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 R2 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 Telmatherina 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 sanguicaua 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).

Potential 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; 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 dH2O until 50 µL of PCR volume. The PCR program includes pre-PCR at 94°C for 5 minutes; PCR was 35 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, elongation at 72°C for 1 minute, and post-PCR at 72°C 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.nlm.nih.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).](image)

**Table 1**

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

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
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 (Hadjiah et al. 2014), *Telmathera ladigesi* was 600 bp (Jayadi et al. 2015), *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*, *Telmathera*, *Tominanga* (Figure 2).

![Figure 2. Dendrogram in the Telmatherinidae family in Lake Towuti.](image)

Phylogenetic results show that three genera from the Telmatherinidae family (*Paratherina*, *Telmathera* and *Tominanga*) were found in Lake Towuti. Species of the genus *Paratherina* are *Paratherina striata*, *Paratherina wolterecki*, *Paratherina cyanea*, and the genus *Telmathera* is represented by *Telmathera celebensis*, *Telmathera bonti*, *Telmathera 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 illustrates that the genus *Telmatherina* including *Telmatherina celebensis*, *T. bonti* and *T. opudi* 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 woltrecki* have similarities between 83 and 84% with *Scorps lineolata*, *Hyporhamphus affinis*, *Cypselurus hiraii*, *Parexocoetus brachypterus*, and *Pristigenys alta*. In addition, genus *Tominanga*: *Tominanga sanguicauda* has 84% similarity to *Hypoatherina tsurugae*, *Cypselurus hiraii*, *Prognichthys sealei*, 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 Tawuti, South Sulawesi Indonesia. The research finding shows that the genus *Paratherina* is represented by the species *Paratherina striata*, *Paratherina woltrecki*, *Paratherina cyanea*, the genus *Telmatherina* - *Telmatherina celebensis*, *T. bonti*, *T. opudi*, and the genus *Tominanga* - *Tominanga sanguicauda*.

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