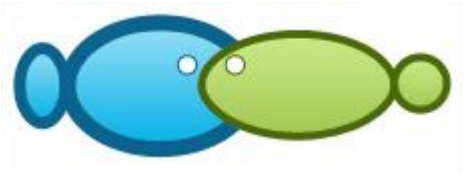

DNA barcoding of Telmatherinidae family in Lake Towuti, South Sulawesi, Indonesia

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Abstract. Telamtherinidae is a family of endemic fish in South Sulawesi. The study is aimed at identifying the molecular gene of the endemic fish of the Telmatherinidae family in Towuti Lake, South Sulawesi, to identify and analyze genetic diversity, genetic marking, genetic distances, genetic characterization, and dendrogram of the fish. DNA barcoding in this research used the Cytochrome c Oxidase I (COI) gene. Amplification of mitochondrial COI gene regions was conducted by using COI Fish F2 and COI Fish R 2 primer. Data analysis, total isolation of DNA, Polymerase Chain Reaction (PCR), Electrophoresis, gel purification, and sequencing Basic Local Alignment Search Tool (BLAST) were performed. The results showed that DNA sequence was 681 bp. Meanwhile, analysis of dendrogram suggested that the fish of Telmatherinidae family in Towuti Lake are similar to the fish in the *Paratherina, Telmatherina* and *Tominanga* genera. The genus *Telmatherina* including *Telmatherina celebensis*, *Telmatherina bonti,* and *Telmatherina opudi* showed 85% significant shared similarity with *Atherina* sp. Only 84% similarity of genus *Telmatherin*a was found in *Pristigenys alta*, *Parexocoetus brachypterus*, *Cypselurus hiraii,* and *Hypoatherina tsurugae*. Meanwhile, genus *Paratherina*: *Paratherina striata* and *Paratherina wolterecki* have 83-84% similarity with *Scorpis lineolata*, *Hyporhamphus affinis*, *Cypselurus hiraii*, *Parexocoetus brachypterus*, and *Pristigenys alta*. In addition, genus *Tominanga*: *Tominanga sanguicauda* has 84% similarity with *Hypoatherina tsurugae*, *Cypselurus hiraii*, *Prognichthys sealei*, *Hyporhamphus affinis*, and *Pristigenys alta*.

Key Words: genetic diversity, Telmatherinidae, COI gene, dendrogram, Lake Towuti.

Introduction. Lake Towuti is located in Nuha sub-district, East Luwu regency South Sulawesi, Indonesia, and is interconnected with Lake Matano and Lake Mahalona. These three lakes are included in an ancient Lake Complex (Wijaya et al 2009; Samuel et al 2009; Nasution et al 2010; Umar et al 2012). Lake Towuti has been an important habitat for several endemic freshwater fish (Kottelat et al 1993; Wirjoatmodjo et al 2003; Samuel et al 2009; Parenti 2011; Parenti & Ebach 2013) such as the fish families from Telmatherinidae, Gobidae, Adrianichthyidae and Hemiramphidae (Herder et al 2006; Nasution et al 2007; Stelbrink et al 2014; Hutama et al 2016). They are economically important for many local communities around Lake Towuti (Wijaya et al 2009).

Potencial productivity of fish in Lake Towuti is equal to ±195 ton year^{-1} (Wijaya et al 2009). Some endemic fish species are utilized for consumption and ornamental purposes. Consequently, the endemic fish population continues to decline yearly, and conservation measures have not been carried out properly (Mamangkey et al 2007; Wijaya et al 2009; Samuel et al 2009; Nasution et al 2010; Umar et al 2012). The uncontrolled exploitation of endemic fish can lead endemic fish into extinction, even in their natural habitat (Jayadi et al 2015, 2016; Nasution et al 2015).

Another threat that causes a decline of endemic fish populations in Lake Towuti is water pollution from domestic waste disposal. Pollution detrimentally affects the environment as well as endemic fish. Moreover, land clearing for settlement and agriculture around the lake leads to erosion and sedimentation. This is exacerbated by introducing invasive fish species into Lake Towuti (Prianto et al 2014).

Several endemic fish species included in the category of vulnerable species in Lake Towuti include Termatherinidae family of genera *Telmatherina*, *Paratherina,* and *Tominanga* (Kottelat et al 1993; IUCN 2003; Suwelo 2005; Herder et al 2006; Nasution et al 2007; Stelbrink et al 2014; Hutama et al 2016). The Telmatherinidae family was referred to as sailfin silversides fish (Kottelat 1991; Stelbrink et al 2014; Hutama et al 2016). Therefore, it is necessary to conduct sustainable management, such as genetic conservation of species and habitat.

Genetic conservation is one of sustainable management measures that can be applied by analyzing the genetic diversity of particular species (Mamangkey et al 2007; Hadijah et al 2014; Jayadi et al 2015). Genetic diversity analysis provides the short and long term information related to a particular species' population (Ferguson et al 1995). For instances, genetic diversity can be used in considering biological resource management (Yusron 2005; Jayadi et al 2015; Nugroho et al 2017). Furthermore, it can be used for improving fish stock (Islam et al 2011), domestication, and aquaculture (Lante et al 2011; Iskandariah et al 2015; Lorenzen et al 2012; Jayadi et al 2016). To analyze the genetic diversity, barcoding DNA using COI can be applied (Ward et al 2005; Muchlisin et al 2013; Jusmaldi et al 2014; Hubert et al 2015; Nuryanto et al 2017; Pramono et al 2017; Abbas et al 2017). The purpose of this study is to determine genetic diversity using molecular identification such as the COI DNA gene in the endemic species of the Telmatherinidae family in Lake Towuti as well as genetic marking, genetic characters, and dendrogram.

Material and Method

Fish sampling. Fish sampling for Telmatherinidae fish family was conducted in Lake Towuti, South Sulawesi, Indonesia from January to June, 2018, using fishing net size of 1 mm. Morphological identification was carried out according to Herder et al (2006), Kottelat (1991), Kottelat et al (1993), and Said & Hidayat (2015). In addition, fish muscle (from 15 fish) in the tail was taken (1 cm x 1 cm) and then placed into a 96% ethanol solution and stored in the freezer.

Total DNA isolation. D NEasy Blood and tissue kit (cat. No. 69504 and 69506 Qiagen) was applied for total DNA. The total DNA isolation process was conducted based on the existing protocol. The electrophoresis technique was performed to measure the quality and the quantity of the total DNA.

Polymerase Chain Reaction (PCR). Total DNA was amplified by PCR technique using a pair of universal primers for COI genes in fish, namely Fish F2: 5'-TCG ACT AAT CAT AAA GAT ATC GGC AC-3 'and FishR2: 5' TCA ACT GGG TGA CCG AAG AAT CAG AA -3' (Ward et al 2005). The PCR components include 1X of Supreme NZY Taq, 2X Green Master Mix, 2.4 µM of forward primary, 2.4 µM reverse primer, 1 µL of total DNA, and dH_2O until 50 µL of PCR volume. The PCR program includes pre-PCR at 94^oC for 5 minutes; PCR was 35 cycles consisting of denaturation at 94^oC for 45 seconds, annealing at 50^oC for 45 seconds, elongation at 72^oC for 1 minute, and post-PCR at 72^oC for 10 minutes. PCR success was detected by electrophoresis techniques.

Electrophoresis. Electrophoresis was performed to detect the success of DNA and PCR isolation. Total DNA and PCR products were moved to a 1% agarose gel in a 1X TBE buffer at 50 voltage for 45 minutes. DNA tape was colored using 5 µg mL^{-1} of ethidium bromide, visualized on a UV transilluminator lamp, and then 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 1st Base in Malaysia. The PCR product was 40 µL and each primer was 30 µL.

Data analysis. DNA sequence data was used forward and reverse 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/BLAST (Madden 2013) was carried out on DNA sequences from each fish sample to determine its similarity to DNA COI sequences in the GenBank database. Accessions were of high similarity, and sequences were downloaded to make phylogenetic trees using the MEGA (molecular evolutionary genetics analysis) version 6.06 (build #: 6140226) (Tamura et al 2013) based on the Kimura 2-parameter model and UPGMA (Unweighted Pair Group Method with Arithmetic mean) with 1000 bootstrap.

Results and Discussion. Amplification of fish samples from the Telmatherinidae family using primary Fish_F2/Fish_R2 produced 750 bp of DNA tape (Figure 1). The DNA band that has been produced indicates that the sequencing process was done properly. The DNA sequence obtained from 20 samples was 681 bp and registered in Gen Bank (Table 1).

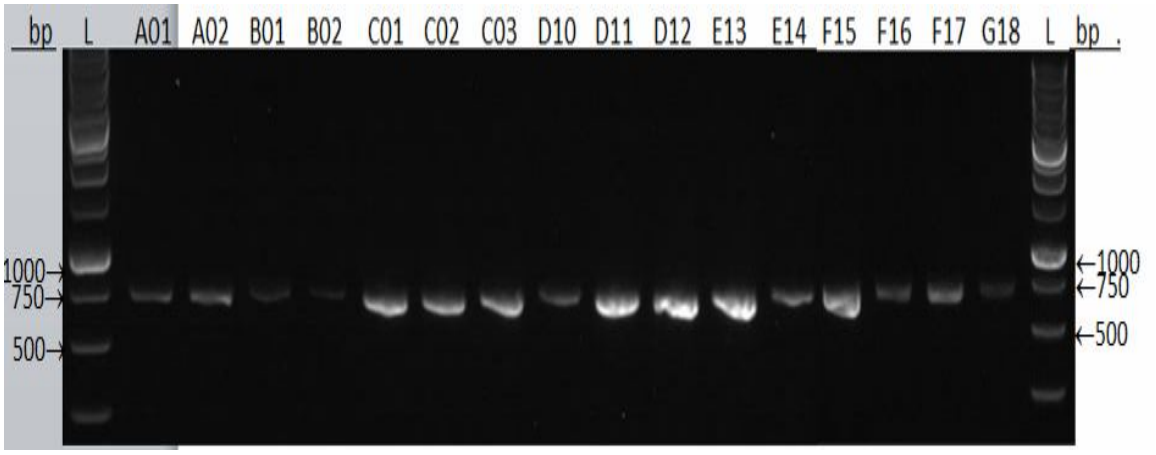

Figure 1. Profiles of DNA bands that use Fish_F2 / Fish_R2 primers. L = 1 kb DNA Ladder (Thermo Scientific).

Table 1

Registration number of DNA sequence results of 20 fish samples from the family of Telmatherinidae in Lake Towuti

No	*Fish spesies*	*Sample label*	*Registration number*
1	*Telmatherina celebensis*	A01	MH568798
2	*Telmatherina celebensis*	A02	MH568799
3	*Paratherina striata*	B01	MH568800
4	*Paratherina striata*	B02	MH568801
5	*Tominanga sanguicauda*	CO1	MH568802
6	*Tominanga sanguicauda*	CO2	MH568803
7	*Tominanga sanguicauda*	CO3	MH568804
8	*Paratherina wolterecki*	D10	MH568805
9	*Paratherina wolterecki*	D11	MH568806
10	*Paratherina wolterecki*	D12	MH568807
11	*Telmatherina bonti*	E13	MH568808
12	*Telmatherina bonti*	E14	MH568809
13	*Paratherina cyanea*	F15	MH568810
14	*Paratherina cyanea*	F16	MH568811
15	*Paratherina cyanea*	F17	MH568812
16	*Telmatherina opudi*	G18	MH568813

DNA barcodes of endemic fish in the Telmatherinidae family in Lake Towuti using primary COI Fish_F2 / Fish_R2 found long DNA band fragments after mt-DNA amplification to around 681 bp. The COI primer has been applied to identify the genus *Thunnus* and

genus *Squalus* with a fragment length of 655 bp (Ward et al 2005), the genus *Kryptopterus* around 707 bp (Jusmaldi et al 2014), and the genus *Mystus* at 697 bp (Pramono et al 2017). The length of fragments in endemic fish in South Sulawesi such as *Glossogobius matanensis* was 500 bp (Mamangkey et al 2007), *Glossogobius aureus* was 600 bp (Hadijah et al 2014), *Telmatherina ladigesi* was 600 bp (Jayadi et al 20015), *Pterygoplichthys* sp. was 650 bp (Rosnaeni et al 2017), Family Sparinidae was 650 bp (Abbas et al 2017), and *Harpadon nehereus, Harpadon microchir* and *Harpadon squamosus* were 618 bp (Nugroho et al 2017).

DNA barcoding is used to assign a biological specimen to a species (Ardura et al 2010; Fahmi et al 2016; Nuryanto et al 2017; Pramono et al 2017). Furthermore, DNA barcoding contributes to science and promotes more sustainable practices in taxonomy and the development of new molecular tools for species identification (Hubert et al 2015).

The results of the Telmatherinidae family dendrogram analysis obtained three genera, namely: *Paratherina, Telmatherina, Tominanga* (Figure 2).

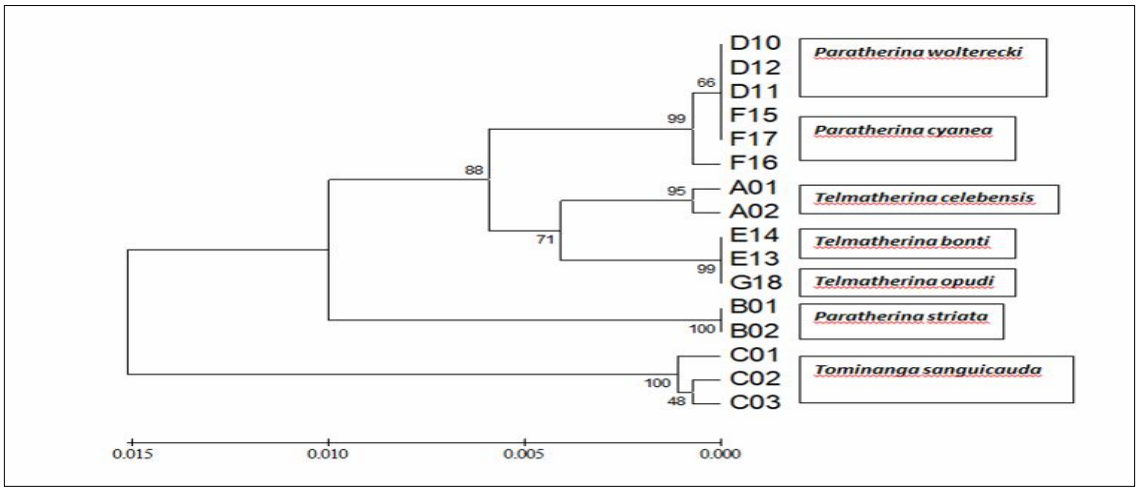

Figure 2. Dendrogram in the Telmatherinidae family in Lake Towuti.

Phylogenetic results show that three genera from the Telmatherinidae family (*Paratherina*, *Telmatherina* and *Tominanga)* were found in Lake Towuti. Species of the genus *Paratherina* are *Paratherina striata*, *Paratherina wolterecki*, *Paratherina cyanea*, and the genus *Telmatherina* is represented by *Telmatherina celebensis*, *Telmatherina bonti*, *Telmatherina opudi*, and the genus *Tominanga* is represented by *Tominanga sanguicauda* (Figure 2). All types of fish have been registered in Gen Bank (Table 1). Those fish population are known as native and endemic in Lake Towuti (Kottelat 1991; Parenti 2011; Stelbrink et al 2014; Hutama et al 2016).

The population of fish species from the Telmatherinidae family in Lake Towuti has decreased (Kottelat et al 1993; Suwelo 2005; Herder et al 2006; Nasution et al 2007; Stelbrink et al 2014; Hutama et al 2016) and have become vulnerable species (IUCN 2003). Consequently, inbreeding in the population leads to lower genetic variation of Telmatherinidae family. Low value of genetic diversity shows that a narrow level of migration can provide opportunities for limited gene exchange with other populations (Sugama et al 1996). Genetic variation of fish populations in nature can reduce due to damaged habitat, limited migration, isolated from other populations, depression inflation and decreased reproductive ability (Jayadi et al 2015). Changes in genes can occur due to individual gene migration in a population (Ezilrani & Christopher 2015).

In addition, genetic variation is important for evaluating fish resources in the wild. Genetic variations have a direct or indirect potential impact on populations, communities, and ecosystems (Hughes et al 2008). As said by Ezilrani & Christopher (2015), the genetic structure of a fish species is an illustration of the long changes due to biological and environmental factors. The results of the BLAST-GEN analysis for endemic fish of Telmatherinidae family in the Gen Fish Bank are presented in Table 2.

Table 2

Analysis of BLAST-GEN in the Telmatherinidae family

Fish species	*Description*	*Max score*	*Total score*	*Query cover*	*E value*	*Indent*	*Accession*
Telmatherina celebensis	Atherinidae sp.	673	673	95%	0.0	85%	MF409492.1
	Scorpis lineolata	685	685	99%	0.0	84%	AP011063.1
	Pristigenys alta	673	673	99%	0.0	84%	MG856600.1
	Hypoatherina tsurugae	673	673	99%	0.0	84%	AP004420.1
	Cypselurus hiraii	667	667	99%	0.0	84%	AB182653.1
Paratherina striata	*Scorpis lineolata*	673	673	99%	0.0	84%	AP011063.1
	Hyporhamphus affinis	656	656	98%	0.0	84%	KJ013045.1
	Cypselurus hiraii	656	656	99%	0.0	83%	AB182653.1
	Parexocoetus brachypterus	650	650	99%	0.0	83%	KY067947.1
	Pristigenys alta	644	644	99%	0.0	83%	MG856600.1
Tominanga sanguicauda	*Hypoatherina tsurugae*	685	685	99%	0.0	84%	AP004420.1
	Cypselurus hiraii	679	679	99%	0.0	84%	AB182653.1
	Prognichthys sealei	673	673	99%	0.0	84%	KY067951.1
	Hyporhamphus affinis	667	667	98%	0.0	84%	KJ013045.1
	Pristigenys alta	662	662	99%	0.0	84%	MG856600.1
Paratherina wolterecki	*Pristigenys alta*	685	685	99%	0.0	84%	MG856600.1
	Cypselurus hiraii	685	685	99%	0.0	84%	AB182653.1
	Hypoatherina tsurugae	679	679	99%	0.0	84%	AP004420.1
	Cheilopogon doederleinii	673	673	99%	0.0	84%	AP017897.1
	Cheilopogon arcticeps	673	673	99%	0.0	84%	KU360728.1
Telmatherina bonti	*Scorpis lineolata*	673	673	95%	0.0	85%	MF409492.1
	Hyporhamphus affinis	679	679	99%	0.0	84%	MG856600.1
	Cypselurus hiraii	679	679	99%	0.0	84%	KY067947.1
	Parexocoetus brachypterus	673	673	99%	0.0	84%	AB182653.1
	Pristigenys alta	673	673	99%	0.0	84%	AP004420.1
Paratherina cyanea	*Pristigenys alta*	685	685	99%	0.0	84%	MG856600.1
	Cypselurus hiraii	685	685	99%	0.0	84%	AB182653.1
	Hypoatherina tsurugae	679	679	99%	0.0	84%	AP004420.1
	Cheilopogon doederleinii	673	673	99%	0.0	84%	AP017897.1
	Cheilopogon arcticeps	673	673	99%	0.0	84%	KU360728.1
Telmatherina opudi	Atherinidae sp.	673	673	95%	0.0	85%	MF409492.1
	Pristigenys alta	679	679	99%	0.0	84%	MG856600.1
	Parexocoetus brachypterus	679	679	99%	0.0	84%	KY067947.1
	Cypselurus hiraii	673	673	99%	0.0	84%	AB182653.1
	Hypoatherina tsurugae	673	673	99%	0.0	84%	AP004420.1

Table 2 illustrates that the genus *Telmatherina* including *Telmatherina celebensis*, *Telmatherina bonti* and *Telmatherina oputi* showed 85% shared significant similarity to Atherinidae sp. Only 84% similarity of genus *Telmatherina* was found in *Pristigenys alta*, *Parexocoetus brachypterus*, *Cypselurus hiraii,* and *Hypoatherina tsurugae*. Meanwhile, genus *Paratherina*: *Paratherina striata*, *Paratherina wolterecki* have similarities between 83 and 84% with *Scorpis lineolata*, *Hyporhamphus affinis*, *Cypselurus hiraii*, *Parexocoetus brachypterus*, and *Pristigeny alta*. In addition, genus *Tominanga*: *Tominanga sangicauda* has 84% similarity to *Hypoatherina tsurugae*, *Cypselurus hiraii*, *Prognichthys sealei*, *Hyporhamphus affinis*, and *Pristigenys alta*.

Conclusions. The use of the cytochrome c oxidase I (COI) DNA has been successful in identifying the family of endemic fish from Telmatherinidae family in Lake Towuti, South Sulawesi Indonesia. The research finding shows that the genus *Paratherina* is represented by the species *Parathrina striata*, *Paratherina wolterecki*, *Paratherina cyanea*, the genus *Telmatherina* - *Telmatherian celebensis*, *Telmatherina bonti*, *Telmatherina opudi*, and the genus *Tominanga* - *Tominanga sanguicauda*.

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