Determination of species boundaries of Selais fish from Arut River, Central Kalimantan based on 16S mitochondrial gene using Bayesian approach

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Abstract. Selais fish is difficult to discriminate with other Silurid fish species based on morphological characters. As a result, the valid species of selais fish is uncertain. Therefore, a molecular phylogenetic study was needed to clarify species boundaries and to addresses genetic relationships of the selais fish. In this study, *16S* mitochondrial gene of ten selais samples collected from Arut River (Central Kalimantan) were sequenced, from which a Bayesian trees was generated. Result revealed monophyletic of selais fish which is revealed as a single species. The Bayesian inference showed that the selais fish clade is distinguished with two other genus, *Kryptopterus* and *Ompok,* by its sequence differences. This finding can address species boundaries of selais fish using Bayesian approach, but the name of the selais species has not been clarified.
Keywords: 16S gene, Bayesian approach, selais fish, species boundaries

1 Introduction

Indonesia which is on a crossroad between two oceans and bridge two continents contributes significantly to two of the world's 25 biodiversity hotspots, Sundaland and Wallacea [1, 2]. The Sundaland hotspot covers the western half of the Indo-Malayan Archipelago and is dominated by Borneo and Sumatra. The hotspot is considered to be the centers of fauna diversity including freshwater fishes [1]. One of the freshwater fish commonly found in Arut River, Central Kalimantan is selais fish which has market demands as a human consumption. The fish is included in the ordo Siluriformes and family Siluridae [3]. Selais fish is inhabited at lowland slowly water such as rivers, streams, and lakes consuming small fishes, prawns, and crustaceans. The fish reaches about 30 cm to 45 cm in length with light colouring body and has maxillary and mandibular barbels [3].

The taxonomic status and classification of selais fish is still remain unclear due to similar morphology with other Silurid fish species. No previous study has described the taxonomic status and classification of selais fish collected from Arut River, Central Kalimantan. The previous studies used *Ompok hypophthalmus* to name the selais fish [4–6] from Kampar River (Riau, Sumatra). In addition, Jusmadi et al. [7] described 6 species of selais fish inhabited in Mahakam River consisting 2 species which are grouped in genus *Ompok* (*O. hypophthalmus* and *O. miostoma*) and 4 species categorized in genus *Kryptopterus* (*K. apogon, K. microneme, K. lompok*, and *K. bicirrhis*). Those previous studies were just used morphological analysis which is insufficient to classify the selais fish. Furthermore, no *16S* mitochondrial sequence data of selais fish from Indonesia recorded at the GenBank. Therefore, *16S* mitochondrial data of selais fish has to be investigated in order to clarify the taxonomic status of the fish. This is due to the use of *16S* mitochondrial DNA as a molecular marker has several advantages such as maternal mode of inheritance, limited recombination, and high conserve which can be used to study species identification and interspecies relationship [8]. The use of *16S* mitochondrial marker had been successfully applied to identify species identification [9–11].

It is not only the choice of the molecular markers but also the use of molecular approaches can give better results for determining the species boundaries of the selais fish. The application of Bayesian approach to describe phylogeny and the interspecies relationship has been conducted in many fishes [12–14]. This is due to the Bayesian approach computationally more efficient than traditional methods. In addition, it quantifies and addresses the source of uncertainty and can incorporate complex models of evolution [15]. Therefore, the application of Bayesian Inference through BEAST software is expected to describe species boundaries and interspecies genetic relationship of selais fish in this study. Furthermore, the *16S* sequence data obtained from selais fish investigated from this study can be used to arrange the *16S* mitochondrial library of the selais fish from Indonesia. The *16S* mitochondrial library is important data not only to help identification of

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uncertain fish species but also to describe the genetic diversity of a species.

2 Materials and methods

2.1 Sample collection and storage

Ten selais fish samples were collected from Arut River, Central Kalimantan (2°40'10.6"S and 111°38'08.3"E) (Figure 1). Selais fish samples were then documented (Figure 2) and 50 mg muscle tissue of each fish sample was put into a 1.5 mL tube containing 99 % ethanol absolute immediately after sampling collection. Next, the preserved muscle tissue fish samples were labelled and sent to the Laboratory of Genetics and Breeding, Faculty of Biology UGM and were kept at −20 °C for further investigation.

Fig. 1. Map of collection site for collecting selais fish from Arut River, Central Kalimantan (Number 1 shows the location of sampling area)

Fig. 2. Selais fish samples collected from Arut River, West Kotawaringin, Central Kalimantan (Photo: Lukman Hakim)

2.2 Extraction, amplification, and sequencing

Approximately 50 mg to 100 mg of muscle tissue of each preserved fish sample was extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, USA) following the manufacturer's protocol. The two universal primers, 16Sar (5'-CGCCT GTTTATCAAAAACAT-3') and 16Sbr (5'-CCGG TCTGAACTCAGATCACGT-3') [16], were used to amplify the partial mitochondrial *16S* gene using the polymerase chain reaction (PCR) method. The PCR amplification used MyTaq HS Red Mix PCR Kit (Bioline) and was carried out in a 50 µl volume containing 10-100 ng of genomic DNA, 25 µl MyTaq HS Red Mix PCR, 2mM MgCl2, 0.6 µM of each primer, and 11 µl ddH2O. PCR thermal cycling followed the instruction in Arisuryanti et al. [17]. Then, PCR products were visualized using 1 % agarose gels after staining with FluoroSafe. The PCR products were then sent to First Base (Malaysia) through P.T. Genetika Science (Jakarta) for purification and sequencing bidirectionally using similar primers for amplification. The sequencing was conducted using ABI 3730XL Genetic Analyzer (Applied Biosystem).

2.3 Data analyses

All sequences of the fish samples were initially inspected visually for reading errors and edited manually using SeqMan and EdiSeq (Lasergene, DNASTAR). The correct sequences were then converted into Fasta format. All sequences were then aligned in MESQUITE 3.51 package [18] using opal multiple sequence alignment and MEGA X [19] using Clustal W. Phylogenetic relationships were estimated using a Bayesian approach. The Akaike Information Criterion (AIC) implemented in jModelTest ver 2.1.10 [20] was used to determine the best fit evolutionary model. Bayesian inference (BI) was performed with BEAST ver 1.10 [15] under the best-fit model. Two simultaneous Markov chain analyses (MCMC) were run for 10^6 generations to estimate the posterior probabilities distribution with a sampling frequency set to every 1,000 generations. The analysis was done until the standard deviation of split frequencies was below 0.01. The analysis used a relative burn-in of 25% for diagnostics. Consensus trees were visualised in FigTree 1.4.4 [21]. Genetic variation and genetic distance within the selais samples was calculated using DnaSP ver 6.0 [22] and MEGAX [19]. In this study, 11 additional sequences from GenBank were used for comparative purposes and outgroup. The 11 additional sequences were *Ompok bimaculatus* (GQ469606 & GQ469616), *Ompok pabo* (GQ469605 & GQ469598), *Ompok pabda* (GQ469553 & GQ469569), *Ompok malabaricus* (FJ432680), *Kryptopterus vitreolus* (NC_035419), *Kryptopterus bicirrhis* (NC_034999), *Pangasius pangasius* (GQ411088), and *Clarias batrachus* (MF769377).

3 Results and discussions

The result revealed that the *16S* mitochondrial gene of the five samples of selais fish (with code SLA-01, SLA-02, SLA-04, SLA-05, SLA-06, SLA-07, SLA-08, SLA-09, SLA-10, and SLA-11) from Arut River was able to be amplified (Figure 3). The length of aligned sequences of the partial *16S* mitochondrial gene of those selais fish after excluding the ambiguous positions was 605 bp. From the 605 bp fragment length, the average rate of *16S* nucleotide composition of the selais fish is A=30.47 %, T=23.18 %, G=22.26 % and C=24.09 %.

The analysis result using Nucleotide BLAST showed that all 10 samples of selais fish from Arut River had similarity as much as 95.80 % (query cover 92 %) with *Ompok bimaculatus* and 94.17 % (query cover 99 %) with *Kryptopterus bicirrhis* samples from GenBank database. This indicates that the 10 samples of selais fish from Arut River might not be verified as *Ompok bimaculatus* or *Kryptopterus bicirrhis* due to low similarity percentage. Therefore, species boundaries of the fish samples have to be determined using Bayesian approach to know the species boundaries and taxonomic status of the selais fish samples.

The optimal model of nucleotide sequence substitution for the *16S* matrix including the outgroup samples was the GTR+I model with gamma-distributed rate variation across sites as inferred by the jModelTest 2.1.10 under the Akaike information criterion (AIC) [20]. Using *16S* mitochondrial gene conducted by a Bayesian approach, we have found strong evidence that the selais fish is represented in the monophyletic group (Figure 4). The monophyletic of the selais fish identified in the phylogenetic analysis represent possible species according to Phylogenetic Species Concept. From the Figure 4, the selais fish samples represents a distinct species by examining phylogenetic relationships through Bayesian Inference. In addition, the selais fish is more closely related to the two *Kryptoterus* species (*K. bicirrhis* and *K. vitreolus*) supported by posterior probability of 0.96 compared to *Ompok* spp. This data indicated that the selais fish might be included in the genus *Krytoterus* rather than the genus *Ompok*. This finding has also been supported by genetic distance between selais and those two genus groups. Mean percentage nucleotide sequence divergence of the *16S* mitochondrial gene between Selais and the genus *Ompok* is higher (65.8 %) than between selais and the genus *Kryptopterus* (44.1 %). However, limited *16S* mitochondrial sequence data of the *Kryptopterus* species recorded at GenBank is difficult to clarify the name of the selais fish species. Therefore, it is challenging to study more comprehensive integrative between molecular data and morphological analysis of selais fish collected from Indonesia to determine more preciously the species boundaries of the selais species and identify accurate species name of selais fish from Arut River, Central Kalimantan.

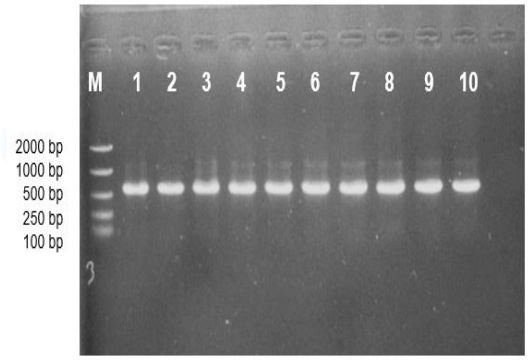

Fig. 3. DNA bands resulted from the PCR amplification of 16S mitochondrial gene of the selais fish samples from Arut River, West Kotawaringin, Central Kalimantan. (M=marker, 1=SLA-01, 2=SLA-02, 3=SLA-04, 4=SLA-05, 5=SLA-06, 6=SLA-07, 7= SLA-08, 8=SLA-09, 9=SLA-10, and 10=SLA-11).

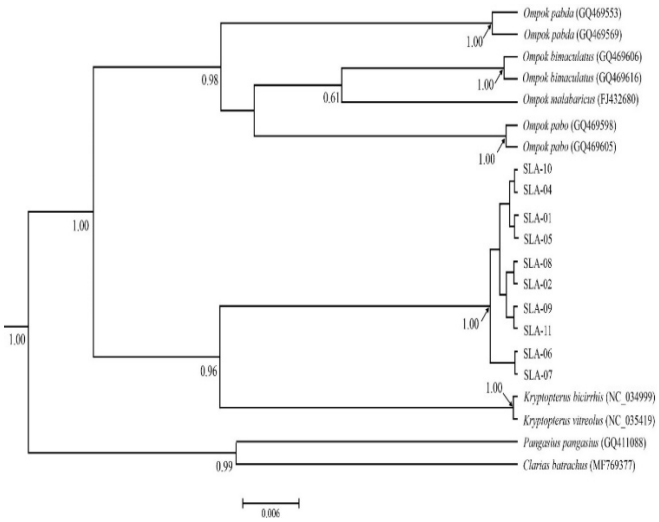

Fig. 4. Bayesian tree inferred from *16S* mitochondrial gene sequences. Tree produced from 10^6 generations using GTR+I+G model. Number of each node represent posterior probabilities and scale correspond to substitutions/site.

This study also found polymorphism within the selais fish investigated in this study. The result revealed two haplotypes with oly one polymorphic site was detected without parsimony informative site. The polymorphic site consisted a transition. In addition, three indels were found in the partial *16S* sequence of the selais fish (Tabel 1). The genetic distance between the selais samples was between 0 % to 0.17 % (average 0.033 %).

Table 1. Summary of nucleotide variations in the partial *16S* mitochondrial gene of the selais fish from Arut River. Only variable sites are shown. Dots indicate identity with the sample with code SLA-01. The number above corresponds to nucleotide base pair position

Samples	Polymorphic sites			
	1	1	1	5
	1	1	3	7
SLA-01	T	T	T	-
SLA-02	.	.	.	-
SLA-04	C	.	.	-
SLA-05	.	.	.	-
SLA-06	.	.	.	-
SLA-07	.	-	.	-
SLA-08	.	-	.	-
SLA-09	.	.	.	-
SLA-10	.	.	.	-
SLA-11	.	.	-	T

The finding of *16S* mitochondrial gene polymorphism in the selais fish from Arut River indicated genetic variation at intra-population level. This is due to *16S* mitochondrial gene is a conserved gene, so the the change of few nucleotide within the samples can be used to indicate intra-population genetic variation.

4 Conclusion

Bayesian analyses based on partial fragment of the *16S* mitochondrial DNA gene cover monophyletic clade of the selais fish. The selais fish investigated in this study is considered to be a single species. However, we still cannot clarified the name of the species of selais species. Further study is needed to examine selais fish specimens and collect vouchered genetic samples from the type areas of the fish species. Those efforts together with the revised identifications and geographic distributions will be important in guiding efforts to conserve the taxonomic, genetic, and morphological diversity of this important fish.

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