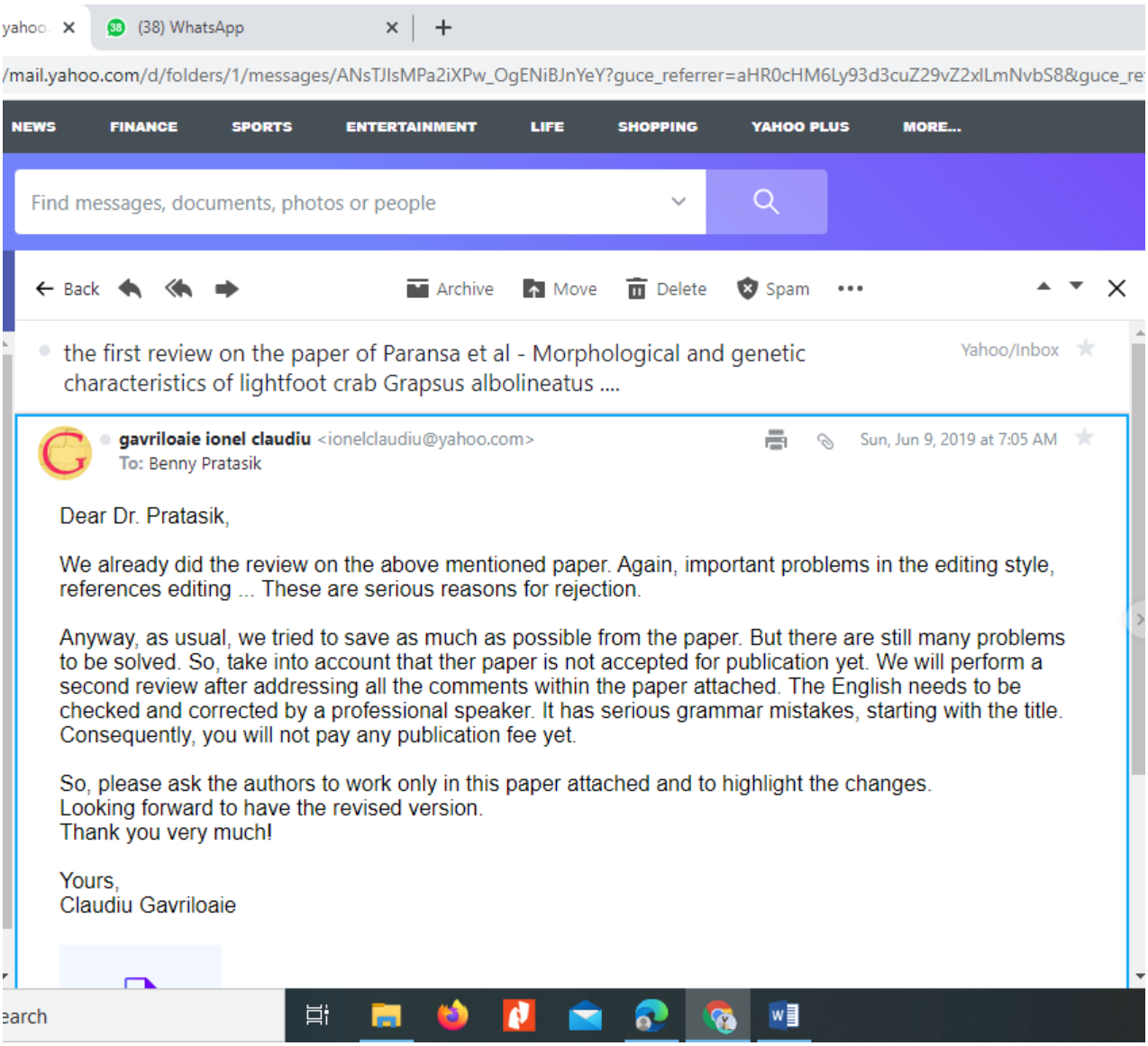

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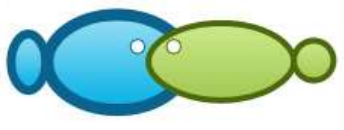

Morphological and genetic characteristics of lightfoot crab of *Grapsus albolineatus* (Lamarck, 1818) from Manado Bay, North Sulawesi

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Abstract. Crustaceans possess the specificity on their carapace, diverse color. Crabs of family Grapsidae, especially genus Grapsus, occur in high numbers in the coast of Manado Bay, Ranowangko village, Tombariri district, Minahasa, North Sulawesi, but cannot be consumed. This genus feeds on microalgae attaching on the rocks where they live. They are, in general, greenish black, reddish black, reddish dark green and have greenish white longitudinal line, four pairs of feet and a pair of claws with purple tip. Based on shape and carapace color, this crab was morphologically identified as *Grapsus albolineatus* (Lamarck) 1818. Molecular identification also confirmed that the crab sample (DP3) had 99% similarity to *Grapsus albolineatus*.

Key Words: crustacean, *Grapsus*, similarity, rocky shore crab.

Introduction. Crabs are crustacean groups that possess typical carapace of diverse colors. Crabs of family Grapsidae, particularly genus Grapsus, comprise *Grapsus albolineatus* Latreille *in* Milbert, 1812, *G. adscensionis* (Osbeck, 1765), *G. fourmanoiri* Crosnier, 1965, *G. grapsus* (Linnaeus, 1758), *G. granulosus* H. Milne-Edwards, 1853, *G. intermedius* De Man, 1888, *G. longitarsus* Dana, 1851, and *G. tenuicrustatus* (Herbst, 1783) (https://en.wikipedia.org/wiki/Grapsus). They occur in abundance in the rocky shore of Manado Bay coast, North Sulawesi, but they are not edible. Carbs of family Grapsidae are opportunistic feeders (Fratini et al 2018). *Grapsus* sp. feeds on microalgae attached on the rocks where they live. According to Denny & Gaines (2007), these crabs are greenish-black, reddish black, reddish dark green, and have white longitudinal line. Several species of this family have nearly similar carapace color. This interesting color visualization occurs due to the role of pigment distribution in the entire crab body tissues (Maoka 2011). Thin layered chromatographic separation, according to Abdullah et al (2018), has indicated that *G. albolineatus* contains carotenoid, especially β-carotene, β-cryptoxanthin, and astaxanthin.

Morphological species identification is still done especially at the early stage, but for theorganisms with similar body shape, color, and size characteristics, it is difficult enough to directly identify. However, it finds limitations and needs relatively long time (Kolondam 2014). Species identification process using the morphological characters has limitation, since body shape, size, and color often change due to the influence of environmental conditions (Reed et al 2013).

Revolution in molecular field pioneered by Paul Hebert suggested "DNA barcoding" as technique of species identification in 2003, and has developed up to now. DNA analysis method can be conducted faster, and with only small amount of body tissue, a species of organism can be determined (Walker & Rapley 2009). Genetic characters-based species identification has been recently considered more accurate. These are

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In this form, the Abstract is only some short description, with general remarks, on the genus *Grapsus*

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useful to explain species diversity and distribution (Bucklin et al 2007). Information on genetic diversity can be obtained through protein-coding gene analysis of mitochondrial DNA (Purnama et al 2019).

Cytocrome oxidase subunit 1 (CO1) is one of the coding genes. It is the most conservative protein-coding gene in the genome of animal's mitochondria (Folmer et al 1994). Wilson & Walker (2010) stated that CO1 gene could be used as DNA barcode because it had numerous benefits including editing in small sequence. Therefore, this study aims to identify the shore crab of genus *Grapsus* with molecular DNA and gene amplification using Cytochrome Oxidase Subunit 1 (CO1).

Material and Method. Shore crabs were collected from the coast of Ranowangko village, Tombariri district, Minahasa regency in July 2018. Ranowangko coast belongs to Manado Bay (Figure 1). Sampling location was rocky shore, submerged in seawater at the highest tide, and exposed to sunlight at low tide, and fertile algal growth occurred on the rocks. According to Denny & Gaines (2007), rocky shore has stable and permanent substrates and is occupied by many kinds of organisms, such as mollusks, shrimps, crabs, worms, and benthos. Crab samples were collected from the rocky shore and brought to the Laboratory of Faculty of Fisheries and Marine Science for further analyses. Species identification was done using morphological and genetic characters. The former followed Carpenter (1998) covering carapace shape and color and claw.

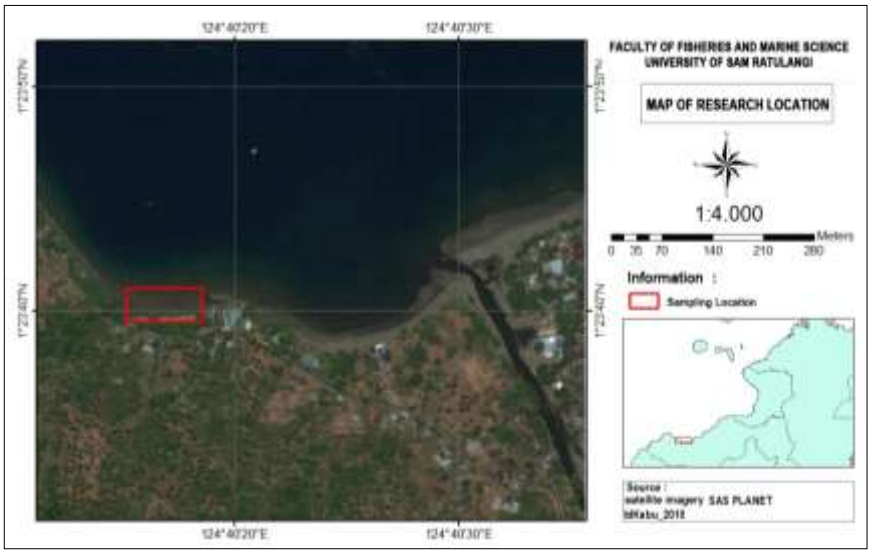

Figure 1. Map of study site.

The latter started with DNA extraction. It employed modified procedure of Plant Genomic DNA Mini Kit (100 Preps) (Geneaid). Muscle of the crab was taken as sample. Gene amplification used Cytochrome c Oxidase 1 (CO1) and universal primer LCO1490 (5'-ggt caa caa atc ata aag ata ttg g-3') and HC02198 (5'-taa act tca ggg tga cca aaa aat ca-3') (Folmer et al 1994). Polymerase chain reaction (PCR) was carried out in 35 cycles at 95°C (30 sec.), 50°C (30 sec.), 72°C (50 sec.). The PCR product was visualized in 1% (b/v) agarosa gel electrophoresis. Bi-directional sequencing was done by First Base CO (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer). The sequenced nucleotide was analyzed further using Geneous software and the nucleotide sequence was fitted to genetic database situs (NCBIBLAST). The chromatogram obtained was edited using Geneious v5.6 (Drummond et al 2012). The sequences were then compared with GenBank data using BLAST (Basic Local Alignment Search Tools) method (Altschul

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et al 1997) and BOLDSystems (Ratnasingham & Hebert 2007). The phylogenetic tree was built using Neighbor-Joining Method (Saitou & Nei 1987).

Results and Discussion

Morphological identification. This crab was morphologically identified as lightfoot crab *Grapsus albolineatus* (Lamarck) 1818, is species whose dorsal part of the carapace is circular convex-shape, has blackish green longitudinal line, has greenish white-striped longitudinal line, and has greenish white parallel longitudinal lines. The carapace size ranged from 2 to 4 cm and had orange circle on the middle.

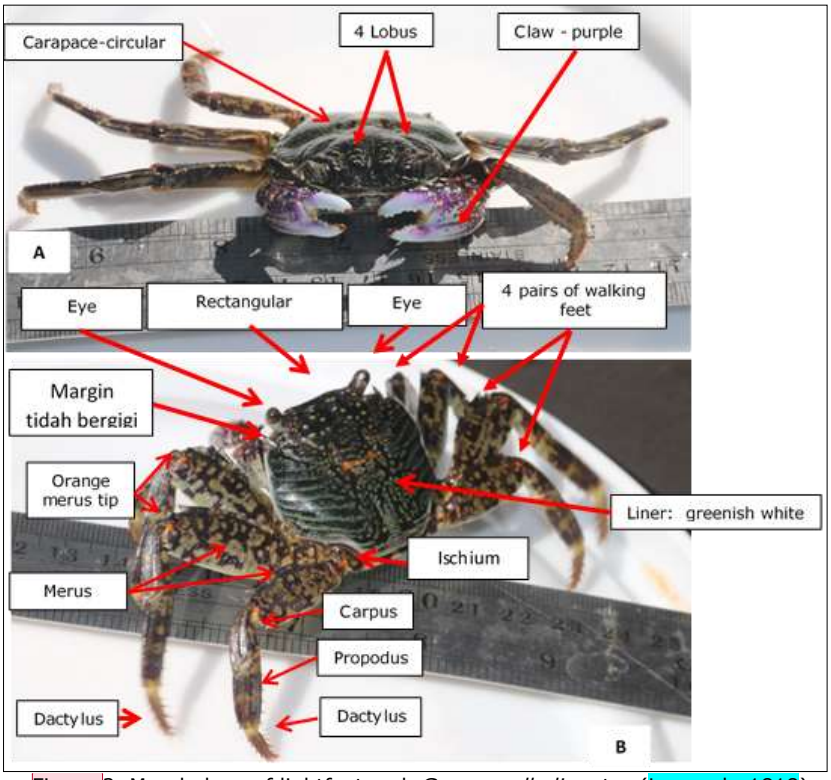

Figure 2. Morphology of lightfoot crab *Grapsus albolineatus* (Lamarck, 1818).

Long foot pairs are equipped with nail, and no swimming leg. The first foot pair is the shortest among feet and has hairs on the parapodia and a pair of small purple claw tips. This crab has also 4 pairs of feet and a pair of claws with purple tip and orange claw base Colin & Arneson (1995). Walking feet have randomly round patterns of greenish orange black or reddish orange black.

Molecular genetic species identification. Crab sample amplification showed the presence of clear single DNA band on the gel cycle. Sample DNA was observed at the amplicon length position of about 600-750 bp and close to 725 bp using 10.000 bp DNA ladder Primer LCO1490 and HC02198 as comparison. The amplicon length position is in line with that of CO1 gene base mostly used as universal marker in animal's species identification based on Folmer et al (1994) and Tang et al (2003). Moreover, DNA marker primer for PCR of 710 bp fragment CO1 gene is available for Echinoderm, Mollusk,

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Annelida, Pogonophora, Arthropoda, Nemertinea, Echiura, Sipuncula, Platyhelminthes, Tardigrada, and Coelenterata (Folmer et al 1994). According to Tang et al (2003), factors affecting the single band and multiple bands on the electrophoretic visual performance come from the condition (quality and quantity) of DNA sample. Crab sample (DP3) was marked with the appearance of thick and clear band on passing path of gel. The PCR product DNA was visualized employing UV-Transiluminator and the success of PCR is detected with the presence of single DNA band of 725 bp (DP3) as shown in Figure 1.

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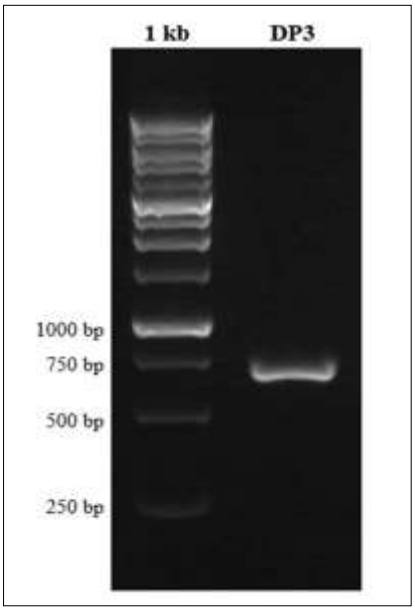

Figure 2. Electrophoresis of CO1 gene amplification of muscle tissue of lightfoot crab muscle (crab DNA/DP3) with Primer LCO1490 and HC02198.

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According to Folmer et al (1994), factor affecting the presence of thin and thick bands on the electrophoretic visual image comes from the DNA sample condition (quality and quantity). The PCR product and the two primers used was then sent to the First Base CO (Malaysia) for sequencing. DNA sequence obtained was presented as chromatogram (Table 1). The nucleotide sequence of 725 bp was then cut the reverse and forward primers and obtained 658 bp.

Table 1

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Nucleotide sequence of CO1 gene of crab sample (DP3) muscle specimen

CRAB SAMPLE
Nucleotide base pair sequence
TACATTATATTTCATCTTTGGTGCCTGAGCGGGAATAGTAGGAACCTCCCTAAGTTTAATTATCCG
AGCAGAATTAAGCCAGCCAGGTAGTCTTATTGGAAATGATCAAATTTACAATGTTGTAGTTACAGC
TCACGCCTTTGTAATGATCTTTTTTATGGTTATACCAATCATAATTGGAGGTTTTGGTAACTGACTT
GTACCCCTTATACTAGGAGCTCCAGATATAGCATTCCCCCGTATAAACAACATAAGATTCTGACTT
TTACCCCCTTCTCTATCCCTTCTTCTTACAAGTAGTATAGTTGAAAGAGGAGTTGGTACCGGATGA
ACTGTTTATCCGCCTCTAGCAGCTGCTATCGCTCACGCAGGAGCCTCGGTTGATCTTGGTATCTTC
TCTTTACATTTAGCTGGTGTGTCATCAATCCTAGGAGCAGTTAATTTTATAACTACAGTTATTAACA

TACGATCCTACGGGATAACAATGGATCAGATACCATTATTTGTCTGAGCTGTATTTATCACCGCTA TCCTACTCCTCTTATCTCTTCCAGTCCTAGCAGGGGCTATTACTATACTCTTAACAGATCGTAACTT GAATACTTCTTTCTTTGACCCAGCGGGAGGCGGAGACCCAGTACTTTATCAACATCTC
Note: 658 bp

The shore crab recorded in this study is presented in Table 2. It is indicated with the maximum score and the level of identity using CO1 genetic characteristic that brought the crab specimen (DP3) belonging to *G. albolineatus* identified through genbank.

Table 2

Identification of *Grapsus albolineatus* using Blast method

Description	*Max. score*	*Identity (%)*	*Access no.*
Grapsus albulineatus Cytochrome c oxidase subunit 1 (CO1) gene, partial cds mitochondrial gene for mitochondrial product.	1061	99	AF317338.1
Grapsus sp PG-2015 mitochondrial CO1 gene for chitochrome oxidase subunit 1. Partial cds isolate: A.	869	92	LT081187.1
Eriocheir cytochrome oxidase subunit 1 (CO1) gene, partial cds: mitochondrial gene for mitochondrial product.	597	83	AF 279269.1

Crab sample collected in the coast of Ranowangko village, Minahasa, based on GenBank, was *Grapsus albolineatus*. This result was obtained through comparison with several groups of crustaceans, such as crabs, hermit crab, and shrimp. Based on the phylogenetic tree using Geneious v5.6. software and genbank data (www.ncbi.nlm.nih.gov), after compared with algoritma Neighbor-Joining, this study found that sample specimen of DP3 sample had very close kinship with lightfoot crab *Grapsus albolineatus*, with similarity of 99 %.

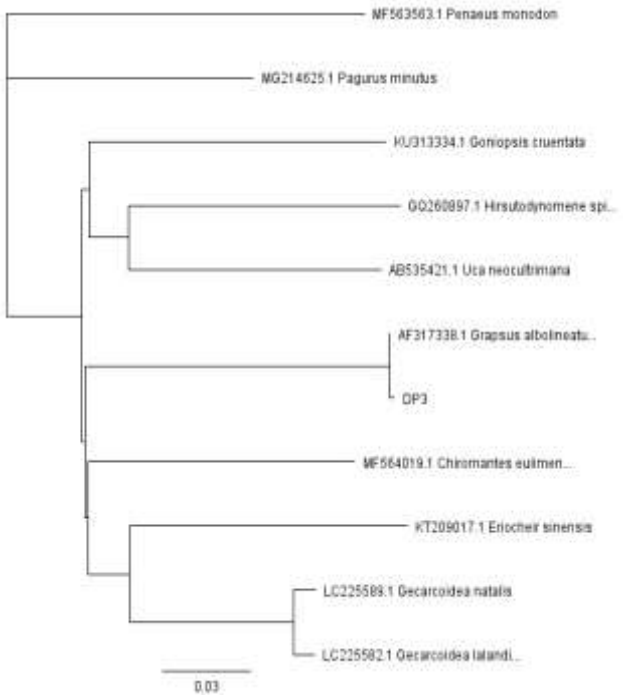

Figure 3. Crab sample (DP3) using Geneious v5.6 software - Algoritma Neighbor - Joining.

Figure 3 demonstrates that all compared NCBI specimens of crabs and shrimp used, but *Grapsus albolineatus,* are in separate clades as the sample specimen meaning that they do not belong to the species of the sample specimen (DP3). The compared crab and shrimp specimens were taken to represent individuals from different habitat types, such as mangrove, land, mud, and sand. This study clearly showed that DP3 was in the same clade as *G. albolineatus* reflecting its strong genetic relationship with this species and confirmed that DP3 sample was *G. albolineatus*.

Conclusions. Crab *Grapsus* sp. is the species abundantly found in Manado Bay coast and lives on the rocky shore. Morphological characters have brought the crab belonging to lightfoot crab *G. albolineatus*. This determination was reconfirmed through DNA analysis using CO1 genetic identity. These data could become information on crab species diversity in Manado Bay, North Sulawesi.

Acknowledgements. We would like to appreciate Mr. Makalalag for crab sampling activities. Our thanks are also addressed to Ms. Diasasthisa and Ms. Seak Werianty for their laboratory help and Mr. Kolondam for handling the molecular works.

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