

Molecular Characterization of *Anguilla* from Cibereum and Sapuregel Rivers Segara Anakan Watersheds Cilacap, Central Java

Agus Nuryanto*, Dian Baghawati, Kusbiyanto, Moh Husein Sastranegara, Farida Nur Rachmawati
Faculty of Biology, Universitas Jenderal Soedirman
Jl. Dr. Soeparno No. 63 Purwokerto, Central Java, Indonesia. 53122
*Email: agus.nuryanto@unsoed.ac.id

ABSTRACT. The taxonomic status of *Anguilla* species' in river watershed that empties into Segara Anakan Cilacap is uncertain, thereby making it difficult for further studies to be carried out to determine its genetic in that area. Therefore, this study evaluates *Anguilla*'s taxonomic status and population genetic in Cibereum and Sapuregel River watersheds. Data were obtained from molecular characterization study using cytochrome c oxidase 1, with fourteen *Anguilla* specimens collected from two sequenced watersheds. Taxonomic status was determined based on homology and divergence values and monophyly of the samples to the reference species. Meanwhile, genetic divergences among samples to the reference species were calculated based on the Juke-Cantor substitution model in DnaSP6. A homology test was performed using a basic local alignment search tool, with monophyly inferred from the cladogram, which was developed using neighbor-joining and maximum likelihood algorithms in MEGAX with 1000 pseudoreplicates and out-group comparison. Furthermore, population genetic was analyzed through polymorphism, haplotype, nucleotide diversity within the population, divergence, and genetic differences. All calculations conducted in Arlequin 3.5 had *Anguilla* samples comprising of high (99.23% to 99.84%) to low genetic divergences (0.224% to 1.127%). The result shows that cladogram with all *Anguilla* samples formed a monophyletic clade with *A. bicolor*, separated from their taxa. Furthermore, *Anguilla* samples from both watersheds have low genetic polymorphisms with medium to high haplotype and nucleotide diversity. The population's comparison proved that both populations have low genetic divergence, and no genetic difference based on variance analysis ($p=0761$). Therefore, *Anguilla* resources in river watersheds that empty into Segara Anakan are a single genetic conservation unit.

Keywords: diversity; eel; genetic; population; Segara Anakan

Article History: Received 26 August 2020; Received in revised form 26 September 2020; Accepted 23 November 2020; Available online 30 December 2020

How to Cite This Article: Nuryanto A, Baghawati D, Kusbiyanto K, Sastranegara MH, Rachmawati FN. 2020. Molecular characterization of *Anguilla* from Cibereum and Sapuregel Rivers Segara Anakan Watersheds Cilacap, Central Java. *Biogenesis: Jurnal Ilmiah Biologi*. vol 8(2): 145-156. doi: <https://doi.org/10.24252/bio.v8i2.15532>.

INTRODUCTION

An eel is a snake-like fish with a slender, elongated body and poorly developed fins, proverbial for its slipperiness, it belongs to the genus *Anguilla*, and locally known as ikan sidat in Indonesian. It is a catadromous fish, which resides in fresh water and goes to the ocean to spawn (Aoyama, 2009). *Anguilla* spp. are economically valuable commodities which are frequently exported to Japan. The market demands, particularly for *A. bicolor* are met based on culture. Its cultivation is primarily supported by naturally collecting *Leptocephalus* larvae or glass eels (Tanaka *et al.*, 2014), although it leads to overexploitation when excessively carried out (Rachmawati & Susilo, 2011). In addition, it is also proven by the alteration of its conservation status from Least Concern to Nearly Threatened in 2010, 2017, and 2020 respectively, as reported by the

IUCN (Jacoby *et al.*, 2014; Pike *et al.*, 2020). According to Jacoby & Gollock (2014), sequentially, the conservation status of *A. marmorata* and *A. nebulosa* is Least Concern, and Not Evaluated as stated in the 2013 version of IUCN Red List.

These alterations were based on several reasons, including the natural harvesting of *A. bicolor* (Jacoby *et al.*, 2014). Therefore, conservation plans and actions are urgently needed to ensure its sustainability. Jacoby *et al.* (2014), reported that it is crucial to carry out taxonomic research on the conversion of *A. bicolor*. This is due to the fact that useful information is only obtained when the taxonomic status is valid (Nuryanto *et al.*, 2019).

Globally, a total of 18 *Anguilla* species has been identified (Aoyama, 2009). In Indonesia waters, approximately five to seven species and

subspecies were discovered (Wouthuyzen *et al.*, 2009). However, some other studies reported that nine of them inhabit these waters, namely *A. celebesensis*, *A. marmorata*, *A. borneensis*, *A. interioris*, *A. obscura*, *A. bicolor bicolor*, *A. bicolor pacifica*, *A. nebulosa nebulosa*, and *A. megastoma* (Sugeha, 2010; Sugeha & Arai, 2010; Sugeha & Genisa, 2015).

An entirely different number was detected in the Western and Eastern Indonesia Region. According to Sugeha *et al.* (2008), *A. nebulosa nebulosa* and *A. bicolor bicolor* inhabit the Western waters, while *A. marmorata*, *A. celebesensis*, *A. bicolor pacifica*, and *A. interiori* are found in the Eastern part (Sugeha, 2010; Sugeha & Arai, 2010). However, a recent study carried out in the Aceh waters by Muchlisin *et al.* (2017) reported an additional species, namely *A. bengalensis*. Therefore, in total, three *Anguilla* species, *A. bicolor*, *A. marmorata* and *A. bengalensis* were discovered in the Aceh waters. Fahmi (2015), also discovered three *Anguilla* species, although the third one was different from that reported by Muchlisin *et al.*, (2017). Conversely, instead of *A. bengalensis*, *A. nebulosa* was discovered.

A different number of species were also reported in the studies carried out in the Southern Areas of West and Central Java (Rachmawati & Susilo, 2011; Minegishi *et al.*, 2012; Tanaka *et al.*, 2014; Fahmi, 2015). Fahmi (2015) discovered three *Anguilla* species in Pelabuhan Ratu, *A. bicolor*, *A. marmorata*, and *A. nebulosa*. Furthermore, Tanaka *et al.* (2014) discovered only one eels species, *A. bicolor*. Rachmawati & Susilo (2011) and Fahmi (2015) reported that one of the species inhabit the Cilacap waters. In addition, Fahmi (2015) collected *Anguilla* from the same site. However, such a minimal number of sample sites is considered unreliable for scientific purposes and eel fisheries management.

Therefore, critical problems related to eel fishery management in Cilacap, such as taxonomic status and population genetics of *Anguilla* in rivers across Cilacap Regency, particularly those belonging to the Segara Anakan watersheds need to be resolved. These issues are assessed by studying molecular characterization using cytochrome *c* oxidase

subunit 1 (COI) gene as a marker. Although, previous studies have proven that COI gene is a reliable marker for molecular identification (Matarese *et al.*, 2011; Hubert *et al.*, 2012; Pereira *et al.*, 2013; Nuryanto *et al.*, 2017; Bhagawati *et al.*, 2020). In addition, phylogeographic research also reported a similar discovery (Song *et al.*, 2013; Zhang *et al.*, 2014; Nuryanto *et al.*, 2019). It was considered a reliable marker for eel's molecular characterization, *Anguilla* (Fahmi, 2015; Muchlisin *et al.*, 2017).

This study aims to determine the taxonomic status and molecular characteristics of *Anguilla* specimens collected at Sapuregel and Cibeureum Rivers, as well as Segara Anakan watersheds Cilacap, Central Java.

MATERIALS AND METHODS

Sampling time and sites. Samples were bought in June 2020, from the first collectors residing at Kubangkangkung Hamlet, District of Kawunganten, and Pondok Lombok Hamlet, Kaliwungu Village, District of Kedungreja, Cilacap Regency (Fig. 1). The first collector from Kubangkangkung Hamlet bought it from the fishermen that captured it in Sapuregel River Watersheds. In contrast, the one from Pondok Lombok Hamlet obtained it from fishermen that caught it in Cibeureum River Watersheds. Subsequently, both sampling sites empty into the Segara Anakan watersheds.

Genomic DNA isolation, marker amplification, and sequencing.

Approximately 2 x 2 millimeter square of dorsal fin tissue samples were sent to PT. Genetika Science Indonesia for DNA analysis. The total genomic DNA was isolated using the gSYNCTM DNA extraction kit (Geneaid, GS050) by adhering to the company's directives. The amplification of the COI fragment was carried out using KOD FXNeo (Toyobo, KFX201). The COI marker was amplified using the following primer sets, VF2_t1

```
TGTAAAACGACGGCCAGTCAACCAACCACAA
AGACATTGGCAC, FishF2_t1
TGTAAAACGACGGCCAGTCGACTAATCATAA
AGATATCGGCAC, FishR2_t1
CAGGAAACAGCTATGACACTTCAGGGTGACC
```

GAAGAATCAGAA, and FR1d_t1
CAGGAAACAGCTATGACACCTCAGGGTGTCC
GAARAAFCARAA. The final volume of PCR

reactions was obtained as 25µl, and each reagent's composition is presented in Table 1.

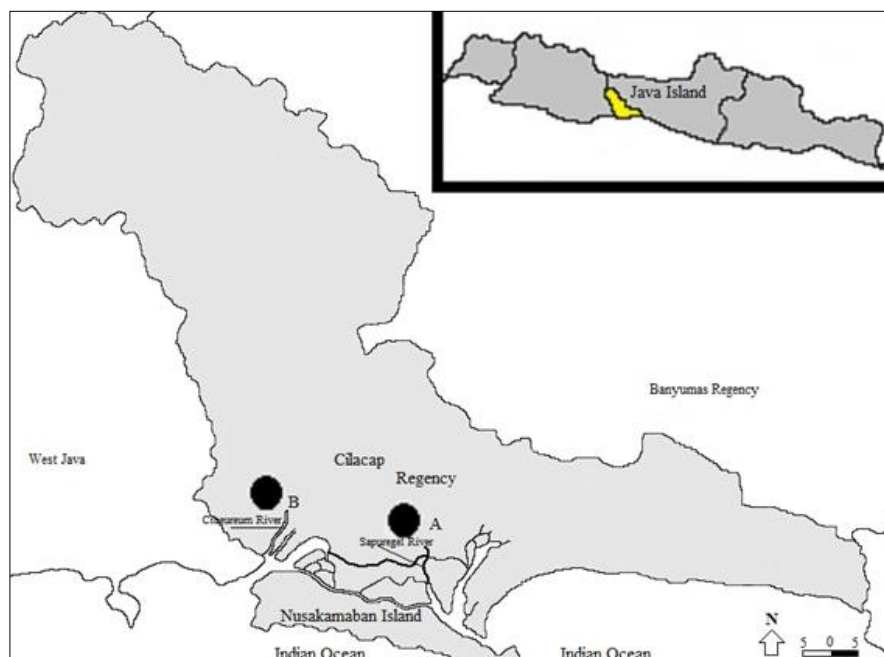


Fig. 1. Sampling sites for *Anguilla* collection.

The thermal conditions for PCR amplification involved an initial denaturation realized at 96°C, which lasted for 3 min. It was further carried out at 94°C for 10 s, annealing at 52°C for 30 s, and extension at 72°C for 45 s. It was repeated in a circular motion 35 times, and this was ended by holding the cyclor at 4°C. Conversely, the PCR amplification was carried out on Agilent Surecycler 8800. Furthermore, the clear and strong products were subjected to bidirectional sequencing.

Table 1. Reagent composition for COI marker amplification.

No	Reagent	Volume in 25 µl
1	ddH ₂ O	4
2	KOD FX Neo	1
3	2x PCR Buffer KOD FX Neo	12.5
4	2 mM dNTPs	2.5
5	10 pmol/µl Primer VF2_t1	1
6	10 pmol/µl Primer FishF2_t1	1
7	10 pmol/µl Primer FishR2_t1	1
8	10 pmol/µl Primer FR1d_t1	1
9	Template DNA	1

Sequence editing and data analysis. The forward and reverse sequences of each sample were contiged and edited in Bioedit version 7.0.4.1 (Hall, 2011). This was carried out in to obtain a consensus sequence. Its alignment was performed using ClustalW in Bioedit version 7.0.4.1 (Hall, 2011). The COI sequences of the samples were deposited in GenBank (accession number MT883612-MT883625).

Taxonomic status. Conservation statuses of the samples were determined based on homology, genetic divergences, and the distance to the reference species deposited in GenBank, including their monophyly. The value of the homology is obtained by comparing the available reference species in the GenBank. Genetic divergence was calculated based on the Juke-Cantor substitution model in DnaSP6 (Rozas *et al.*, 2017). The homology test was carried out using a basic local alignment online search tool (BLAST) NCBI. The monophyly of *Anguilla* samples in accordance with the reference species was inferred from the cladogram, which was developed using neighbor-joining (NJ) and maximum likelihood (ML) algorithms in

MEGA X (Kumar *et al.*, 2018). The reliability of tree topology was obtained from 1000 bootstraps pseudoreplicates. Subsequently, the branching pattern's polarity was realized from the comparison, of the out-group specimen *Muraenesox bagio* (MK041057).

Population genetics. The population genetics of the species recovered in Cibeureum and Sapuregel Rivers watersheds were assessed based on genetic divergence, polymorphic sites, haplotype, and nucleotide diversity. Polymorphism and genetic divergences within populations were estimated using the Juke-Cantor substitution model implemented in DnaSP version 6 (Rozas *et al.*, 2017).

Haplotype and nucleotide diversity were calculated using Arlequin 3.5 (Excoffier & Lischer, 2010).

RESULTS AND DISCUSSION

Taxonomic status. The results from BLAST showed that the samples used in this study have sequence identity values ranging from 99.23% to 99.84%. The *Anguilla bicolor* in GenBank encompasses sequences queries ranging from 96% to 99% and an error value of 0.00. The detailed BLAST parameter of the samples in accordance with the reference species is shown in Table 2.

Table 2. BLAST parameters of *Anguilla* samples to the reference species.

Code	Accession Number	Max Score	Total Score	Query Cover	E-value	Identity	Reference Species	Accession number
Kja01	MT883612	1162	1162	98	0.00	99.38	<i>Anguilla bicolor</i>	AP007236
Kja02	MT883613	1162	1162	98	0.00	99.38	<i>Anguilla bicolor</i>	AP007236
Kja03	MT883614	1168	1168	99	0.00	99.53	<i>Anguilla bicolor</i>	AP007236
Kja04	MT883615	1162	1162	99	0.00	99.84	<i>Anguilla bicolor</i>	AP007236
Kja05	MT883616	1173	1173	98	0.00	99.69	<i>Anguilla bicolor</i>	AP007236
Kja06	MT883617	1164	1164	96	0.00	99.84	<i>Anguilla bicolor</i>	KM875505
Kja07	MT883618	1168	1168	98	0.00	99.53	<i>Anguilla bicolor</i>	AP007236
Kja08	MW080739	1140	1140	100	0.00	99.84	<i>Anguilla bicolor</i>	KM875504
Kja09	MW078537	1134	1134	99	0.00	99.84	<i>Anguilla bicolor</i>	MG675613
Kja11	MW078538	1134	1134	100	0.00	99.68	<i>Anguilla bicolor</i>	KP979655
Kja12	MW078539	1147	1147	100	0.00	100	<i>Anguilla bicolor</i>	KU692247
Kja13	MW080740	1155	1155	100	0.00	99.53	<i>Anguilla bicolor</i>	AP007236
Kja15	MW078540	1123	1123	100	0.00	99.35	<i>Anguilla bicolor</i>	KP979655
Kwt01	MW078541	1096	1096	100	0.00	100	<i>Anguilla bicolor</i>	KY618776
Kwt03	MW080741	1153	1153	99	0.00	99.68	<i>Anguilla bicolor</i>	AP007236
Kwt04	MT883619	1173	1173	98	0.00	99.38	<i>Anguilla bicolor</i>	AP007236
Kwt06	MT883620	1168	1168	99	0.00	99.23	<i>Anguilla bicolor</i>	AP007236
Kwt07	MW080742	1127	1127	99	0.00	99.84	<i>Anguilla bicolor</i>	KU692247
Kwt08	MT883621	1162	1162	99	0.00	99.84	<i>Anguilla bicolor</i>	AP007236
Kwt09	MT883622	1234	1234	98	0.00	99.41	<i>Anguilla bicolor</i>	AP007236
Kwt10	MT883623	1173	1173	98	0.00	99.38	<i>Anguilla bicolor</i>	AP007236
Kwt12	MT883624	1173	1173	98	0.00	99.38	<i>Anguilla bicolor</i>	AP007236
Kwt13	MW078542	1129	1129	100	0.00	99.52	<i>Anguilla bicolor</i>	KP979655
Kwt14	MW078543	1092	1092	99	0.00	99.83	<i>Anguilla bicolor</i>	KY618771
Kwt16	MT883625	1229	1229	98	0.00	99.26	<i>Anguilla bicolor</i>	AP007236

The BLAST parameters of the samples, shown in Table 2, absolutely align with the *Anguilla bicolor*. Therefore, it was concluded that the eel samples collected at Cibeureum and Sapuregel River watersheds are categorized as *Anguilla bicolor*. This decision was made based on the fact that all the samples' values were higher than 97%, which commonly serves as the threshold for genetic identity (Ko *et al.*, 2013). In some instances, it tends to be lower

than 97% depending on the fish groups (Pereira *et al.*, 2013; Ratnasingham & Hebert, 2013).

The present research realized a similar number of *A. bicolor* species from Segara Anakan estuary. However, this is consistent with the previous studies carried out by Fahmi (2015), and Rachmawati & Susilo (2011). These similarities confirm and strengthen the hypotheses reported in previous studies that the species of eel discovered in Segara Anakan

waters is *A. bicolor* and also indicates as dominant species that inhabits Cilacap waters, particularly the Cibeureum and Sapuregel River watersheds which empty into Segara Anakan. The result from this analysis is reasonable hence *A. bicolor* is commonly restricted to Cibeureum and Sapuregel, which are lowland river systems and are rarely found in large upland streams (Froese & Pauly, 2019).

Subsequently, when compared to studies carried out in the Indian Ocean near Java and Sumatera, it was discovered that the present research obtained a different number of species as reported by Fahmi & Hirnawati (2010) and

Muchlisin *et al.* (2017). This is due to the differences in the study areas. Conversely, this research is focused on Cibeureum and Sapuregel rivers watersheds which empty into the Segara Anakan estuary, while Fahmi & Hirnawati (2010) carried out a study on the species composition at Cimandiri River, Sukabumi. These rivers possess different characteristics. Cibeureum and Sapuregel Rivers are typically short (40 km) lowlands with its spring in Gunungsari Hill, which is only 260 masl, and they are typical habitats for *A. bicolor*.

Table 3. Genetic divergences among samples and reference species (*A. bicolor*).

No	Sample Code	The accession number of reference species (%)							
		KM875504	KP979655	KY618776	KU692247	KY618771	AP007236	MG675613	KM875505
1	Kja01	1.1475	0.1897	0.9544	0.9544	0.1897	0.38022	0.1897	0.9544
2	Kja02	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
3	Kja03	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
4	Kja04	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
5	Kja05	1.1475	0.1897	0.9544	0.9544	0.1897	0.000	0.1897	0.9544
6	Kja06	0.3802	0.5706	0.1897	0.1897	0.5706	0.7622	0.5706	0.1897
7	Kja07	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
8	Kja08	0.1897	1.1475	0.3802	0.3802	1.1475	1.3413	1.1475	0.3802
9	Kja09	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
10	Kja11	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
11	Kja12	0.1897	0.7622	0.0000	0.0000	0.7622	0.9544	0.7622	0.0000
12	Kja13	1.1475	0.1897	0.9534	0.9534	0.1897	0.3798	0.1897	0.9534
13	Kja15	1.3413	0.3802	1.1475	1.1475	0.3802	0.5714	0.3802	1.1475
14	Kwt01	0.1897	0.7622	0.0000	0.0000	0.7622	0.9544	0.7622	0.0000
15	Kwt03	1.1475	0.1897	0.9544	0.9544	0.1897	0.3802	0.1897	0.9544
16	Kwt04	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
17	Kwt06	1.1475	0.1897	0.9544	0.9544	0.1897	0.3802	0.1897	0.9544
18	Kwt07	0.3802	0.9544	0.1897	0.1897	0.9544	1.1475	0.9544	0.1897
19	Kwt08	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
20	Kwt09	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
21	Kwt10	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
22	Kwt12	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
23	Kwt13	1.1475	0.1897	0.9544	0.9544	0.1897	0.3802	0.1897	0.9544
24	Kwt14	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622
25	Kwt16	0.9544	0.0000	0.7622	0.7622	0.0000	0.1897	0.0000	0.7622

In contrast, Cimandiri is regarded both as a lowland and upland river running from several mountains (Gede, Pangrango, Halimun, and Salak) at the height of approximately 1200 masl. It is a suitable habitat for several *Anguilla* species, and this type of characteristics is also similar to the Aceh rivers (Muchlisin *et al.*, 2017). According to Froese & Pauly (2019), *A. marmorata*, *A. nebulosa*, and *A. bengalensis* are found in such rivers. The differences in characteristics are ideal, and the present study

was only able to discover *A. bicolor*. Fahmi & Hirnawati (2010) and Muchlisin *et al.* (2017) discovered three *Anguilla* species.

The BLAST results are supported by the values of the genetic or molecular divergence of the samples and *A. bicolor* in GenBank, which ranges from 0.224% to 1.127%. A detailed description of these values for all individual species and the reference *A. bicolor* sequences are shown in Table 3. They were discovered to be within the range of 0.1% to

6.0% which is below the genetic divergences threshold for fish species identity in Canada and Australia (Ratnasingham & Hebert, 2013). The values obtained are also within the range reported by Pegg *et al.* (2006), which is from 1% to 3%. Furthermore, the genetic divergences realized in this research are far below the maximum value reported by Pereira *et al.* (2013), which was 8.5%. In addition, this

is also consistent with previous studies (Muchlisin *et al.*, 2017; Nuryanto *et al.*, 2017a; Nuryanto *et al.*, 2017b; Nuryanto *et al.*, 2018). Therefore, it is compelling that the low genetic divergence values of eel samples and *A. bicolor* sequences in GenBank prove that the species from Cibeureum and Sapuregel River watersheds belong to the *A. bicolor*.

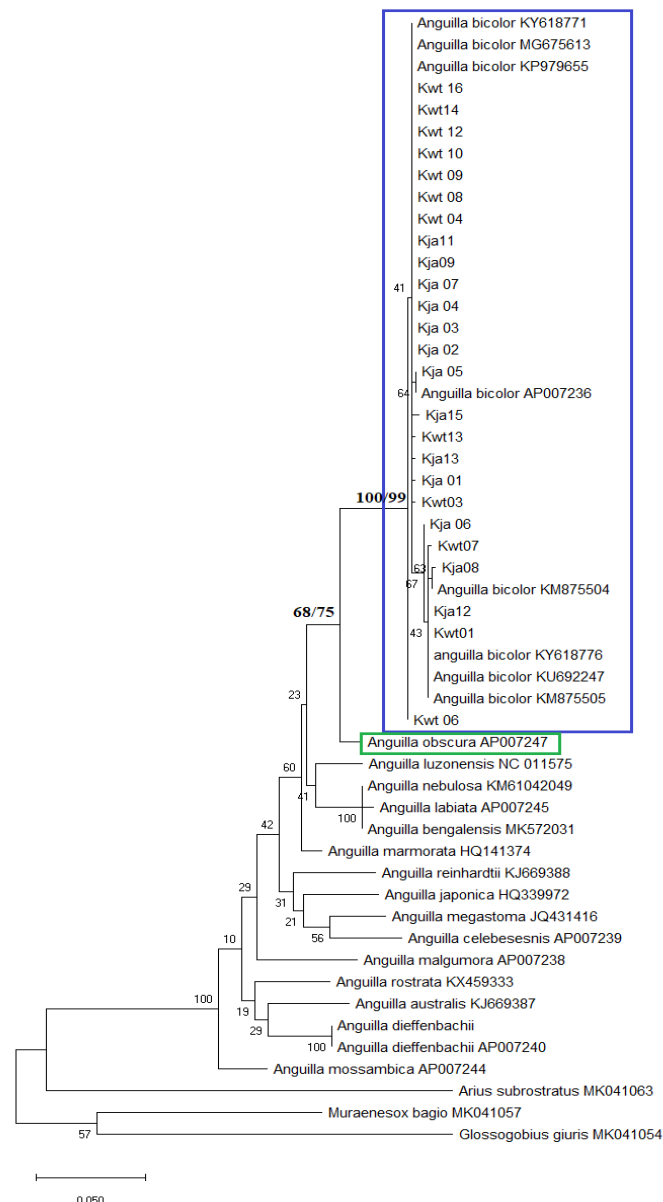


Fig. 2. Taxonomic tree showing the monophyly of the samples to the references species in GenBank and separation to sister taxon (*A. obscura*). Left: ML bootstrap values; Right: NJ bootstrap values.

The cladogram in Fig. 2 showed that the eel samples collected from Cibeureum and Sapuregel River watersheds belonged to the monophyletic, clade or *Anguilla bicolor* group retrieved from the GenBank (Blue box). It is

also evident in Fig. 2 that these samples are separated from its sister taxon, *A. obscura* (green box). Its monophyly, in accordance with their reference species, was supported by high bootstrap values (ML= 99; NL= 100). The

separation of the sample clade from their sister taxon was also triggered by high bootstraps values (ML= 81; NJ=78).

The monophyly of the samples and *A. bicolor* from GenBank shows extremely low genetic distances (the branch length in Fig. 2). A clear separation of the sample clade from its sister taxon (*A. obscura*) provides additional data which stated that species from Cibeureum and Sapuregel River watersheds are genetically identified as *bicolor*. The result also showed that the COI gene is a reliable marker for

species identification. Its reliability also serves as a barcode marker which varies among species, due to its high mutation rate (Sachithanandam *et al.*, 2012). This is also consistent with the research carried out by Muchlisin *et al.* (2017), or studies related to the strain in fish species (Nuryanto *et al.*, 2018) and also in Crustacea (Bhagawati *et al.*, 2020).

Based on the BLAST parameters, genetic divergence, and monophyly of the samples, was categorized as *Anguilla bicolor*. The taxonomic status of eel samples is shown in Table 4.

Table 4. Taxonomic status of the *Anguilla* samples.

Code	Ordo	Familia	Genus	Species
Kja01	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja02	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja03	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja04	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja05	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja06	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja07	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja08	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja09	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja11	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja12	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja13	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kja15	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt01	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt03	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt04	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt06	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt07	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt08	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt09	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt10	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt12	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt13	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt14	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>
Kwt16	Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla bicolor</i>

Population Genetics. Molecular identification categorized all samples as *A. bicolor*. This served as the basis for the analysis of population genetics in both sampling sites. Multiple sequence alignment resulted in 630 base pairs (bp) fragment of the COI gene. However, 7 of them are polymorphic sites with a value of 1.111%. This simply means that the most common allele has a frequency of 98.99%. In addition, this value shows a decline in the polymorphism of the COI gene of *Anguilla bicolor* collected at the two river watersheds that empty into the Segara Anakan Estuary. It is because the value is less than 5%, which

implies that the most common allele has a higher frequency than 95%. According to Nuryanto *et al.* (2019), a gene is said to be polymorphic supposing it has a common allele with a maximum frequency of 95%.

The overall haplotype and nucleotide diversities were 0.681 ± 0.132 , and 0.160 ± 0.124 , respectively. The total haplotype diversity shows that the COI gene of *A. bicolor* collected at the river watersheds that empty into Segara Anakan has a medium level of genetic diversity. According to Nei (1987), its values range between 0.50 and 0.7. In contrast, the value of nucleotide diversity

realized is classified as low. It is reasonable that a low value was discovered in *A. bicolor*'s COI gene in the Cibereum and Sapuregel watersheds because it encompasses only seven polymorphic sites total of 630 locations that were analyzed. Nei (1987) reported that the nucleotide diversity value below 1% is categorized as low.

Genetic diversity analysis showed that the Cibereum and Sapuregel River watershed populations have haplotype diversities of 0.857 ± 0.137 , and 0.524 ± 0.209 , respectively. This shows that the *A. bicolor* from Cibereum and Sapuregel watersheds has high haplotype and medium nucleotide diversities. This was because the value of the haplotype diversity in the Cibereum watershed was slightly higher than 0.8, while in Sapuregel, it was approximately 0.5 and 0.7. According to Nei (1987), the values of haplotype diversity range between 0.5 to 0.7 in the medium category. On the contrary, a high value lies within the range of 0.8 and 1.0. The result showed that a complex pattern of genetic diversity is a common phenomenon in the natural population. The High and low haplotype genetic diversity levels have been reported in various animal groups ranging from bivalve, gastropod, insects to fish (Díaz-Ferguson *et al.*, 2010; Dorn *et al.*, 2011; Pavesi *et al.*, 2011; Sabando *et al.*, 2011; Dung *et al.*, 2013; Song *et al.*, 2013; Zhang *et al.*, 2014; Liu *et al.*, 2017; Parmaksiz & Eksi, 2017).

Based on the nucleotide level, the Cibereum and Sapuregel watershed populations have a diversity of $0.245\% \pm 0.185$, and $0.082\% \pm 0.085$, respectively. Subsequently, these values show that both populations had low nucleotide diversity. This was due to the fact that the values obtained were below 1%. According to Kochzius & Nuryanto (2008), it depicts low nucleotide diversity.

The values obtained imply that *A. bicolor* from Cibereum watershed has higher genetic diversity than those from Sapuregel. It is due to the fact that both rivers have different physical characteristics, such as the width and length of the watersheds, which leads to diverse riverscape. It was reported that a positive correlation exists between genetic diversity and

the riverscape of various organisms (Cook *et al.*, 2011; Olsen *et al.*, 2011; Cooke *et al.*, 2014; Brauer *et al.*, 2016; Davis *et al.*, 2018). Based on this observation, Cibereum watersheds are broader and longer as well as have more scapes than Sapuregel. Therefore, it is reasonable to state that *A. bicolor* population in Cibereum watersheds has higher genetic diversity than the species in Sapuregel.

Sequentially, the decline in polymorphisms, medium and low haplotype and nucleotide diversities of *Anguilla*'s COI gene in this research indicate that there are no genetic differences between the two watersheds. This is proven by the low level of genetic divergence between the samples from Sapuregel and Cibereum Rivers watershed, which was obtained as 0.175%. It is due to the fact that historically both populations were reproduced from a similar maternal *A. bicolor* which tend to spawn at a particular site in the Indian Ocean. There is a high possibility that individual species returned to both watersheds were their eggs were hatched by a single maternal *A. bicolor*. Therefore, they were reported to have similar molecular constituents in their COI gene because it is inherited. According to Hebert *et al.*, (2003), COI is a mitochondrial genome inherited from the maternal line. Conversely, it is used for maternity analysis and shows high similarity in closed populations.

Table 5. The amova analysis.

Source of variation	df	Sum of Squares	Var. Component	% of Variation
Among populations	1	0.372	-0.004 Va ^{ns}	-1.03
Within populations	23	9.788	0.426 Vb	101.03
Total	24	10.160	0.430	
Fixation Index FST	=	-	0.0103 ^{ns}	
p-value	=	0.535 ± 0.016		

Notes: ns= non significant

However, genetic differences were not discovered in both populations, as shown in the amova analysis (Table 5). Based on Table 4, the variance component (Va) among the populations was realized as -0.009 (p-value=

0.761) while the genetic fixation index (F_{st}) was -0.025 (p -value = 0.761). Both values show that no genetic structure or difference was discovered between Cibereum and Sapuregel Watersheds. It simply means that *A. bicolor* in the Segara Anakan Cilacap is a single population.

Surprisingly, the present study was unable to observe genetic differences between the two river systems. Generally, riverine populations tend to show significant genetic differences (Hughes *et al.*, 2009) which is also observed among tributaries without physical barriers (Kanno *et al.*, 2011). However, since *A. bicolor* from both river watersheds spawned in the same areas in the Indian Ocean, therefore the absence of genetic differences is a normal phenomenon.

Furthermore, genetic differences were not observed in the common anchovy across the Segara Anakan estuary (Nuryanto *et al.*, 2019). This is consistent with the studies related to the *Hemibagrus nemurus*, while the other populations showed significant genetic structure (Nuryanto *et al.*, 2019). Significant genetic differences among populations were discovered in some fish species (Pavesi *et al.*, 2011; Dung *et al.*, 2013). Some studies that adopted microsatellite markers which are believed to be more sensitive than the COI gene also showed variable results. On the contrary, genetic differences among the fish populations were not detected (Damerau *et al.*, 2012; Basharat *et al.*, 2016). Conversely, studies using microsatellite also showed significant genetic differences among populations (Bartáková *et al.*, 2013; Esa & Rahim, 2013; Abbas *et al.*, 2017; Yan *et al.*, 2018). However, both present and previous studies reported that genetic differentiation among the natural populations is complex, irrespective of the adopted genetic markers.

The medium to relatively high genetic diversity and the absence of genetic differences in the watersheds have important implications for the conservation of *A. bicolor*. It is suggested that *A. bicolor* resources in the Segara Anakan watershed need to be managed as an enormous single population, however, this means that Segara Anakan Cilacap needs to

be conserved as a unit. Nevertheless, additional samples and genetic markers are required to provide more reliable information on *A. bicolor*'s molecular characterization in the Segara Anakan watershed which tends to be used as the scientific basis for the conservation of *Anguilla* in Segara Anakan. Moreover, additional information, such as catchment areas and rates of *A. bicolor*, social-economic, and ecological data, is required to provide the actual number of this species in Segara Anakan. Therefore, conservation policy needs to be made based on a firm scientific basis.

CONCLUSION

In conclusion, *Anguilla bicolor* is the only eel species that inhabit river watersheds around Segara Anakan Cilacap. It has medium to high genetic diversity, lacks genetic differences and formed a single genetic conservation unit across Segara Anakan.

ACKNOWLEDGEMENTS

The authors are grateful to the Universitas Jenderal Soedirman for funding this study through the research scheme Riset Unggulan Terapan Unsoed (contract number: P/234/UN23.18/PT.01.03/2020). The authors are also grateful to the Research and Public Service Institute and Biology Faculty of Universitas Jenderal Soedirman for the facilities provided for the research to be carried out and to the editors as well as reviewers for valuable input and comments provided in this manuscript.

REFERENCES

- Abbas K, Xiaoyun Z, Weimin W. 2017. Microsatellite markers reveal genetic differentiation of Chinese dojo loach *Misgurnus anguillicaudatus* in the Yangtze River Basin. *Turkish Journal of Fisheries and Aquatic Sciences*. vol 17: 1167–1177. doi: <https://doi.org/10.4194/1303-2712-v17>.
- Aoyama J. 2009. Life history and evolution of migration in catadromous eels (Genus *Anguilla*). *Aqua-BioScience Monographs*. vol 2(1): 1–42. doi: <https://doi.org/10.5047/absm.2009.00201.0001>.
- Bartáková V, Reichard M, Janko K, Polačik M, Blažek R, Reichwald K, Cellerino A, Bryja J. 2013. Strong population genetic structuring in an annual fish, *Nothobranchius furzeri*, suggests multiple savannah refugia in southern Mozambique. *BMC*

- Evolutionary Biology*. vol 13(1): 1–15. doi: <https://doi.org/10.1186/1471-2148-13-196>.
- Basharat H, Ghafoor A, Chavhan A, Zafar M, Abbas K, Parveen J. 2016. Microsatellite markers revealed poor genetic structure of Wallago Attu in Punjab, Pakistan. *International Journal of Life Sciences*. vol 4(3):385–393.
- Bhagawati D, Winarni ET, Nuryanto A. 2020. Molecular barcoding reveals the existence of mole crabs *Emerita emerita* in the north coast of Central Java. *Biosaintifika: Journal of Biology & Biology Education*. vol 12(1): 104–110. doi: <https://doi.org/10.15294/biosaintifika.v12i1.20497>.
- Brauer CJ, Hammer MP, Beheregaray LB. 2016. Riverscape genomics of a threatened fish across a hydroclimatically heterogeneous river basin. *Molecular Ecology*. vol 25(20): 5093–5113. doi: <https://doi.org/10.1111/mec.13830>.
- Cook BD, Kennard MJ, Real K, Pusey BJ, Hughes JM. 2011. Landscape genetic analysis of the tropical freshwater fish *Mogurnda mogurnda* (Eleotridae) in a monsoonal river basin: importance of hydrographic factors and population history. *Freshwater Biology*. vol 56(5): 812–827. doi: <https://doi.org/10.1111/j.1365-2427.2010.02527.x>.
- Cooke GM, Landguth EL, and Beheregaray LB. 2014. Riverscape genetics identifies replicated ecological divergence across an Amazonian ecotone. *Evolution*. vol 68(7): 1947–1960. doi: <https://doi.org/10.1111/evo.12410>.
- Damerau M, Matschiner M, Salzburger W, Hanel R. 2012. Comparative population genetics of seven notothenioid fish species reveals high levels of gene flow along ocean currents in the Southern Scotia Arc, Antarctica. *Polar Biology*. vol 35(7): 1073–1086. doi: <https://doi.org/10.1007/s00300-012-1155-x>.
- Davis CD, Epps CW, Flitcroft RL, Banks MA. 2018. Refining and defining riverscape genetics: how rivers influence population genetic structure. *Wiley Interdisciplinary Reviews: Water*. vol 5(2): 1–20. doi: <https://doi.org/10.1002/wat2.1269>.
- Díaz-Ferguson E, Haney R, Wares J, Silliman B. 2010. Population genetics of a trochid gastropod broadens picture of Caribbean Sea connectivity. *PLoS One*. vol 5(9):1–8. doi: <https://doi.org/10.1371/journal.pone.0012675>.
- Dorn A, Ng'oma E, Janko K, Reichwald K, Polačik M, Platzer M, Reichard M. 2011. Phylogeny, genetic variability and colour polymorphism of an emerging animal model: the short-lived annual *Nothobranchius* fishes from southern Mozambique. *Molecular Phylogenetics and Evolution*. vol 61(3): 739–749. doi: <https://doi.org/10.1016/j.ympev.2011.06.010>.
- Dung DT, Hop NT, Thaenkham U, Waikagul J. 2013. Genetic differences among Vietnamese Haplorchis taichui populations using the COI genetic marker. *Journal of Helminthology*. vol 87(1): 66–70. doi: <https://doi.org/10.1017/S0022149X12000041>.
- Esa YB, Rahim KAA. 2013. Genetic structure and preliminary findings of cryptic diversity of the Malaysian mahseer (*Tor tambroides Valenciennes*: Cyprinidae) inferred from mitochondrial DNA and microsatellite analyses. *BioMed Research International*. vol 2013: 1–15. doi: <https://doi.org/10.1155/2013/170980>.
- Excoffier L, Lischer HEL. 2010. Arlequin suite Ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*. vol 10(3): 564–567. doi: <https://doi.org/10.1111/j.1755-0998.2010.02847.x>.
- Hubert N, Meyer CP, Bruggemann HJ, Guérin F, Komono RJL, Espiau B, Causse R, Williams JT, Planes S. 2012. Cryptic diversity in indo-pacific coral-reef fishes revealed by DNA-barcoding provides new support to the centre-of-overlap hypothesis. *PLoS One*. vol 7(3): 1–8. doi: <https://doi.org/10.1371/journal.pone.0028987>.
- Jacoby D, Gollock M. 2014. *Anguilla marmorata*. The IUCN red list of threatened species 2014: E.T166189A45832585. doi: <https://www.iucnredlist.org/> doi: <https://doi.org/http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T166189A45832585.en>.
- Jacoby D, Harrison IJ, Gollock M. 2014. *Anguilla bicolor*. The IUCN red list of threatened species 2014: E.T166894A67015710. doi: <https://www.iucnredlist.org/> doi: <https://doi.org/http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T166894A67015710.en>.
- Fahmi MR. 2015. Short communication: Conservation genetic of tropical eel in Indonesian waters based on population genetic study. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*. vol 1(1): 38–43. doi: <https://doi.org/10.13057/psnmbi/m010106>.
- Fahmi MR, Hirnawati R. 2010. Keragaman ikan sidat tropis (*Anguilla* sp.) di perairan Sungai Cilandir, Pelabuhan Ratu, Sukabumi. *Prosiding Forum Inovasi Teknologi Akuakultur*. vol 1(1): 1–8.
- Froese R, Pauly D. 2019. World wide web electronic publication. www.fishbase.org. Assessed August 2020.
- Hall T. 2011. BioEdit: an important software for molecular biology. *GERF Bulletin of Biosciences*. vol 2(1): 160–161. doi: <https://doi.org/10.1017/S0317167100012865>.
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*. vol 270: 313–321. doi: <https://doi.org/10.1098/rspb.2002.2218>.
- Hughes JM, Schmidt DJ, Finn DS. 2009. Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. *BioScience*. vol 59(7): 573–583. doi: <https://doi.org/10.1525/bio.2009.59.7.8>.

- Kanno Y, Vokoun JC, Letcher BH. 2011. Fine-scale population structure and riverscape genetics of brook trout (*Salvelinus fontinalis*) distributed continuously along headwater channel networks. *Molecular Ecology*. vol 20(18): 3711–3729. doi: <https://doi.org/10.1111/j.1365-294X.2011.05210.x>.
- Ko HL, Wang YT, Chiu TS, Lee MA, Leu MY, Chang KZ, Chen WY, Shao KT. 2013. Evaluating the accuracy of morphological identification of larval fishes by applying DNA barcoding. *PLoS One*. vol 8(1): 3–9. doi: <https://doi.org/10.1371/journal.pone.0053451>.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*. vol 35 (6): 1547–1549. doi: <https://doi.org/10.1093/molbev/msy096>.
- Liu X, Cao Y, Xue T, Wu R, Zhou Y, Zhou C, Wu X. 2017. Genetic structure and diversity of *Nodularia douglasiae* (Bivalvia: Unionida) from the middle and lower Yangtze River drainage. *PLoS One*. vol 12(12): 1–23. doi: <https://doi.org/10.1371/journal.pone.0189737>.
- Matarese AC, Spies IB, Busby MS, Orr JW. 2011. Early larvae of *Zesticelus profundorum* (Family Cottidae) identified using DNA barcoding. *Ichthyological Research*. vol 58(2): 170–174. doi: <https://doi.org/10.1007/s10228-010-0198-7>.
- Minegishi Y, Gagnaire PA, Aoyama J, Bosc P, Feunteun E, Tsukamoto K, Berrebi P. 2012. Present and past genetic connectivity of the Indo-Pacific tropical eel *Anguilla bicolor*. *Journal of Biogeography*. vol 39(2): 408–420. doi: <https://doi.org/10.1111/j.1365-2699.2011.02603.x>.
- Muchlisin ZA, Batubara AS, Fadli N, Muhammadar AA, Utami AI, Farhana N, Siti-Azizah MN. 2017. Assessing the species composition of tropical eels (Anguillidae) in Aceh Waters, Indonesia, with DNA barcoding gene Cox1. *F1000Research*. vol 6: 1–10. doi: <https://dx.doi.org/10.12688/2Ff1000research.10715.1>.
- Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.
- Nuryanto A, Pramono H, Sastranegara MH. 2017a. Molecular identification of fish larvae from east plawangan of Segara Anakan, Cilacap, Central Java, Indonesia. *Biosaintifika: Journal of Biology & Biology Education*. vol 9(1): 33–40. doi: <https://doi.org/10.15294/biosaintifika.v9i1.9191>.
- Nuryanto A, Qonita NH, Pramono H, Kusbiyanto K, Soedibja PHT. 2017b. Genetic Variation In Cytochrome B-Hinfl And -AluI Gene Correlated To Body Size In Soang Gourami (*Osphronemus Goramy* Lacepede, 1801) From Single Spawning. *Biosaintifika: Journal of Biology & Biology Education*. vol 9(2): 185–192. doi: <https://doi.org/10.15294/biosaintifika.v9i2.9301>.
- Nuryanto A, Amalia G, Khairani D, Pramono H, Bhagawati D. 2018. Molecular Characterization Of Four Giant Gourami Strains From Java And Sumatra. *Biodiversitas*. vol 19(2): 578–584. doi: <https://doi.org/10.13057/biodiv/d190228>.
- Nuryanto A, Komalawati N, Sugiharto. 2019. Genetic diversity assessment of *Hemibagrus nemurus* from rivers in Java Island, Indonesia using COI gene. *Biodiversitas*. vol 20(9): 2707–2717. doi: <https://doi.org/10.13057/biodiv/d200936>.
- Olsen JB, Crane PA, Flannery BG, Dunmall K, Templin WD, Wenburg JK. 2011. Comparative landscape genetic analysis of three Pacific salmon species from subarctic North America. *Conservation Genetics*. vol 12(1): 223–241. doi: <https://doi.org/10.1007/s10592-010-0135-3>.
- Parmaksiz A, Eksi E. 2017. Genetic diversity of the cyprinid fish *Capoeta trutta* (Heckel, 1843) populations from Euphrates and Tigris Rivers in Turkey based on mtDNA COI sequences. *Indian Journal of Fisheries*. vol 64(1): 18–22. doi: <https://doi.org/10.21077/ijf.2017.64.1.62396-03>.
- Pavesi L, de Mattheis E, Tiedemann R, and Ketmaier V. 2011. Temporal population genetics and COI phylogeography of the sandhopper *Macarorchestia remyi* (Amphipoda: Talitridae). *Zoological Studies*. vol 50(2): 220–229.
- Pegg GG, Sinclair B, Briskey L, Aspden WJ. 2006. MtDNA barcode identification of fish larvae in the southern Great Barrier Reef, Australia. *Scientia Marina*. vol 70(2): 7–12. doi: <https://doi.org/10.3989/scimar.2006.70s27>.
- Pereira LHG, Hanner R, Foresti F, Oliveira C. 2013. Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? *BMC Genetics*. vol 14: 1–14. doi: <https://doi.org/10.1186/1471-2156-14-20>.
- Pike C, Crook V, Jacoby D, Gollock M. 2020. *Anguilla bicolor* (amended version of 2019 assessment). The IUCN red list of threatened species 2020: e.T166894A176494582. doi: <https://www.iucnredlist.org/> doi: <https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T166894A176494582.en>.
- Rachmawati FN, Susilo U. 2011. Profil hormon dan kinerja reproduksi ikan sidat (*Anguilla bicolor* McClelland) yang tertangkap di perairan Segara Anakan Cilacap. *Journal of Biota*. vol 16(2): 221–226. doi: <https://doi.org/10.24002/biota.v16i2.103>.
- Ratnasingham S, Hebert PDN. 2013. A DNA-based registry for all animal species: the barcode index number (BIN) system. *PLoS One*. vol 8(8): 1–16. doi: <https://doi.org/10.1371/journal.pone.0066213>.
- Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, and Sanchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*. vol 34(12): 3299–3302. doi: <https://doi.org/10.1093/molbev/msx248>.
- Sabando MC, Vila I, Peñaloza R, Véliz D. 2011. Contrasting population genetic structure of two

- widespread aquatic insects in the Chilean high-slope rivers. *Marine and Freshwater Research*. vol 62(1): 1–10. doi: <https://doi.org/10.1071/MF10105>.
- Sachithanandam V, Mohan PM, Muruganandam N, Chaitanya IK, Dhivya P, Baskaran R. 2012. DNA barcoding, phylogenetic study of *Epinephelus* spp. from Andaman Coastal Region, India. *Indian Journal of Geo-Marine Sciences*. vol 41(4): 203–211.
- Song LM, Munian K, Rashid ZA, Bhassu S. 2013. Characterisation of Asian snakehead murrel *Channa striata* (Channidae) in Malaysia: an insight into molecular data and morphological approach. *The Scientific World Journal*. vol 2013: 1–17. doi: <https://doi.org/10.1155/2013/917506>.
- Sugeha HY, Bataragoa NE, Wouthuyzen S. 2008. Inshore migration of the tropical Anguillid Glass Eels recruited in the estuary of Dumoga River (North Sulawesi, Indonesia). Prosiding Seminar Nasional Ikan V. June 3, 2008. Bogor: Masyarakat Iktiologi Indonesia. ISBN 979-3688-89-0. hal 225–237.
- Sugeha HY. 2010. Recruitment mechanism of the tropical glass eels genus *Anguilla* in the Poso Estuary, Central Sulawesi Island, Indonesia. *Jurnal Perikanan*. vol XII(2): 86–100.
- Sugeha HY, Arai T. 2010. Contrasting morphology, genetic, and recruitment season of *Anguilla marmorata* glass eels from northern, western, and central Sulawesi Island, Indonesia. *Ilmu Kelautan*. vol 1: 1–19.
- Sugeha HY, Genisa MU. 2015. External and internal morphological characteristics of glass eels *Anguilla* bicolor bicolor from the Cibaliung River Estuary, Banten, Indonesia. *Oceanologi dan Limnologi di Indonesia*. vol 41 (1): 37–48.
- Tanaka C, Shirotori F, Sato M, Ishikawa M, Yoshinaga T, Shinoda A, Aoyama J. 2014. Genetic identification method for two subspecies of the Indonesian short-finned eel, *Anguilla bicolor*, using an allelic discrimination technique. *Zoological Studies*. vol 53(1): 1–7. doi: <https://doi.org/10.1186/s40555-014-0057-8>.
- Wouthuyzen S, Aoyama J, Sugeha HY, Miller MJ, Kuroki M, Minegishi Y, Suharti SR, Tsukamoto K. 2009. Seasonality of spawning by tropical anguillid eels around Sulawesi Island, Indonesia. *Naturwissenschaften*. vol 96(1): 153–158. doi: <https://doi.org/10.1007/s00114-008-0457-x>.
- Yan RJ, Zhang GR, Guo XZ, Ji W, Chen KC, Zou GW, Wei KJ, and Gardner JPA. 2018. Genetic diversity and population structure of the northern snakehead (*Channa argus* Channidae: Teleostei) in Central China: implications for conservation and management. *Conservation Genetics*. vol 19(2): 467–480. doi: <https://doi.org/10.1007/s10592-017-1023-x>.
- Zhang GH, Yuan ZJ, Zhang CX, Yin KS, Tang MJ, Guo HW, Fu JY, Xiao Q. 2014. Detecting deep divergence in seventeen populations of tea geometrid (*Ectropis obliqua* Prout) in China by COI MtDNA and cross-breeding. *PLoS One*. vol 9(6): 1–9. doi: <https://doi.org/10.1371/journal.pone.0099373>.