Determination of organic carbon content and molecular biology of mud-skipper species in acidic substrate mangrove ecosystem in Cawan Island Riau, Indonesia

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Abstract. Mangrove trees grow in tropical and sub-tropical coastal areas, which experience inundation and dry periods during high and low tides. Mangroves have essential functions in ecological, physical, and economic aspects, also as potential carbon storage. The study aimed to explore mangrove species, the substrate's total organic carbon (TOC) and DNA of the associated mud-skippers in the Cawan island, Riau, Indonesia. The study was approached using a new paradigm where two aspects were combined: allometric equations and DNA methods. *Rhizophora apiculata*, *Rhizophora mucronata*, *Bruguiera gymnorrhiza*, *Lumnitzera racemosa*, *Acrostichum aureum*, and *Nypa fruticans* were six species found in the ecosystem. The dominant species is *R. apiculata*, which can reach a height of 15 m, much taller than in other parts of Indonesia. The mangroves' substrate acidity ranges between 5-7, with a density range of 390-690 trees ha^{-1}. The content of the substrate organic carbon is 6.793-16.323 tons C ha^{-1}. The study revealed that only two species of mud-skipper survive in this specific acidic mangrove substrate: *Periopthalmodon schlosseri* and *Parapocryptes serperaster*, which was confirmed by DNA analysis. The results of this study could be beneficial for managing and conserving the mangrove ecosystem and for developing coastal ecotourism on Cawan Island and in other regions.
Key Words: total organic carbon, allometric equation, DNA analysis, acidic peat-substrate.

Introduction. Mangrove forests have essential environmental functions for the associated organisms. Maintaining the stability of the coastal ecosystem provides regulating services such as protection, symbiotic functions and carbon storage. Mangrove forests have a remarkable carbon-dioxide absorption capacity, reducing global warming. Mangrove ecosystems are unique and play an essential role in the environment and socio-economic functions (Hapsari et al 2020; Rospita et al 2017). The sediment redox potential values are a governing variable in an acidic mangrove substrate and the oxygenation processes. Sediments can originate either from the transition zone or from the oxidation zone. Environmental factors can also determine variations of the redox potential. Syahrial et al (2018) stated that factors affecting the redox potential are rainfall, tides, location, depth of sampling and sediment texture. Redox value will decrease with the distance to the estuary or coastal area. The natural presence of many mangrove seedlings at almost all stations indicated that mangrove trees breed and grow in suitable environmental conditions. The parent trees can recruit seedlings for the species' survival (Istomo & Afriyani 2018).

The mangrove ecosystem is a type of forest that grows in tidal areas that are flooded at high tide and free from inundation at low tide; the plant community is tolerant to salt (Asyiawati & Akliyah 2014; Hartoko et al 2021). According to Wiakanti (2016), mangrove forests are the primary ecosystem supporting life in coastal and marine areas. Mangroves have various functions, namely (i) ecological, as a nutrient provider for aquatic biota, spawning grounds and nursery grounds for various kinds of biota; (ii) physical functions, as a buffer for coastal erosion, waves, raging winds and tsunamis, sewage absorbers, preventing seawater intrusion, (iii) economic functions, as providers of wood, medicines, fishing tools and techniques (Alongi 2009; Muskananfola et al 2020a,2020b). Mangrove ecosystems are also crucial to the issue of climate change because, providing the storage of huge carbon stocks (Donato et al 2011; Latifah et al 2018). Mangroves are a habitat for marine organisms such as shellfish, shrimp, crabs, fish, and mudskippers.

Mudskippers are a group of the fish family of Gobiidae, Perciformes order and genus of *Periophthalmus* (Murdy 1989), and inhabiting the coast, river, estuary and mangrove ecosystems (Ansari et al 2014; Gosal et al 2013; Bidawi et al 2010). They count several subfamilies, with about 2,000 species. Numerous species of mudskipper have been found in various aquatic ecosystems. Seven species were found in Singapore coastal waters: *Periophthalmodon schlosseri, Periophthalmus argentilineatus, Periophthalmus chrysospilos, Periophthalmus gracilis, Periophthalmus malaccensis, Periophthalmus novemradiatus, Periophthalmus variabilis, Periophthalmus walailakae* (Larson et al 2016). Ten species were documented in Selangor waters, Malaysia (Khaironizam & Norma-Rashid 2002), and four species in the waters of Brunai Darusalam, namely *P. malaccensis, P. gracilis*, and *P. vulgaris*. There is limited research on mudskipper in Indonesian coastal waters, such as on the *P. schlosseri* from the Barito estuary of South Kalimantan (Polgar & Lim 2011). The protein of mudskipper is a biomarker for seawater heavy metal contamination (Hidayaturrahmah et al 2019). Four species of *Boleophthalmus boddarti*, *P. chrysospilos*, *P. gracilis and P. schlosseri* were found in the mangrove areas of the coast of North Sumatra (Muhtadi 2016). Following their observation of the Sumatra Island, Murdy & Takita (1999) and Murdy (1989) identified the species *P. pilotus* sp., *B. pectinirostris, B. boddarti, Periophthalmus takita* and *P. schlosseri.* The Payumb beach of Meraukeis inhabited by *P. gracilis*, while *P. pusing* was found on the Lesser Sunda Island (Sunarni & Maturbongs 2016). Mudskippers are an essential source of protein for people, and they have a role in the food chain of aquatic organisms in mangrove ecosystems (Hui et al 2019). They predate on snails, fish and others. Keeping mudskippers in the mangrove ecosystem could increase the tourism potential of the mangrove ecosystem. Mudskippers are very sensitive to their ambient. More research would be beneficial, especially on the ecological importance of this species in detecting pollution levels in coastal water ecosystems (Ansari et al 2014). Therefore, an environmental quality programme could be established using these organisms as bio-indicators of pollution.

Generally, most mangrove ecosystems in the Cawan Island Indragiri Hilir are in good condition. Four families of mangroves were identified at Cawan Island (Fadlian et al 2018). However, to the present, there is no accurate data on organic carbon and the exact species of its associate mud-skipper in an acidic mangrove peat substrate, especially in tropical regions such as Indonesia. The field sampling difficulties consist of collecting a sample of mud-skipper in a remote area, as well as in obtaining a good condition of mud-skipper samples for morphological identification. This study aimed to explore and analyze the organic carbon in this particular acid peat substrate and to identify the exact species of the associated mud-skipper using DNA analysis and the molecular biology method. The results will be beneficial for managing and conserving the mangrove ecosystem and its related mudskippers, and developing mangrove ecotourism.

Material and Method

Study area. The study was conducted in Cawan Island Riau (Figure 1). Cawan Island is located in the Mandah district, Indragiri Hilir regency, Riau province Sumatera. According to the Office of Forestry Riau Province (2013), the Indragiri Hilir regency has a wide area, of 11.605 km^2, and about 93.31% of this regency is a peat swamp with mangrove vegetation. Geographically, Cawan Island is located in the coordinate of 103°33’18’’E and 0˚05’59’’N (Lubis et al 2018). According to the Bureau of Statistics and Planning and Development Office of Indragiri Hilir (2013), Cawan Island has a wide area of 36.30 km^2.

The vast extent of the mangrove ecosystem in Cawan Island is 1,000 hectares consisting of about 60% of mangrove trees. The tree diameter, on average, is 40 cm. Surprisingly and especially on this island, the mangrove tree reaches a height up to 20 m and grows in an acidic peat substrate with a pH range of 5-7 and a salinity range of 19–21 ppt (Office of Environment Indragiri Hilir Regency 2009). On the Cawan Island there is the most expansive mangrove area of the Riau province. A community-based mangrove forest ecotourism was developed by the local government of Indragiri Hilir regency (Ritung & Kartawisastra 2016).

Field sampling. The study uses a purposive sampling method with five stations representing the Cawan Island (Figure 1). Samples of mangrove substrate were collected using a cylinder coring with a diameter of 10 cm, with a depth of 30 cm (Aini et al 2016). According to Mahasani et al (2016), the sample of mangrove substrate used for analyzing organic carbon should be collected at a depth of 30-50 cm, in order to give a better organic carbon content representation. The weight of the substrate sample from each station was 200 g, as indicated by Indah et al (2008). Mangrove substrate samples were then put into a labelled plastic zipper, as stated by Amin et al (2015).

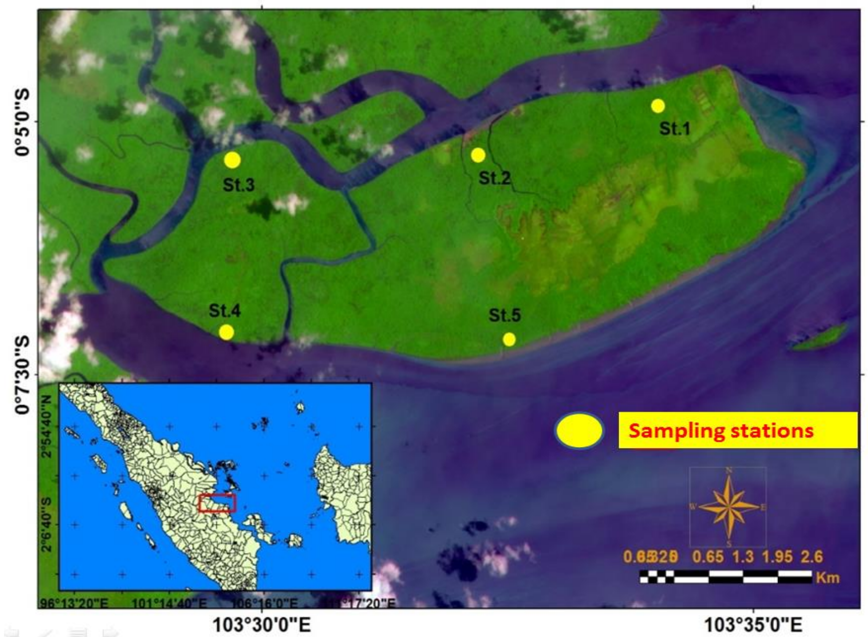

Figure 1. Sampling stations at Cawan Island, Riau, Indonesia.

Data analysis

Mangrove biomass. The mangrove biomass content was calculated based on the field measurement of the diameter at the breast height (DBH) of mangrove trees, as proposed by Kaufman & Donato (2012). A series of the mangrove biomass allometric equations were presented in Table 1, for the dominant species of *Rhizopora apiculata, R. mucronata* and *Bruguiera gymnorrhiza*. The DBH was measured at a height of 1.3 m (Cintron & Novelli 1984). Table 1 describes the mangrove biomass allometric equation of three mangrove species covering trunk biomass, branch biomass and leaf biomass using the allometric equation (Komiyama et al 2005):

$$Y = a + bX$$

Where:

X - the explanatory variable (measured variable);

Y - the carbon biomass as the dependent variable.

The slope of the line is b, and a is the intercept (the value of y when x = 0).

Table 1

Mangrove biomass allometric equations by species

No	Species	*Trunk biomass (WT)*	*Branch biomass (WB)*	*Leaf biomass (WL)*
1	*R. mucronata*	$0.0882\ (DBH)^{2.5621}$	$0.012726\ (DBH)^{2.6844}$	$0.013896\ (DBH)^{2.1072}$
2	*R. apiculaa*	$0.08855\ (DBH)^{2.5621}$	$0.01272\ (DBH)^{2.6844}$	$0.01389\ (DBH)^{2.1072}$
3	*B. gymnorrhiza*	$0.224801\ (DBH)^{2.1407}$	$0.031535\ (DBH)^{2.2789}$	$0.013896\ (DBH)^{1.4914}$

Organic carbon. The carbon stock analysis in mangrove substrate samples was conducted in the Laboratory of Ecology and Plant Production, in the Department of Agriculture, Faculty of Animal Husbandry and Agriculture, Diponegoro University Semarang. The analysis of the carbon, using the ash method, of 2 g of mangrove substrate sample, was performed in the furnace at a temperature of 550°C for 6 hours (Agus et al 2011). Total organic content in the substrate is calculated using the following formula (Habibi et al 2014):

$$LOI = \frac{Wo - Wt}{Wo} x\ 100\%$$

Where:
LOI - loss on ignition (%);
W_0 - initial weight (g);
W_t - final weight (g).

The value of organic content (OC): LOI (%) x 2 g of substrate sample. The OC is converted to total organic carbon (TOC) using a conversion constant of 1.724 (Agus et al 2011; Chmura et al 2003).

$$TOC\ (ton\ C\ ha^{-1}) = TOC\ in\ each\ sampling\text{-}area / 0.2$$

The organic carbon per hectare is calculated using the formula (Agus et al 2011):

$$Organic\ C\text{-}hectare = C\text{-}substrate \times 100\ (ton\ ha^{-1})$$

DNA analysis by molecular biology method

Sample collection. A sample of mud-skipper fish was collected from the substrate of the mangrove at Cawan island. A slice of body tissue of mud skipper fish was stored in a cool box. DNA extraction, electrophoresis purification and PCR were done in the Laboratory of Molecular Biology, in the Faculty of Fisheries and Marine Science, Semarang. Further processing with the Basic Local Alignment Search Tool (BLAST) was done in the Genetica Science Indonesia laboratory, Jakarta, for reconstructing the phylogenetic tree, based on the complete mitochondrial genome, by the National Center for Biotechnology Information (NCBI).

DNA extraction, amplification and sequencing. The high molecular weight genomic DNA size was isolated (Sulardiono et al 2022). The PCR gene amplification used a Cytochrome C-Oxidase subunit-1 primer with 669 bp length, comparing to the genes of the reference species. The sample was directly placed into a ZR BashingBead ™ Lysis Tube and was processed. The DNA from the samples was isolated and purified with the Fast-Spin technology. The genomic DNA extraction was carried out using ZR Tissue and Insect DNA MiniPrep. The PCR amplification utilized MyTaq Red Mix (Bioline) BIO-25047. PCR Master Mix consisted of several components (25 µL), namely: dd H_2O reagent, MyTaq Red Mix (Bioline) BIO-25047, VF2_t1: TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC, FishF2_t1: TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC, FishR2_t1: CAGGAAACAGCTATGA

CACTTCAGGGTGACCGAAGAATCAGAA, FR1d_t1: CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA and template DNA. The sequencing process was carried out using an ABI PRISM 3730xl Genetic Analyzer developed by Applied Biosystems, USA. The kit used for the sequencing purpose is BigDye® Terminator v3.1 Cycle Sequencing.

Homology BLAST method. The sample DNA sequence was compared with the sequence in the DNA database. The search was carried out using the BLAST database in NCBI, National Institute for Health, USA (Pearson 2013) to determine the match (in percentage) of species, based on the nucleotide base sequence's homology level. The series of nucleotide bases is compared with a GenBank database. Visual qualitative data, DNA isolation and amplification profiles, and electropherograms will be presented descriptively. PCR Primer: COI. PCR Products: Species Barcoding (~700). Genomic DNA extraction with ZR Tissue and Insect DNA Miniprep Kit-Zymo Research, D6016 (Pearson 2013; Hartoko et al 2020).

Results. This study found four mangrove families and six species in the Cawan island, Indragiri Hilir, Riau. The most dominant species always found at all five sampling stations is *R. apiculata* (Figure 2). The five other species are *Rhizophora mucronata, B. gymnorrhiza, Lumnitzera racemosa, Acrostichum aureum*, *Nypa fruticans.*

Figure 2. Most dominant species *Rhizophora apiculata* at Cawan Island Indragiri Hilir, Riau.

The study revealed that the mangrove organic carbon is different due to mangrove species and tree diameter. Above-ground biomass and carbon were calculated using an allometry equation based on a function of mangrove tree diameter, as presented in Table 2. The mangrove tree organic biomass at stations 1 to 5 was in the range of 66.31–89.17 ton ha^{-1} with an organic carbon level in the field between 33.16 and 44.59 ton C ha^{-1}. The lowest mangrove tree organic carbon is 33.16 ton C ha^{-1}, found at station 1, and the highest organic carbon is 44.59 ton C ha^{-1} at station 4.

Table 2

Biomass, carbon, organic carbon and mangrove density at Cawan Island Indragiri Hilir Riau

Station	*Biomass (ton ha^{-1})*	*Carbon (ton C ha^{-1})*	*Organic carbon (ton C ha^{-1})*	*Density (tree ha^{-1})*
I	66.31	33.16	14.53	690
II	69.21	34.61	16.32	390
III	78.58	39.29	6.79	470
IV	89.17	44.59	13.85	620
V	78.25	39.12	6.91	520
Total	381.53	190.77	58.41	2690
Average	76.31	38.15	19.47	896.67

The lowest mangrove density was found at station II with 390 tree ha^{-1} but with the highest organic carbon of 16.32 ton C ha^{-1}, while the highest mangrove density was found at station 1 with 690 tree ha^{-1} (Table 2). The result of gel electrophoresis on the sample of big mud-skipper (sample no. 2) and small mud-skipper (sample no. 3) is presented in Figure 4. The quality of the extracted DNA can be determined by the electrophoresis process (Sulardiono et al 2022). The result of sequence assembly of the big mud-skipper sample AH_Gld_BS was presented in Table 3. The effect of the sequence assembly of the small mud-skipper sample sequence assembly on a piece of AH_Gld_Kc was showed in Figure 4 and Figure 5, and Table 4. The result of the BLAST analysis is presented in Table 5. Based on the DNA analysis on the sample AH-Gld-Bs, this belongs to the species *P. schlosseri* with a similarity percentage of 99%, ascession no. KX35324.1, and not to *Periopthalmus barbarous,* with a similarity percentage of 87.52% or *Periopthalmus modestus*, with a similarity percentage of 87.26% (Table 4), and phylogenetic tree in Figure 7.

Figure 3. (a) Sample of dried big mud-skipper, and (b) Sample of dried small mud-skipper from the Cawan island, Indragiri Hilir, Riau.

According to Rannala & Yang (1996), if the bootstrap value in the parsimony analysis is more than 70%, the branch will be constant. The phylogenetic tree on a sample of small mud-skipper from the Cawan island, Indragiri Hilir, Riau confirmed as *Parapocryptes serperaster* with a similarity of 98.01% and ascession no. KT965855.1, as presented in Table 4 and Figure 6.

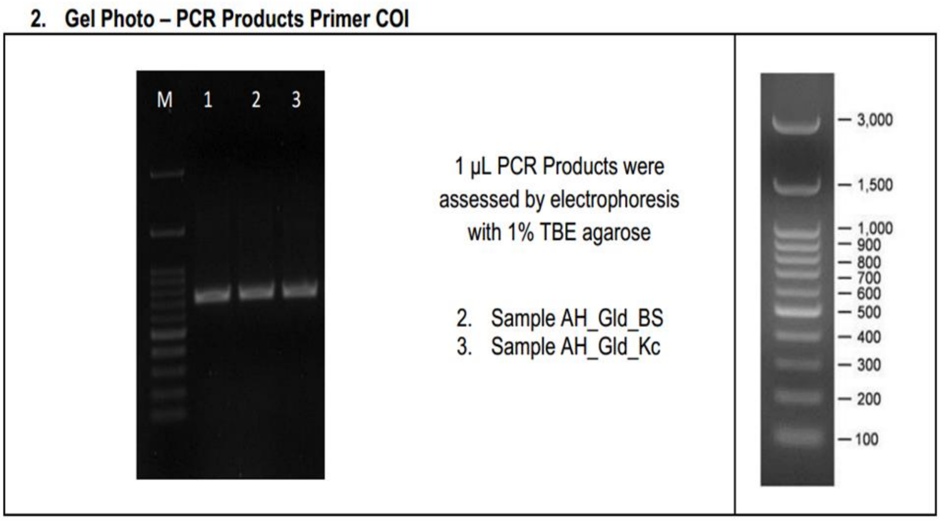

Figure 4. Result of the gel electrophoresis on a sample of big (no. 2) and small (no. 3) mudskipper from the Cawan island, Indragiri Hilir, Riau.

Sequence Assembly
Big mud skipper
GCACCCTTTATCTTGTATTTGGTGCTTGAGCCGGAATGGTAGGAACCGCCCTAAGCCTCCTAA TTCGTGCTGAACTAAGCCAACCCGGTGCCCTTCTTGGGGATGACCAGATTTATAATGTAATTGT AACAGCTCATGCTTTTGTAATAATCTTCTTTATAGTAATACCAATTATGATTGGAGGATTTGGGA ACTGACTAGTGCCCTTAATAATTGGAGCCCCTGATATGGCCTTCCCACGAATAAACAACATAAG CTTCTGACTCCTCCCACCATCTTTCCTTCTCCTCCTAGCATCTTCTGGCGTTGAAGCAGGGGCT GGAACTGGATGAACAGTTTACCCCCCTCTTGCAGGCAACCTCGCACATGCTGGAGCCTCTGTA GATTTAACAATCTTCTCCCTTCATTTAGCAGGTATCTCTTCAATTTTGGGTGCTATTAATTTTATC ACAACAATTTTAAACATAAAACCCCCAGCAATCTCACAATATCAAACCCCCCTTTTTGTATGAGC TGTATTAATTACAGCTGTTCTCTTACTGCTTTCTCTACCAGTTCTAGCTGCTGGCATTACAATAC TTCTCACAGACCGAAACCTAAACACAACCTTCTTTGACCCTGCAGGAGGGGGAGATCCAATTC TCTATCAACACCTTTTCTGATTCTTCG
Small mud skipper
TGGCACCCTCTACCTTGTAATTCGGTGCCTGAGCTGGAATAGTAGGCACAGCCCTAAGCCTCC TAATCCGAGCCGAGCTAAGTCAACCAGGTGCCCTCCTTGGGGACGATCAAATTTACAATGTTA TTGTTACAGCCCATGCCTTTGTAATAATTTTCTTTATAGTAATACCTGTCATGATCGGGGGATTT GGAAACTGACTAATCCCCTTGATAATCGGTGCTCCAGACATGGCCTTCCCTCGAATAAACAATA TAAGCTTCTGACTCCTTCCCCCCTCATTCCTCCTCCTCCTCGCATCCTCAGGGGTAGAGGCCG GGGCTGGTACTGGATGAACAGTCTACCCCCCACTTGCAGGCAACCTTGCCCATGCAGGGGCT TCTGTAGACCTAACCATCTTCTCCCTCCACCTGGCTGGGGTTTCTTCAATTCTTGGGGCCATCA ACTTTATCACAACAATTCTTAATATGAAACCCCCAGCAATTTCACAATATCAAACCCCCCTCTTT GTCTGAGCTGTCCTTATTACAGCTGTCCTTCTCCTTCTTTCCCTTCCAGTTCTAGCTGCCGGCA TCACAATACTTTTAACAGACCGAAATTTAAACACAACCTTCTTTGACCCGGCAGGAGGAGGAG ACCCCATCCTATACCAGC

Figure 5. The result of sequence assembly of mud-skipper sample on AH_Gld_BS

Table 3

Result of BLAST NCBI analysis on a sample of AH_Gld_BS

Description	*Max score*	*Total score*	*Query score*	*E value*	*Per. ident*	*Accession*
Periophthalmodon schlosseri mitochondrion, complete genome	1199	1199	99%	0.0	99.85%	KX355324.1
Periophthalmus barbarus isolate PERIBAR cytochrome c oxidase subunit I gene. Partial cds mitochondrial	826	826	99%	0.0	87.52%	AF391339.1
Periophthalmus modestus haplotype C06 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	820	820	99%	0.0	87.26%	JNO33339.1
Periophthalmus modestus haplotype C16 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	816	816	99%	0.0	87.11%	JNO33349.1
Periophthalmus modestus haplotype C15 cytochrome c oxidase subunit I (COI) gene partial cds, mitochondrial	816	816	99%	0.0	87.11%	JNO33348.1
Periophthalmus modestus haplotype C11 cytochrome c oxidase subunit I (COI).gene. partial cds; mitochondrial	816	816	99%	0.0	87.11%	JNO33344.1
Periophthalmus magnuspinnatus mitochondrion, complete genome	816	816	99%	0.0	87.11%	KT284931.1

Description	Max score	Total score	Query score	E value	Per. ident	Accession
Scartelaos histophorus mitochondrion complete genome	816	816	99%	0.0	87.11%	JQ654459.1
Periophthalmus modestus mitochondrial DNA. complete.genome	811	811	99%	0.0	86.96%	AP019406.1
Periophthalmus modestus OCF-P-4026 mitochondrial DNA complete sequence	811	811	99%	0.0	86.96%	AP019358.1
Periophthalmus modestus haplotype C12 cytochrome c oxidase subunit I (COI).gene partial cds mitochondrial	811	811	99%	0.0	86.96%	JN033345.1
Periophthalmus modestus haplotype C08 cytochrome c oxidase subunit I (COI).gene partial cds mitochondrial	811	811	99%	0.0	86.96%	JN033341.1
Periophthalmus modestus haplotype C07 cytochrome c oxidase subunit I (COI).gene partial cds mitochondrial	811	811	99%	0.0	86.96%	JN033340.1
Periophthalmus modestus haplotype C05 cytochrome c oxidase subunit I (COI).gene partial cds mitochondrial	811	811	99%	0.0	86.96%	JN033338.1
Periophthalmus modestus haplotype C04 cytochrome c oxidase subunit I (COI).gene partial cds mitochondrial	811	811	99%	0.0	86.96%	JN03337.1

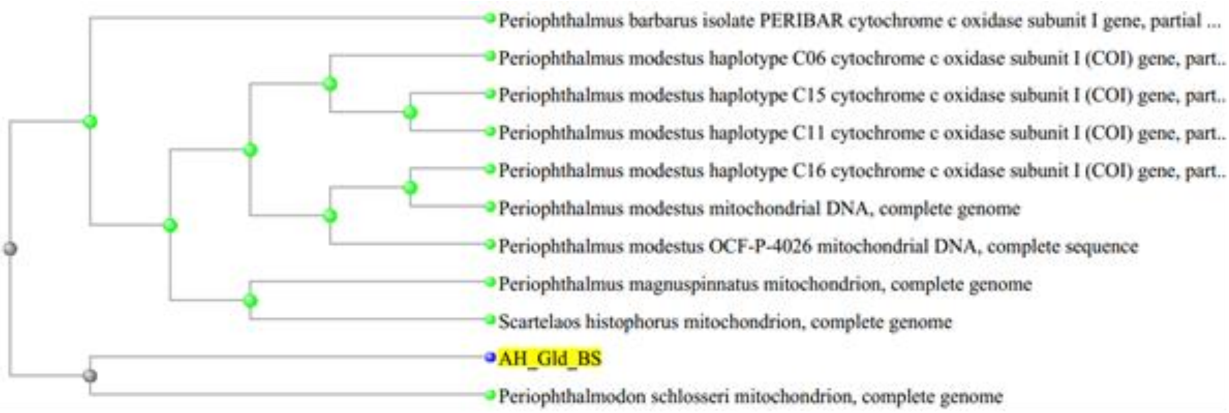

Figure 6. The phylogenetic tree based on the sample AH_Gld_BS of big mud-skipper from the Cawan island, Indragiri Hilir, Riau, confirmed as *Periopthalmodon schlosseri.*

Table 4

Result of BLAST NCBI analysis on a sample of AH_Gld_Kc

Description	Max score	Total score	Query score	E value	Per. ident	Accession
Parapocryptes serperaster mitochondrion, complete genome	1118	1118	100%	0.0	98.01%	KT965855.1
Parapocryptes serperaster from	1081	1081	98%	0.0	97.21%	MK572460.1

Description	Max score	Total score	Query score	E value	Per. ident	Accession
Bangladesh cytochrome oxidase subunit 1 (COI) gene partial cds, mitochondrial						
Pseudapocryptes lanceolatus voucher 1v cytochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial	1070	1070	98%	0.0	96.90%	JX260938.1
Parapocryptes serperaster mitochondrial COI gene for cytochrome oxidase subunit I. partial cds, isolate adult 17	1050	1050	88%	0.0	100%	LC010490.1
Parapocryptes serperaster mitochondrial COI gene for cytochrome oxidase subunit I. partial cds, isolate adult 13	1046	1046	88%	0.0	99.83%	LC010486.1
Parapocryptes serperaster mitochondrial COI gene for cytochrome oxidase subunit I. partial cds, isolate adult 12	1046	1046	90%	0.0	99.49%	LC010485.1
Parapocryptes serperaster mitochondrial COI gene for cytochrome oxidase subunit I. partial cds, isolate adult 18	1041	1041	88%	0.0	99.66%	LC010491.1
Parapocryptes serperaster mitochondrial COI gene for cytochrome oxidase subunit I. partial cds, isolate adult 16	1036	1036	88%	0.0	99.65%	LC010489.1
Parapocryptes serperaster mitochondrial COI gene for cytochrome oxidase subunit I. partial cds, isolate adult 14	1036	1036	88%	0.0	99.65%	LC010487.1

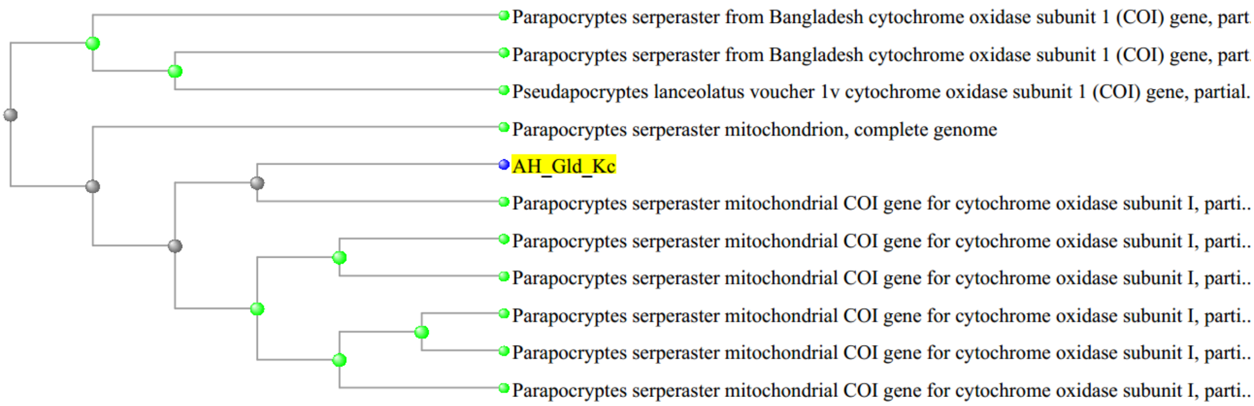

Figure 7. The phylogenetic tree based on the sample AH_Gld_Kc of small mud-skipper from the Cawan island, Indragiri Hilir, Riau, confirmed as *Parapocryptes serperaster.*

Discussion. This study revealed that mangrove biomass and organic carbon are not always due to mangrove tree density. As shown by the data presented in Table 2, the highest mangrove density in station 1 has the lowest organic carbon compared to other stations. On the contrary, station 2, with the lowest mangrove density of 390 tree ha^{-1}, has a higher organic carbon than other stations. In comparison, mangrove density in the Karimunjawa Islands north Central Java is 1,021-2,829 trees ha^{-1} (Hapsari et al 2020), almost double the mangrove density in Cawan Island. The TOC was in the range of

6.793-16.323 ton C ha^{-1} and the highest mangrove density was found at station 1 with 690 trees (Table 1). Thus, the organic carbon depends on the mangrove tree diameter, by species. *R. apiculata* was found at all the stations, while at station 1 this was the only type of mangrove found. The species *B. gymnorrhiza* was found at stations 2 and 3, which have a dry clay substrate. The species of *R. mucronata* were found only at station 4 and 5, which have the deepest sandy clay substrate compared with other stations. Another point of view is that sandy porous substrate at tidal flushing zones is suitable for benthic crustaceans like *Emerita emeritus* in the south Java coastal area, where the primary organic matter supplied by tidal flush (Hartoko et al 2021).

The variation of mangrove substrate organic carbon, as presented in Table 2, shows that the low organic carbon found at station 3 is 6.793 ton C ha^{-1} and at station 5 is 6.912 ton C ha^{-1}. In comparison, a higher organic carbon in the substrate was found in station 1, 2 and 4. In this specific case, the variation of the organic carbon in the substrate was affected by the mangrove species, tree diameter, height and age. Older mangrove trees are higher, generating more leaf litter which falls on the substrate and more organic decomposition. The other effect is the type of substrate; this study found that substrates less organic, as in station 3 and 5, are correlated with more sand composition in the clay substrate, whereas at stations 1, 2 and 4 there were found more clay-dominated substrates. Ati et al (2014) stated that sediments with more clay and less sand fraction in the substrate would have higher organic carbon. Finer sediment particles such as clay and silts have a wider surface area enabling to contain more organic matters (Muskananfola et al 2020a,2020b). Sari et al (2017) stated that organic carbon in mangrove substrate sources is due to the decomposition from mangrove leaves and associated bushes vegetation. This study revealed that the organic carbon in mangrove substrate correlated to the mangrove tree species, diameter, height and age. The mangrove area of Cawan island have an acidic substrate with a pH of 5-7, a low nutrient content and a low substrate fertility. Mangrove substrate of Cawan island, which is classified as peat type substrate, has a combination of estuarine acidic seawater mixing with tidal water mass, which has a high mineral content enrichment from open seawater mass and which is relatively more fertile than those typical peat coastal land. Mangrove substrate in this area, in general, is positioned directly next to the coastline. This condition had been considered the benefit factor for mangrove vegetation which still grows well in this peat substrate. This fact has become the main factor of the extended mangrove area of 3,867,423 ha in the Riau province (Ritung & Kartawisastra 2016).

This study revealed that the two species of mud-skipper that can be found in the acidic mangrove substrate of Cawan island are *Periopthalmodon shlosseri* and *P. serperaster*, able to survive to the mangrove organic carbon of the acidic substrate. Environmental conditions determine morphological and behavioral adaptation, such as skin color and feeding habits of the mudskipper. According to Rumahlatu et al (2020), the main difference between the mudskippers from the Rutong beach and those from Ambon, resides in the skin color. The skin of mudskipper *P. argentilineatus* is browner than that of the same mudskipper species from other places. The difference of skin colours are determined by the environment, namely the substrate, in the mangrove ecosystem. Pavlidis et al (2008) explained that environmental factors such as water temperature, light intensity, and substrate color significantly influence the brightness of the skin, especially the back skin. As reported by Leclercq et al (2010) the carotenoid, the pigments of trout that lives on a bright substrate, will in turn, affect the skin to flesh. The substrate in the mangrove area is composed of sand and mud, while bright colorful fish inhibit the sand and gravel substrate. Dinh (2017) concluded that an environment providing food and conditions suitable for mudskipper life affects the growth and development of fish. Environmental factors, together with genes, can influence the phenotype expression in more extreme conditions (Rake & Sullivan 2015). Other factors that influence differences in fish morphology are food availability, environmental conditions, and fish maturity stages. A study of Daud et al (2005) revealed that the morphometrics and meristic of *P. minutus*, *P. argentilineatus*, *and P. kalolo* have variation in measurements. A study in Merauke Papua estuary by Sunarni & Maturbongs (2016) found four species of mudskipper *B. boddarti*, *P. takita*, *Boleophthalmus pectinirostis and*

S. histophorus. High organic matter in mangrove substrate will accompany an increased abundance of mudskipper (Sisca & Sunarni 2018). A study on the Batubara coastal area of North Sumatera found that the mangrove area is dominated by *Periophthalmus gracilis*, and the dominant species on the river are *B. boddarti* and *Periophthalmodon schlosseri* (Muhtadi et al 2016).

Conclusions. Six species of mangrove *R. apiculata*, *R. mucronata*, *B. gymnorrhiza*, *L. racemosa*, *A. aureum*, *N. fruticans* were found in Cawan island, Indragiri Hilir, Riau. The most dominant species, which is found at all five sampling stations is *R. apiculata,* can reach a height of 15 m, much taller than in most parts of Indonesia. Mangrove tree density range is 390 to 690 trees ha^{-1}, and the organic carbon in the acidic substrate at Cawan Island range is from 6.793 to 16.323 ton C ha^{-1}. There are two species of mud-skipper found in the acidic mangrove substrate of Cawan Island, which have been confirmed by DNA analysis *P. shlosseri* and *P. serperaster.* In this case, the relevant assumption is the possibility of the adaptation of their digestive system to an acidic substrate condition.

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