Morphological and genetic analysis of batak fish (Tor soro) in North Sumatera

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Abstract. Research on the analysis of morphological and genetic variations of Batak fish (Tor soro) in North Sumatra has been conducted. The Batak fish samples were captured from flowing rivers at several districts in North Sumatera namely: Bahorok River (Langkat), Alian River (Toba Samosir), Batang Toru River (South Tapanuli). Truss method was used to analyze morphological parameters by using SPSS ver. 16.0 computer program, while the genetic parameters were analyzed by scoring amplified DNA strands visualized in electrophoresis using Polymerase Chain Reaction – Random Amplified Polymorphism DNA (PCR-RAPD) method. Primers used in this study were: OPA-02, OPA-03, OPA-04, OPC-01 and OPC-02. The PCR amplicons were then analyzed by using Numerical Taxonomy and Multivariate Analysis System (NTSYS) ver. 2.02. computer program. Dendrogram analysis showed that the level of morphological variability was at 1–25 euclidean distance. Dendogram analysis also showed that genetic diversity level was within coefficient of 0.23–0.71 in which obtained a diversity level of 48%. Based on the morphological and genetic results, it may be indicated that the Batak fishes originating from Batang Toru had a close similarity with Alian’s rather than Bahorok’s.

1. Introduction

Batak fish is a species of local fish in North Sumatera, with religious value, long known being used in traditional ceremonies for the Butaknese tribe. Furthermore, the fishes are currently of high economic value. In Indonesia, especially Sumatera, Batak fish consists of two genera: genus Neolissochilus and Tor. Depicting from their natural habitat, Tor species are facing serious decline to their population due to deforestation and overfishing by the locals. One species, Tor soro are mostly found in the nature and considered as native fish species of North Sumatra. The species can be found in some rivers, like Batang Toru, Alian and Bahorok rivers. These different habitats may allow them to differentiate in terms of morphological and genetic characteristics.

Recent prospect of Batak fish is the domestication efforts, which is by collecting genetic data from wild species as the initial requirement in determining its genetic variations. To meet the requirements, morphological and genetic variations of living Tor must be evaluated. The most common genetic analysis is by differentiating specific genetic identity between organisms through the use of genetic markers. Random Amplified Polymorphism DNA (RAPD) technique is a combination technique of PCR by using random primers to determine the genetic relationship between related species. Both morphological and genetic parameters can be correlated to draw some conclusion and we are hoping
to obtain further information regarding the genetic relationship between *Tor soro* from various habitats in North Sumatera.

2. Materials And Methods

2.1 Morphological Measurement

Measurement of fish morphology followed Truss method [1], each truss lines produced six main characters, then from ten truss points will produce another 21 characters (Figure 1). Standard lengths were measured by using ruler and vernier caliper.

![Figure 1. Morphological characters based on Truss method](image)

2.2 DNA Extraction

Fish DNA was extracted using Phenol-Chloroform method. Part of the fish body to be extracted was the fin and cut with a weight of 5–10 mg. The fin was inserted into a 1.5 ml tube containing 500 ul of TNES urea solution. Then 15 µg/ mL of protein kinase was added and incubated at 55°C for 1 hour. After cooling at room temperature, 1,000 solution of Phenol-Chloroform-Isoamylalcohol was added. Then centrifuged at 10,000 rpm for 10 minutes. The supernatant layer was transferred into a new tube, and then 1000 µL 90% ethanol solution together with 10 µL sodium acetate solution (CH₃COONa) were added. The solution was homogenized until white precipitate or crude DNA extract appeared. The crude DNA extract was centrifuged at a rate of 10,000 rpm for 10 minutes, then the supernatant layer was discarded. The DNA pellets were dried at room temperature and dissolved in 50–100 µL Tris-EDTA (TE) buffer then stored in 4 °C.

2.3 DNA Amplification

DNA amplification by using Promega GoTaq® Green Master Mix with RAPD primers such as: OPA-02, OPA-03, OPA-04, OPC-01 and OPC-02. The amplification reaction is then carried out by inserting the reaction tubes into the PCR (eppendorf vapo protect) block with specifications: denaturation of 95 °C for 30 seconds, then denaturation 95 °C for 1 minute, annealing (primary optimum temperature) 350 °C for 30 seconds, and extension at 72 °C for 1 minute, followed by the final extension at 72 °C for 5 minutes, cooling or termination was completed at 4°C. The PCR reaction was performed for 40 cycles. The PCR results were then subjected to electrophoresis for 90 minutes at 70 volts and 100 A. The electrophoresis results were then stained by immersion in Ethydium Bromide solution (EtBR 1%) for 15 minutes then washed in aquadest for 10 minutes. The DNA quality of electrophoresis results was observed under the UV transluminator and documented within gel-doc.
2.4 Data Analysis
For morphological analysis, samples with similar characters were clustered into same groups which were tested using SPSS ver. 16.0 to obtain several discriminate variables of tested subjects. For genetic analysis, qualitative data resulting from visualised DNA bands were scored binary as follow: presence of DNA band (1), and no presence (0). The numerical data were then analyzed to construct a dendrogram showing genetic relationship between *Tor soro* based on similarity index by using NTSYS ver. 2.02 [2].

3. Results and Discussion
Batak fishes (*T. soro*) sampled from Alian are morphological distinct with samples from Batang Toru and Bahorok rivers. Alian specimens had darker body color compared to the others which had brighter body color. Alian specimens also had reddish-colored operculum while other specimens had gray-colored operculum. Alian specimens tend to have more flattened body than other specimens. Variations in tail color are also notable in which Bahorok, Batang Toru and Alian specimens had reddish-black, yellowish-gray and transparent-gray color, respectively. Differences in the shape of the Batak fish are assumed to be influenced by the current velocity while color differences such as the operculum, body and tail by temperature factors. These morphological characters can be used to determine the relationship between Batak fish originating from different places. Dendrogram is constructed from 21 morphological characters of Batak fishes with symbols representing the localities: B (Bahorok), BT (Batang Toru) and A (Alian) (Figure 2).

![Dendrogram analysis from morphological characters of *Tor soro*](image.png)

The dendrogram showed similarity based on morphology which is divided into three main groups: group 1 consists of four fish samples from Bahorok (B1, B2, B3 and B4), group 2 consists of seven samples from Alian and Batang Toru (A1, A2, A3, BT2, A4, BT3 and BT4). Group 1 and 2 met at euclidean distance at point 19. Group 3 consists of only one sample namely BT1, groups 1, 2 and 3 met at euclidean distance at point 25. Then, BT1 sample possessed similar morphological characters with Alian samples. Furthermore, it may be indicated that *Tor soro* originating from the three locations belonged to one fish population from the same upstream of Lake Toba, the original habitat of Batak fish. This may be due to the different geographical location causing distance of morphological similarity level from Batak fish that leads to limited migration between Batak fish populations.

Morphometric variations that occurred in between related species can be caused by environmental factors such as habitat conditions, distances between populations and geographical isolation. The greater the distance between populations, the higher the difference in phenotypic characters [3,4]. A sufficient level of isolation within limited geographic areas may produce significant morphometric and genetic differences between populations because there is no gene flow occurring between populations [5]. The most influential environmental factors for the occurrence of morphological variation in one species are the physical factors, especially the river flows [6]. To assess the genetic characters, RAPD
method was chosen to display distinctive DNA genes, amplified with several random primers. The number of DNA bands in base pairs (bp) are showed in Table 1.

Table 1. Number of polymorphic bands (\textit{Tor soro}) assessed with five RAPD primers

| Primers | Band Sizes (bp) | Number of Bands | Number of Polymorphic Bands | Percentage of Polymorphic (%) |
|---------|----------------|-----------------|-----------------------------|-------------------------------|
| OPA-02  | 250-1000       | 11              | 11                          | 100                           |
| OPA-03  | 100-600        | 8               | 8                           | 100                           |
| OPA-04  | 100-1000       | 15              | 15                          | 100                           |
| OPC-01  | 100-1000       | 10              | 10                          | 100                           |
| OPC-02  | 100-1000       | 21              | 21                          | 100                           |
| **Total** |                | **65**          | **65**                      | **100**                       |
| **Average** |              | 100-1000        |                             |                               |

Number of polymorphic bands also revealed the level of genetic variations within species. Other study found about 11–28 polymorphic bands within species \textit{Tor douronensis} from West Sumatera, which indicated a high level of genetic variations in the population [7]. Other study found a low level of genetic variations within species \textit{Tor putitora} as showed from the number of polymorphic bands about 2–6 bands [8]. In this study, genetic variations of \textit{Tor soro} are considered low because no polymorphic bands were formed during amplification. Genetic similarity relationship in each \textit{Tor soro} population based on RAPD bands profile can be analyzed by creating a matrix similarity or dice coefficient within individual from the three rivers.

Table 2. Genetic similarity matrix based on Dice Coefficient

| Samples | BT1 | BT2 | BT3 | BT4 | A1  | A2  | A3  | A4  | B1  | B2  | B3  | B4  |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| BT1     | 1.00          |     |     |     |     |     |     |     |     |     |     |     |
| BT2     | 0.21          | 1.00|     |     |     |     |     |     |     |     |     |     |
| BT3     | 0.33          | 0.43| 1.00|     |     |     |     |     |     |     |     |     |
| BT4     | 0.32          | 0.27| 0.60| 1.00|     |     |     |     |     |     |     |     |
| A1      | 0.07          | 0.00| 0.00| 0.00| 1.00|     |     |     |     |     |     |     |
| A2      | 0.07          | 0.00| 0.00| 0.00| 0.33| 1.00|     |     |     |     |     |     |
| A3      | 0.18          | 0.33| 0.20| 0.21| 0.33| 0.33| 1.00|     |     |     |     |     |
| A4      | 0.00          | 0.00| 0.00| 0.00| 0.17| 0.17| 0.44| 1.00|     |     |     |     |
| B1      | 0.15          | 0.21| 0.30| 0.23| 0.00| 0.11| 0.32| 0.16| 1.00|     |     |     |
| B2      | 0.06          | 0.00| 0.10| 0.21| 0.00| 0.00| 0.11| 0.00| 0.08| 1.00|     |     |
| B3      | 0.06          | 0.00| 0.11| 0.22| 0.00| 0.00| 0.00| 0.00| 0.08| 0.71| 1.00|     |
| B4      | 0.06          | 0.00| 0.20| 0.21| 0.00| 0.00| 0.11| 0.00| 0.23| 0.42| 0.44| 1.00|

The highest genetic similarity found in this matrix was between B2 and B3 with the percentage of 71% (0.71) while the lowest was from the pairs of BT1-B2, BT1-B3 and BT1-B4 with percentage of 6% (0.06). According to these results, then diversity level within species of \textit{Tor soro} samples in this study are considered low. Genetic relationship analysis was then performed by constructing dendrogram by using NTSYS (Numerical Taxonomy and Multivariate Analysis System). The analysis from all RAPD DNA profiles produced a dendrogram with similarity coefficient between 0.23–0.71 (Figure 3). Based on the dendrogram, the twelve samples of \textit{Tor soro} are clustered into three main groups at coefficient of 0.32. The first group consisted of 8 samples (BT1, BT2, BT3, BT4, A1, A2, A3, A4). The second group consisted of only one sample (B1) while the third group consisted of three samples (B2, B3, B4).
The dendrogram showed similarity based on genetic characters. The results supported the previous morphological analysis by showing the Bahorok group diverging out from Batang Toru and Alian groups. The result was assumed to be the effect of geographical distinction between Bahorok and the other two rivers, which limit the migration pattern from *Tor soro* in Bahorok. Furthermore, Batang Toru and Alian groups still showed close genetic relationship with each other in which, again emphasized that they were once belonged to the same *Tor soro* population originating from Lake Toba. Geographical isolation may occur in certain distances that affect the gene flow between adjacent populations, leading to genetic variations [9]. The low genetic variation indicated the slow migration rate and limited access of population to exchange genes with others [10]. A limited genetic exchange may cause inbreeding, leading to high homogeneity. Other than that, habitat degradation is also affecting population to survive in the wild. Therefore, a conservation effort is needed to preserve the natural habitat of genus *Tor*, especially rivers in North Sumatera as the important habitats for Cyprinids [11].

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