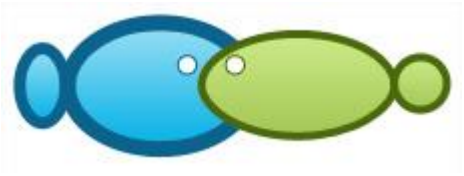

Molecular identification of the freshwater prawn *Macrobrachium idea* in Tempe Lake, South Sulawesi, Indonesia

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Abstract. Lake Tempe is one of the tectonic lakes in South Sulawesi and it is a source of freshwater shrimp fisheries such as *Macrobrachium idea*. The local name of this shrimp is urang salo or udang puce. This research aimed to analyze the DNA barcoding of *M. idae* from Tempe Lake, South Sulawesi, Indonesia. Polymerase Chain Reaction (PCR) primers used were LCO1490 (5'-GGTCAACAA ATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACC AAAAAATCA-39). Data were collected using a local tool known as Bubu, while genomic DNA was isolated to determine PCR, electrophoresis, gel purification, and DNA sequencing. Phylogenetic analysis using the Neighbor-Joining Method and sequencing Basic Local Alignment Search Tool (BLAST) was based on the NCBI database. The results showed that the BLAST analysis of *M. idae* was based on data from NCBI obtained 93.37-100% with a DNA fragment size of 684 bp. Furthermore, the Neighbor-Joining method showed that *M. idae* is different from *Macrobrachium idella*, *Macrobrachium meridionalis* and *Macrobrachium rosenbergii.*
Key Words: DNA barcoding, Orana river prawn, COI gene l, phylogenetic tree, BLAST.

Introduction. Sultan Tempe Lake is a freshwater fishery resource with important economic value, such as urang salo or puce shrimp (Yusuf 2006). For centuries, urang Salo has been used as a source of livelihood for local communities in Tempe Lake (Priyatna & Sumartono 2008). Kusmini & Hadie (2000) stated that based on morphometric identification urang salo is a species of *Macrobrachium idea*. The trade name of *M. idae* according to FAO is Orana river prawn (Holthuis 1980).

The demand for urang salo by consumers is only fulfilled from the catch in Tempe Lake because its production is not available throughout the year due to the limited qualities and seasonality (Yusuf et al 2018). The urang salo species are shrimps with important economic values, therefore, their demand increases yearly, thereby causing more intensive and non-selective fishing (Yusuf 2006). It is also associated with the problem of flooding in every rain in the upstream area, which carries sediment from the mud, thereby causing the lake to become shallower and damaging its ecosystem (LIPI 2012). This tends to affect the given impact on the sustainability of the lake's environment (Rosyidah 2011).

According to Yusuf et al (2018), these problems have decreased the *M. idae* population with a decrease in body size due to a faster reproductive process. The sustainability of the *M. idae* population in Lake Tempe is being threatened due to the inability to carry out conservation, leading to a decrease in pollution. According to the International Union for Conservation of Nature (IUCN) report, the *M. idae* population is in the endangered category (IUCN 2013; DeGrave et al 2013). Although the information on *M. idae* species in Indonesia is limited, it was found in other areas by Winarni et al (2011), while Yusuf (2006) analyzed its ecology, reproduction, and morphometric

variance. Furthermore, Wahidah et al (2015) studied the gonad maturity level and index (Yusuf et al 2018), as well as the morphometric character ratio (Wahidah et al 2019).

Research on the molecular indentification of *M. idae* in Tempe Lake has not been conducted. The genetic diversity of *M. idae* species is identified using DNA barcodes with LCO1490 and HCO2198 primers. The mt-COI gene has also been used for the population genetic variability of many freshwater shrimps (Udayasuriyan et al 2015).

According to Jayadi et al (2019), it is important to carry out DNA barcoding for sustainable management, such as genetic conservation of species and habitat. Therefore, this study aimed to analyze the DNA barcoding of *M. idae* from Tempe Lake, South Sulawesi, Indonesia.

Material and Method

Shrimp sampling. *M. idae* samples were collected from Tempe Lake, Wajo Regency, South Sulawesi, Indonesia, using a shrimp catching tool locally known as Bubu. The prawn sampling was conducted in January 2020 with the specimens preserved in a 96% ethanol solution and stored in the freezer until it was sent to the PT Genetika Science in Jakarta.

Total DNA isolation. Genomic DNA extractions with Quick-DNA Tissue Miniprep Kit (Zymo Research, D6016) were applied for total isolation. DNA purity eluted with elution buffer is well suited for PCR amplification with KOD FX Neo (Toyobo, KFX-201). The electrophoresis technique was performed to measure the quality and the quantity of the total DNA.

Polymerase Chain Reaction (PCR). The primary types used were LCO1490 (5'-GGTC AAAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-39) (Folmer et al 1994). The PCR components included 5 µL of double-distilled water (ddH2O), 12.5 µL of 2x PCR buffer for KoD FX neo, 5 µL of 2 mMdNTPs, 10 pmol/0.5 µL of LCO 1490, 10 pmol/0.5 µL of HCO 2198, 1 µL of DNA template, 0.5 µL of KOD FX Neo (1.0 U μL^{-1}). The step of PCR condition optimization included an initial denaturation at 950C for 3 minutes, and 1 cycle, a denaturation at 98^0C for 10 minutes, an annealing at 500C for 50 minutes, and an extension at 68^0C for 45 minutes. Eventually, the template was hold at 98^0C for 1 cycle. The PCR product purification was carried out using a ZymocleanTM Gel DNA Recovery Kit (Zymo Research, D 4001). This kit provides a hassle-free method for recovering a high yield of pure DNA from agarose gels. Furthermore, the PCR's success was detected by electrophoresis techniques.

Electrophoresis. Electrophoresis determines the outcome of DNA and PCR isolation. Total DNA and PCR products were moved to a 1% agarose gel in a 1X TBE buffer at 50 V for 45 minutes. DNA tape was colored using 5 µg mL^{-1} of ethidium bromide, visualized on a UV transilluminator lamp, and photographed using a UV filtered digital camera.

DNA sequencing. DNA sequencing was performed at PT Genetika Science in Jakarta as a channeling agent. Gene purification and sequencing were carried out at 1^{st} Base in Malaysia. The product of the PCR was of 40 µL, and of 30 µL for each primer.

Data analysis. DNA sequence data used forward and reversed primers which were put together or aligned using BioEdit7 software. The BLAST (Basic Local Alignment Search Tool) analysis at http://www.ncbi.nih.nlm.gov/ was carried out to determine the similarity of each prawn sample to DNA COI sequences in the Gen Bank database (Madden 2013). Furthermore, phylogenetic analysis was carried out using the Neighbor-Joining Method with a bootstrap of 1000x.

Results. The produced DNA band indicates that the sequencing process is carried out properly. Amplification of *M. idae* sample using primary LCO1490 and HCO2198 produced 684 bp of DNA tape, as shown in Figure 1. Therefore, the sequence of COI gene is strongly used as a standard barcode for the identification of *M. idae* in Tempe Lake.

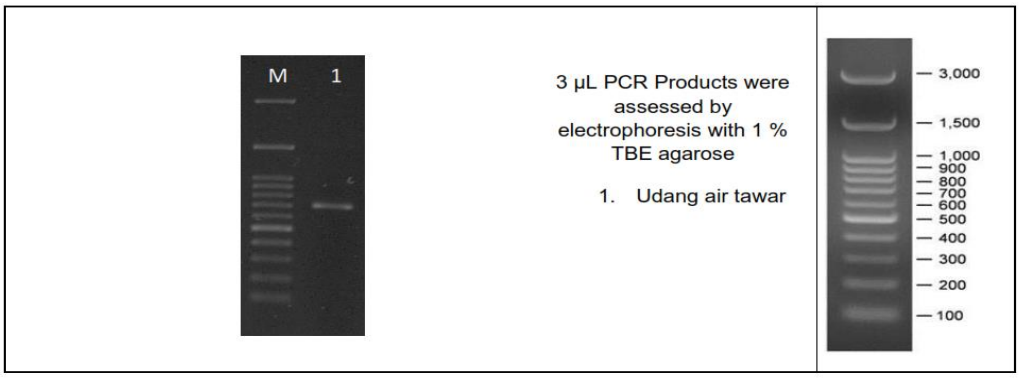

Figure 1. Profiles of DNA bands that use LCO1490/HCO2198 primers. L=1 kb of DNA ladder.

Table 1 shows the results of the BLAST analysis based on data from NCBI. The level of similarity obtained in *M. idae* is 93.37-100%, while mounted *Macrobrachium idella*, *Macrobrachium meridionalis*, and *Macrobrachium rosenbergii* were at 89.1, 88.28, and 87.64%, respectively. The result of the BLAST regarding urang salo in Lake Tempe was a species of *M. idae*.

Table 1

Analysis BLAST based on the NCBI database in the *Macrobrachium idae* from Tempe Lake

Prawn species	*Description*	*Max score*	*Total score*	*Query cover*	*E value*	*Indent*	*Accession*
Macrobrachium idae	*Macrobrachium idae* mitochondrial COI gene for cytochrome oxidase subunit I, partial cds	741	741	100%	0.0	100%	AB235262.1
	Macrobrachium idae isolate 10 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	737	737	81%	0.0	93.37%	GU205062.1
	Macrobrachium idella voucher NBFGR:CHN MI 2.1 cytochrome oxidase subunit I-like (COI) gene, partial sequence; mitochondrial	717	717	95 %	0.0	89.1%	JF774068.1
	Macrobrachium meridionalis mitochondrial COI gene for cytochrome oxidase subunit I, partial cds, haplotype: ML, from mainland China	726	726	99%	0.0	88.28%	AB235283.1

The reconstruction of *M. idae* phylogenetic tree information is shown in Figure 2. The phylogenetic trees based on DNA sequences using the Neighbor-Joining method showed that *M. idae* is different from *M. idella*, *M. meridionalis*, and *M. rosenbergii.* The results of DNA barcode analysis of *M. idae* in Tempe Lake were first reported in this research.

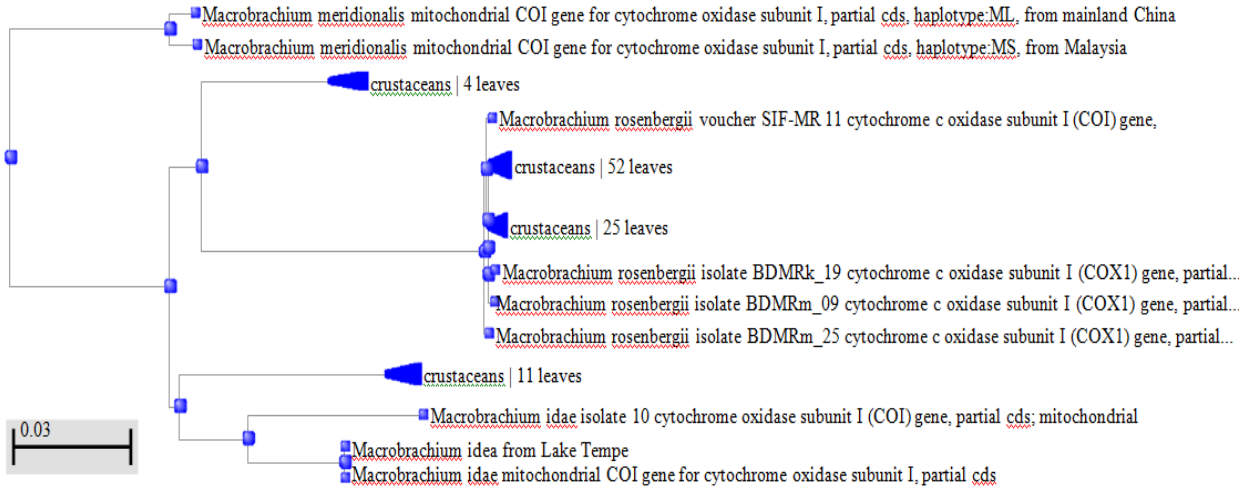

Figure 2. The phylogenetic tree of *Macrobrachium idae* in Tempe Lake, South Sulawesi, based on mtCO1 with Neighbor Joining.

Discussion. DNA barcoding aims to provide an efficient method for species-level identifications using an array of species-specific molecular tags derived from COI genes (Pradhan et al 2015). The primary types of DNA barcodes used in this research are LCO1490 and HCO2198 (Costa et al 2007; Bilgin et al 2014; Udayasuriyan et al 2015). The DNA band that has been produced indicates that the sequencing process is carried out properly. The technique of DNA barcoding plays an essential role as a taxonomic tool for exposing genetically distinct and separated species quickly and accurately (Nursyahra et al 2019). The use of DNA barcoding is performed with short gene sequences, such as the cytochrome oxydase (COI) gene (Hebert et al 2003). Therefore, the sequence of the COI gene is very strongly used as a standard barcode for the identification of *M. idae* in Tempe Lake.

The level of similarity obtained in *M. idae*, *M. idella, M. meridionalis,* and *M. rosenbergii* are 93.37-100%, 89.1, 88.28 and 87.64%, respectively. According to Stackebrandt & Goebel (1994), these samples are identical at the species level, assuming the identity percentage value is above 97.5%. The BLAST result of Urang Salo in Tempe Lake identified the *M. idae* species.

M. idae has morphological characteristics, such as 9-12 and 3-4 rostrum teeth on the top and bottom (Yusuf 2006) as well as three rostrum teeth at the top and behind the orbit (Holthuis 1955). The chest has five pairs of pereiopods, each being distributed to the five segments of the abdomen. Pereiopods grow very long in male prawns and short in females. According to Hadie et al (2001), female prawn incubates their eggs in the pleopods. Winarni et al (2011) stated that pereiopod II is longer than the merus and slender on the right and left sides of the pelopods, while chela's pereiopod II is tubercles, hairless, and has no teeth.

Approximately 30 species of freshwater are prawn from the genus *Macrobrachium* found in Indonesia (Holthuis 1955). *Macrobrachium* was characterized by the extreme enlargement of the second pair of male pereiopods, which in many species tends to exceed the body length. One of these genera is the *M. idea,* found in Tempe Lake, Kawung River and LukUlo River Kebumen Regency (Winarni et al 2011), Banjaran River, Pelus River and Logawa River Banyumas District (Siregar et al 2001) and Halmahera (Cai & Peter 2001), Tamil, Nadu India (Arumugam 2011). The species of *M. idae* is widespread in the Indo-West Pacific region, from east Africa to the Philippines, New Guinea, and Australia (Short 2004). It is very abundant in lowland areas, including estuaries and stagnant fresh water. *Macrobrachium* species are widely distributed in

heterogeneous or geographically isolated environments with phenotype variations because they are prone to show plastic responses to different environmental influences (Schwander & Leimar 2011). The use of DNA barcoding to illustrate phylogeographic patterns was useful in the research carried out by Koizum et al (2012) due to its ability to successfully determine a population's genetic structure using COI sequences.

DNA barcode is used as a reference in the development of domestication of freshwater prawns to maintain their sustainability through conservation. Identification of the species of prawn is important in order to obtain information for applications in biodiversity conservation and aquaculture (Munasinghe 2010). This research is the first to report the DNA barcode analysis of *M. idae* in Tempe Lake. Therefore, further research needs to be conducted on the genetic diversity in several locations in the Lake Tempe.

Conclusions. The use of LCO1490 and HCO2198 primers has successfully determined molecular identification of *M. idae* from Tempe Lake, South Sulawesi, Indonesia, with a DNA fragment size of 684 bp. Furthermore, the BLAST analysis of Urang Salo in Tempe lake showed the highest similarity *M. idae* (100%).

Conflict of interest. The authors declare no conflict of interest.

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