Morphology and genetic characteristics of sea snail, *Littoraria pallescens* based on different colors from Batu Nona Beach, Kema Tiga, North Minahasa Regency, North Sulawesi Province, Indonesia

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Abstract. The snail, *Littoraria pallescens* (R. A. Philippi, 1846) is widely distributed in the Indo-Pacific region. This species lives in mangroves. *L. pallescens* displays various colors, namely bright colors (yellow and red) and dark colors (black and brown). The purpose of this study was to determine the morphology and genetic identification of sea snail with different colors. The sampling method was based on a site survey in the mangrove area and then grouped by color and distribution of sea snail in the mangrove tree. Morphological identification of sea snail was carried out by observing the shape and color of the shell, color of the operculum and the shape of the genital organs based on the identification book. Molecular genetic identification followed the DNeasy Blood & Tissue kit procedure and the sequencing process was carried out using the Sanger method. The results of this research showed that this species lives in mangrove trees, and scattered in the leaves, branches, and roots. It was found that 33.57% of sea snail shells discovered on mangrove leaves had dark brown spots. Meanwhile, 2.91% of red and 4.37% yellow with dark spots snail shells were also found living on mangrove leaves. Molecular analysis of sea snails with red, yellow, brown with dark spots, and yellow with dark spots identified the snail species as *L. pallescens.*

Key Words: brown, gastropod, mangrove, red, yellow.

Introduction. Marine biodiversity is supported by the existence of coastal ecosystems in which there are mangrove forests, seagrass beds and coral reefs, as well as various marine biota that live in association with each other. One of them is a gastropod from the mollusk phylum that lives on mangrove trees, namely *Littoraria* sp. known as sea snail (Boneka 1994; Nurrudin et al 2015).

The genus *Littoraria* has 39 species and is distributed throughout the tropics (Reid 1986, 1989, 1999, 2001; Stuckey & Reid 2002). *Littoraria* species are often found in mangroves where there are abundant microalgae as a food source. These snails are mostly found exclusively in the roots, branches and leaves of mangrove, but some species as *L. irrorata* and *L. scabra* can be found in salt marsh plants, driftwood and rocks along the coast, or in various substrates. In wood and plant substrates the diet of this genus is known to include a high proportion of fungal hyphae (Kohlmeyer & Bebout 1986; Newell & Bärlocher 1993), as well as some leaf material (Reid 1986; Boneka 2013). One species of *Littoraria* genus is *L. pallescens*. This species measures up to 31 mm and is ovoviviparous. *L. pallescens* is usually found on the leaf margins of mangroves, less often on branches, roots and rocks, from the seaside to the upper estuary, 1 to 4.5 m above ground level (Reid 1986). This species is mainly found on the edge of the sea and in the middle of the *Rhizophora* zone (Torres et al 2008). This species with orange shell appears at low frequency in almost all populations of *L. pallescens*. This species lives in the habitat of mangrove trees. This species is always

polymorphic but with relatively unchanged frequency, dark colors being the most common form, yellows comprising one-fourth to one-third of the population, and orange about five percent. These orange shell individuals are usually smaller in size than dark or yellow shell individuals (Cook & Kenyon 1993). Global distribution of *L. pallescens* is throughout the tropical Indo-Pacific, from Central East Africa to Sri Lanka, the Ryukyu Islands, the Marshall Islands, and Samoa (Reid 1986). Based on the research results of Boneka (1994), species of *L. pallescens* and *L. scabra* were found living in mangrove forests. These two species are often found in these forests because of the abundant availability of food, especially micro algae).

This species has a planktonic larval stage in its life cycle and at the juvenile stage will live permanently in mangrove trees. The gastropod body consists of four main parts, namely the head, legs, entrails and mantle. This sea snail is very interesting because it consists of various shell colors, namely light colors (red and yellow) and dark colors (black and brown) (Reid 1986; Boneka 1994; Sumampouw et al 2018). The genus *Littoraria* breathes with gills located in the front, and an operculum attached to its feet. This operculum functions as a cover, when the body enters the shell. The aim of this research was to determine the morphologic and genetic characteristics of sea snail *L. pallescens* with different colors.

Material and Method. This research was conducted from November 2022 to February 2023. Samples of *L. pallescens* were taken from the waters of Batu Nona Beach, Kema Tiga, North Minahasa Regency, North Sulawesi Province (Figure 1). Based on the data from a site survey, around the coastal waters of Batu Nona Beach there were two genera of mangroves, namely, *Rhizophora* sp. and *Avicennia* sp. *L. pallescens* samples were taken from the mangrove tree *Rhizophora* sp. on leaves, branches and roots. The process of taking samples by hand was at low tide. When the sea water recedes, the sea snails were still in a resting phase after eating until just before the tide starts. The samples were put into a plastic bottle and then were taken to the Laboratory of Molecular Biology and Marine Pharmacy, Faculty of Fisheries and Marine Science, Sam Ratulangi University for morphological identification. Meanwhile, the molecular identification was carried out at the Indonesian Biodiversity Laboratory, Denpasar Bali.

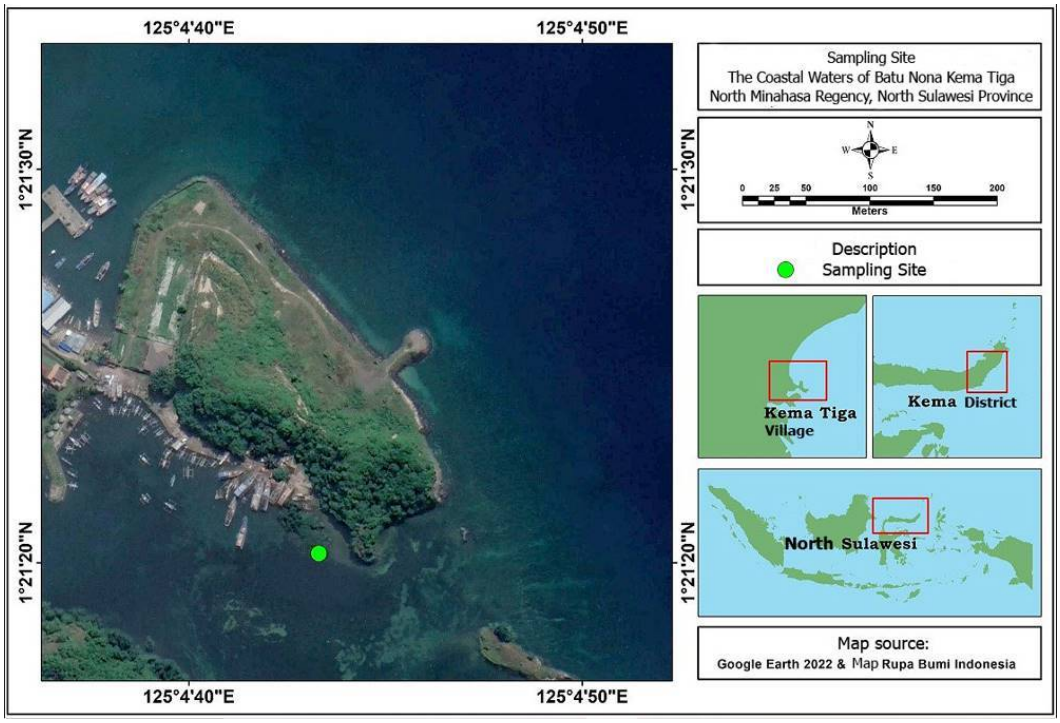

Figure 1. Sampling site.

Sea snails were taken from leaves, branches and twigs, as well as roots of mangroves, then grouped based on shell color and site in the mangrove. Color types were grouped by image

color application, https://imagecolorpicker.com/id. Snail samples were identified based on Reid (1986) and Dharma (1988). External anatomy and shell were briefly described.

The extraction of *L. pallescens* genomic DNA was carried out by following the DNeasy Blood & Tissue kit (www.qiagen.com) procedure and the sequencing process was carried out using the Sanger method. The Sanger method was a DNA sequencing method with the principle of using dideoxyribonucleotide triphosphates (ddNTPs) as a stopper for DNA synthesis at random positions. Genetic analysis was carried out to determine the four different shell colors (dark brown, dark yellow, yellow, and red). The samples sent to the Indonesian Biodiversity Laboratory were the body flesh tissue taken from individual sea snails from four groups of sea snails with different shell colors. DNA replication at the Cytochrome c oxidase I (COI) locus was done following the BIONESIA PCR protocol using LCO1490 / HCO2198[18] primers.

Data analysis was conducted by using BLAST, and phylogenetic tree analysis was carried out by comparing with the database from GenBank (NCBI). Phylogenetic analysis was performed using the Neighbor-joining (NJ) method with bootstrap replication 1,000 times. The value of genetic distance was analyzed using p-distance.

Results and Discussion

Micro habitat and shell color. The results of this study showed that *L. pallescens* live in mangrove trees and spread on the leaves, branches, and roots. This species lives together with another species, namely *L. scabra*, but this species is found only in mangrove roots. According to Reid (1986), the genus *Littoraria* is usually abundant and occurs in mangrove areas, spending all of its adult life on the trunks, roots, and leaves of mangrove trees.

The dark shell of *L. pallescens* found on the branches and roots was used to trick the predators, which are generally crabs. The light color of the shell was used as a strategy for survival as well (Boneka 1994; Boneka et al 1997). In our study, bright colors sea snails were not only found on the leaves but also on the trunks and roots of mangrove trees; on the contrary, dark colors sea snails were also found on the leaves and branches. So, the distribution of sea snail shell color was not concentrated in certain parts of the mangrove tree. The number of sea snails found in the mangrove tree *Rhizophora* sp. reached 191 individuals. The sea snail individuals number found by Salawati et al (2022) on the mangrove tree *R. mucronata* was 124 individuals spread on the leaves, branches, and roots and 2 times more than that found on the mangrove tree *Avicennia marina* because of the position of the roots of *R. mucronata* which makes it easier for sea snail to climb and ascent onto the mangrove branches or leaves.

The shell colors of *L. pallescens* (Figure 2) obtained were categorized into 4 groups, namely yellow, red, yellow with dark spots, and brown with dark spots. The red shell was thinner than the dark colored shell. This is in line with the findings of Boneka et al (1997) where the dark colored shell was thicker than the light-colored shell.

Yellow | Red | Yellow with dark spots | Brown with dark spots

Figure 2. Shell shape and color of *Littoraria pallescens*
(Source: authors' personal archive and photos taken by authors, 2023).

The species *L. pallescens* on the mangrove trees in Mokupa Village, Tombariri Sub-district and Basaan Village, Ratatotok Sub-district had various shell colors which were grouped

into seven color categories (Sumampouw et al 2018). The pigments contained in the shell of *L. pallescens* of different colors were carotenoids, namely ß-carotene and melanin (Sumampouw et al 2018). Carotenoid pigments can enter through foods (Mantiri et al 2004). *L. pallescens* is an animal that feeds on microalgae (Dharma 1988) and epiphytes on mangrove leaves which are rich in carotenoid pigments. Carotenoid pigments from food are then distributed into the sea snail's body through the mantle into the shell (Mantiri et al 2021). In other organisms such as the crustacean *Homarus gammarus*, feeding with feed rich in carotenoids can change the color of the carapace due to metabolic processes (Mantiri et al 1995, 1996).

The number of *L. pallescens* scattered on leaves, branches, and roots of mangrove in coastal waters of Batu Nona Beach, Kema Tiga, North Minahasa Regency, North Sulawesi Province is shown in Figure 3. The results showed that there were 33.57% of *L. pallescens* individuals with brown with dark spots on the leaves. The colors that were the least dispersed on the leaves are red 2.91% and yellow with darks spots 4.37%.

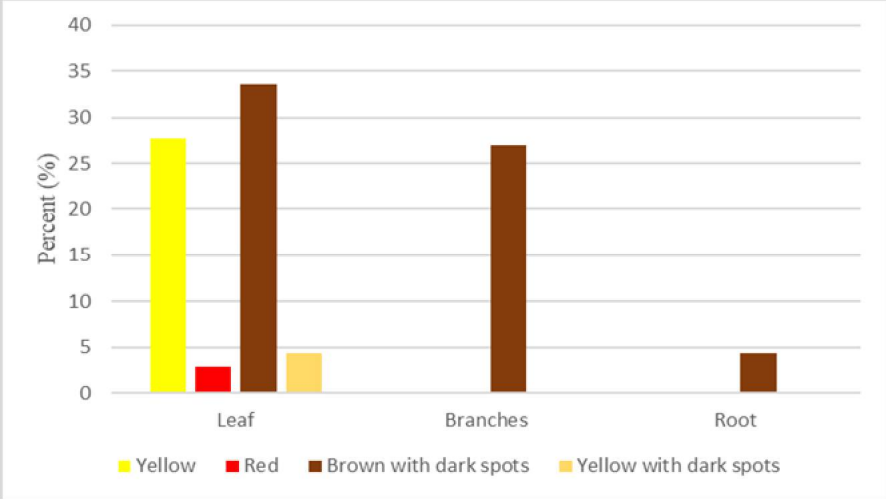

Figure 3. Color distribution of *L. pallescens* on parts of mangrove trees (Source: authors' analyses, 2023).

Morphology. The shell of *L. pallescens* was elongated and tapered at the ends. The tapered shape at the end was very small and very soft. This shell also resembles a thread roll up and forms a pyramid (Figure 4). As in the subclass Prosobrancia, *L. pallescens* have a shell cover or referred to as a purplish operculum. This species is sometimes almost similar to other *Littoraria* species, but can be distinguished by determining the color of the shell cover or operculum of this species.

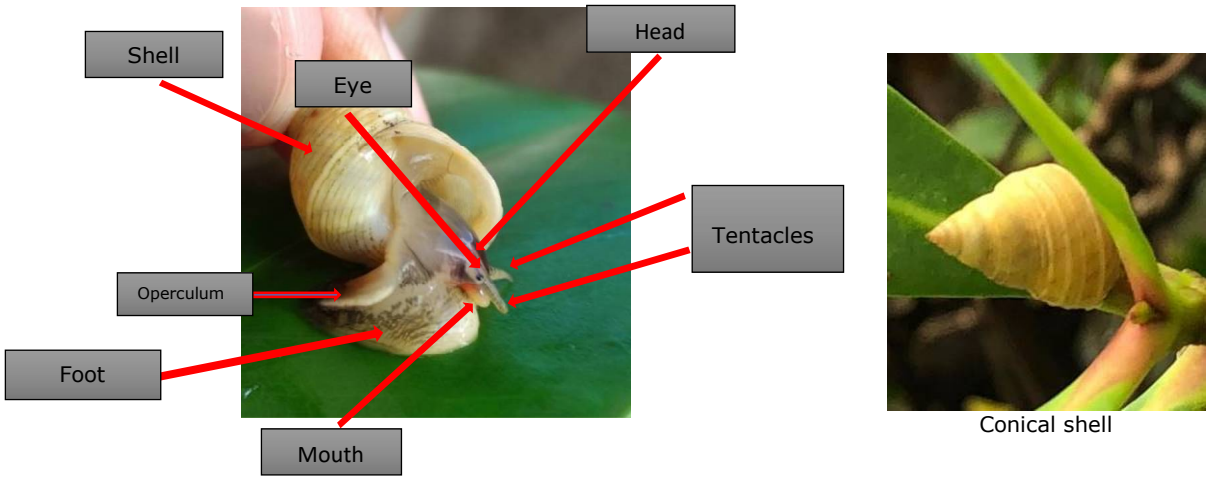

Figure 4. Sea snail *L. pallescens* morphology (Source: authors' personal archive and photos taken by authors, 2023).

Molecular identification. Amplification of sea snail sample showed a clear single band in the gene cycle. Sample DNA was observed at 600-750 bp. The result of the nucleotide length sequence of the *L. pallescens* sample obtained was ±713 bp (Figure 5) for the sample with:

code BIOSUB157.001 (Brown with dark spots)
AACATTATACATTTTATTTGGAATATGATCTGGCCTGGTAGGTACAGCTTTAAGCCTCCTTATTCGA
GCTGAATTAGGACAACCAGGCGCCCTACTAGGAGATGATCAGCTTTATAACGTTATTGTAACAGC
TCATGCATTTGTTATAATCTTTTTTTTAGTTATACCAATGATAATTGGAGGCTTCGGAAATTGATTA
GTCCCTTTAATGCTAGGTGCACCCGACATAGCATTCCCTCGACTAAATAACATAAGCTTTTGACTT
CTTCCTCCCGCACTTTTACTTCTACTCTCCTCAGCTGCAGTAGAAAGCGGTGTAGGAACTGGATGA
ACTGTTTATCCTCCTCTCGCAGGCAATTTAGCTCACGCCGGAGGCTCCGTAGATCTAGCAATTTTT
TCGCTTCATCTAGCTGGTGTTTCCTCTATTTTAGGAGCTGTAAACTTTATTACAACCATCATCAATA
TGCGATGACGTGGTATGCAGTTTGAACGCCTACCTCTTTTCGTTTGATCGGTAAAAATTACAGCCA
TTCTTCTTCTTTTATCCCTCCCAGTTTTAGCTGGTGCAATTACAATACTCCTAACAGATCGAAACTT
TAATACTGCTTTCTTTGATCCTGCCGGAGGGGAGACCCCATTCTTTACCAACATTTGTTT;

code BIOSUB157.003 (Yellow with dark spots)
AACATTATACATTTTATTTGGAATATGATCTGGCCTGGTAGGTACAGCTTTAAGCCTCCTTATTCGA
GCTGAATTAGGACAACCAGGCGCCCTACTAGGAGATGATCAGCTTTATAACGTTATTGTAACAGC
TCATGCATTTGTTATAATCTTTTTTTTAGTTATACCAATGATAATTGGAGGCTTCGGAAATTGATTA
GTCCCTTTAATGCTAGGTGCACCCGACATAGCATTCCCTCGACTAAATAACATAAGCTTTTGACTT
CTTCCTCCCGCACTTTTACTTCTACTCTCCTCAGCTGCAGTAGAAAGCGGTGTAGGAACTGGATGA
ACTGTTTATCCTCCTCTCGCAGGCAATTTAGCTCACGCCGGAGGCTCCGTAGATCTAGCAATTTTT
TCGCTTCATCTAGCTGGTGTTTCCTCTATTTTAGGAGCTGTAAACTTTATTACAACCATCATCAATA
TGCGATGACGTGGTATGCAGTTTGAACGCCTACCTCTTTTCGTTTGATCGGTAAAAATTACAGCCA
TTCTTCTTCTTTTATCCCTCCCAGTTTTAGCTGGTGCAATTACAATACTCCTAACAGATCGAAACTT
TAATACTGCTTTCTTTGATCCTGCCGGAGGAGGAGACCCCATTCTTTACCAACATTTGTTT;

code BIOSUB130.001 (Yellow)
AACACTATACATTTTATTTGGAATATGATCTGGCCTGGTAGGTACAGCTTTAAGCCTCCTTATTCG
AGCTGAATTAGGACAACCAGGCGCCCTACTAGGAGATGATCAGCTTTATAACGTTATTGTAACAG
CTCATGCATTTGTTATAATCTTTTTTTTAGTTATACCAATGATAATTGGAGGCTTCGGAAATTGATT
AGTCCCTTTAATGCTAGGTGCACCCGACATAGCATTCCCTCGACTAAATAACATAAGCTTTTGACT
TCTTCCTCCCGCACTTTTACTTCTACTCTCCTCAGCTGCAGTAGAAAGCGGTGTAGGAACTGGATG
AACTGTTTATCCTCCTCTCGCAGGCAATTTAGCTCACGCCGGAGGCTCCGTAGATCTAGCAATTTT
TTCGCTTCATCTAGCTGGTGTTTCCTCTATTTTAGGAGCTGTAAACTTTATTACAACCATCATCAAT
ATGCGATGACGTGGTATGCAGTTTGAACGCCTACCTCTTTTCGTTTGATCGGTAAAAATTACAGCC
ATTCTTCTTCTTTTATCCCTCCCAGTTTTAGCTGGTGCAATTACAATACTCCTAACAGATCGAAACT
TTAATACTGCTTTCTTTGATCCTGCCGGAGGAGGAGACCCCATTCTTTACCAACATTTGTTT;

code BIOSUB130.002 (Red)
AACATTATACATTTTATTTGGAATATGATCTGGCCTGGTAGGTACAGCTTTAAGCCTCCTTATTCGA
GCTGAATTAGGACAACCAGGCGCCCTACTAGGAGATGATCAGCTTTATAACGTTATTGTAACAGC
TCATGCATTTGTTATAATCTTTTTTTTAGTTATACCAATGATAATTGGAGGCTTCGGAAATTGATTA
GTCCCTTTAATGCTAGGTGCACCCGACATAGCATTCCCTCGACTAAATAACATAAGCTTTTGACTT
CTTCCTCCCGCACTTTTACTTCTACTCTCCTCAGCTGCAGTAGAAAGCGGTGTAGGAACTGGATGA
ACTGTTTATCCTCCTCTCGCAGGCAATTTAGCTCACGCCGGAGGCTCCGTAGATCTAGCAATTTTT
TCGCTTCATCTAGCTGGTGTTTCCTCTATTTTAGGAGCTGTAAACTTTATTACAACCATCATCAATA
TGCGATGACGTGGTATGCAGTTTGAACGCCTACCTCTTTTCGTTTGATCGGTAAAAATTACAGCCA
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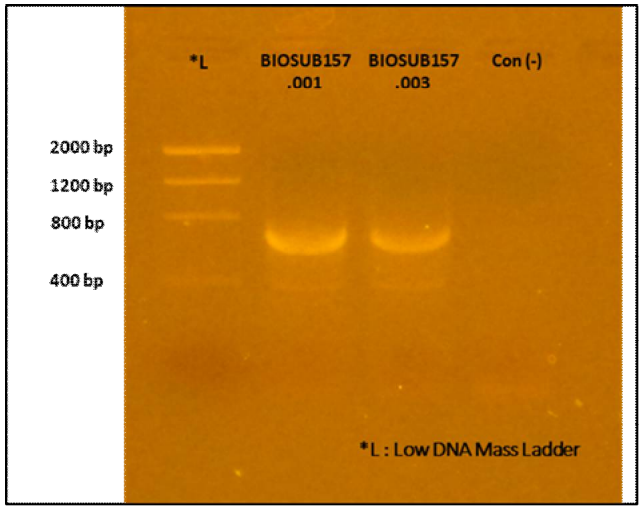
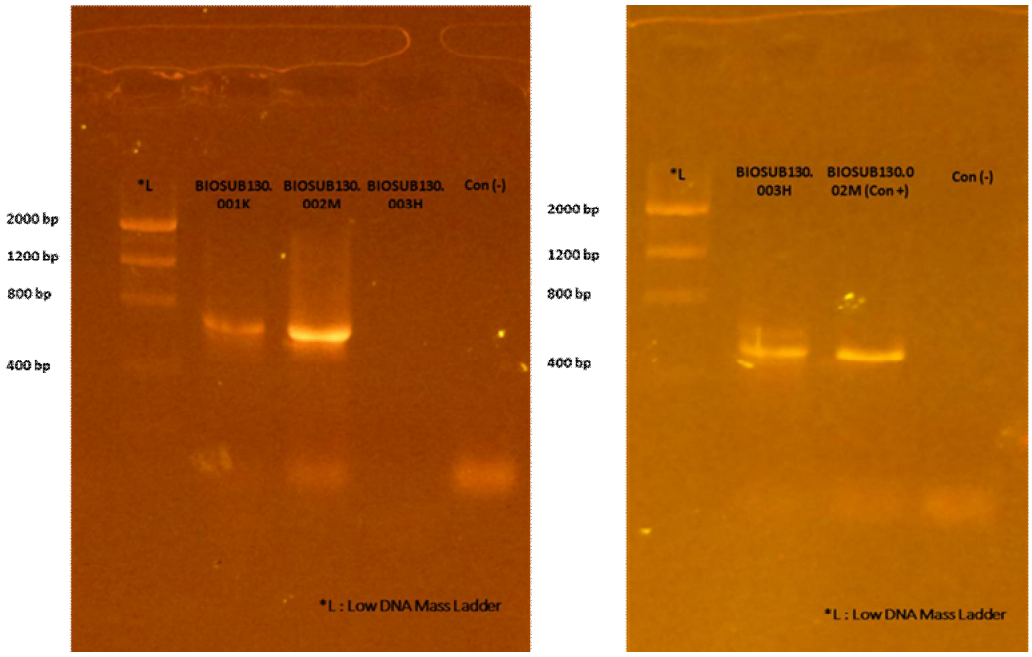


(Sample code electrophoresis results of BIOSUB157.001 and BIOSUB157.003)



(Sample code electrophoresis results of BIOSUB130.001 and BIOSUB130.002)
Figure 1. Electrophoresis results.

The results of identification of *L. pallescens* using the blast method are shown in Table 1.

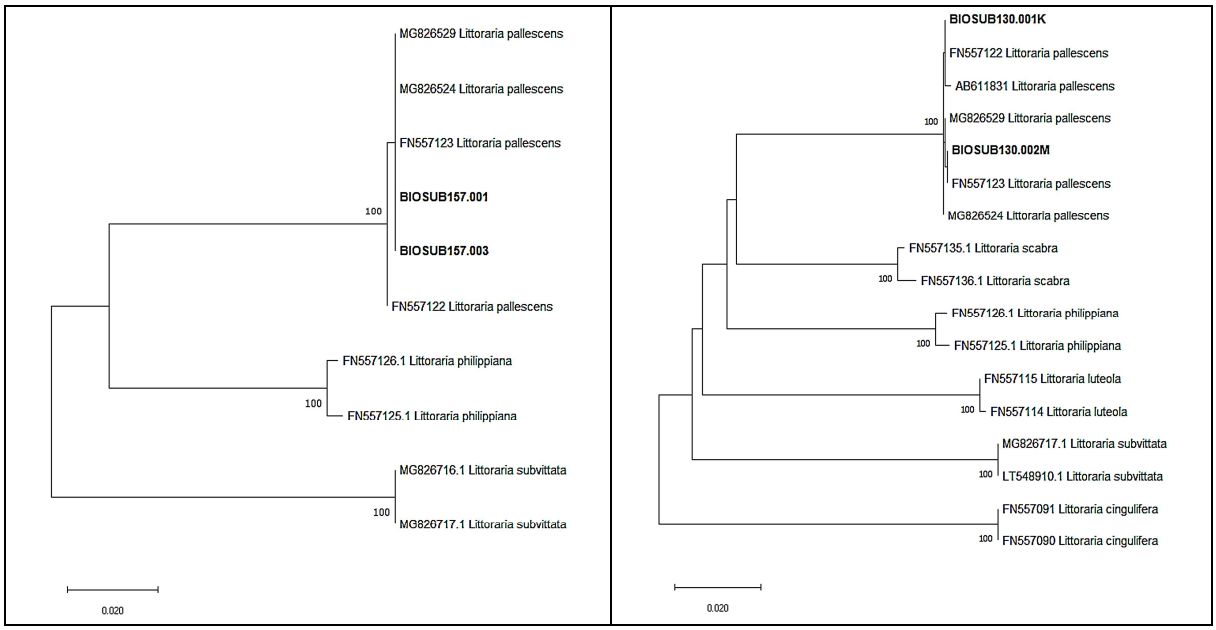
Table 1

Identification of *L. pallescens* using the blast method

Sample	*Max score*	*Identity (%)*	*Gene*	*Accession number*
Brown with dark spots	1216	100	COI	FN557123.1
Yellow with dark spots	1216	100	COI	FN557123.1
Yellow	1216	100	COI	FN557122.1
Red	1216	100	COI	FN557123.1

Compared to GenBank data, the samples taken from Batu Nona Beach, Kema Tiga, North Minahasa Regency, North Sulawesi Province were identified as *L. pallescens*. This result was obtained from comparison with other *Littoraria* species. Based on the results of nucleotide matching and the sample neighbor tree against the GenBank database, the phylogenetic tree was performed using the Neighbor-joining (NJ) method with bootstrap replication 1,000 times, finding similarities that BIOSUB157.001, BIOSUB157.003,

BIOSUB130.001, and BIOSUB130.002 are species *L. pallescens* with a similarity index of 100% (Figure 6).



BIOSUB157.001 & BIOSUB157.003 BIOSUB130.001 & BIOSUB130.002

Figure 2. Genetic neighbor tree.

Conclusions. This study found that the sea snail, *L. pallescens*, which is widely distributed in the Indo-Pacific, was found in the coastal waters of Batu Nona Beach, Kema Tiga, North Minahasa Regency, North Sulawesi Province. This species lives in any species of mangrove tree, and scattered on the leaves, branches and roots. The shell color of this sea snail can indicate where this species grows and matures. Morphologically this sea snail has a tapered shape with two differences, namely bright and dark. The results of DNA analysis stated that although the shell color of the sea snails was different, they were the same species, namely the *L. pallescens.*

Acknowledgements. We would like to appreciate Sam Ratulangi University through the Institute for Research and Community Service who have funded this research.

Conflict of interests. The authors declare that there is no conflict of interest.

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Received: 02 October 2023. Accepted: 29 October 2023. Published online: 13 December 2023.
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How to cite this article:
Mantiri D. M. H., Kepel R. C., Boneka F. B., Londo R., 2023 Morphology and genetic characteristics of sea snail, *Littoraria pallescens* based on different colors from Batu Nona Beach, Kema Tiga, North Minahasa Regency, North Sulawesi Province, Indonesia. AACL Bioflux 16(6):3209-3216.