST-n results, the species level identification of the species of the *Macrobrachium* shrimp recruiting to Palu River remains elusive. Accessions from two taxa (*M. australe* and *M. indicus*) had 100% identity with the Palu shrimp barcode, while the phylogenetic analysis of GenBank

Macrobrachium accessions highlighted other ambiguities in the taxonomy of this genus. These results reinforce the need for further research on diadromous taxa, including shrimps, in the Palu River and across the Wallacea biodiversity hotspot.

ACKNOWLEDGMENT

The authors thank all who assisted in any way during this research.

REFERENCES

- [1] R. Ambo-Rappe and A. M. Moore, "Sulawesi Seas, Indonesia," in *World Seas: an Environmental Evaluation*, Second Edi., C. Sheppard, Ed. London, United Kingdom: Elsevier, 2019, pp. 559–581.
- [2] F. W. Miesen, F. Drollmann, S. Hüllen, R. K. Hadiaty, and F. Herder, "An annotated checklist of the inland fishes of Sulawesi," *Bonn Zool. Bull.*, vol. 64, no. 2, pp. 77–106, 2016.
- [3] N. Serdiati, S. Ndobe, A. Moore, and D. Wahyudi, "Species composition of glass eels (*Anguilla* spp.) recruiting to the Palu River, Central Sulawesi," *J. Iktiologi Indones.*, vol. 13, no. 2, pp. 109–123, 2013.
- [4] S. Ndobe et al., "DNA barcoding detects resurrected taxon *Giuris laglaizei* (Sauvage 1880) in Sulawesi, Indonesia: Bolano Sau Lake payangka phylogeny, phenotypic characters and implications for *Giuris* spp. conservation," *F1000Research*, vol. 11, p. 295, 2022.
- [5] A. I. Burhanuddin et al., "Additional records of *Sicyopus discordipinnis* (Watson, 1995) (Oxudercidae: Sicydiinae) in Central Sulawesi, Indonesia," *Cybiium*, vol. 46, no. 6, pp. 41–43, 2022.
- [6] D. Wowor and J. W. Short, "Two new freshwater prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea: Decapoda: Palaemonidae) from the Kelian River, East Kalimantan, Indonesia," *Raffles Bull. Zool.*, vol. 55, no. 1, pp. 77–87, 2007.
- [7] Nurjirana, A. I. Burhanuddin, P. Keith, A. Haris, and M. Afrisal, "Amphidromous goby postlarvae (penja) migration seasons and fisheries in West Sulawesi, Indonesia, preliminary data," *Biodiversitas*, vol. 23, no. 1, pp. 375–380, 2022.
- [8] N. Hubert et al., "DNA Barcoding Indonesian freshwater fishes: challenges and prospects," *DNA Barcodes*, vol. 3, no. 1, pp. 144–169, 2015.
- [9] J. M. Leis, "Taxonomy and systematics of larval Indo-Pacific fishes: a review of progress since 1981," *Ichthyol. Res.*, vol. 62, pp. 9–28, 2015.
- [10] M. R. Gostel and W. J. Kress, "The Expanding Role of DNA Barcodes: Indispensable Tools for Ecology, Evolution, and Conservation," *Diversity*, vol. 14, no. 3, p. 213, Mar. 2022, doi: 10.3390/d14030213.
- [11] S. Behrens-Chapuis, F. Herder, and M. F. Geiger, "Adding DNA barcoding to stream monitoring protocols – What's the additional value and congruence between morphological and molecular identification approaches?," *PLoS One*, vol. 16, no. 1, p. e0244598, 2021.
- [12] P. D. N. Hebert, A. Cywinska, S. L. Ball, and J. R. DeWaard, "Biological identifications through DNA barcodes," *Proc. R. Soc. London. Ser. B Biol. Sci.*, vol. 270, no. 1512, pp. 313–321, Feb. 2003.
- [13] M. Hajibabaei, G. A. C. Singer, P. D. N. Hebert, and D. A. Hickey, "DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics," *Trends Genet.*, vol. 23, no. 4, pp. 167–172, Apr. 2007.
- [14] F. O. Costa et al., "Biological identifications through DNA barcodes: the case of the Crustacea," *Can. J. Fish. Aquat. Sci.*, vol. 64, no. 2, pp. 272–295, Feb. 2007.
- [15] Abinawanto, M. D. Bayu Intan, W. Wardhana, and A. Bowolaksono, "DNA barcoding reveals underestimated species diversity of mantis shrimp larvae (stomatopods) in Banten Bay, Indonesia," *Biodiversitas J. Biol. Divers.*, vol. 20, no. 6, pp. 1758–1763, May 2019.
- [16] J. Geller, C. Meyer, M. Parker, and H. Hawk, "Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys," *Mol. Ecol. Resour.*, vol. 13, no. 5, pp. 851–861, 2013.
- [17] J. W. Short, "A Revision of Australian River Prawns, *Macrobrachium* (Crustacea: Decapoda: Palaemonidae)," *Hydrobiologia*, vol. 525, no. 1–3, pp. 1–100, Sep. 2004.
- [18] I. Dore and C. Frimodt, *An Illustrated Guide to Shrimp of the World*. Boston, MA: Springer US, 1991.
- [19] K. E. Carpenter and V. H. Niem, Eds., *The living marine resources of the Western Central Pacific*, volume 2. Cephalopods, crustaceans, holothurians and sharks., vol. 2. Rome: Food and Agriculture Organization of the United Nations, 1998.
- [20] A. Annawaty, N. H. E. Lapasang, P. Rahayu, H. Hairul, F. Rahman I. Tadeko, and D. Dwiyanto, "Checklist of the freshwater shrimps (Crustacea, Decapoda, Caridea) from the Banggai Archipelago, Central Sulawesi, Indonesia," *Check List*, vol. 18, no. 2, pp. 341–355, Apr. 2022.
- [21] "Global Biodiversity Information Facility." <https://www.gbif.org/> (accessed Nov. 13, 2022).
- [22] K. Tamura, G. Stecher, and S. Kumar, "MEGA11: Molecular Evolutionary Genetics Analysis Version 11," *Mol. Biol. Evol.*, vol. 38, no. 7, pp. 3022–3027, Jun. 2021.
- [23] N. Saitou and M. Nei, "The neighbor-joining method: a new method for reconstructing phylogenetic trees," *Mol. Biol. Evol.*, vol. 4, no. 4, pp. 406–425, Jul. 1987.
- [24] K. Tamura, M. Nei, and S. Kumar, "Prospects for inferring very large phylogenies by using the neighbor-joining method," *Proc. Natl. Acad. Sci.*, vol. 101, no. 30, pp. 11030–11035, Jul. 2004.
- [25] M. Kimura, "A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences," *J. Mol. Evol.*, vol. 16, no. 2, pp. 111–120, 1980.
- [26] I. Letunic and P. Bork, "Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees," *Nucleic Acids Res.*, vol. 44, no. W1, pp. W242–W245, 2016.
- [27] I. Letunic and P. Bork, "Interactive Tree Of Life (iTOL) v4: recent updates and new developments," *Nucleic Acids Res.*, vol. 47, no. W1, pp. W256–W259, 2019.
- [28] L. Plaisance, K. Matterson, K. Fabricius, S. Drovetski, C. Meyer, and N. Knowlton, "Effects of low pH on the coral reef cryptic invertebrate communities near CO2 vents in Papua New Guinea," *PLoS One*, vol. 16, no. 12, p. e0258725, Dec. 2021.
- [29] M. Ulfah et al., "Identification of Caridae Cryptic organism (Crustacea) on the Pocillopora dead coral in Sabang, Aceh," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 674, no. 1, p. 012008, Feb. 2021.
- [30] R. Hernawati et al., "Exploring community assembly among Javanese and Balinese freshwater shrimps (Atyidae, Palaemonidae) through DNA barcodes," *Hydrobiologia*, vol. 847, no. 2, pp. 647–663, Jan. 2020.
- [31] M. S. Ajina, K. V. Jayachandran, A. T. Landge, Y. Gladston, and A. Pavan-Kumar, "Establishment of taxonomic status of *Macrobrachium indicum* Jayachandran & Joseph (Decapoda : Palaemonidae) through molecular characterization with a note on related species," *Zootaxa*, vol. 4652, no. 1, pp. 174–182, Aug. 2019.
- [32] M. Castelin, V. De Mazancourt, G. Marquet, G. Zimmerman, and P. Keith, "Genetic and morphological evidence for cryptic species in *Macrobrachium australe* and resurrection of *M. ustulatum* (Crustacea, Palaemonidae)," *Eur. J. Taxon.*, vol. 2017, no. 289, pp. 1–27, Mar. 2017.
- [33] K. V. Jayachandran and N. I. Joseph, "On a New Species of *Macrobrachium* (Decapoda, Palaemonidae) from the South-West Coast of India," *Crustaceana*, vol. 50, no. 2, pp. 217–224, 1986.
- [34] F. E. Guérin-Méneville, "Crustacés et Arachnides," in *Voyage Autour du Monde, exécuté par Ordre du Roi, sur la Corvette de Sa Majesté, La Coquille, pendant les années 1822, 1823, 1824 et 1825*, vol. Volume 2, L. I. Duperrey, Ed. Paris: Imprimerie de A Firmin Didot, 1883, p. i–xii + 9–56.
- [35] L. B. Holthuis, *The Decapoda of the Sibolga Expedition Part X: The Palaemonidae collected by the Siboga and Snellius expeditions with remarks on other species I. Subfamily Palaemoninae*. Leiden: E. J. Brill, 1950.
- [36] N. P. Murphy and C. M. Austin, "Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): biogeography, taxonomy and the convergent evolution of abbreviated larval development," *Zool. Scr.*, vol. 34, no. 2, pp. 187–197, Mar. 2005.

- [37] M. Castelin et al., "New insight on population genetic connectivity of widespread amphidromous prawn *Macrobrachium lar* (Fabricius, 1798) (Crustacea: Decapoda: Palaemonidae)," *Mar. Biol.*, vol. 160, no. 6, pp. 1395–1406, Jun. 2013.
- [38] W. A. Nabil and R. Eprilurahman, "Karakterisasi Udang Air Tawar Anggota Genus *Macrobrachium* Bate, 1868 (Decapoda: Palaemonidae) dari Air Terjun Tegenungan, Gianyar, Bali Berbasis Data Morfologi dan Molekular," *Biog. J. Ilm. Biol.*, vol. 6, no. 2, p. 131, Nov. 2018.
- [39] A. Annawaty, D. Wowor, A. Farajallah, D. Setiadi, and B. Suryobroto, "Habitat preferences and distribution of the freshwater shrimps of the genus *Caridina* (Crustacea: Decapoda: Atyidae) in Lake Lindu, Sulawesi, Indonesia," *HAYATI J. Biosci.*, vol. 23, no. 2, pp. 45–50, 2016.
- [40] F. Herder et al., "More non-native fish species than natives, and an invasion of Malawi cichlids, in ancient Lake Poso, Sulawesi, Indonesia," *Aquat. Invasions*, vol. 17, no. 1, pp. 72–91, 2022.
- [41] D. Wowor, V. Muthu, R. Meier, M. Balke, Y. Cai, and P. K. L. Ng, "Evolution of life history traits in Asian freshwater prawns of the genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae) based on multilocus molecular phylogenetic analysis," *Mol. Phylogenet. Evol.*, vol. 52, no. 2, pp. 340–350, 2009.
- [42] H. Madduppa et al., "eDNA metabarcoding of decapod crustaceans across Indonesian seas has implications for biodiversity conservation and fisheries sustainability," *Front. Mar. Sci.*, vol. 9, p. 918295, Sep. 2022.
- [43] W. Giesen, M. Baltzer, and R. Baruadi, *Integrating conservation with land-use development in Wetlands of South Sulawesi*. Bogor: Asia Wetland Bureau Indonesia, 1991.
- [44] D. Swiyanto, Fahri, and Annawaty, "Keanekaragaman Udang Air Tawar (Decapoda: Caridea) di Sungai Batusuya, Sulawesi Tengah, Indonesia," *Ser. Biol.*, vol. 5, no. 2, pp. 65–71, 2018.
- [45] J. Jurniati, D. Arfiati, S. Andriyono, A. M. S. Hertika, A. Kurniawan, and W. A. Tanod, "The morphological characters and DNA barcoding identification of sweet river prawn *Macrobrachium esculentum* (Thallwitz, 1891) from Rongkong watershed of South Sulawesi, Indonesia," *Biodiversitas J. Biol. Divers.*, vol. 22, no. 1, pp. 113–121, Dec. 2021.
- [46] N. Nursyahrhan, K. Kariyanti, J. Jayadi, A. Yusuf, and S. Wahidah, "Molecular identification of the freshwater prawn *Macrobrachium idea* in Tempe Lake, South Sulawesi, Indonesia," *AACL Bioflux*, vol. 14, no. 4, pp. 2174–2180, 2021.
- [47] Wahida, S. B. A. Omar, D. D. Trijuno, E. Nugroho, and Amrullah, "The morphological characteristics of South Sulawesi's giant freshwater prawn *Macrobrachium rosenbergii*," *AACL Bioflux*, vol. 10, no. 4, p. 2017, 2017, [Online]. Available: http://www.bioflux.com.ro/docs/2017.820_829.pdf
- [48] D. Dwiyanto, F. Fahri, and A. Annawaty, "First Record on Freshwater Prawn *Macrobrachium Scabriculum* (Heller, 1862) From Batusuya, Donggala, Sulawesi, Indonesia," *Nat. Sci. J. Sci. Technol.*, vol. 6, no. 3, pp. 254–262, 2017.
- [49] S. De Grave, C. H. J. M. Fransen, and T. J. Page, "Let's be pals again: major systematic changes in Palaemonidae (Crustacea: Decapoda)," *PeerJ*, vol. 3, no. 8, p. e1167, Aug. 2015.
- [50] J. W. Martin, K. A. Crandall, and D. L. Felder, Eds., *Decapod Crustacean Phylogenetics*. Boca Raton: CRC Press, 2016.
- [51] W. Siriwut, E. Jeratthitikul, S. Panha, R. Chanabun, P. B. Ngor, and C. Sutcharit, "Evidence of cryptic diversity in freshwater *Macrobrachium* prawns from Indochinese riverine systems revealed by DNA barcode, species delimitation and phylogenetic approaches," *PLoS One*, vol. 16, no. 6, p. e0252546, Jun. 2021.
- [52] L. J. Robe, S. Machado, and M. L. Bartholomei-Santos, "The DNA Barcoding and the caveats with respect to its application to some species of Palaemonidae (Crustacea, Decapoda)," *Zoolog. Sci.*, vol. 29, no. 10, p. 714, Oct. 2012.
- [53] M. Thiel and L. Watling, Eds., *The Natural History of the Crustacea Series Volume 2: Lifestyles and Feeding Biology*. New York: Oxford University Press, 2015.
- [54] K. Anger, "Adaptation to Life in Fresh Water by Decapod Crustaceans: Evolutionary Challenges in the Early Life-History Stages," in *A Global Overview of the Conservation of Freshwater Decapod Crustaceans*, T. Kawai and N. Cumberlidge, Eds. Cham: Springer International Publishing, 2016, pp. 127–168.
- [55] I. S. Wehrtmann and L. R. Lara, "Reproductive biology of the freshwater shrimp *Macrobrachium carcinus* (L.) (Decapoda: Palaemonidae) from Costa Rica, Central America," *J. Crustac. Biol.*, vol. 29, no. 3, pp. 343–349, Jan. 2009.
- [56] R. T. Bauer and J. Delahoussaye, "Life history migrations of the amphidromous river shrimp *Macrobrachium Ohione* from a continental large river system," *J. Crustac. Biol.*, vol. 28, no. 4, pp. 622–632, Jan. 2008.
- [57] T. Kawai and N. Cumberlidge, *A global overview of the conservation of freshwater decapod crustaceans*. Cham: Springer International Publishing, 2016.
- [58] B. E. Hunter, "Population genetic structure and patterns of dispersal in the giant long-armed prawn, *Macrobrachium lar* (Fabricius, 1798) (Decapoda: Palaemonidae)," *Queensland University of Technology*, 2011.
- [59] A. Rizal et al., "Tracking the stranded area of marine debris in Indonesian coasts by using floating drifter," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 925, no. 1, p. 012034, Nov. 2021.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits any noncommercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

