Global Ecology and Conservation 26 (2021) e01493

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: http://www.elsevier.com/locate/gecco

Original Research Article

Genetic diversity, population structure and demographic history of the tropical eel *Anguilla bicolor pacifica* in Southeast Asia using mitochondrial DNA control region sequences

Melfa Marini [a, b, *], Ivane R. Pedrosa-Gerasmio [c], Mudjekeewis D. Santos [d], Takuro Shibuno [a], Ayu Daryani [a], Maria Rowena R. Romana-Eguia [e], Arif Wibowo [a, b]

[a] *Inland Fishery Resources Development and Management Department, Southeast Asian Fisheries Development Center, Jl. Gubernur H.A Bastari No 08, Jakabaring Palembang, 30252, Indonesia*
[b] *Research Institute for Inland Fishery and Extension, Ministry of Marine Affairs and Fisheries, Jl. Gubernur H. A. Bastari No. 08, Kel. Silaberanti, Kec. Seberang Ulu I, Palembang, South Sumatera, 30252, Indonesia*
[c] *Department of Marine Sciences, College of Science and Mathematics, Mindanao State University – Iligan Institute of Technology, Iligan City, Philippines*
[d] *Genetic Fingerprinting Laboratory, National Fisheries Research and Development Institute, 101 Mother Ignacia St., Quezon City, Philippines*
[e] *Aquaculture Department, Southeast Asian Fisheries Development Center, Binangonan Freshwater Station, Binangonan, 1940, Rizal, Philippines*

ARTICLE INFO

Article history:
Received 19 May 2020
Received in revised form 7 February 2021
Accepted 7 February 2021

Keywords:
Tropical eel
Conservation
Haplotypes
Gene flow
Panmixia

ABSTRACT

The tropical catadromous eel, *Anguilla bicolor pacifica,* an important fishery resource in Southeast Asia, is under threat due to overexploitation (especially of its glass eel phase) and the limited information on their current genetic status which is necessary for resource management. Mitochondrial DNA (mtDNA) control region sequences, a useful marker for population genetic studies in many aquatic organisms, were used to investigate the genetic diversity, population structure and demographic history of *A. bicolor pacifica* in the region. A total of 151 specimens were collected from three sites in Southeast Asia, namely: Phu Yen, Vietnam (n = 48); General Santos, Philippines (n = 52); and, Palu, Indonesia (n = 51). A total of 138 haplotypes were identified using the mtDNA control region sequences. In spite of the lack of shared haplotypes, low and non-significant F_{ST} values, high haplotype diversity in concurrence with relatively low nucleotide diversity, a haplotype network with no phylogeographic structuring indicate no significant genetic population structuring among the eel samples from Vietnam, Philippines and Indonesia. Population expansion of *A. bicolor pacifica* was also suggested based on the results of the neutrality tests, mismatch distribution analysis and Bayesian skyline plot. Taken together, a joint management strategy for *A. bicolor pacifica* must involve countries in Southeast Asia particularly Vietnam, Philippines and Indonesia for its sustainable use.

* Corresponding author. Jl. Gubernur H.A Bastari No. 08, Jakabaring, 8 Ulu, Kecamatan Seberang Ulu I, Kota Palembang, Sumatera Selatan, 30267, Indonesia.
E-mail address: melfa.marini@kkp.go.id (M. Marini).

https://doi.org/10.1016/j.gecco.2021.e01493

1. Introduction

The tropical anguillid eel, such as the shortfin eel, *Anguilla bicolor*, is one of the most economically important species emerging in Southeast Asia (Edge, 1939; Arai et al., 1999; Suryati et al., 2019). *Anguilla bicolor* is the preferred second choice to *A. japonica* due to its similar texture and taste, hence it is considered economically important in terms of market demand (Arai 2014 as cited in Shiraishi and Crook, 2015). It has a relatively wide geographic distribution compared to most of the 19 species and subspecies of the genus *Anguilla* (Watanabe et al., 2005a; Fahmi et al., 2015a, b). *Anguilla bicolor* was reported to inhabit sub-tropical to tropical regions, mostly in the waters around Indonesian archipelagos (Aoyama et al., 2001; Watanabe et al., 2005a; Minegishi et al., 2012; Sugeha and Suharti, 2008).

Anguilla bicolor has two valid subspecies; *A. bicolor bicolor* and *A. bicolor pacifica* (Edge, 1939; Aoyama et al., 2001; Watanabe et al., 2005a; Sugeha and Suharti, 2008; Tanaka et al., 2014), which have very similar characteristic morphologies but vary in geographical distribution. *Anguilla bicolor bicolor* is restricted to the Indian Ocean while *A. bicolor pacifica* occurs in natural waters of the Indo-Pacific area (Tseng, 2012), the Western Pacific Ocean, all the seas around the northern part of Indonesia (Edge, 1939; Sugeha and Suharti, 2008; Fahmi et al., 2015a, b), and the coast of China, Vietnam, Philippines, Borneo Island, Sulawesi Island and New Guinea Island (Watanabe et al., 2005a, b).

Hence of the two subspecies, *Anguilla bicolor pacifica* is considered an important fishery resource around Southeast Asia, particularly in the Philippines, Vietnam, and Indonesia (Crook, 2014; Gollock et al., 2018). In order to meet the requirements of eel farmers in these countries, large numbers of glass eels are caught from the wild since production of hatchery-bred seedstock has not yet been successful (Tanaka et al., 2014). According to the Food and Agriculture Organization (FAO) data, wild-caught juvenile eels (glass eels or eel fry) constitute 90% of the total anguillid production worldwide (Shiraishi and Crook, 2015). At present, only a few studies on the population differentiation within the subspecies of *A. bicolor* are available (Sugeha and Suharti, 2008; Fahmi et al., 2015a, b). *Anguilla bicolor pacifica* is more widely distributed (Aoyama et al., 2018), but there is still little evidence about the location of their spawning areas (Kuroki et al., 2019).

Since the supply of glass eels for aquaculture depends exclusively on wild populations, eel fishery will inevitably decrease drastically. Freshwater eels have shown drastic decreases worldwide for the last three decades (Aoyama, 2009). Nonetheless, future strategies for the production of eel seedstock in hatcheries require data on the genetic diversity and population structure of wild stocks for successful broodstock and genetic resources management. In the future, efforts to sustainably use and improve eel stocks may rely on managing fisheries at various life history stages, to include in particular, restocking (Cucherousset et al., 2007). Should restocking be considered, it may be referenced as obtaining glass eels in Asian countries and stocking them into ponds, lakes or rivers of different countries, which is understood as a rather debatable approach. Any plan of introducing eel stocks into a water body must always be preceded by an investigation of potential genetic impacts and should be conducted with the objectives of minimizing or if possible, being devoid of any harmful genetic consequences (Grant et al., 2017).

Many studies on the population structure of anguillids are mainly for temperate eel species, i.e. *A. japonica* (Ishikawa et al., 2001; Tseng et al., 2006), *A. rostrata* (Côté et al., 2013), and *A. anguilla* (Daemen et al., 2001; Wirth and Bernatchez, 2001). Only a few studies have been done on the population structure of the tropical eel species, such as *A. marmorata* (Robinet et al., 2003; Ishikawa et al., 2004; Maes et al., 2006; Minegishi et al., 2008; Gagnaire et al., 2011); *A. bicolor* (Minegishi et al., 2012; Fahmi et al., 2015a); *A. bicolor bicolor* (Watanabe et al., 2005b); and *A. megastoma* (Gubili et al., 2019). Currently, there is no detailed region-wide study on the population structure of *A. bicolor pacifica*.

This study aims to reveal the genetic differences between *A. bicolor pacifica* collected from three countries in Southeast Asia (Vietnam, Philippines and Indonesia) and to determine the population structure of *A. bicolor pacifica* using mitochondrial DNA (mtDNA) control region sequences, which has been a useful marker for population genetic studies in many aquatic organisms in the region (Pedrosa-Gerasmio et al., 2015; Willette et al., 2015; Santos et al., 2010). The mtDNA control region is a non-coding region that includes signals necessary for replication of a molecule and is the most rapidly evolving region of mtDNA (Heyer et al., 2001). MtDNA evolves four times faster than the average nuclear gene. Hence, mtDNA can be used to follow divergence in very closely related taxa and even within species (De Salle et al. 2017). The results of this study will be helpful for resource protection and sustainable utilization of *A. bicolor pacifica*.

2. Materials and methods

2.1. Sampling, DNA extraction and sequencing

A total of 151 individuals of *A. bicolor pacifica* were collected from three sites in Southeast Asia (Fig. 1): Phu Yen, Vietnam (n = 48); General Santos, Philippines (n = 52); and Palu, Indonesia (n = 51). The samples were gathered from 2018 to 2019 by using a fyke net. Samples collected from Indonesia and Vietnam comprised of elvers, while the Philippine samples were glass eels. Approximately 1.0 g of muscle tissue was obtained from the dorsal portion of each fish and placed in individual 1.5 ml Eppendorf tubes (containing 95% ethanol) prior to extraction using a Quick-DNA™ Miniprep Plus Kit (Zymo Research), following the manufacturer's protocol.

The hypervariable site of the mtDNA control region for *A. bicolor pacifica* was amplified using the forward and reverse primers L15774 (5′-ACA TGA ATT GGA GGA ATA CCA GT-3′; Shields and Kocher 1991) and H16498aj (5′-CCT GAA ATA GGA ACC AAA TG-3′ (Tanaka et al., 2014), respectively. This universal primer pair was previously used to amplify a partial DNA

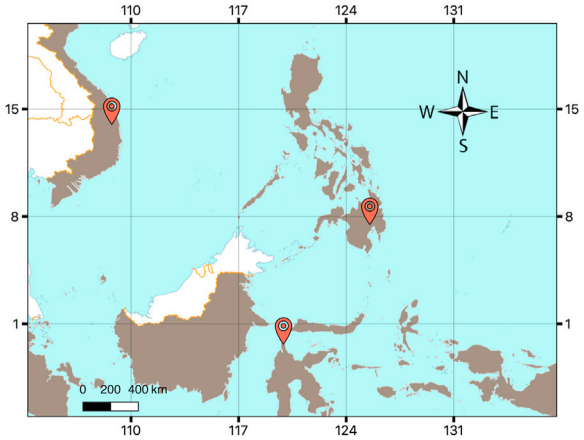

Fig. 1. Sampling sites (red pointer): Vietnam (Phu Yen), Philippines (General Santos) and Indonesia (Palu). This map was generated using QGIS v3.10 software (QGIS Development Team, 2016). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

nucleotide sequence of the mtDNA control region in many organisms (Gunduz et al., 2007; Saarma and Kojola, 2007), including eel (Tanaka et al., 2014, with modified reverse primer). The mtDNA control region was amplified in 50 μl polymerase chain reactions containing 20 μl H_2O, 25 μl Quick Taq® HS DyeMix of Toyobo, 1 μl forward primer, 1 μl reverse primer and 3 μl of DNA template.

The PCR condition consisted of an initial denaturation at 94 C for 2 min, followed by 35 cycles of 94 °C denaturation for 30 s, 50 °C annealing for 30 s, 68 °C extension for 1 min, and final extension of 72 °C for 30 s. After amplification, the PCR products were evaluated for quality and quantity using 1% agarose gels stained with SYBR Safe. PCR amplicons and the same primers (L15774 and H16498aj) were used for DNA sequencing at the Sequencing Centre of the 1st base (http://www.base-asia.com/dna-sequencing-services). All sequences were stored in the public domain database in GenBank; the registered sequences were attributed to the accession numbers MW079028 – MW079178.

2.2. Data analyses

2.2.1. Genetic diversity and population structure

Consensus sequences were created using BioEdit v7.2.5 (Hall, 2013) and aligned and edited using MEGA v6 (Tamura et al., 2013). The number of haplotypes and polymorphic sites were determined using DnaSP v5 (Librado and Roxas, 2009). Using the program Arlequin v3.5 (Excoffier and Lischer 2010), the following were estimated: a) haplotype or gene diversity (hd) and nucleotide diversity (π), and their corresponding variances; b) analysis of molecular variance (AMOVA) to obtain the genetic differentiation indices (F_{ST}) and genetic variation partitioning within and among populations (Vietnam, Philippines and Indonesia); and, c) pairwise population comparisons following the Tamura and Nei distance methods (Tamura and Nei, 1993). This method was developed for the control region of the mtDNA and takes into account excess transitions, inequality of nucleotide frequencies and differences in substitution rate among nucleotides (Tamura and Nei, 1993). Using the program NETWORK v4.6.1.0 (copyright 2004–2012, Fluxus Technologies Ltd.; http://www.fluxus-engineering.com), median joining haplotype networks were drawn to visually illustrate haplotype variability.

2.2.2. Demographic history

To test for demographic expansion, Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) tests were implemented in Arlequin v3.5 (Excoffier and Lischer 2010), and *p*-values were generated using 1000 simulations. Tajima's D (Tajima, 1989) makes use of the frequency of the number of variable positions (segregating or polymorphic nucleotide sites), while Fu's Fs (Fu, 1997) uses the distribution of haplotypes. These neutrality tests can be used to investigate demographic history and detect selection in cases where DNA polymorphism deviates from those predicted by the Wright-Fisher neutral model of evolution (Tajima, 1989; Fu, 1997).

Mismatch distribution analysis was also employed to further test for demographic history and expansion. All the figures illustrating the frequency of pairwise comparison (y-axis) between individuals with corresponding number of pairwise differences (x-axis) were generated using DnaSP v5 (Librado and Roxas, 2009). Expected demographic parameters (τ, θ_0, θ_1, Harpending's raggedness index (Hri) and sum of squared deviation (SSD)) were estimated in Arlequin v3.5 (Excoffier and Lischer 2010), based on the sudden expansion model (Rogers and Harpending, 1992). Additionally, changes in female effective population size (N_{ef}) across time were inferred using Bayesian skyline plot analysis implemented in BEAST 2.6.2 with XML files prepared using BEAUti v2 (Bouckaert et al., 2014). MEGA was used to determine the best-fit nucleotide substitution

model (i.e. HKY + G) based on Bayesian Information Criterion (BIC). A standard Markov chain Monte Carlo (MCMC) analysis was run for 10 million generations (with a burn-in of 1 million), sampled every 1000 iterations under the HKY + G model. The mutation rate was set at 3.6% per million years, based on the average mutation rate previously identified using mtDNA control region sequences in teleosts (Donaldson and Wilson, 1999). Convergence of the parameters' values was visualized in Tracer v1.7.1 (Rambaut et al., 2018).

3. Results

3.1. Genetic diversity

Based on the 470-bp fragment of the mtDNA control region of 151 samples, 138 haplotypes of *A. bicolor pacifica* (47, 52, and 39 haplotypes from Vietnam, Philippines and Indonesia, respectively) were identified. For Vietnam, only one haplotype is composed of two samples, while the rest appear as singletons (Supplementary Figure 1; Supplementary Figure 2). For Philippines, all haplotypes were unique (Supplementary Figure 3), appearing as singletons in the haplotype network (Supplementary Figure 1). In the case of Indonesia, 12 haplotypes are composed of two samples each and the remaining are singletons (Supplementary Figure 1; Supplementary Figure 4). No haplotypes were shared among the three sampling sites. The haplotype diversities (hd) in all sampling locations were high, with the lowest hd found in samples from Indonesia (hd = 0.9906 + 0.0054 SD), and the highest hd from samples collected in the Philippines (hd = 1.0000 + 0.0038 SD; Table 1). For the nucleotide diversity values (π), samples collected in Vietnam showed the lowest π (π = 0.0429 + 0.0214) while the highest π was from samples collected in the Philippines (π = 0.0469 + 0.0233; Table 1).

3.2. 2.4. population genetic structure

AMOVA showed that the percentage of variation within population constituted 99.65%, while the variation among populations was only 0.35% (Table 2). The F_{ST} value is low (F_{ST} = 0.00347) and not significant (Table 2), which indicates no geographic population structuring of *A. bicolor pacifica* from the three sampling sites. Furthermore, population pairwise F_{ST} results were low and all comparisons were not significant (Table 3).

The median-joining haplotype network (Supplementary Figure 1) generated for *A. bicolor pacifica* from the three sampling sites showed many haplotypes, with very low frequency (the highest haplotype frequency is 2). The data sets did not separate into geographic locations. The high haplotype diversity values previously mentioned were clearly reflected in this haplotype network (Supplementary Figure 1).

3.3. Demographic history

Fu's Fs and Tajima's D values (Table 4) were negative in all sampling sites and most of the *p*-values were statistically significant (except for Indonesia, Tajima's D), which is suggestive of population growth or expansion. Significant negative values indicated an excess of rare haplotypes and rejection of the null hypothesis of neutral evolution (Fu, 1997; Tajima, 1989).

The mismatch distributions of *A. bicolor pacifica* from Vietnam, Philippines and Indonesia exhibited a unimodal distribution (Fig. 2a–c), which were not significantly different (measured by the SSD having $p > 0.05$) from that predicted by the growth expansion model (Rogers and Harpending, 1992). Hri values were low for all sampling sites, ranging from 0.00191 to 0.00427, which indicates a good fit between the observed and expected values and thus supporting population expansion. Estimated tau (τ) values of *A. bicolor pacifica* were nearly similar for all sampling sites, indicating that population expansion in all sites may date back to about the same historical periods (Harpending, 1994).

Using the mismatch parameters θ_0 and θ_1 (Harpending, 1994), the mean female effective population size after expansion (θ_1) was estimated to be approximately 300 times higher than before expansion (θ_0; Table 5). The resulting output of the MCMC analysis of A. *bicolor pacifica* using the Bayesian skyline plot model, showed that *A. bicolor pacifica* has experienced two events of rapid increase of effective population size: from 880,000 years ago to 690,000 years before present (Fig. 3) and from 600,000 years ago until about 520,000 years before the present (Table 5) assuming a lineage mutation rate of 3.6% per million years based on the average mutation rate previously identified in teleosts using mtDNA control region sequences (Donaldson and Wilson, 1999). From around 520,000 years ago until present, *A. bicolor pacifica* has a relatively stable effective population size.

Table 1
Sample size (n), number of haplotype (k), number of polymorphic sites (PS), clusters, haplotype diversity (hd) ± SD and nucleotide diversity (π) ± SD.

Sampling site	n	k	PS	hd±SD	π±SD
Vietnam (Phu Yen)	48	47	151	0.9991 ± 0.0045	0.0429 ± 0.0214
Philippines (General Santos)	52	52	172	1.0000 ± 0.0038	0.0469 ± 0.0233
Indonesia (Palu)	51	39	141	0.9906 ± 0.0054	0.0439 ± 0.2187

Table 2
Analysis of Molecular Variance (AMOVA) results for *A. bicolor pacifica*.

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variation
Among populations	2	24.635	0.03652 Va	0.35
Within populations	148	1551.113	10.48050 Vb	99.65
Fixation index (*FST*)	0.00346 (*p*-value = 0.18866)			

4. Discussion

This is the first population genetic structure study based on the mtDNA control region of *A. bicolor pacifica* inhabiting waters of Southeast Asia. These data are necessary for regional and/or local management and conservation initiatives for this valuable resource in terms of both biodiversity and economic development (Fahmi et al., 2015a, 2015b). Key objectives in population genetics provide an adequate description of the genetic structure of populations, reconstruct a genetic architecture and could deduce evolutionary factors (Avise, 1986).

4.1. Genetic diversity

Here, the analysis of the mtDNA control region indicated high haplotype (gene) diversity for all the sampling sites (Table 1), which could be attributed to the large effective population size of *A. bicolor pacifica* (Fig. 3). Populations having larger effective population size will have more haplotypes compared to those with smaller effective population size (Ewens, 1972). High haplotype diversity values in this study (i.e., overall hd = 0.9966, n = 151) concur with previous studies on several *Anguilla* species using mtDNA control region, including European eel, *A. anguilla* (hd = 0.9965, n = 125; Butkauskas *at al*., 2009), Japanese eel, *A. japonica* (hd = 0.9160, n = 273; Tseng et al., 2012) and the tropical giant mottled eel *A. marmorata* (hd = 0.9820 to 1.000; Ding et al., 2012). Being catadromous, *A. bicolor pacifica* migrate flexibly among fresh, brackish and marine water environments, but was reported to prefer high salinity waters and thus, they were either marine or brackish water residents (Arai and Chino, 2019). The levels of genetic variability of most marine fish species tend to be higher than those of freshwater fish species (Hauser and Carvalho, 2008).

On the other hand, the nucleotide diversities for all the sampling sites in this study (ranging from 0.0429 to 0.0469, n = 151) were higher than that reported for *A. australis* from the western South Pacific (WSP) islands ($\pi = 0.0380$, n = 2; Gubili et al., 2019) and *A. bicolor* from the Indian Ocean ($\pi = 0.0340$, n = 70; Minegishi et al., 2012) and lower compared to *A. marmorata* ($\pi = 0.0650$, n = 259), *A. megastoma* ($\pi = 0.0560$, n = 42), *A. reinhardtii* ($\pi = 0.0650$, n = 68) and *A. obscura* ($\pi = 0.0690$, n = 33) from WSP islands using mtDNA control region sequences (Gubili et al., 2019).

4.2. Population genetic structure

Based on the results of AMOVA (low and nonsignificant F_{ST} value) and neighbor-joining haplotype network (no geographical structuring), there is no significant population genetic structuring for *A. bicolor pacifica* in the three sampling areas. It is generally accepted that an F*ST* value > 0.15 indicate significant genetic differentiation among sampling groups (Frankham et al., 2002). In this study, the F*ST* value was almost zero (F*ST* = 0.00347), which supports the hypothesis of no genetic structuring and panmixia. Similarly, based on partial nucleotide sequences of the mtDNA control region, haplotypes did not also assort by sample location in five species of sympatric Pacific eels, i. e *A. marmorata*, *A. megastoma*, *A. obscura*, *A. reinhardtii* and *A. australis* (Gubili et al., 2019). Eel species populations showed no distinct isolation by geographic area which suggests high levels of connectivity among their sampling locations in the western South Pacific (Gubili et al., 2019). Additionally, no significant genetic variation was observed within the Pacific Ocean populations (Philippines and Hitu) of *A. bicolor* (Minegishi et al., 2012).

The median-joining haplotype networks of the samples *A. bicolor pacifica* showed a bush-like shape mostly by singletons (Supplementary Figure 1), a pattern characteristic of DNA hyperdiversity and its F*ST* and median-joining haplotype networks showed no genetic partitioning based on geographic location. Such results were comparable to the *A. megastoma* and *A. marmorata* populations (Gubili et al., 2019) indicating panmixia in *A. bicolor pacifica* as well. The high gene flow of *A. bicolor pacifica* populations may be explained by its life cycle, migratory history and habitat use. Catadromous eels of the genus *Anguilla* have long spawning migrations (Tesch, 2003) and spawn in the offshore ocean (Chino and Arai, 2010). They have pelagic eggs and larvae that are spread or drift passively by ocean currents (Miller and Tsukamoto, 2017; Gong et al., 2019) and randomly choose their environments, which could possibly lead to local differences in genetic diversity. *A. bicolor pacifica* leptocephali (larvae) are believed to take around five to six months to migrate from their spawning site to their recruitment sites. The exact spawning area of *A. bicolor pacifica* is unknown (Miller and Tsukamoto, 2017; Aoyama et al., 2018), however it is suggested that spawning of *A. bicolor pacifica* occurs in an area of interconnecting currents and eddy systems (Miller and Tsukamoto, 2017) that could favor the wide distribution of their larvae. The recruitment and spawning seasons of tropical eels, including *A. bicolor pacifica*, were reported to extend throughout the year, which would lead to interconnectedness (Arai et al., 2001). Previous studies have reported that both *A. bicolor pacifica* and *A. marmorata* leptocephali collected around Indonesian waters were large, i.e., >30 mm long (Kuroki et al., 2006; Wouthuyzen et al., 2009) which indicates that these

Table 3
Population pairwise F_{ST} results with corresponding *p*-values in parenthesis.

	Vietnam	Philippines	Indonesia
Vietnam	–		
Philippines	−0.00132 (0.513)	–	
Indonesia	0.00696 (0.144)	0.00482 (0.126)	–

Table 4
Tajima's D and Fu's F_S with corresponding *p*-values in parenthesis.

Sampling site	Tajima's D	Fu's F_S
Vietnam (Phu Yen)	−1.59039 (0.03400*)	−24.18200 (0.00000**)
Philippines (General Santos)	−1.63435 (0.02300*)	−24.14552 (0.00000**)
Indonesia (Palu)	−1.35243 (0.05700)	−19.57791 (0.00400**)

Statistical significance $*p < 0.05$; $**p < 0.01$.

came from the North Pacific or the South Pacific and not from local spawning grounds (Aoyama et al., 2018). *A. bicolor pacifica* and *A. marmorata* glass eel recruits in Indonesian waters appear to come from two to three different spawning seasons or sources (Sugeha and Arai, 2010). Unlike *A. marmorata* and *A. bicolor pacifica*, other tropical eels species like *A. celebesensis, A. borneensis* and *A. interioris* appear to spawn locally after short migrations (Aoyama et al., 2018). High levels of gene flow, long stage larval durations, egg and larval dispersal and panmictic populations were also observed in other anguillid eels, both temperate and tropical (Arai and Kadir, 2017; Barth et al., 2020; Gubili et al., 2019).

While there is no genetic structuring of *A. bicolor pacifica* attributed to geographic locations based on the mtDNA control region sequences used in this study, it is interesting to note that the haplotypes seem to form two haplogroup clusters with representatives distributed almost equally from the three sampling locations (Supplementary Figure 1). It is not yet clear whether this was caused by the limitation of only one marker used in the study and thus, further investigations are needed, including the use of nuclear markers.

4.3. Demographic history

The result for neutrality tests suggests that the populations of *A. bicolor pacifica* in the three areas is expanding because of the strongly significant negative values from both Fu's F*s* indices and Tajima's D. Fu's F*s* is more sensitive in the detection of population expansion (Fu, 1997); hence, the results generally suggest population expansion for all the three sampling sites of *A. bicolor pacifica*. Moreover, standard deviations from the mean (τ) were low for all three studied sites which suggest a nearly identical distribution per sampling area with the implication that the expansion happened at approximately the same timeline for all sites. This could also be used as supporting evidence for panmixia since it appears that the distributions generated describes one identical population for all the three sampling areas of *A. bicolor pacifica*.

The Bayesian skyline plot (Fig. 3) also suggests population expansion of *A. bicolor pacifica* populations, initiating around the Pleistocene era, which was about 1.8 million years ago to 10,000 years before present (Hofreiter and Stewart, 2009). During this era, there were repeated glaciations and deglaciations causing sea-level and temperature fluctuations (Graham et al., 2003) and changes in current patterns and upwelling intensities (Lambeck et al., 2002). Since *A. bicolor pacifica* populations have expanded despite several environmental fluctuations, it is suggested that this fish is very resilient over evolutionary timescales. Patterns of population expansion in the Pleistocene era have also been reported in other eel species such as *A. marmorata, A. megastoma, A. obscura and A. reinhardtii* and *A. japonica* (Gubili et al., 2019; Tseng et al., 2012). However, despite a generally stable and large effective population size of *A. bicolor pacifica*, it should also be noted that a slight recent decrease in effective population size can be seen in the Bayesian skyline plot (Fig. 3). The population decline may possibly be attributed to habitat losses and anthropogenic barriers as shown in *A. rostrata* (Verreault et al., 2004) and *A. megastoma* (Gubili et al., 2019) populations. The use of more markers in future studies could verify this result.

5. Conclusion

No significant genetic structure of *A. bicolor pacifica* was observed among the three sampling areas in the Southeast Asian region using mtDNA control region sequences, suggesting that the populations are panmictic. These results, however, is only limited to the use of a single marker and given the pronounced genetic divergence, the genetic mosaic may indicate cryptic species, thus, the use of other strategies like nuclear markers, such as microsatellites, or next generation sequencing, which may be more sensitive in detecting genetic population structure, is suggested.

Proper management is deemed necessary since the supply of glass eels for aquaculture is still dependent on wild populations, which is subject to overexploitation. With the absence of genetic structuring among the sampling areas investigated in this study, future strategies for the sustainable management of *A. bicolor pacifica* must be the same in Southeast Asian region sharing this resource, particularly Vietnam, Philippines and Indonesia.

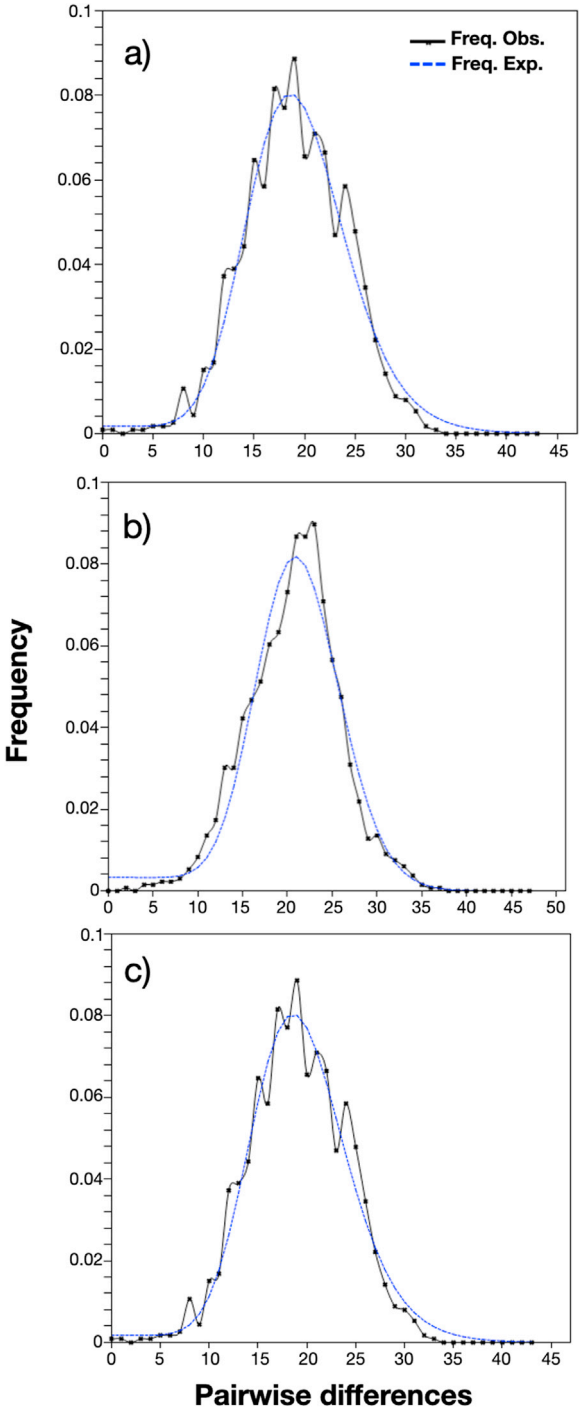

Fig. 2. Mismatch distribution of *A. bicolor pacifica* for three sampling sites: a) Vietnam; b) Philippines; and, c) Indonesia.

Table 5
Demographic parameters of *Anguilla bicolor pacifica* based on mtDNA control region sequence data: tau (τ), theta at time 0 (θ_0), theta at time 1 (θ_1), Harpending's raggedness index (Hri), and sum of squared differences (SDD).

Sampling site	τ	θ_0	θ_1	Hri	SSD
Vietnam (Phu Yen)	17.19531	2.47676	567.96875	0.00346	0.00101
Philippines (General Santos)	21.39648	0.05801	301.18164	0.00191	0.00112
Indonesia (Palu)	19.17773	1.19707	335.31250	0.00427	0.00159
Mean	19.25651	1.24395	401.48763	0.00321	0.00124
s.d.	1.71603	0.98801	118.54170	0.00098	0.00025

Statistical significance $*p < 0.05$.

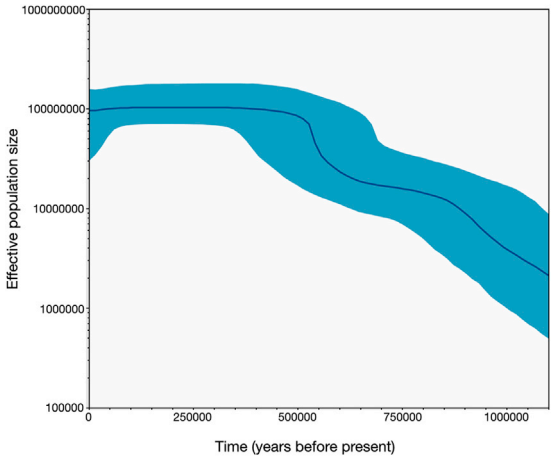

Fig. 3. Bayesian skyline plot of *A. bicolor pacifica* mtDNA control region sequences showing effective population size (Ne*f*) through time assuming a lineage mutation rate of 3.6% per million years. The solid line indicates the median estimate while the purple area reflects the standard deviation values of the estimated Ne*f*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study could not have been carried out without the help of the following contacts, especially during the sample collection: Dr. Maria Lourdes C. Aralar (SEAFDEC/AQD, Philippines), Dr. Evelyn Ame (Philippine Bureau of Fisheries and Aquatic Resources (BFAR)), Dr. Macmod Mamalangkap (BFAR-ARMM), Ying Parinthon (SEAFDEC Secretariat, Thailand), Ni K. Suryati (SEAFDEC/IFRDMD, Indonesia), Yanu Pamungkas (SEAFDEC/IFRDMD, Indonesia), Yeni S. Mulyani (SEAFDEC/IFRDMD, Indonesia), Dr. Nguyen Thanh Binh (Directorat of Fisheries, Viet Nam), and Pham Truong Giang (RIA-3, Viet Nam). The authors are also grateful to Prof. Husnah, M. Phil for her invaluable comments in the paper. This research received financial support from the Japan ASEAN Integration Fund Project entitled "*Enhancing Sustainable Utilization and Management Scheme of Tropical Anguillid Eel Resources*".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2021.e01493.

References

Aoyama, J., Nishida, M., Tsukamoto, K., 2001. Molecular phylogeny and evolution of the freshwater eel, genus *Anguilla*. Mol. Phylogenet. Evol. 20 (3), 450–459.
Aoyama, J., 2009. Life history and evolution of migration in catadromous eels (Genus Anguilla). Aqua-BioSci Monograps 2 (1), 1–42.
Aoyama, J., Wouthuyzen, S.S., Miller, M.J., Sugeha, H.Y., Kuroki, M., Watanabe, S., Syahailatua, A., Tantu, F.Y., Hagihara, S., Triyanto, Otake, T., Tsukamoto, K., 2018. Reproductive ecology and biodiversity of freshwater eels around Sulawesi Island Indonesia. Zool. Stud. 57, 30. https://doi.org/10.6620/ZS.2018.57-30.
Arai, T., 2014. Do we protect freshwater eels or do we drive them to extinction? SpringerPlus 3, 534, 2014.

Arai, T., Chino, N., 2019. Variations in the migratory history of the tropical catadromous eels *Anguilla bicolor bicolor* and *A. bicolor pacifica* in south-east Asian waters. J. Fish. Biol. 94 (5), 752–758.
Arai, T., Kadir, S.R.A., 2017. Opportunistic spawning of tropical anguillid eels *Anguilla bicolor bicolor* and A. *bengalensis bengalensis*. Sci. Rep. 7 (1), 1–17.
Arai, T., Limbong, D., Otake, T., Tsukamoto, K., 2001. Recruitment mechanisms of tropical eels Anguilla spp. and implications for the evolution of oceanic migration in the genus Anguilla. Mar. Ecol. Prog. Ser. 216, 253–264.
Arai, T., Limbong, D., Otake, T., Tsukamoto, K., 1999. Metamorphosis and inshore migration of tropical eels *Anguilla* spp. in the Indo-Pacific. Mar. Ecol. Prog. Ser. 182, 283–293.
Avise, J.C., 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. Philos. Trans. R. Soc. Lond. B Biol. Sci. 312 (1154), 325–342.
Barth, J.M., Gubili, C., Matschiner, M., Tørresen, O.K., Watanabe, S., Egger, B., et al., 2020. Stable species boundaries despite ten million years of hybridization in tropical eels. Nat. Commun. 11 (1), 1–13.
Bouckaert, R.R., Heled, J., Kuehnert, D., Vaughan, T.G., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. Beast 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10 (4), e1003537.
Butkauskas, D., Ragauskas, A., Sruoga, A., Ložys, L., Tzeng, W.N., 2009. Current knowledge about European eel *Anguilla Anguilla* (L.) mtDNA D-loop region haplotypic variety. Acta Zool. Litu. 19 (4) https://doi.org/10.2478/v10043-009-0040-5.
Chino, N., Arai, T., 2010. Occurrence of marine resident tropical eel *Anguilla bicolor bicolor* in Indonesia. Mar. Biol. 157 (5), 1075–1081.
Côté, C.L., Gagnaire, P.A., Bourret, V., Verreault, G., Castonguay, M., Bernatchez, L., 2013. Population genetics of the American eel (*Anguilla rostrata*): F ST= 0 and North Atlantic Oscillation effects on demographic fluctuations of a panmictic species. Mol. Ecol. 22 (7), 1763–1776.
Crook, V., 2014. Slipping Away: International Anguilla Eel Trade and the Role of the Philippines. TRAFFIC and ZSL, UK.
Cucherousset, J., Paillisson, J.M., Carpentier, A., Thoby, V., Damien, J.P., Eybert, M.C., Robinet, T., 2007. Freshwater protected areas: an effective measure to reconcile conservation and exploitation of the threatened European eels (*Anguilla anguilla*)? Ecol. Freshw. Fish 16 (4), 528–538.
Daemen, E., Cross, T., Ollevier, F., Volckaert, F.A.M.J., 2001. Analysis of the genetic structure of European eel (*Anguilla anguilla*) using microsatellite DNA and mtDNA markers. Mar. Biol. 139 (4), 755–764.
De Salle, R., Schierwater, B., Hadrys, H., 2017. MtDNA: the small workhorse of evolutionary studies. Frontiers in Bioscience, Landmark 22, 873–887.
Ding, X., Qi, X., Yin, S.W., 2012. Genetic variation and population evolutionary history of the giant mottled eel (*Anguilla marmorata*) based on the mitochondrial D-loop gene. Mar. Sci. (5), 20.
Donaldson, K.A., Wilson, R.R., 1999. Amphi-panamaic geminates of snook (Percoidei: centropomidae) provide a calibration of the divergence rates in the mitochondrial DNA control region of fishes. Mol. Phylogenet. Evol. 13, 208–213.
Edge, V., 1939. A revision of the genus Anguilla Shaw: a systematic, phylogenetic and geographical study. Dana-Report 16, 1–256.
Ewens, W.J., 1972. The sampling theory of selectively neutral alleles. Theor. Popul. Biol. 3 (1), 87–112.
Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of program to perform population genetics analysis under Linux and windows. Molecular Ecology Resources 10, 564–567.
Fahmi, M.R., Solihin, D.D., Shao, Z., Pouyaud, L., Berrebi, P., 2015a. Population genetic structure of the tropical eel *Anguilla bicolor* in Indonesian waters based on microsatellite markers. Folia Zoologica - Praha. https://doi.org/10.2525/fozo.v64.i2.a1.2015.
Fahmi, M.R., Solihin, D.D., Shao, Z., Pouyaud, L., Berrebi, P., 2015b. Molecular phylogeny and genetic diversity of freshwater Anguilla eels in Indonesian waters based on mitochondrial sequences. Vie Et Milieu-Life and Environment 65 (3), 139–150.
Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.
Fu, Y.X., 1997. Statistical test of neutrality of mutation against population growth, hitchhiking and background selection. Genetics 147, 915–925.
Gagnaire, P.A., Minegishi, Y., Zenboudji, S., Valade, P., Aoyama, J., Berrebi, P., 2011. Within-population structure highlighted by differential introgression across semipermeable barriers to gene flow in Anguilla marmorata. Evolution: International Journal of Organic Evolution 65 (12), 3413–3427.
Graham, M.H., Dayton, P.K., Erlandson, J.M., 2003. Ice ages and ecological transitions on temperate coasts. Trends Ecol. Evol. 18 (1), 33–40.
Grant, W.S., Jasper, J., Bekkevold, D., Adkison, M., 2017. Responsible genetic approach to stock restoration, sea ranching and stock enhancement of marine fishes and invertebrates. Rev. Fish Biol. Fish. 27 (3), 615–649.
Gollock, M., Shiraishi, H., Carrizo, S., Crook, V., Levy E-TRAFFIC Report, 2018. Status of non-CITES listed anguillid eels (cites.org).
Gong, X., Davenport, E.R., Wang, D., Clark, A.G., 2019. Lack of spatial and temporal genetic structure of Japanese eel (*Anguilla japonica*) populations. Conserv. Genet. 20 (3), 467–475.
Gubili, C., Schabetsberger, R., Poellabauer, C., Bates, B., Wagstaff, R.M., Woodward, L.M., Acou, A., 2019. High genetic diversity and lack of pronounced population structure in five species of sympatric Pacific eels. Fish. Manag. Ecol. 26 (1), 31–41.
Gunduz, I., Jaarola, M., Tez, C., Yeniyurt, C., Polly, P.D., Searle, J.B., 2007. Multigenic and morphometric differentiation of ground squirrels (*Spermophilus, Scuiridae, Rodentia*) in Turkey, with a description of a new species. Mol. Phylogenet. Evol. 43 (3), 916–935.
Harpending, H.C., 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum. Biol. 66, 591–600.
Hauser, L., Carvalho, G.R., 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 9 (4), 333–362.
Hall, T., 2013. BioEdit 7.2.5—Biological Sequence Alignment Editor. Ibis Biosciences, vol. 2251. An Abbott company, Faraday Avenue, Carlsbad, CA, 92008, 760-476-3375.
Heyer, E., Zietkiewicz, E., Rochowski, A., Yotova, V., Puymirat, L., Labuda, D., 2001. Phylogenetic and familial estimates of mitochondrial substitution rates: study of control region mutations in deep-rooting pedigrees. Am. J. Hum. Genet. 69 (5), 1113–1126.
Hofreiter, M., Stewart, J., 2009. Ecological change, range fluctuations and population dynamics during the Pleistocene. Curr. Biol. 19 (14), R584–R594.
Ishikawa, S., Aoyama, J., Tsukamoto, K., Nishida, M., 2001. Population structure of the Japanese eel *Anguilla japonica* as examined by mitochondrial DNA sequencing. Fish. Sci. 67, 246–253.
Ishikawa, S., Tsukamoto, K., Nishida, M., 2004. Genetic evidence for multiple geographic populations of the giant mottled eel *Anguilla marmorata* in the Pacific and Indian oceans. Ichthyol. Res. 51 (4), 343–353.
Kuroki, M., Aoyama, J., Miller, M.J., Wouthuyzen, S., Arai, T., Tsukamoto, K., 2006. Contrasting patterns of growth and migration of tropical anguillid leptocephali in the western Pacific and Indonesian Seas. Mar. Ecol. Prog. Ser. 309, 233–246.
Kuroki, M., Miller, M.J., Feunteun, E., Sasal, P., Pikering, T., Han, Y.S., et al., 2019. Distribution of anguillid leptocephali and possible spawning areas in the South Pacific Ocean. Prog. Oceanogr. 180, 102234.
Lambeck, K., Esat, T.M., Potter, E.K., 2002. Links between climate and sea levels for the past three million years. Nature 419 (6903), 199–206.
Librado, P., Roxas, J., 2009. DnaSP v5: a sofware for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Maes, G.E., Pujolar, J.M., Raeymaekers, J.A.M., Dannewitz, J., Volckaert, F.A.M., 2006. Microsatellite conservation and Bayesian individual assignment in four Anguilla species. Mar. Ecol. Prog. Ser. 319, 251–261.
Miller, M.J., Tsukamoto, K., 2017. The ecology of oceanic dispersal and survival of anguillid leptocephali. Can. J. Fish. Aquat. Sci. 74 (6), 958–971.
Minegishi, Y., Gagnaire, P.A., Aoyama, J., Bosc, P., Feunteun, E., Tsukamoto, K., Berrebi, P., 2012. Present and Past genetic connectivity of the Indo-Pacific tropical eel *Anguilla bicolor*. J. Biogeogr. 39, 408–420.
Minegishi, Y., Aoyama, J.U.N., Tsukamoto, K., 2008. Multiple population structure of the giant mottled eel, *Anguilla marmorata*. Mol. Ecol. 17 (13), 3109–3122.
Pedrosa-Gerasmio, I.R., Agmata, A.B., Santos, M.D., 2015. Genetic diversity, population genetic structure, and demographic history of *Auxis thazard* (Perciformes), *Selar crumenophthalmus* (Perciformes), *Rastrelliger kanagurta* (Perciformes) and *Sardinella lemuru* (Clupeiformes) in Sulu-Celebes Sea inferred by mitochondrial DNA sequences. Fish. Res. 162, 64–74.
QGIS Development Team, 2016. QGIS geographic information system. Open source geospatial foundation Project. http://qgis.osgeo.org.
Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67, 901–904.

Robinet, T., Sbaihi, M., Guyet, S., Mounaix, B., Dufour, S., Feunteun, E., 2003. Advanced sexual maturation before marine migration of *Anguilla bicolor bicolor* and *Anguilla marmorata* at Réunion Island. J. Fish. Biol. 63 (2), 538–542.
Rogers, A.R., Harpending, H., 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9 (3), 552–569.
Saarma, U., Kojola, I., 2007. Matrilineal genetic structure of the brown bear population in Finland. Ursus 18 (1), 30–37.
Santos, M.D., Lopez, G.V., Barut, N.C., 2010. A pilot study on genetic variation of eastern little tuna (*Euthynnus affinis*) in Southeast Asia. Philipp. J. Sci. 139 (1), 43–50.
Shiraishi, H., Crook, V., 2015. Eel Market Dynamics: an Analysis of Anguilla Production. TRAFFIC, Tokyo, Japan.
Shields, G.F., Kocher, T.D., 1991. Phylogenetic relationships of NorthAmerican ursids based on analysis of mitochondrial DNA. Evolution 45 (1), 218–221.
Sugeha, H.Y., Arai, T., 2010. Contrasting morphology, genetic, and recruitment season of Anguilla marmorata glass eels from northern, western, and central Sulawesi Island, Indonesia. Ilmu Kelautan 1, 1–19.
Sugeha, H.Y., Suharti, S.R., 2008. Discrimination and distribution of two tropical short-finned eels (*Anguilla bicolor bicolor* and *Anguilla bicolor pacifica*) in the Indonesian waters. ResearchGate. https://doi.org/10.5134/144634. The NAGISA WESTPAC Congress: 1-14, 2008.
Suryati, N.K., Pamungkas, Y.P., Muthmainnah, D., 2019. Addressing the issues and concerns on Anguillid eel fisheries in Southeast Asia. Fish for the People 17 (1), 19–24.
Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.
Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA7: molecular evolutionary genetics analysis version 7.0. Mol. Biol. Evol. 30, 2725–2729.
Tanaka, C., Shirotori, F., Sato, M., Ishikawa, M., Shinoda, A., Aoyama, J., Yoshinaga, T., 2014. Genetic Identification method for two subspecies of the Indonesian short-finned eel, *Anguilla bicolor*, using an allelic discrimination technique. Zool. Stud. 53, 57.
Tesch, F.W., 2003. The Eel. Biology and Management of Anguillid Eels. Chapman and Hall, London.
Tseng, M.C., 2012. Evolution of microsatellite loci of tropical and temperate Anguilla eels. Int. J. Mol. Sci. 13, 4281–4294. https://doi.org/10.3390/ijms13044281.
Tseng, M.C., Kao, H.W., Hung, Y.H., Lee, T.L., 2012. A study of genetic variation, population size, and population dynamic of the catadromous Japanese eel *Anguilla japonica* (Pisces) in northern Taiwan. Hidrobiologia (Buchar.) 683, 203–216.
Tseng, M.C., Tzeng, W.N., Lee, S.C., 2006. Population genetic structure of the Japanese eel *Anguilla japonica* in the northwest Pacific Ocean: evidence of non-panmictic populations. Mar. Ecol. Prog. Ser. 308, 221–230.
Verreault, G., Dumont, P., Mailhot, Y., 2004. Habitat Losses and Anthropogenic Barriers as a Cause of Population Decline for American Eel (Anguilla Rostrata) in the St. Lawrence Watershed. Canada. *International Council for the Exploration of the Sea. CM Document*.
Watanabe, S., Aoyama, J., Nishida, M., Tsukamoto, K., 2005a. A molecular genetic evaluation of the taxonomy of eels of the genus *Anguilla* (Pisces: anguilliformes). Bull. Mar. Sci. 76 (3), 675–690.
Watanabe, S., Aoyama, J., Nishida, M., Tsukamoto, K., 2005b. Evaluation of the population structure of *Anguilla bicolor bicolor* using total number of vertebrae and the mtDNA control region. Coastal Marine Science 29 (2), 165–169.
Willette, D.A., Santos, M.D., Leadbitter, D., 2015. Longtail tuna *Thunnus tonggol* (Bleeker, 1851) shows genetic partitioning across, but not within, basins of the Indo-Pacific based on mitochondrial DNA. J. Appl. Ichthyol. 1–6. https://doi.org/10.1111/jai.12991.
Wirth, T., Bernatchez, L., 2001. Genetic evidence against panmixia in the European eel. Nature 409 (6823), 1037–1040.
Wouthuyzen, S., Aoyama, J., Sugeha, H.Y., Miller, M.J., Kuroki, M., Minegishi, Y., Suharti, S., Tsukamoto, K., 2009. Seasonality of spawning by tropical anguillid eels around Sulawesi Island, Indonesia. Naturwissenschaften 96 (1), 153–158.