Multiple population structure of the giant eel *Anguilla marmorata* in Thua Thien Hue, Vietnam base on *COI* sequences

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Abstract

The giant mottled eel Anguilla marmorata is a species of great economic, ecological, and conservative values in the Southeast Asian region. The research aims to conservation and evaluation of the genetic diversity of A. marmorata populations living in Thua Thien Hue (TTH), Vietnam. The sequencing of the barcode region of the cytochrome c oxidase subunit 1 gene was carried out for 48 individuals of A. marmorata elvers, which were collected from five different ecosystem regions. The sequences were analyzed using various genetic, phylogenetic, and population analyses to assess their variability. A total of 20 polymorphic sites and 17 haplotypes were identified. Extremely low fixation (Fst = -0.073 – 0.003; < 0.05) was found for A. marmorata populations in the region. The populations showed signs of recent populations' expansion, besides negative Tajima's D test, Fu's Fs, Fu and Li's D* and F* test values. The genetic evolution of eel occurs in a randomized pattern over a large population, with the likelihood of rare alleles appearing in the population. In TTH (Viet Nam) territories, we indicated the five areas for conservation units with moderate eels' populations' diversity.

Introduction

In Viet Nam, there were 5 out of 19 species belong to the genus *Anguilla* (Watanabe et al. 2004; 2005), *A. marmorata* (Quoy & Gaimard, 1824) was identified to appear in the central region of Vietnam from Nghe An to Khanh Hoa (Nguyen et al. 2018). In This province, *A. marmorata* is widely distributed, exploited and cultivated with a high economic value (Huyen and Linh 2019). The species has a catadromous life-history strategy with distances from several hundred to thousands of kilometers when they were white grass eels from North ocean to South East Asian sea (Linh et al. 2010). During migration between oceans and freshwater during special stages of the life cycle, strong environmental changes have shaped not only their physiological characteristics (Wang et al. 2014) but also the genetic structure of eels (El-Nabi et al. 2017). Besides, upheaval conditions of river management, water containment, overfishing, and pollution may have influenced the movement of upstream eels (Wasserman et al. 2011) and downstream (Huyen and Phu 2015) led to the increase of population decline risk, on way back to oceans with mature and reproduction (Linh et al. 2010). Although in the world *A. marmorata* is ranked at LC level (least concern) (Pike et al. 2019), but it has been listed in the Viet Nam Red Data Book as VU (Vulnerable) lever since 2007 in Vietnam (Ministry of Science Technology and Environment 2007).

DNA barcoding provides an efficient method for species-level identifications (Hubert et al. 2008), to leading to the ecology and evolution of natural system was mentioned by (Kress et al. 2015), based on short, standardized gene regions. For animals, markers located on the mitochondrial genome, such as *cytochrom b (cytb*) gene, *cytochrom c oxyase 1 (COI)* gene, and *D-loop* region…. often have been recommended using by scientists (Kress et al. 2015). In which, a short DNA sequence in the mitochondrial gene encoding *COI* with 600 base pairs (bp) has been accepted as a practical, standardized, species-level DNA barcode for animals (Kress et al. 2015). To assess the genetic diversity of the *A. marmorata* population has distributed in TTH, Vietnam regarding the distribution environment and examining the evolution and development of populations, identifying conservation units for *A. marmorata* in TTHue, we designed this study basing on the amplification and sequencing of polymerase chain reaction (PCR) for the *COI* subunit of *A. marmorata* collected in 5 different ecological regions.

Materials And Methods

Ethics statement

All animal protocols were approved by the Committee on the Ethics of Animal Experiments of Hue University, Vietnam (permit No. DHH2019-02-113), and were performed strictly with the Guide for fishing capture and animals of Institute of Biotechnology. Animals were fishing capture by fishermen and allowed the Provincial Department of Fisheries.

Collection of specimens

Wild adult specimens of *A. marmorata* were collected from 5 localities in TTH, Viet Nam, including Phong Dien (PD), Thao Long dam (DTL), Truoi dam (DTR), Nam Dong (ND), Bu Lu and Lang Co (PL) from October 2017 to October 2018 (Fig. 1). *A. marmorata* species were identified using the keys developed by Watanabe et al. (2004). Tissues from the adductor muscle were dissected from fresh specimens, preserved in 95% ethanol, and frozen at -80 °C until DNA extraction.

Genomic DNA isolation and amplification

Total genomic DNA was extracted by a description of (Kumar et al. 2007) with a modification for *A. marmorata*. The primer used to amplify the *COI* gene segment was designed based on the nucleotide sequence of the two gene bank codes: *A. marmorata* - AP007242.1 and *A. marmorata* - HQ141374.1. The nucleotide sequences of the primers are A. marFw-1: 5'-GCACTAAGCTTCTAATCCG- 3' and A. marRv-1: 5'-GATGATTATTGTGGCAGAAG-3' (Huyen and Linh 2020). The PCR amplifications were performed in 35 µL reaction volumes: 1 µL DNA, 1 µL F-primer (10 mM), 1 µL R-primer (10 mM), 7 µL PCR buffer (10X), 0.5 µL dNTP (10 mM), 0.2 µL Enzyme Taq (5 UI/ µL) and sterile distilled water addition to full volume 35 µL. PCR amplification was performed on the machine (MJ-Mini™ Personal Thermal Cycle, Bio-Rad) according to the following thermal cycling: 95 ° C/5 minutes; followed by 30 cycles: 95 °C / 45 seconds, 51 °C / 30 seconds, and 72 °C / 1 minute; The last is 72^{o}C / 7 minutes. PCR product quality was checked by running electrophoresis on 1% agarose gel with buffers used as TAE 1X (Tris - acetate 40 mM + EDTA 1 mM) and stained with ethidium bromide fluorescent dye (EtBr 0, 5 µg / L) while washing the gel with distilled water for 10 minutes. The gel is observed by the Gel Documentation image analysis system.

Sequence alignment and molecular phylogenetic analysis

For all sequence analyses, *COI* genetic similarities were evaluated using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST) to identify *A. marmorata* sequences. The raw DNA sequences were edited using BioEdit (Hall 1999) and the pairwise, as

well as multiple alignments of sequences, which was performed using ClustalW (Larkin et al. 2007) alignment editor. Multiple sequence alignment was also checked manually, and the consensus sequences were obtained. Molecular phylogenetic analyses were performed in MEGA. X software (Kumar et al. 2018). Sequence data were subsequently analyzed for Neighbor Joining methods with bootstraps of 1000 replicates (Felsenstein 1985). Nucleotide composition analysis was carried out using BioEdit. Transition and transversions were equally weighted. The final consensus sequences were submitted to the National Center for Biotechnology Information (NCBI) database with accession number - MN067923 to MN067970. Standard genetic diversity indices, such as the number of haplotypes, polymorphic sites (S), haplotype numbers (h), haplotype diversity (Hd), nucleotide diversity (π), population mutation rates based on the number of segregation sites (θω) and mean the number of pairwise differences (θπ) was calculated with DnaSP 5.0 (Librado and Rozas 2009). Statistical analysis to distinguish DNA sequences evolving randomly (neutrality) with those evolving under a non-random process was done using Tajima's D (Tajima 1989); Fu's Fs tests; Fu & Li's Fs test and Fu & Li's D test (Tamura and Nei 1993). The Network version 5.0 software was implemented to estimate phylogenetic relationships among the unique haplotypes. Genetic differentiation, genetic distance, and migration rate among the populations were estimated by calculating the F statistic (Fst) between the populations and testing their significance with 1000 permutations.

Results

Genetic variation

Sequences of the 843 bp *COI* gene were determined in 48 specimens, 20 polymorphic sites, and 17 haplotypes were detected. 12 haplotypes were found in only one population, 2 (H1 and H9) were found equally between two populations, only 1 (H7) was found in three populations, and 2 (H4 and H5) were found in all five populations (Table 1 and Fig. 2). Haplotype positions were showed in Table 1.

Table 1
Variable position of 17 haplotypes of *COI* of *A. marmorata* in Thua Thien Hue, Vietnam

Haplo-type	GenBank accession number	Nucleotide position beginning from 5′ end																		
		39	75	171	174	189	210	237	270	342	387	420	582	585	611	657	690	747	804	808
H1	MN067923, MN067925, MN067938	A	C	C	T	T	C	T	G	G	A	G	T	A	T	A	C	C	A	A
H2	MN067924	*	*	*	*	*	T	C	*	*	*	A	*	*	*	*	*	*	*	*
H3	MN067926	*	*	*	*	*	*	C	*	A	*	A	*	*	C	*	T	*	*	*
H4	MN067927, MN067928, MN067929, MN067930, MN067932, MN067934, MN067937, MN067939, MN067943, MN067946, MN067947, MN067956, MN067958, MN067959, MN067960, MN067963, MN067964, MN067966, MN067967, MN067970	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*
H5	MN067931, MN067935, MN067940, MN067942, MN067950, MN067954, MN067962, MN067965	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	G	*
H6	MN067933	*	*	*		C		C	*	*	*	*	*	*	*	G	*	*	*	*
H7	MN067936, MN067945, MN067957	*	*	*	*	*	*	C	*	*		A	*	*	*	*	*	*	*	*
H8	MN067941	*	*	T	*	*	*	C	A	*	*	*	*	*	*	*	*	*	*	*
H9	MN067944, MN067969	*	*	*	*	*	*	C	*	*	G	*	*	*	*	*	*	*		*
H10	MN067948	C						C	*	*	*	*	*	*	*	G	*	*	*	*
H11	MN067949	*	*	*	*	*	*	C	*	*	*	*	G	G	*	G	*	*	G	*
H12	MN067951	C	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*
H13	MN067952	C	*	*	*	*	*	C	*	*	*	A	*	*	*	*	*	*	*	*
H14	MN067953	C	T	T	*	*	*	C	A	*	*	*	*	*	*	*	*	*	*	*
H15	MN067955	*	*	*	*	*	*	C	*	*	*	*	*	*	*	G	*	*	*	*
H16	MN067961	*	*	*	C			C	*	*	*	*	*	*	*	*	*	T	*	*
H17	MN067968	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	C

Haplotype diversity (h) and nucleotide diversity (π) based on *COI* genes for the 5 values are listed in Table 2. Haplotype diversity (h) and nucleotide diversity (π) values for the whole population in TTHue are 0.801 and 0.00196, respectively. Haplotype diversity of 5 populations in different eco-regions ranges from 0.7556 to 0.9000. In particular, the DTR population has the highest haplotype diversity value. The lowest belongs to DTL and PL. Nucleotide diversity values in these populations ranged from 0.00161 to 0.00242 with the highest in the PD population.

Concerning demographic estimators, methods of Tajima's D test, Fu's Fs, Fu, and Li's D* and F* test were used to test population neutrality (Tajima 1989), (Tamura and Nei 1993). The results in Table 2 show that all exposed exhibited significant negative values for all neutrality tests with $p < 0.05$. These indicators support standing 'expansion in these areas. The population of eel in TTH, evolved by a random selection expanded population, and rare alleles appear in the population with high frequency. Also, the value of Fu and Li's D * = -2.03402 ($p < 0.05$) indicated that in the study population, many individuals were showing a big difference compared to other individuals in the population. Besides, when considering the values of Tajima's D test, Fu's Fs, Fu and Li's D * and F * test in 5 individual populations according to the sampling area, the trend was similar but the value $p > 0.05$ except for Fu's Fs results for the *COI* gene in the PL. This shows that the difference in the individual eel is only significant when selected with large populations (14 individuals or more).

Table 2
Neutrality test results for *COI* data obtained for *A. marmorata* in TTH, Viet Nam

Population	DTL	DTR	ND	PD	PL	TTHue
No. of sample	10	5	9	14	10	48
H	5	4	6	9	5	17 (35.42%)
S	7	4	6	9	6	20
Hd	0.75556 ± 0.0168	0.9 ± 0.0259	0.88889 ± 0.00828	0.87912 ± 0.00621	0.75556 ± 0.01678	0.801 ± 0.00267
Π	0.00185	0.00190	0.00178	0.00242	0.00161	0.00196
θ	2.47	1.92	2.21	2.83	2.12	4.51
Θπ	1.56	1.60	1.50	2.04	1.36	1.65
Tajima's D	-1.573**	-1.0938	-1.3984	-1.06599	-1.4929	-2.03402*
Fu's Fs	-1.181*	-1.405*	-2.978*	-4.742*	-1.507	-12.228192*
Fu and Li's Fs	-1.818	-1.0938	-1.5509	-1.07521	-1.6893	-2.93225*
Fu and Li's D	-1.634	-1.0938	-1.3904	-0.8946	-1.51	-2.70391*

*Abbreviations: h haplotype numbers, S number of segregation sites, Hd haplotype diversity, π nucleotide diversity, θ Watterson's theta based on S, θπ theta based on π. *P < 0.05*

Population genetic structure

Significant F_{st} values were observed in all pairwise comparisons between populations for the *COI* gene ($p < 0.001$; Table 3). Pairwise F_{ST} values ranged from -0.073 to 0.048, indicating weak genetic differentiation between populations (F_{st}<0.05). The biggest difference occurred in two populations DTL and PD (F_{st} =0.04558), while Pairwise Nm value ranged from – 0.01961 to 0.011456 (< 1) indicating extremely low gene flow among the populations.

Table 3. Fst value and gene flow among 5 populations of *A. marmorata* in Thua Thien Hue, Viet Nam

Population	DTL	DTR	ND	PD	PL
DTL	–	0.012	-0.012	-0.011	0.001
DTR	-0.038*	–	0.003	-0.012	0.011
ND	-0.042*	-0.073*	–	-0.020	-0.011
PD	0.046*	-0.045*	0.012*	–	-0.010
PL	0.003*	-0.041*	-0.045*	0.048*	–

Data allow the diagonal is F_{st}; Nm values are data above the diagonal.

** indicates the significance level of F_{st} value at $p < 0.001$.*

Haplotype network analysis

The median-joining network (Figure 2) illustrates the polymorphic sites, including the number and frequency of the haplotypes for *COI* sequences. The *COI* network was radial-like with a high number of unique haplotypes closely related to 1 central haplotype (H4). Dominant haplotype H4 accounted for 27.87% (20/48) of all 48 specimens. This also indicated that H4 is an ancestral haplotype. H5 also showed the presence of all sub-populations in the study area but with little correlation with other haplotypes, which indicated the specificity of it. The haplotypes tended to be dispersed in comparison to the central haplotype, revealing a tendency to generate discrepancies among independent individuals. The giant mottled eel *A. marmorata* populations in TTH are strongly correlated with each other, and there seems no separation of an individual population.

The evolutionary history was inferred using the Unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal 1973) in figure 3 with 1000 replicates of the bootstrap test (Felsenstein 1985). The sum of the branch length is 0.07028378. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Nei-Gojobori method (Nei and Gojobori 1986) in MEGA X (Kumar et al. 2018). The UPGMA tree showed that most of the haplotypes were weakly associated (less than 50% bootstrap support) or unresolved, the differences among which were possibly due to low nucleotide. Haplotypes of *COI* of *A. marmorata* in TTHue were clustered into 2 obvious branches. H8 and H14 stand in the same independent branches with the remaining 14 haplotypes with bootstrap support of 56%. H5 and H15 together with H12 and H13 were grouped into 2 subgroups in branch 2 with the highest correlation (bootstrap> 60%). H4, H7, and H17 also form a group but have an extremely low bootstrap support rate of only 17%. The remaining haplotypes have a discrete distribution in the branch.

Discussion

A. marmorata with long life and long migrate distance could have a strong population genetic structure. Genetic diversity within and between populations provides a potential genetic resource for future adaptation and can be vital for the fitness of a population (Xu et al. 2012). It is mainly explained by several historical and contemporary processes, such as genetic drift, effective migration, natural selection, fragmentation, and range expansion (Slatkin 1985). Earlier works have analyzed eel population genetics-based mainly on allozymes (Pantelouris et al. 1970, 1971) and demonstrated genetic differentiation between geographical locations. The genetic structure of *A. marmorata* was later reassessed based on the region of the *Cyt b*, mitochondrial (mt) DNA, mitochondria control region (*mtCR*), *COI* and *16S rRNA* genes. In which, *COI* gene barcode region exhibited a credible population-based diversity that exceeded that obtained with sequencing the mitochondrial *Cytb* gene, showing a percentage of haplotypes from the total sequences equaling 45% for *COI*, versus 15% for *Cytb* (El-Nabi et al. 2017). For the first time in Viet Nam, we could identify the level of genetic diversity of *A. marmorata* based on *COI* basic.

Results of the *COI* gene in the region showed high levels of haplotype diversity (h = 0.7556–0.9000) and low levels of nucleotide diversity (π = 0.00161–0.00242). 17 haplotypes were identified in the 48 samples analyzed (35.42%). It is a similar trend with some other researchers on *Anguilla* species. For example, in *A. marmorata*, Fahmi et al. (2015) were found 44 haplotypes, h = 0.937 ± 0.013 and π = 0.861 ± 0.002 (%) in Indonesia base on analyzed the *Cyt b* gene (Fahmi et al. 2015) and 14 haplotypes, nucleotide diversity (p = 2.156%) and haplotype diversity (h = 0.780) from *mtCR* sequences in Pohnpei and Kosrae (Donovan et al. 2012). And in other species, 129 haplotypes were also identified with h = 0.92–1.00 and π = 0.13–1.06% for 6 other species of tropical eels (*A. interior, A. nebulosi nebulosi, A. bicolor pacific, A. bicolor bicolor, A. celebesensis, A. borneensis*) in Indonesia determined by Famil et al. (2015); And 44 segregating nucleotide sites, 33 haplotypes, haplotype diversity (h = 0.94), and nucleotide diversity (π = 0.008) were found when analyzed the *COI* of *A. anguilla* with 525 nucleotides in length by El-Nabi et al. (2017) base on DNA barcode technique.

Researchers often use Fst to assess gene flow, a higher Fst value indicates a lower level of gene flow (Nm) and higher genetic differentiation among populations (Hedrick 2005). Fst reflects the level of inbreeding within populations (Wright 1984) or the extent to which populations are differentiated (Hartl and Clark 2007). The presence of genetic structure is an outcome of limited gene flow and a high level of genetic drift within each reproductively isolated group. Fst values below 0.05 indicate negligible genetic differentiation, whereas values greater than 0.25 indicate high genetic differentiation within the analyzed population (Weir 1996). Fst values of *A. marmorata* in TTHue, Viet Nam were significant but weak (Fst = -0.073–0.003; $p < 0.05$) and high Nm values were detected among the populations. This may be because primitive and highly conservative of the *COI* gene in this species. However, DTR and ND showed high and significant Fst values (Fst = 0.073, $p > 0.05$); this indicated a high degree of genetic differentiation among 2 populations, which endorsed a signal of isolation due to distance. Populations may be divided by major geographic barriers such as land barriers and oceanographic patterns. The negative Tajima's d test and Fu's Fs neutrality test values were obtained for all tested populations, thus rejecting the null hypothesis of neutral evolution of the *COI* marker. This indicated that most populations of *A. marmorata* have been in expansion. These results also showed a similar trend of variation when compared with Famil et al. (2015) and El-Nabi et al. (2017) studies.

Results of the median-joining network of the *COI* gene showed several ambiguous connections among the 5 populations. The demographic analyses using UPGMA for all haplotypes show 2 similar branches one branch contains two types of haplotypes *(COI)* from two populations ND and PD and the other branch contains most of haplotypes from the remaining 5 populations. The ramification of the haplotypes has a low Boostrasp's support value. This can be explained by several reasons. The first, Anguilid have a catadromous life-history strategy, spawning in remote tropical seas with larvae that are transported back by currents to their nursery grounds in freshwater or coastal areas (Arai 2016). Intense migration and long-lived habitats in the inland ecosystems, about six to 20 years or more in golden eel stage (Williamson and Boëtius 1993), have driven the formation of haplotypes independently of individual species. Secondly, *A. marmorata*, a catadromous eel, migrates upstream on nights, following the lunar cycle (Huyen et al. 2012). The dramatic changes of environmental between ocean and freshwater during migration shape their physiological features, e.g. visual sensitivity, olfactory ability, and salinity tolerance (Wang et al. 2014). This can also be proposed as a cause of the occurrence of rare genes and single haplotypes. This induces then isolation by time (IBT) of spawning groups. IBT causes a restriction in gene flow, taking place between early and late spawners (Hendry and Day 2005). And the last, based on analyzed three different phylogenetic trees of *A. marmorata* population in Thua Thien Hue, Vietnam, Huyen and Linh (2020) showed that there was the high genetic similarity of

individuals in eel populations in Thua Thien Hue and it was divided into two separate groups that are guided by the migration process and specific ecological (Huyen and Linh 2020).

Declarations

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Authors' contributions

Both authors discussed and designed the experiments. Kieu Thi Huyen conducted the main experiments and data analysis. All authors wrote, read, and approved the final manuscript.

Availability of data and materials

The datasets of COI sequences analyzed during the current study are available on the GenBank with accession number from MN067923 to MN067970. The data were simultaneously made available to ENA in Europe and the DNA Data Bank of Japan.

Code availability (software application or custom code)

Not applicable

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Ethics approval and consent to participate

All animals and samples applied to international, national, and regional and Institutional guidelines for animal care and rules in Vietnam.

Consent for publication

Not applicable

Competing interests

The authors report no conflict of interest.

References

Arai T (2016) Biology and Ecology of *Anguillid* Eels (ed. Arai T.): Taxonomy and Distribution. CRC Press: 1-20 [Accessed on 6 April 2018]. https://www.crcpress.com/Biology-and-Ecology-of-Anguillid-Eels/Arai/p/book/9781482255157.

Donovan S, Pezold F, Chen Y, Lynch B (2012) Phylogeography of *Anguilla marmorata* (Teleostei: Anguilliformes) from the eastern Caroline Islands. Ichthyol Res 59, 70–76. https://doi.org/10.1007/s10228-011-0245-z.

El-Nabi SESH, El-Desoky MS, Mohammed-Geba K (2017) Genetic population diversity of European eel *Anguilla anguilla* elvers in two Egyptian water bodies, Rosetta (Rachid) estuary and Burullus lake. Genes Genom 39: 1035 – 1045. https://doi.org/10.1007/s13258-017-0572-1

Fahmi M R, Solihin DD, Soewwardi K, Pouyaud I, Berrebi P (2015) Molecular phylogeny and genetic diversity of freshwater Anguilla eels in Indonesian waters based on mitochondrial sequences. Life and environment 65(3): 139-150. https://www.documentation.ird.fr/hor/fdi:010066025 [Accessed on 12 May 2019]

Felsenstein J (1985). Confidence-Limits on Phylogenies – an approach using the bootstrap. Evolution 39: 783–791. DOI: 10.1111/j.1558-5646.1985.tb00420.x

Gagnaire PA, Minegishi Y, Aoyama J, Réveillac E, Robinet T, Bosc P, Tsukamoto K, Feunteun E, Berrebi P (2009) Ocean currents drive secondary contact between *Anguilla marmorata* populations in the Indian Ocean. Marine Ecology Progress Series. 379:267–78. DOI: 10.3354/meps07895

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT." In Nucleic acids symposium series, vol. 41, no. 41, pp. 95-98. [London]: Information Retrieval Ltd., c1979-c2000.

Hartl DL, Clark AG (2007). Principles of population genetics, 4th edn. Sunderland, MA, USA: Sinauer Associates

Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59(8):1633–1638. https://doi.org/10.1111/j.0014-3820.2005.tb01814.x

Hendry AP, Day T (2005) Population structure attributable to reproductive time: isolation by time and adaptation to time. Mol Ecol 14:901–916. https://doi.org/10.1111/j.1365-294X.2005.02480.x

Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, Burridge M, Watkinson D, Dumont P, Curry A, Bentzen P, Zhang J, April J, Bernatchez L. (2008) Identifying Canadian Freshwater Fishes through DNA Barcodes. PLoS ONE 3(6): e2490. https://doi.org/10.1371/journal.pone.0002490

Huyen KT, Linh NQ, Hue HT (2012) Resources of grass eel *(Anguilla marmorata)* in Quang Binh estuaries and protected techniques. Journal of Agriculture and Rural Development 2/2012:115–22. (in Vietnamese with English abstract)

Huyen KT, Linh NQ (2019) Characterization of giant mottled eel (*Anguilla marmorata*) gastrointestinal tract that origin from Thua Thien Hue, Vietnam. J Fish Res. 3(2):4-9. **DOI:** 10.35841/fisheries-research.3.2.4-9

Huyen KT, Phu VV (2015) Status of exploitation and management measures and cosnevation development for eel *(Anguilla marmorata)* resources in Huong river system. Hue University Journal of Science 104(5). (in Vietnamese with English abstract)

Huyen KT, Linh NQ (2020) Phylogenetic analysis of *Anguilla marmorata* population in Thua Thien Hue, Vietnam based on the cytochrome C oxidase I (COI) gene fragments. AMB Expr 10, 122. https://doi.org/10.1186/s13568-020-01059-7

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**,** 343–353. https://doi.org/10.1007/s10228-004-0241-7

Kress WJ, Garcia-Robledo C, Uriarte M, Erickson DL (2015) DNA barcodes for ecology, evolution, and conservation. Trends in Ecology & Evolution 30: 25–35. https://doi.org/10.1016/j.tree.2014.10.008

Kumar R, Singh PJ, Nagpure NS, Kushwaha B, Srivastava SK, Lakra WS (2007) A non- invasive technique for rapid extraction of DNA from fish scales. *Indian Journal of Experimental Biology* 45: 992 – 997. [Download on 6 March 2018] https://pdfs.semanticscholar.org/2d3f/616c0f78098c25b74c5e42446d7e20cc586d.pdf

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35 (6): 1547 – 1549. https://doi.org/10.1093/molbev/msy096

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23 (21): 2947 - 2948. https://doi.org/10.1093/bioinformatics/btm404

Librado P, Rozas J (2009) DnaSP v5: A Software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11): 1451 – 1452. https://doi.org/10.1093/bioinformatics/btp187

Linh NQ, Nghia VD, Minh TD, Thanh ND, Lam HV, Tram NDQ, Hue HT (2010) Study of the season, time appears the eel fingerlings in Quang Binh provine. Journal of Agriculture and Rural Development 12/2010: 195 - 200. (in Vietnamese with English abstract).

Ministry of Science Technology and Environment (2007) Viet Nam Red Data Book. Part 1. Animals. Natural Science and Technology Publishing House, Ha Noi.

Nei M, Gojobori T (1986) Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol 3(5): 418 – 426. DOI:10.1093/oxfordjournals.molbev.a040410

Nguyen AT, Tsukamoto K, Lokman PM (2018) Composition and distribution of freshwater eels *Anguilla spp.* in Vietnam Fish Sci 84 (8): 987 - 994. https://doi.org/10.1007/s12562-018-1239-9

Pantelouris EM, Árnason A, Tesch FW (1970) Genetic variation in the eel. II. Transferrins, haemoglobins and esterases in the eastern North Atlantic. Possible interpretations of phenotypic frequency differences. *Genet Res* **16**: 277 – 284. https://doi.org/10.1017/S0016672300002536.

Pantelouris EM, Árnason A, Tesch FW (1971) Genetic variation in the eel. III. Comparisons of Rhode Island and Icelandic populations. Implications for the Atlantic eel problem. Marine Biology 9: 242–249. https://doi.org/10.1007/BF00351386

Pike C, Crook V, Gollock M, Jacoby D (2019) *Anguilla marmorata* (errata version published in 2020). The IUCN Red List of Threatened Species 2019: e.T166189A167699312. [Downloaded on 08 May 2020]. https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T166189A167699312.en.

Slatkin M (1985) Gene flow in natural populations. Annual Review of Ecology and Systematics 16: 393 – 430. https://www.annualreviews.org/doi/pdf/10.1146/annurev.es.16.110185.002141

Sneath PHA, Sokal RR (1973) Numerical Taxonomy. Freeman, San Francisco. https://www.cabdirect.org/cabdirect/abstract/19730310919

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123(3):585–595. https://www.genetics.org/content/123/3/585.short

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526., https://doi.org/10.1093/oxfordjournals.molbev.a040023

Wang FY, Fu WC, Wang IL, Yan HY, Wang TY (2014) The giant mottled eel, *Anguilla marmorata*, uses Blue-shifted rod photoreceptors during upstream migration. PLoS ONE 9(8): 1–11. https://doi.org/10.1371/journal.pone.0103953.

Wasserman RJ, Weyl OLF, Strydom NA (2011) The effects of instream barriers on the distribution of migratory marine-spawned fishes in the lower reaches of the Sundays River, South Africa. Water South Africa 37(4): 495-504. DOI: 10.4314/wsa.v37i4.7.

Watanabe S, Aoyama J, Tsukamoto K (2004) Reexamination of Ege's (1939) Use of taxonomic characters of the genus *Anguilla*. *ulletin of Marine Science* 74: 337–351. [Download on 5 March 2018]. https://www.ingentaconnect.com/content/umrsmas/bullmar/2004/00000074/00000002/art00006

Watanabe S, Aoyama J, Nishida M, Tsukamoto K (2005) A molecular genetic evaluation of the taxonomy of eels of the genus *Anguilla* (Pisces: Anguilliformes). *Bulletin of Marine Science* 76(3): 675–690. [Download on 5 March 2018]. https://www.ingentaconnect.com/content/umrsmas/bullmar/2005/00000076/00000003/art00006

Weir BS (1996) Genetic Data Analysis II: Methods for discrete population genetic data. Sinauer Associates, Inc., Sunderland.

Williamson GR, Boëtius J (1993) The eels *Anguilla marmorata* and *A. japonica* in the Pearl River, China, and Hong Kong. Asian Fish Sci 6:129–138

Wright S (1984) Evolution and the genetics of populations, volume 4: variability within and among natural populations (Vol. 4). University of Chicago press.

Xu X, Wu X, Yu Z (2012) Comparative studies of the complete mitochondrial genomes of four Paphia clams and reconsideration of subgenus Neotapes (Bivalvia: Veneridae). Gene 494:17–23. https://doi.org/10.1016/j.gene.2011.12.002

Figures

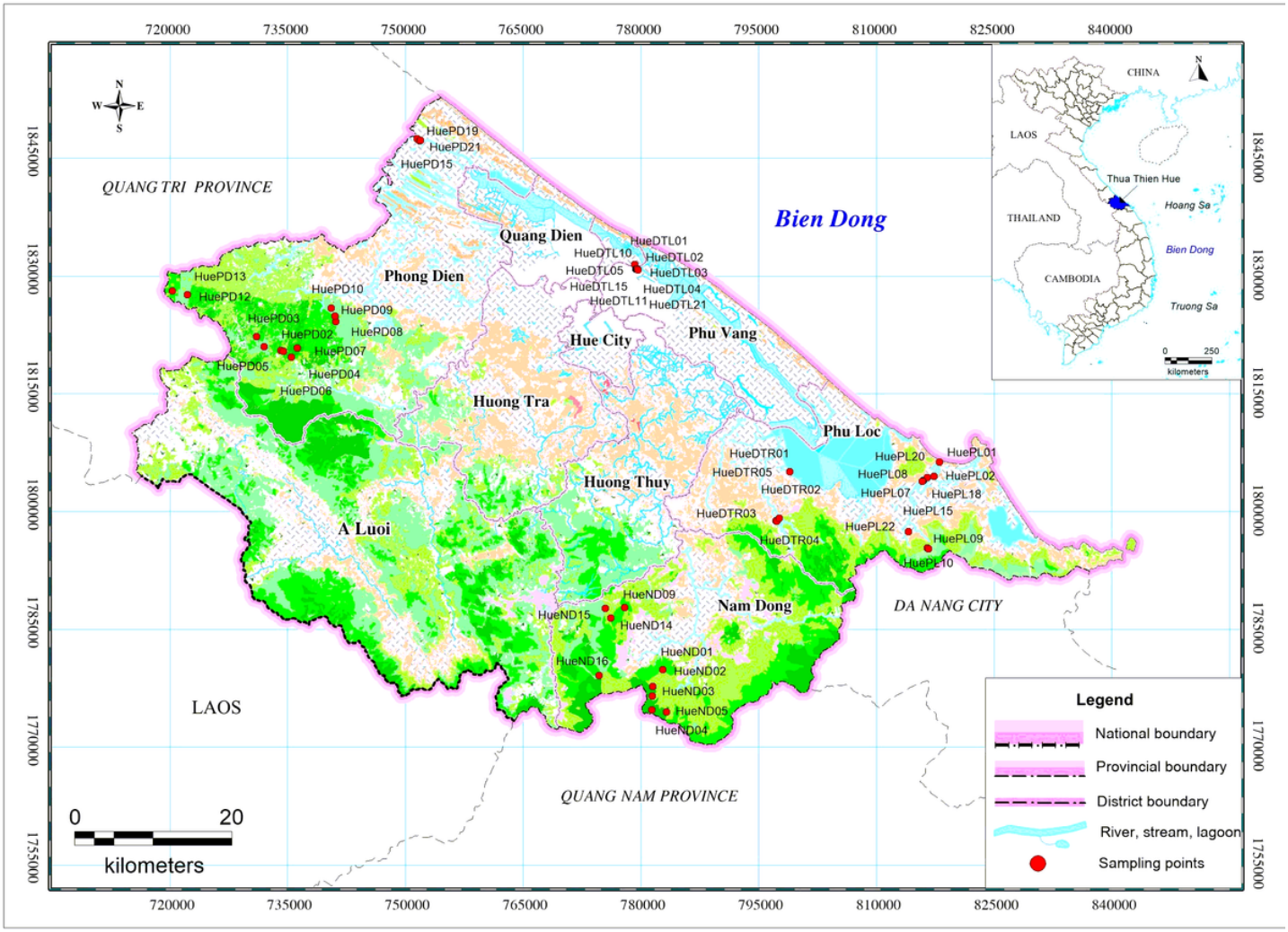

Figure 1

Illustrating sample sites map of A. marmorata populations in TTH, Vietnam

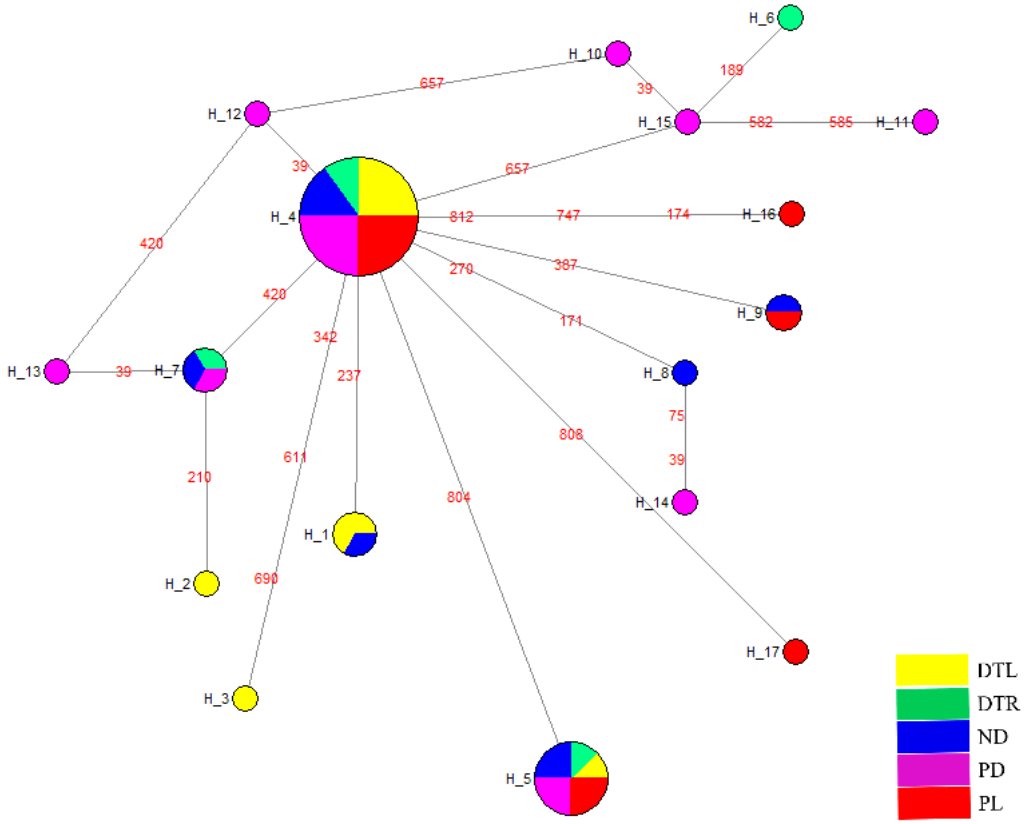

Figure 2

Median-joining network for COI haplotypes of A. marmorata in TTH, Viet Nam. On the connecting lines, red numbers present the variable sites between each haplotype pair. Different colors represent the different populations in the network (Yellor, Green, Blue, and Pink colors represented for DTL - Thao Long Dam, DTR - Truoi Dam, ND - Nam Dong and PD - Phong Dien respectively. PL - Phu Loc was indicated by red color).

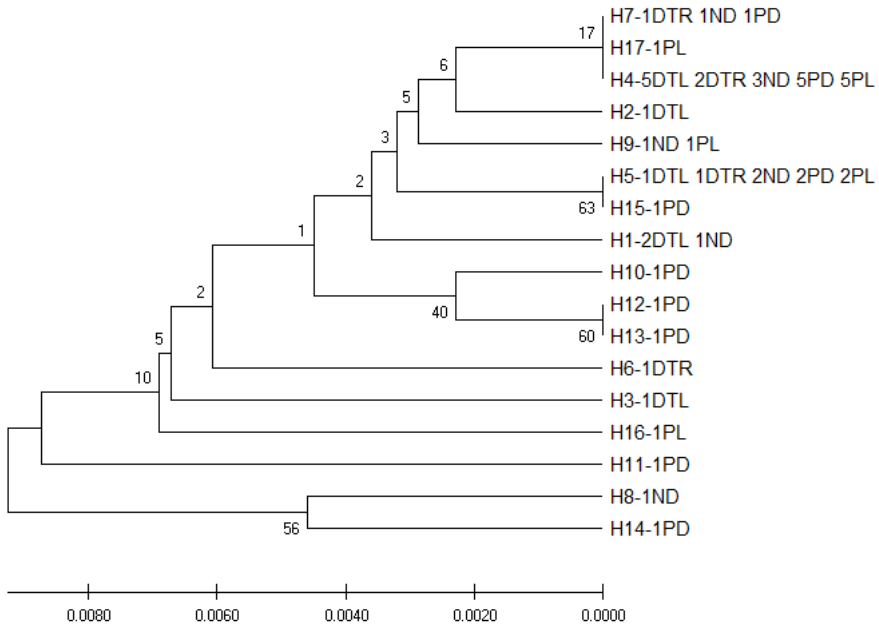

Figure 3

The phylogenetic relationship of A. marmorata among the haplotypes was determined using COI. Figures before population codes, which are behind the haplotypes, indicate that the number of individuals from the population belongs to the haplotype. The scale bar is branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.