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Intraspecific Genetic Variation of Pokea, *Batissa violacea* (Lamarck, 1818), from Southeast Sulawesi Based on *COI* Mitochondrial Gene

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Abstract

Batissa violacea is easily found in Southeast Sulawesi, where it is known locally as Pokea. The declining population of *B. violacea* in Southeast Sulawesi due to limited systematic studies underscores the need for conservation strategies. This study aimed to identify the species and evaluate the intraspecific genetic variation and genetic population structure of *B. violacea* from the rivers of Southeast Sulawesi based on PCR-amplified COI mitochondrial gene sequences. The results revealed that all samples belong to the *B. violacea* species according to both morphological and molecular analyses. Genetic analysis revealed low genetic diversity, with five haplotypes and four variable sites detected across populations. AMOVA results indicated that the primary source of genetic variation occurred within populations. No clear genetic separation was observed, as shared haplotypes suggest ongoing gene flow among populations. However, low diversity may reflect human impacts such as overfishing and habitat degradation, which contribute to genetic decline. These findings highlight a potentially vulnerable genetic structure. Ensuring habitat protection, sustainable practices, and enhancement of gene flow are crucial for conservation management. Overall, this study provides a valuable basis for conservation planning and emphasizes the importance of sustainable management of *B. violacea* in its natural habitat.

Introduction

Batissa violacea is a member of the Corbiculidae family, characterized by its adaptive strategies, migration abilities, good survival in riverine habitats, and tolerance of fast-flowing rivers (Morton, 1989; Ledua et al., 1996; O'connor et al., 2007). It is widely distributed in Tropical India and Indo-Pacific regions, including Malaysia, Northwest Australia, Fiji, Philippines, Papua New Guinea, and Indonesia (Argente, 2016; Mayor and Ancog, 2016). In Indonesia, several rivers in Southeast Sulawesi known as natural habitats for *B. violacea*, which is locally recognized as 'Pokea' (Bahtiar, 2008; Purnama et al., 2019). Pokea offers ecological services and significant economic value, the local community was utilizing Pokea as a source of food and income (Bahtiar et al., 2005). Surprisingly, recent reports indicate that population number of Pokea was declined due to anthropogenic disturbances such as overexploitation and sand mining activities in and around their habitats (Bahtiar et al., 2016; Bahtiar et al., 2022; Basri et al., 2019). These continuous threats may lead to severe consequences, including local extinction, reduced adaptability to environmental changes, genetic drift, and decreased gene flow and genetic diversity (Furlan et al., 2012; Chakraborty and Samanta, 2019; Almeida-Rocha et al., 2020). These issues underscore the urgent need for effective conservation strategies.

Accurate species identification is essential for conservation efforts. Traditionally, shell characteristics are commonly employed for the identification of mussels based on morphology (Hamli et al., 2015). However, systematic studies of Corbiculidae, including *Batissa,* are still confused and limited (Glaubrecht et al., 2007). Subspecies differentiation within this taxon has generated controversy for species delineation (Glaubrecht, 2004). Moreover, the presence of intraspecific conchological variations in *B. violacea,* such as shell shape, color, and erosion in the umbonal, which are attributed to phenotypic plasticity, often make it difficult to distinguish taxa (Lowey, 1997; Fuiman et al., 1999; Akester and Martel, 2000; Renard et al., 2000; Korniushin, 2004). These factors indicate that relying on shell characteristics alone remains dubious in systematic studies (Glaubrecht et al., 2007). In recent years, integrating morphological and molecular data has been widely implemented to address this problem. Molecular markers, particularly mitochondrial DNA, have been effective for robust identification purposes of Corbiculidae. For instance, the populations of Corbicula in Thailand can be accurately distinguished and identified (Ramli et al., 2022), and the sympatry of Corbicula in Lake Toba was clearly delimited by morphological and DNA analysis (Bespalaya et al., 2019).

Study of Pokea in Southeast Sulawesi has primarily focused on its bioecology, biochemical properties, health benefits, and nutritional value. Systematic and genetic studies are still lacking. A prior study using the *18S* rRNA gene analysis showed that Pokea from Pohara River belongs to the Corbiculidae family and has a close relationship with *C. fluminea* (Muzuni et al., 2014). In the present study, we used the mitochondrial gene *COI* (Cytochrome c Oxidase Subunit I) gene. The COI gene has been widely used for Corbiculidae species identification (Renard et al., 2000; Glaubrecht et al., 2006; Bespalaya et al., 2018; Kropotin et al., 2023). Compared to the *18S* rRNA gene, the *COI* exhibits high genetic variability, possesses high resolution at the species level, and reveals intraspecific variation (Hebert et al., 2003; Wu et al., 2015; Leray and Knowlton, 2016). Additionally, *COI* is supported by adequate reference database coverage in almost all animal taxa (Mejías-Alpízar et al., 2024; Velo-Antón et al., 2023).

Therefore, this study aims to identify Pokea from the Southeast Sulawesi Rivers using an integrative approach based on shell morphology and *COI* gene sequences. Furthermore, we examine genetic variation and genetic population structure as the fundamental components for the development of conservation strategies.

Materials and Methods

Sample Collection and Morphological Identification

A total of 30 Pokea mussels were collected during February 2024 from three river sites in Southeast Sulawesi Province, Indonesia: Lasolo, Pohara, and Konaweha River, with 10 individuals sampled from each river, representing one population per site (Figure 1). The sampling was conducted using local traditional fishing gear known as tangge (bamboo basket), which was operated by a local fisherman from a boat. After collection, the samples were cleaned, placed in a zip-lock plastic bag, and stored in a cool box. Morphological identification was carried out by observing shell

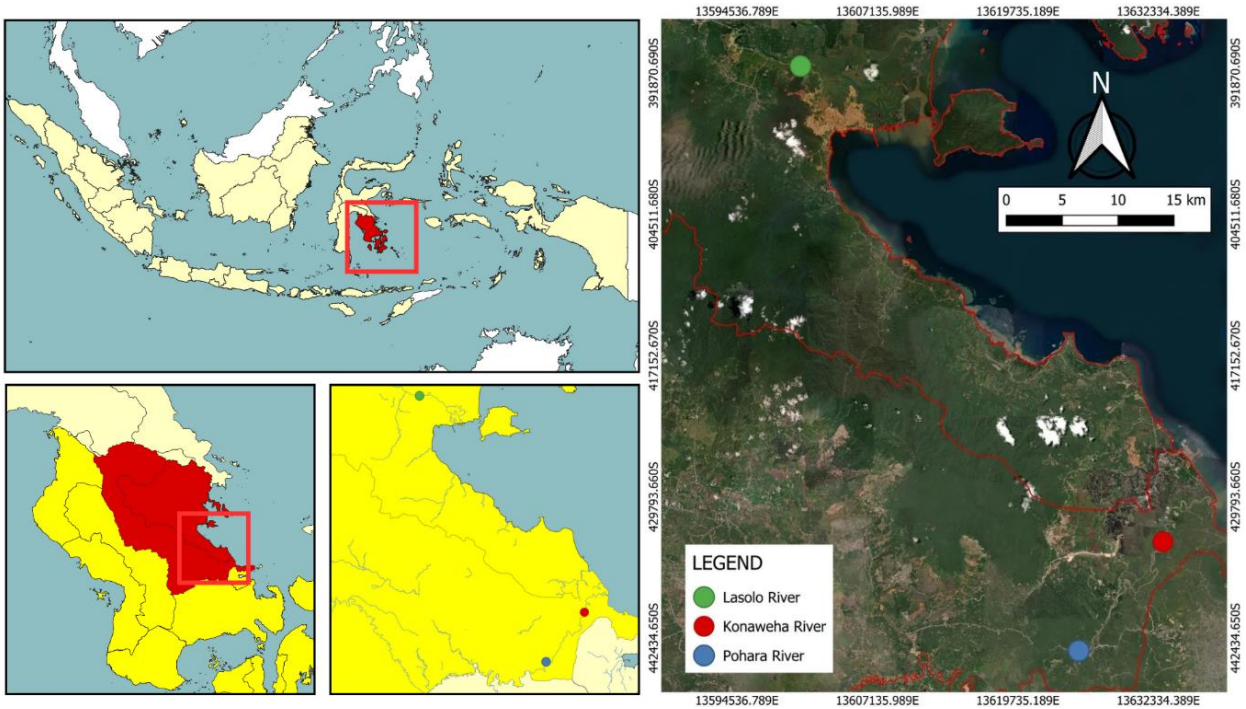

Figure 1. Map illustrating the three river locations of Pokea specimens collected in this study.

characteristics, including shell shape and color, sculpture, umbo, hinge structure, and ligament structures (Bespalaya et al., 2018). Identification process was referred to FAO Species Identification Guide Volume 1 (Carpenter and Niem, 1998), Morton (1989), and Jutting (1953). For molecular analysis, adductor muscle tissues were removed and preserved in a 1.5 mL tube with 96% ethanol and transported to the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, for further analysis.

DNA Extraction, Amplification, Electrophoresis, and Sequencing of *COI* Gene

Total genomic DNA from each tissue was extracted using DNeasy blood and tissue kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocols. A pair of universal primers, LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994), was used to amplify *COI* mitochondrial gene fragments. PCR amplification was conducted using Bio-Rad T100 thermal cycler in a 25 µL reaction volume. The reactions consisted of 10–100 ng genomic DNA, 12.5 µL MyTaqHS Red Mix PCR (Bioline, Meridian Bioscience, USA), 1 mM $MgCl_2$, 0.6 µM of each primer, and 5.5 µL ddH_2O. The thermocycling profiles were as follow: initial denaturation at 95°C for 2 min, followed by 35 cycles of 15 sec denaturation at 95°C, 30 sec annealing at 50°C, and 30 sec extension at 72°C, with a final step of 5 min extension at 72°C, and holding to 4°C (Arisuryanti et al., 2020). PCR products were assessed by electrophoresis on a 1% agarose gel in TAE buffer and stained with FloroSafe (Bioline). Then, amplified products were purified and sequenced at the LPPT-UGM using ABI 3500 Genetic Analyzer (Applied Biosystems). Amplicons were sequenced in both forward and reverse directions.

Data Analysis

Sequence Editing and Alignment

Ambiguous bases were manually edited using GeneStudio and validated by DNASTAR program (DNASTAR Inc, Madison, USA). Chromatograms were inspected for noisy and ambiguous base calling and translated to check for stop codons. Noisy tails were trimmed. For each individual, sequencing reactions were performed using both forward and reverse primers, resulting in a consensus fragment of 564 bp in length. To confirm the species identification, the consensus sequence was individually compared to the GenBank database using the BLAST on NCBI (http://www.ncbi.nlm.nih.gov/BLAST). All sequences were then aligned using both Opal in Mesquite v.3.51 program (Maddison & Maddison, 2018) and ClustalW in MEGA X program (Kumar et al., 2018).

Nucleotide Composition, Phylogenetic and Genetic Distance Analysis

The nucleotide composition of the *COI* gene was analyzed using MEGA X (Kumar et al., 2018). The phylogenetic tree was reconstructed using the Neighbor-Joining (NJ) and Maximum-Likelihood (ML) methods with the Kimura-2 Parameter (K2P) model and 1,000 bootstrap replicates in MEGA X (Kumar et al., 2018). Bayesian Inference (BI) was performed using the BEAST (Suchard et al., 2018). The Akaike Information Criterion (AIC) was defined by jModelTest2 software (Darriba et al., 2012), and GTR + Gamma was chosen as the best-fit model of evolution. The posterior probability was estimated by Markov Chain Monte Carlo (MCMC) with 10,000,000 generations, with the parameters being sampled every 1,000 generations, and discarding the first 25% of sampled trees as burn-in. Then, the phylogenetic trees were visualized using the FigTree 1.4.4 program (Rambaut, 2019). Genetic distance was calculated using the MEGA X program with the Kimura-2-Parameter (K2P) model. The phylogenetic analysis added *B. violacea* with accession numbers DQ837726 and DQ837727 for comparative purposes and used four other species from two genera, *Geloina erosa* (OM791693), *Geloina expansa* (OM791697), *Corbicula africana* (OM912303), and *Corbicula javanica* (AY275668), for outgroups.

Intraspecific Genetic Variation, Haplotype Network, and Principal Coordinate of Analysis (PCoA)

Intraspecific genetic variation was estimated as the number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π), the number of polymorphic sites, parsimony site, and singleton site number, were calculated using DnaSP ver 6.0 software (Rozas et al., 2017). Haplotype network was visualized using PopART v1.7 (Leigh and Bryant, 2015), and Principal Coordinate Analysis (PCoA) as well as Analysis of Molecular Variance (AMOVA) were conducted using GenAIEx 6.51 (Peakall and Smouse, 2006).

Results

Morphological and Molecular Identification

A total of 30 individual Pokea were observed for identification. Morphological identification revealed that all samples had the same shell characters (Figure 2A-D). Pokea has a rounded-oval shell and concentric sculpture. It lacks a lunule and escutcheon. The internal margin is smooth, and the mantle margin is not fused ventrally. The periostracum layer is blackish brown, and the internal nacreous surface is purplish white, especially outside the pallial line. The umbo is prosogyrate, and the layer around the umbo is often eroded. The exterior ligament is large and strong,

oriented opisthodetically, is blackish-greenish brown, and extends posteriorly. The hinge is very strong and has heterodont-type teeth. On each shell valve, there are 3 cardinal teeth and 2 lateral teeth on either side of the cardinal teeth. The lateral tooth was grooved laterally and serrated. The adductor muscle scars are of the isomyarian type, located below the hinge, oval, and connected to the pallial line without sinus. Table 1 for BLAST results using the GenBank database confirmed that the 30 *COI* Pokea mussel sequences from three river sites exhibited a high similarity (99.66-100%) and high query cover (99-100%) with the *B. violacea* species from Indonesia, with accession number DQ837727.

Nucleotide Composition

The average nucleotide composition of the COI sequences across all *B. violacea* samples exhibited notable variation, with a consistent bias toward A+T (64.67%) over C+G (35.32%) (Table 2). On average, the base composition was 45.39% T(U), 19.27% A, 22.91% G, and 12.41% C. Among the three populations, the Lasolo River samples had the highest A+T content (64.70%), whereas the Konaweha River samples showed the highest C+G content (35.35%).

Phylogenetic and Genetic Distance

Phylogenetic trees constructed using Neighbor-Joining (NJ), Maximum Likelihood (ML), and Bayesian Inference (BI) consistently clustered all *B. violacea* sequences from this study into a single monophyletic clade, together with *B. violacea* from GenBank (DQ837727) (Figure 3). This clade received strong statistical support across all methods (NJ= 97%, ML= 96%, BI= 0.97), indicating the robustness of the inferred relationships. Another *B. violacea* reference sequence (DQ837726) branched slightly outside this main cluster but remained within a strongly supported group that included all study samples (NJ= 100%, ML= 100%, BI= 1.00), potentially reflecting intraspecific variation. Samples from Lasolo, Pohara, and Konaweha were intermixed throughout the tree, with no distinct subclades corresponding to individual river populations, suggesting a lack of phylogeographic structure.

Genetic distance analysis using the Kimura 2-Parameter model indicated low overall divergence, with pairwise distances ranging from 0.0178% to 0.0889%. The highest genetic distance was observed between samples from Pohara and Konaweha Rivers, while the lowest was between Lasolo and Pohara Rivers.

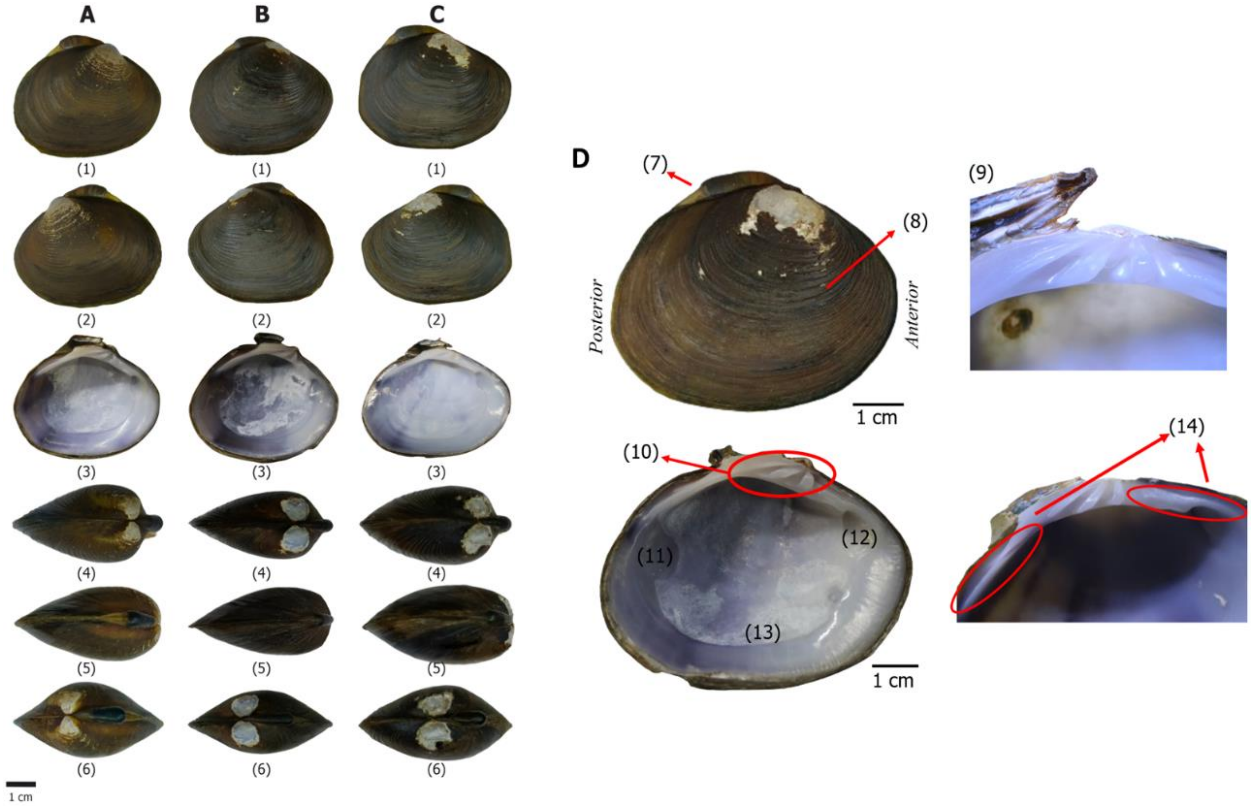

Figure 2. Pokea obtained from Lasolo River (A), Pohara River (B) and Konaweha River (C). External view of right shell (1); External view of left shell (2); Internal view of right shell (3); Anterior view of shell (4); Posterior view of shell (5); Dorsal view of shell (6). Morphological features of *B. violacea* obtained in this study (D). The ligament extends posteriorly (7); concentric sculpture (8); hinge structure (9); cardinal teeth (10); posterior and anterior adductor muscle scar (11,12); pallial line without sinus (13); lateral teeth serrated (14).

Genetic Variation

A total of four variable sites were detected in the COI sequences of *B. violacea*, including one parsimony-informative site and three singleton sites (Table 3). All variations were transitions, with no transversions observed. Most of the variation was found in sequences from the Konaweha River, while no variation was detected in samples from the Lasolo River. Translation of the 564-bp sequences into amino acids revealed no insertions, deletions, or stop codons. However, two nonsynonymous amino acid substitutions were detected in a few individuals from the Konaweha population.

Haplotype Network, Principal Coordinate of Analysis (PCoA), and Analysis of Molecular Variance (AMOVA)

Five haplotypes were detected among the 30 individuals analyzed (Table 3). The Lasolo River population exhibited no variation and was monomorphic, whereas both Pohara and Konaweha Rivers showed greater haplotype diversity. The predominant haplotype (H1) was found across all three rivers and accounted for 87% of the total samples. The haplotype network (Figure 4) depicted H1 as a central, widely shared haplotype, with the remaining haplotypes (H2–H5) radiating from it through one or two mutational steps. These derived haplotypes were restricted to individuals from Pohara and Konaweha Rivers, indicating localized variation. Despite the presence of river-specific haplotypes, the overall network topology did not display clear geographic clustering, suggesting limited phylogeographic structure and potential gene flow among populations. This pattern was further supported by the Principal Coordinate Analysis (PCoA; Figure 5), which showed overlapping clusters of individuals from different rivers. Haplotype diversity ranged from 0.00000 to 0.53300, and nucleotide diversity from 0.00000 to 0.00134, with the Konaweha

Table 1. Result of Species Identification of Pokea using NCBI BLAST

Sample Code	% Similarity	% Query Cover	Accession Number	Species	Locality	Reference
PKL1	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL2	99.84	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL3	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL4	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL5	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL6	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL7	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL8	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL9	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKL10	99.83	99	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP1	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP2	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP3	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP4	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP5	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP6	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP7	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP8	99.83	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP9	99.83	99	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKP10	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK1	99.83	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK2	100	99	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK3	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK4	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK5	100	99	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK6	99.66	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK7	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK8	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK9	100	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)
PKK10	99.83	100	DQ837727	*Batissa violacea*	SE Sulawesi	Glaubrecht et al. (2006)

Table 2. Nucleotide composition percentages of *COI* gene of *B. violacea* from Lasolo, Pohara and Konaweha River

Location	T(U)	C	A	G	A+T	C + G
Lasolo River	45.40	12.40	19.30	22.90	64.70	35.30
Pohara River	45.40	12.40	19.28	22.91	64.68	35.31
Konaweha River	45.38	12.42	19.24	22.93	64.62	35.35
Average	45.39	12.41	19.27	22.91	64.67	35.32

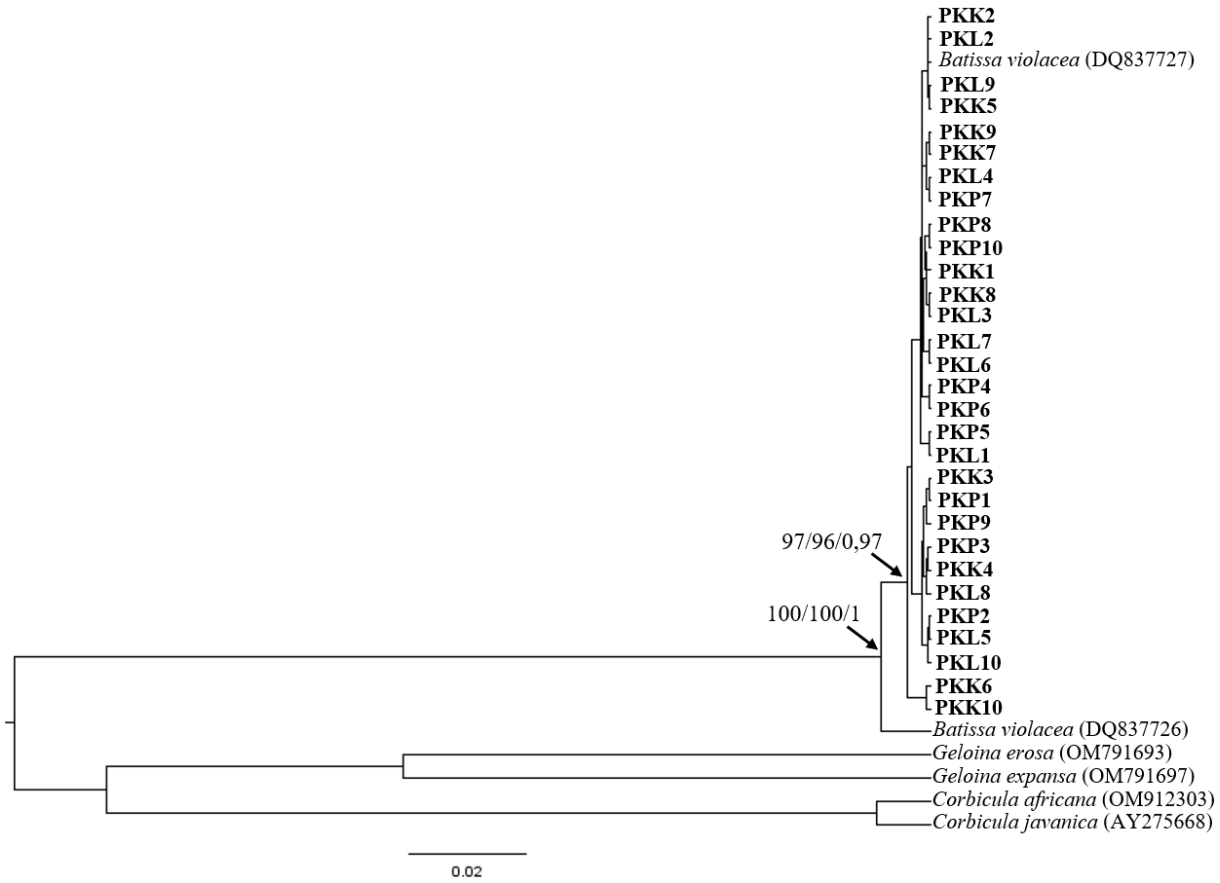

Figure 3. Phylogenetic trees constructed by Neighbor-Joining (NJ), Maximum-Likelihood (ML) and Bayesian Inference (BI) methods. *Corbicula* and *Geloina* species were used as the outgroups. Numbers near the nodes indicate NJ, ML bootstraps and Bayesian posterior probability value. Sequences obtained in this study are bolded.

Table 3. Intrapopulation Genetic Variation of *Batissa violacea* from Lasolo, Pohara and Konaweha Rivers based on mitochondrial *COI* gene

Location	n	Hn	V	P	Hd±SD	π±SD	ts+tv
Lasolo	10	1	0	0	0.000	0.000	0+0
Pohara	10	2	1	0	0.200±0.154	0.00035±0.00027	1+0
Konaweha	10	4	3	1	0.533±0.180	0.00134±0.00054	3+0

**n* number of samples, *Hn* number of haplotypes, *V variable sites, P parsimony informative sites, Hd* haplotype diversity, *π* nucleotide diversity, *ts+tv* number transition and transversion

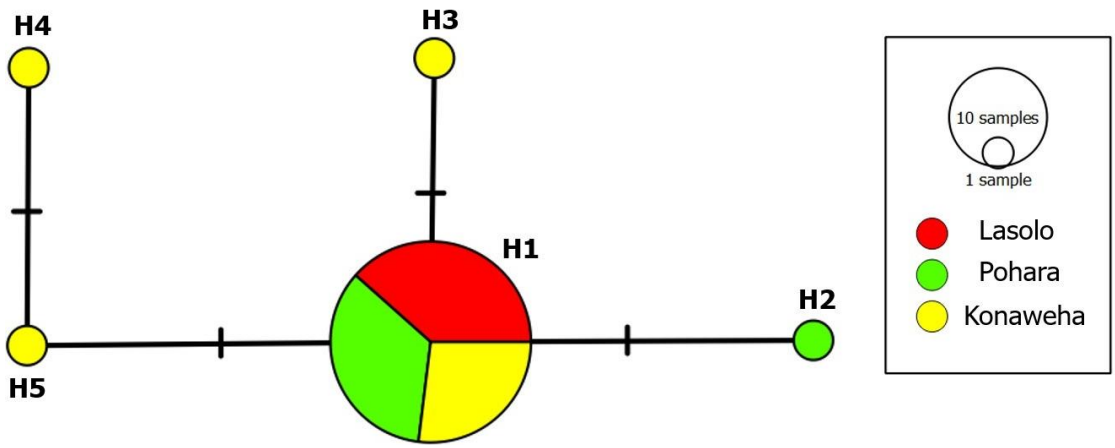

Figure 4. Haplotype network of *COI* gene of *B. violacea* using median-joining network. Each mutation is represented by a hatch mark.

River exhibiting the highest diversity and Lasolo the lowest (Table 4). AMOVA results revealed that 96% of the total genetic variation occurred within populations rather than among them (Table 5).

Discussion

Accurate species identification is a critical foundation for effective conservation planning and management (Quek, 2024; Ferreira-Rodríguez et al., 2019; Kurzel et al., 2021), as misidentification can lead to inaccurate assessments of population status and misguided conservation efforts (Austen, 2016). To resolve species identity in this study, we combined morphological and molecular approaches to identify freshwater mussels from three rivers in Southeast Sulawesi.

Morphological analyses confirmed that all observed specimens belong to *Batissa violacea*, based on shell shape and ornamentation consistent with diagnostic features described by Carpenter and Niem (1998), including non-prominent umbones and transversely striated lateral hinge teeth. Molecular analysis using *COI* barcoding further validated this identification, with all samples showing >99% similarity and full query coverage when compared with *B. violacea* sequences in GenBank.

Our findings also help clarify the inconclusive findings of a previous study by Muzuni et al. (2014), which were unable to assign Pokea to the species level. Their analysis using the *18S* rRNA gene yielded a top BLAST hit of *Corbicula fluminea* with 99.27% similarity, likely due to the absence of *Batissa violacea 18S* sequences in GenBank at that time. Even now, the available *B. violacea* data in GenBank remain limited, consisting of only three *COI* sequences, one *16S* sequence, and one complete mitochondrial genome. Additionally, the *18S* rRNA gene is a highly conserved nuclear marker that evolves too slowly to resolve closely related or recently diverged species (Wu et al., 201). Its limited variability makes it unsuitable for fine-scale or intraspecific discrimination. In contrast, the mitochondrial *COI* gene exhibits faster evolutionary rates and higher resolution at the species level, making it a widely accepted marker for species-level studies in bivalves (David & Savini, 2011; Wu et al., 2015; Papadopoulos et al., 2024). For these reasons, we selected *COI* as a more informative marker, and our results represent the first accurate and comprehensive molecular identification of Pokea (*B. violacea*) from the three Southeast Sulawesi rivers at the species level.

Phylogenetic analyses using NJ, ML, and BI methods consistently resolved the relationships among samples. Bootstrap support values exceeded those reported by Muzuni et al. (2014), indicating strong clade stability. All specimens formed a single clade, supporting their conspecific status. This pattern may reflect geographic proximity among sampling locations, further supported by low genetic distances between populations (Odahara et al., 2006). In line with Hebert et al. (2003), who proposed a 3% *COI* divergence threshold for species delimitation, our findings support the conclusion that Pokea from all three rivers belongs to *B. violacea*.

Base composition analysis of the *COI* gene revealed a marked AT bias, consistent with mitochondrial sequences in other Corbiculidae species (Liao & Liu, 2020; Park & Kwak, 2022). Mao (2011) stated that a high AT base content is commonly observed in the mitochondrial DNA of invertebrates. In our study, we found a transition/transversion ratio of 4:0, indicating that variation in the Pokea *COI* sequence occurs exclusively between purines (A and G) and between pyrimidines (C and T). In general, transitions are more likely than transversions, as transitions do not alter the base type (Stoltzfus and Norris, 2016). Neither the haplotype network nor PCoA showed clear geographic structuring among populations, a result congruent with the phylogenetic tree. The widespread distribution of haplotype H1 across all rivers suggests shared ancestry and limited genetic divergence (Mohd-Yusof et al., 2018; Gwak et al., 2019).

In this study, Pokea populations exhibited low haplotype and nucleotide diversity Hd<0.5, π<0.005) based on Nei and Kumar's (2000) classification. This reduced genetic diversity may hinder the species'

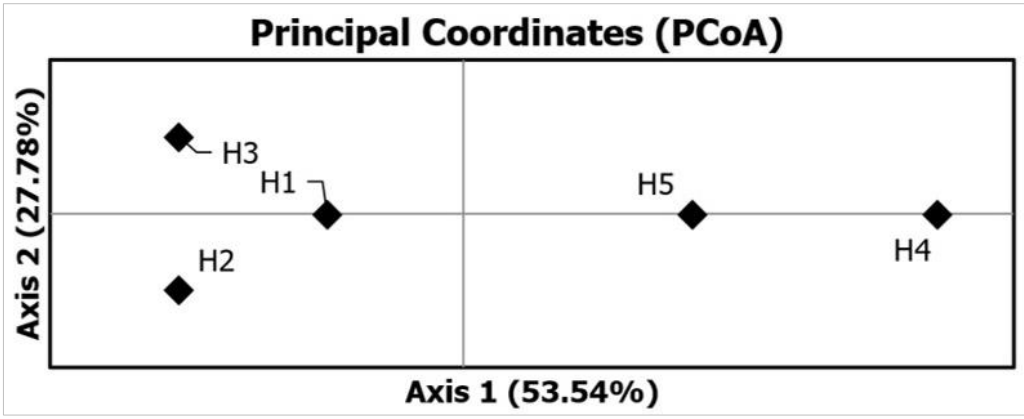

Figure 5. Principal Coordinate Analysis (PCoA) of *COI* gene of *B. violacea* from Lasolo, Pohara and Konaweha Rivers.

Table 4. The Polymorphism sites of mitochondrial *COI* gene of *B. violacea* from Lasolo, Pohara and Konaweha Rivers. Dots indicate nucleotide matching the first sequence

Codon site		30			81			144			174		
Nucleotide site	Haplotype	88	89	90	241	242	243	430	431	432	520	521	522
PKL1	H1	G	C	T	A	T	T	A	A	G	T	T	A
PKL2	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKL3	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKL4	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKL5	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKL6	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKL7	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKL8	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKL9	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKL10	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP1	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP2	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP3	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP4	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP5	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP6	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP7	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP8	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKP9	H2	.	.	.	.	.	.	.	.	.	.	.	**G**
PKP10	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKK1	H3	.	.	.	.	.	.	.	**G**	.	.	.	.
PKK2	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKK3	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKK4	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKK5	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKK6	H4	.	.	**C**	**G**	.	.	.	.	.	.	.	.
PKK7	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKK8	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKK9	H1	.	.	.	.	.	.	.	.	.	.	.	.
PKK10	H5	.	.	.	**G**	.	.	.	.	.	.	.	.

Amino Acid site	Haplotype	30	81	144	174
PKL1	H1	A	I	K	L
PKL2	H1	.	.	.	.
PKL3	H1	.	.	.	.
PKL4	H1	.	.	.	.
PKL5	H1	.	.	.	.
PKL6	H1	.	.	.	.
PKL7	H1	.	.	.	.
PKL8	H1	.	.	.	.
PKL9	H1	.	.	.	.
PKL10	H1	.	.	.	.
PKP1	H1	.	.	.	.
PKP2	H1	.	.	.	.
PKP3	H1	.	.	.	.
PKP4	H1	.	.	.	.
PKP5	H1	.	.	.	.
PKP6	H1	.	.	.	.
PKP7	H1	.	.	.	.
PKP8	H1	.	.	.	.
PKP9	H2	.	.	.	.
PKP10	H1	.	.	.	.
PKK1	H3	.	.	**S**	.
PKK2	H1	.	.	.	.
PKK3	H1	.	.	.	.
PKK4	H1	.	.	.	.
PKK5	H1	.	.	.	.
PKK6	H4	.	**V**	.	.
PKK7	H1	.	.	.	.
PKK8	H1	.	.	.	.
PKK9	H1	.	.	.	.
PKK10	H5	.	**V**	.	.

Table 5. Analysis of Molecular Variance (AMOVA) of *Batissa violacea* in this study

Source	Df	SS	MS	Est. Var.	%
Among Populations	2	0.467	0.233	0.007	4%
Within Populations	27	4.300	0.159	0.159	96%
Total	29	4.767		0.167	100%

adaptive potential, as genetic variation is fundamental for responding to environmental pressures and maintaining long-term population viability (Hoban et al., 2021; Liu et al., 2022; Cruz et al., 2013). Similar low diversity patterns have been reported in other freshwater bivalves, such as Manila clams in China, possibly due to population founder effects (Wei et al., 2023), and *Lampsilis powellii* from Arkansas, USA, where prolonged habitat fragmentation has contributed to genetic isolation (Walters et al., 2021). Additionally, the AMOVA results showed that most genetic variation occurred within rather than among populations, a pattern consistently observed in other bivalve species (Liu et al., 2017; Vikhrev et al., 2022; Gardner et al., 2023).

Low genetic diversity in Pokea may be attributed to anthropogenic pressures, particularly overharvesting. Bahtiar et al. (2022) reported substantial harvest rates of 155 tons/year in the Pohara River and 357 tons/year in the Lasolo River, exceeding the threshold (E>0.5) for sustainable exploitation. Overfishing can reduce population size, limiting genetic diversity over time (Sadler et al., 2023). Moreover, the natural migration of Pokea is likely constrained by physical barriers such as land and mountains, impeding gene flow among rivers. This isolation can lead to inbreeding and genetic drift, resulting in reduced heterozygosity and allelic richness (Storfer, 1999). These factors may increase vulnerability to diseases, reduce ability to adapt to environmental changes, reduce the life expectancy and health, and limit the capacity for population growth, ultimately threatening the survival of a population (Qin et al., 2021; Texeira and Huber, 2021).

Given these findings, conservation strategies should focus on enhancing gene flow, maintaining genetic diversity, and supporting population recovery. Effective measures may include habitat protection, regulatory policies to manage land-use changes, improving river connectivity, translocation of individuals, and the establishment of selective or captive breeding programs (Zhang et al., 2024). Engaging local communities in conservation efforts, particularly in habitat protection and restoration, is also critical. Through these integrative approaches, sustainable ecosystems can be maintained to support both Pokea populations and their habitats in the long term.

Conclusions

This study provides the first comprehensive identification of Pokea (*Batissa violacea*) by combining morphological traits with mitochondrial COI barcoding. All 30 specimens collected from multiple rivers in Southeast Sulawesi were confidently assigned to *B. violacea*, exhibiting close genetic relationships and minimal divergence. Phylogenetic and haplotype analyses revealed no clear geographic structuring, while genetic diversity was low (Hd<0.5, π<0.005), indicating limited adaptive potential and heightened vulnerability to environmental pressures. These findings emphasize the need for conservation strategies that enhance gene flow, preserve genetic diversity, and support population recovery. Recommended actions include habitat protection, regulating land-use changes, improving river connectivity, translocating individuals, and developing selective or captive breeding programs. Engaging local communities in conservation and restoration initiatives is also critical. Such integrative efforts will be essential for maintaining sustainable freshwater ecosystems and safeguarding *B. violacea* populations in the face of ongoing environmental change.

Ethical Statement

This study did not require ethical approval as *Batissa violacea* is an invertebrate bivalve and is not listed under regulations requiring ethical clearance for animal research. Specimens were obtained in a deceased state with the assistance of traditional fishermen, as they are commonly harvested for consumption. The study was conducted in accordance with applicable national and international guidelines for ethical research on non-regulated invertebrate species.

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Author Contribution

AJS: Data Curation, Investigation, Methodology, Visualization, Writing – Original Draft.

ZR: Conceptualization, Supervision, Writing – Review & Editing.

TA: Project Administration, Supervision, Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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