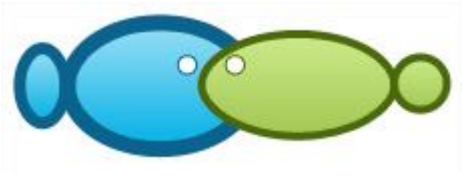

Morphological and genetic characteristics of lightfoot crab *Grapsus albolineatus* Latreille *in* Milbert, 1812 from Manado Bay, North Sulawesi

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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 Ranowangko village, Tombariri district, Minahasa, Manado Bay, North Sulawesi, but cannot be consumed. This genus feeds on microalgae attaching on the rocks where they live. Samples were collected through haphazard search at night along 1 km distance of the rocky shore and taken by hand using a flashlight. Species identification was morphologically done using body characters and confirmed with DNA analysis. For DNA analysis, crab muscle was used. Results showed that, the crab was identified as *Grapsus albolineatus* L. Milbert, 1812. This species is, 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. Molecular identification also confirmed that the crab sample (DP3) had 99% similarity to *G. albolineatus*. Thus, both approaches have strengthened this species occurrence in the coast of Tanahwangko.
Key Words: crustacean, *Grapsidae*, body character, DNA.

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, *Grapsus intermedius* De Man, 1888, *G. longitarsus* Dana, 1851, and *G. tenuicrustatus* (Herbst, 1783). Grapsid crabs living in the rocky shore are also *Pachygrapsus minutus* A. Milne Edwards, 1873, *P. planifrons* De Man, 1888, *P. fakaravensis* Rathbun, 1907, *P. plicatus* (H. Milne Edwards, 1837) (Poupin & Juncker 2010). They occur in abundance in the rocky shore of Manado Bay coast, North Sulawesi, but they are not edible. Crabs 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 the organisms 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 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 (Purnami et al 2010).

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. Lightfoot crabs *G. albolineatus* 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. This area is grown with fertile algae 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. Sampling was carried out in the form of haphazard search along 1 km distance at night using a flashlight and the crabs were hand-collected. Night sampling was selected, since crabs are nocturnal organisms (Poore 2004; Lalli & Parsons 2006). The samples were then brought to the Laboratory of Faculty of Fisheries and Marine Science for further analyses. Species identification was done using morphological and genetic characters. Morphological identification followed Carpenter & Niem (1998) by looking at the general shape of the carapace, the typical shape, and the color. Other observations were done on the leg shape and color, and the claw shape and color. For DNA analysis, sample was preserved in 95% ethanol. Nucleotide characterization of CO1 gene began with DNA genome extraction of the crab's muscle.

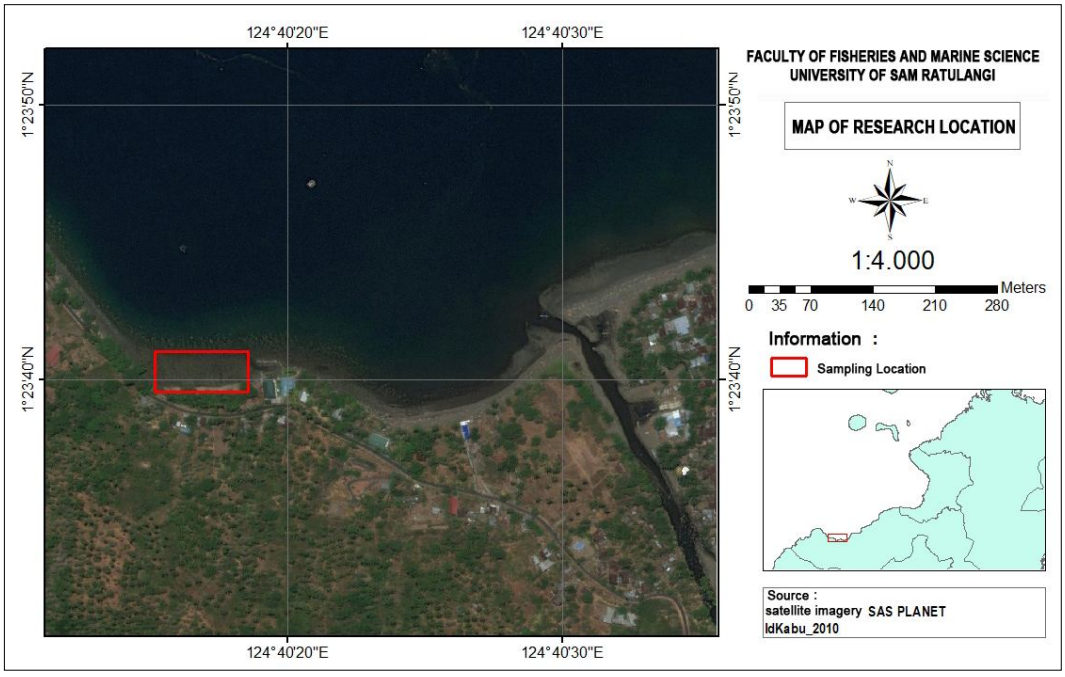

Figure 1. Map of study site.

DNA extraction employed modified procedure of Plant Genomic DNA Mini Kit (100 Preps) (Geneaid). DNA obtained was stored in the freezer. Gene amplification used Cytochrome c Oxidase 1 (CO1) and universal primer LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG-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 for denaturation at 95^{o}C (30 sec.), annealing at 50^{o}C (30 sec.), extension at 72^{o}C (30 sec.). The PCR product

was separated using 1% (b/v) agarosa gel electrophoresis (TBE buffer 1x) and observed using UV-Transluminator. The success of PCR DNA product is detected with the presence of single DNA band of 725 bp. 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 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* L. Milbert 1812, 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 (Figure 2). This finding is consistent with Majchacheep (1989). The carapace size ranged from 2 to 4 cm and had orange circle on the middle, in the range of Majchacheep (1989), 2-5 cm width.

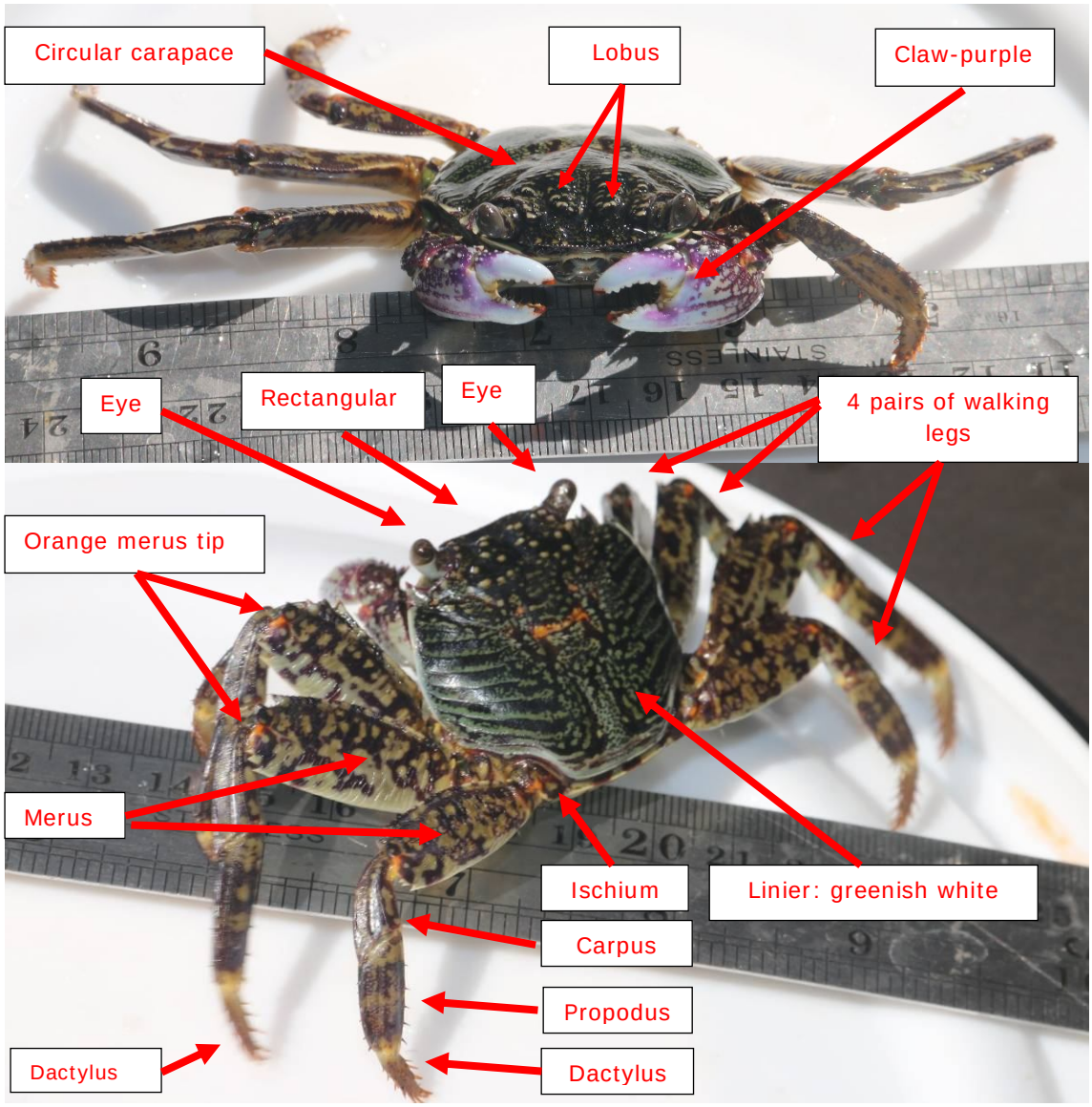

Figure 2. Morphology of lightfoot crab *Grapsus albolineatus* L. Milbert, 1812.

Other characters showed that the anterior part between the eyes occurs 4 blackish green lobes, and a pair of claws is purplish orange. Poupin & Juncker (2010) have characterized that *G. albolineatus* has convex carapace with low tubercles, round lateral margins with length of front equal to length of posterior margin of carapace, and brown-mottled legs with one orange spot at tip of meri. Carpenter & Niem (1998) identified the carapace with green and white transverse markings.

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 legs and a pair of claws with purple tip and orange claw base Colin & Arneson (1995). Walking legs have randomly round patterns of greenish orange black or reddish orange black.

According to Denny & Gaines (2007) and Poore (2004), periopod consists of dactylus, propodus, carpus, merus and ischium, in which merus tip occurs an orange circle and the leg section is greenish brown-speckled. Lateral margins rounded or trapezoid with straight front (rectangular) parts; carapace margin with unclear border, looking almost straight or gently convex with one anterior tooth (Carpenter & Niem 1998).

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, 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 3. 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 were then sent to the First Base CO (Malaysia) for sequencing. DNA sequence obtained was presented as chromatogram. The nucleotide sequence of 725 bp was then edited to obtain 658 bp as
TACATTATATTTCATCTTTGGTGCCTGAGCGGGAATAGTAGGAACCTCCCTAAGTTTAATTATCCG
AGCAGAATTAAGCCAGCCAGGTAGTCTTATTGGAAATGATCAAATTTACAATGTTGTAGTTACAGC
TCACGCCTTTGTAATGATCTTTTTTATGGTTATACCAATCATAATTGGAGGTTTTGGTAACTGACTT
GTACCCCTTATACTAGGAGCTCCAGATATAGCATTCCCCCGTATAAACAACATAAGATTCTGACTT
TTACCCCCTTCTCTATCCCTTCTTCTTACAAGTAGTATAGTTGAAAGAGGAGTTGGTACCGGATGA
ACTGTTTATCCGCCTCTAGCAGCTGCTATCGCTCACGCAGGAGCCTCGGTTGATCTTGGTATCTTC
TCTTTACATTTAGCTGGTGTGTCATCAATCCTAGGAGCAGTTAATTTTATAACTACAGTTATTAACA
TACGATCCTACGGGATAACAATGGATCAGATACCATTATTTGTCTGAGCTGTATTTATCACCGCTA
TCCTACTCCTCTTATCTCTTCCAGTCCTAGCAGGGGCTATTACTATACTCTTAACAGATCGTAACTT
GAATACTTCTTTCTTTGACCCAGCGGGAGGCGGAGACCCAGTACTTTATCAACATCTC.

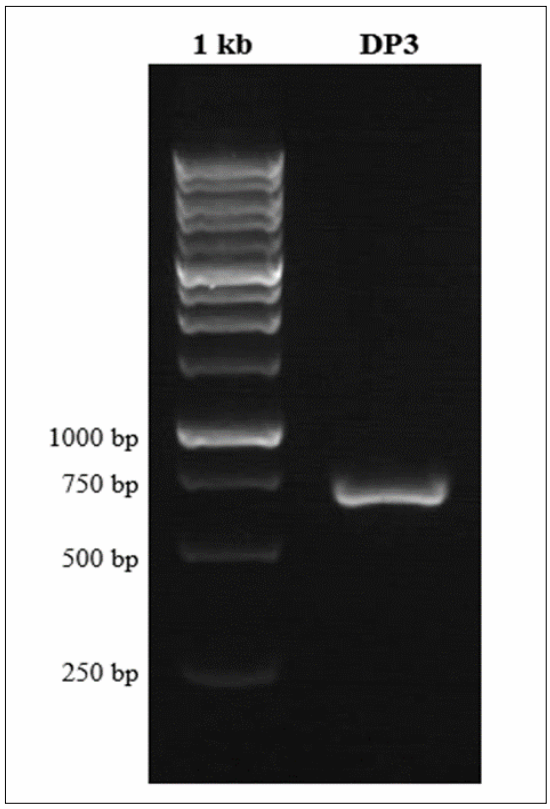

Figure 3. Electrophoresis of CO1 gene amplification of muscle tissue of lightfoot crab muscle (crab DNA/DP3) with Primer LCO1490 and HC02198.

Table 2 demonstrates the shore crabs listed in the genbank data through blast method, the maximum score and the level of identity using CO1 genetic characteristic, in which the crab specimen (DP3) has the highest similarity to the lightfoot crab *Grapsus albolineatus*.

Table 2

Identification of *Grapsus albolineatus* using Blast method

Description	*Max. score*	*Identity (%)*	*Access no.*
Grapsus albolineatus 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
Gegarcoidae natalis mitochondrial CO1 gene for cytochrome oxidase subunit 1 partial cds, isolate: Gen6.	584	83	LC225589.1
Uca neocultrimana mitochondrial CO1 gene for cytochrome oxidase subunit 1, partial cds, isolasi: Uvn5.	582	83	AB535421.1
Gegarcoidae lalandii mitochondrial CO1 gene for cytochrome oxidase subunit 1 partial cds, isolate GEL25.	584	83	LC225577.1

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 had very close kinship with lightfoot crab *Grapsus albolineatus*, with similarity of 99 % (Figure 4).

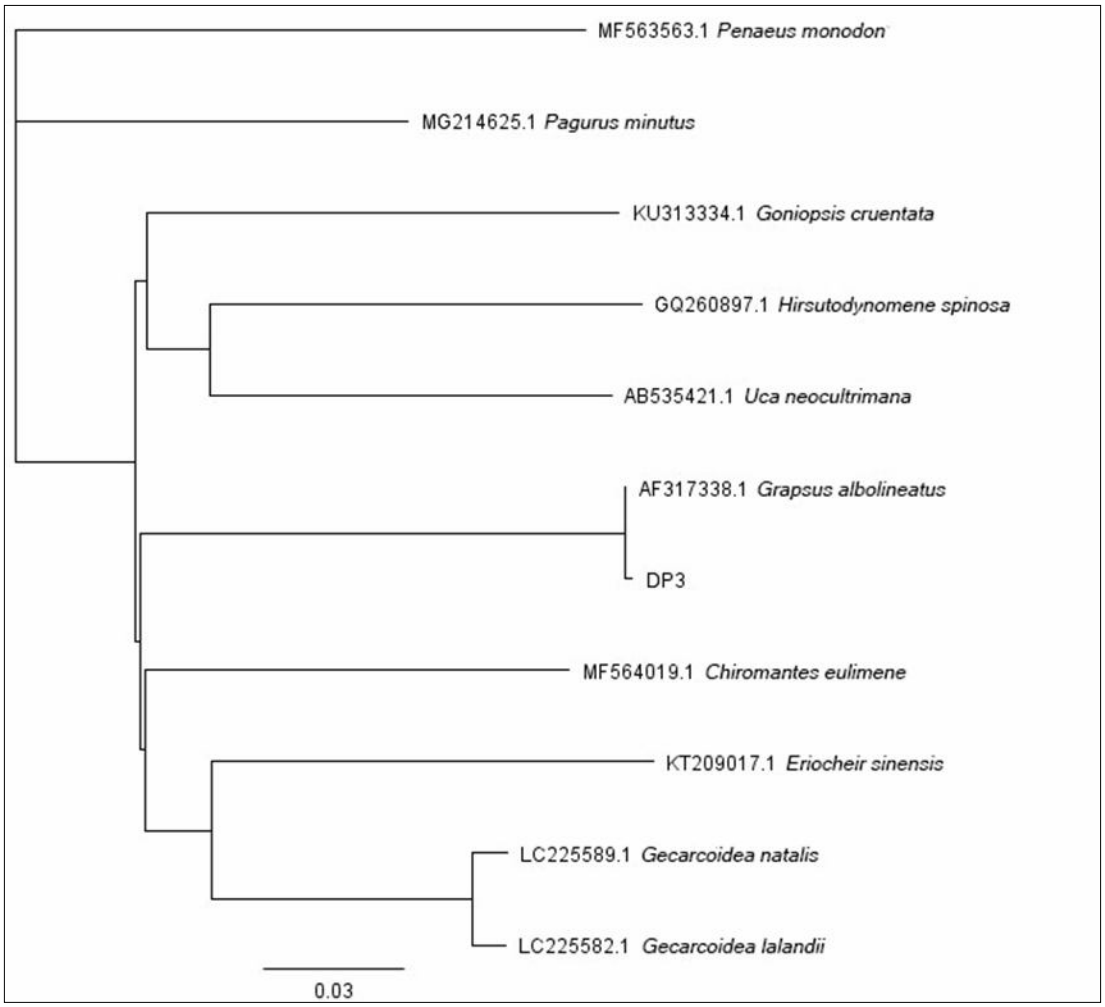

Figure 4. Crab sample (DP3) using Geneious v5.6 software - Algoritma Neighbor - Joining.

Figure 4 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* with 99% similarity confirming DP3 sample as *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.

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