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Journal of Fisheries Science
https://ojs.bilpublishing.com/index.php/jfs

ARTICLE

Occurrence of *Anguilla luzonensis* in the Tributaries along the Lagonoy Gulf, Philippines

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ARTICLE INFO

Article history
Received: 25 March 2021
Accepted: 14 April 2021
Published Online: 23 April 2021

Keywords:
Anguilla
Glass eels
Species composition
PCR-RFLP
DNA sequencing
Diversity

ABSTRACT

Anguillids are a valuable fish commodity worldwide. Although *Anguilla luzonensis* have been abundantly found in the northern Philippines and collected for trade, no available records show that it recruited in the mid-part where Lagonoy Gulf, Bicol is situated. In this study, we investigated the occurrence of *A. luzonensis* in the tributaries along the Lagonoy Gulf, Philippines using molecular tools. Glass eel specimens were collected in 2018–2019 from the Comun river, Albay; the Lagonoy river, Camarines Sur; and the Bato river, Catanduanes. *Anguilla luzonensis* was first reported in Lagonoy Gulf using molecular analysis. *A. luzonensis* was the second most abundant species in the Comun and Lagonoy rivers (9.5 and 22.4 %, respectively). *Anguilla luzonensis* collected from the Comun and Lagonoy rivers did not show a significant difference (FST= 0.00825, p>0.05). *Anguilla marmorata* was the most dominant species in all tributaries (71.1–98.0 %). In the Comun and Lagonoy rivers, *A. bicolor pacifica* was the third most abundant species (7.7 and 6.5 %, respectively). In addition, *Anguilla celebesensis* was only found rarely in the Comun river (0.9 %). This study provides important information for sustainable resource management and effective utilization of the eel species in these regions.

1. Introduction

The Philippines can be considered a species-rich habitat for Anguillid eels, with the total of six species (*A. luzonensis*, *A. marmorata*, *A. bicolor pacifica*, *A. bicolor bicolor*, *A. japonica*, and *A. celebesensis*) among the 19 species/subspecies of the genus *Anguilla* [1-7]. Anguillids from the Philippines are a commercially important commodity for export [8]. *Anguilla* luzonensis was discovered in Cagayan and described as a new species in 2009 using adult specimens (244-682 mm) [9]. *Anguilla* luzonensis has potential as a fishery product since trade to eel-consuming countries in the East Asia has been reported [7, 10-11]. Information on its population dynamics, stock status, and utilization is limited [12]. Due to the limited known range of *A. luzonensis*, it was recently assessed by CITES as a

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DOI: https://doi.org/10.30564/jfsr.v3i1.3010

'Vulnerable' (VU) species under category D2 [13]. Therefore, resource management strategies in support of effective utilization, economic potentials, and sustainability of *A. luzonensis* bioresources are needed.

Anguilla luzonensis may be widely distributed in the Western Pacific with reports of its occurrence in Cagayan [1-5], Mindanao [2-3, 5-6], Taiwan [2-3], and Okinawa, Ryuku archipelago [14]. Abundance of *A. luzonensis* was reported in Cagayan [1, 3]. *Anguilla* luzonensis is presumed to have spawned in the North Equatorial Current (NEC) [3, 15]. One of the NEC bifurcates, the Kuroshio Current [16], transported *A. luzonensis* to Eastern and Northern Luzon and Taiwan [3]. As the Bicol region faces the Kuroshio Current, it is highly possible that these species may also occur in the Lagonoy Gulf in the same region. The Bicol region ranked 2nd for highest municipal fish production [17]. Therefore, the *A. luzonensis* glass eel might be one of the important target species for fishermen, but no fishery data are available. For resource management, the precise occurrence of *A. luzonensis* and its species composition must be clarified.

In our recent study, the species of freshwater eels recruited in the tributaries along the Lagonoy Gulf, Bicol were investigated [18]. Freshwater eel species were identified based on pigmentation patterns using an illustration [19]. Although the illustrations for *A. marmorata*, *A. bicolor pacifica*, and *A. luzonensis* showed that these species have pigmentation, they were difficult to identify. Consequently, among more than 4,000 identified pigmented glass eels, 89.8 % were grouped as *A. marmorata*, 10.1 % as *A. bicolor pacifica*, and none as *A. luzonensis*. No *A. luzonensis* individuals were found to recruit in the Lagonoy Gulf. The use of morphological identification alone was not enough to distinguish the pigmented Anguillid species; hence, molecular identification may be used for precise species identification and confirmation of species composition.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), a fast and reliable technique, could identify 14 known *Anguilla* species [19-23] among the 18 known species and subspecies. Although *A. bicolor* subspecies cannot be distinguished, PCR-RFLP technique was suggested to be refined using a large number of samples to identify any eel species [22-23]. There may be cases where PCR-RFLP could not identify a specimen; hence, DNA sequencing may be used for further species identification [24]. The DNA sequences were also used in investigating molecular evolution [24-25]. Thus, *A. luzonensis*, *A. marmorata*, *A. bicolor pacifica*, *A. bicolor bicolor*, *A. japonica*, and *A. celebesensis*, commonly found in Luzon, Philippines, may be identified by PCR-RFLP and DNA sequencing analysis.

In this study, we clarified the occurrence of *A. luzonensis* in Lagonoy Gulf using molecular tools. The species composition of six target species in the tributaries along Lagonoy Gulf was clarified. *Anguilla* luzonensis population structure was also confirmed.

2. Materials and Methods

2.1 Study Site and Sample Collection

The specimens analyzed in this study were glass eels that were identified on the basis of morphology [18] and collected from three main rivers, namely the Comun river (13°25'09.1" N 123°42'44.6" E) in Albay, the Lagonoy river (13°43'37.9" N 123°35'11.0" E) in Camarines Sur, and the Bato river (13˚35'40.0" N, 124˚17'10.0" E) in Catanduanes (Figure 1).

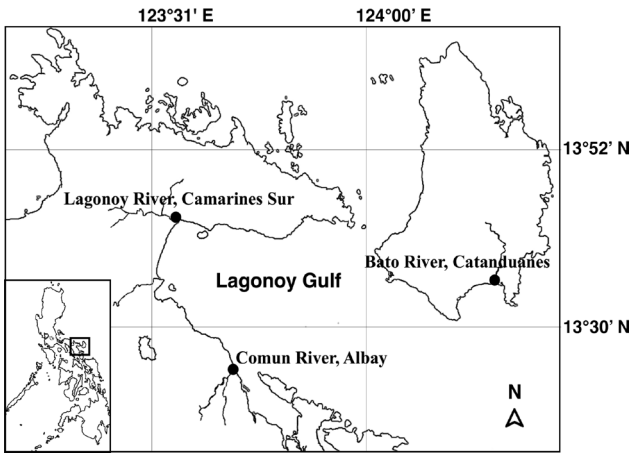

Figure 1. Map showing the study area in Lagonoy Gulf, Philippines, (●) indicates the sampling sites in Albay, Camarines Sur and Catanduanes

The glass eels were collected using a modified fyke net that was 1.15 m in height (H) and 10 m in length (L), with 2-8 m long wings and a 2.5 m-long catching bag [oval-shaped opening dimensions - 1.15 m H and 3 m width (W)] installed near or at a 3-4 m distance from the river mouth at a water depth of 1-1.2 m in the Comun river and Lagonoy river. A push net with dimensions 1.0 m H by 1.15 m W was positioned at a 3-4 m distance from the river mouth and a water depth of 1-1.2 m in the Bato river.

A one-day sample collection was conducted beginning at 18:00 h for 2-4 h during the new Moon phase between August 2018 and August 2019 in the Comun river, July 2018 and July 2019 in the Lagonoy river, and December 2018 and May/July 2019 in the Bato river. The collected glass eels were preserved in 95 % ethanol for subsequent analysis. Among the morphologically identified pigmented glass eels, approximately 10 % (n = 554; Comun= 220,

 DOI: https://doi.org/10.30564/jfsr.v3i1.3010

Lagonoy= 232, Catanduanes= 102) were randomly selected for molecular analysis.

2.2 DNA Extraction and PCR Amplification

Approximately 25 mg of the middle-part of a glass eel was cut into small pieces. These were lysed overnight, and DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) or FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp., MI, USA). The cytochrome c oxidase 1 (COI) target fragment gene was amplified using the designed primers 5503F1 (5'-CCGCTTAAACATTCAGCC-3') and 7138R1 (5'-GGGGGTTCAATTCCTTCC-3'). PCR was performed in a 25 µl mixture containing 1.0 µl DNA template, 2.5 µl 10× buffer [magnesium (Mg^{2+}) plus], 2.0 µl 2.5 mM deoxynucleoside triphosphate, 1.25 µl 10 µM of each primer, 0.125 µl Taq polymerase (Takara Bio Inc., Shiga, Japan), and double-distilled water. The thermal cycler profile included initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, elongation at 72 °C for 60 s, and final extension of 72 °C for 10 min. The PCR amplicons were visualized on 1 % agarose Tris-acetate-EDTA (TAE) gel electrophoresis with GelRed Nucleic Acid Gel Stain (Biotium, CA, USA).

2.3 Restriction Fragment Length Polymorphism Analysis

The simulation of restriction digestion was carried out for the target species *A. marmorata*, *A. bicolor pacifica*, *A. luzonensis*, *A. japonica*, *A. bicolor bicolor*, and *A. celebesensis* found in the Philippines. The complete mitochondrial genome of these six Anguillid species (Accession nos. AB469437, AP007242, AP007237, AP007236, AP007239, AB038556) was downloaded from the National Center for Biotechnology Information (NCBI) and aligned using MEGA X software [26]. Thirty-two restriction enzymes with clear and identifiable cleavage sites within COI fragment genes were used for virtual digestion. Based on its ability to cut DNA into fragments and the patterns produced, *Msp* I was used for species identification. *Dde* I was used to distinguish *A. bicolor pacifica* from *A. bicolor bicolor* since *Msp* I cannot distinguish these two species. Restriction digestion was carried out in a 10 µl reaction mixture with 80-100 ng amplified product according to the *Msp* I and *Dde* I protocol (Takara Bio, Japan). The RFLP patterns were visualized in a 2 % agarose Tris-Borate-EDTA (TBE) gel electrophoresis then photographed. Expected RFLP patterns for the specimens were used for species identification. The PCR-RFLP profiles of several samples were visualized using DNA1000 LabChips (Agilent Technologies, Inc.) with the 2100 Bioanalyzer microchip capillary electrophoresis system according to the manufacturer's instructions. The obtained RFLP patterns were analyzed using 2100 expert software.

2.4 DNA Sequencing and Phylogenetic Analysis

The designed four oligonucleotide primers, 5511F2 (5'-ACATTCAGCCATCTTACC-3'), 6468R3 (5'-TGCRATGATTATTGTGGC-3), 6126F3 (5'-VCCAGTCCTAGCTGCAGG-3'), and 7131R2 (5'-CAATTCCTTCCTTTCTTG-3') were used to sequence the COI target fragment gene. The PCR product purified by Agencourt AMPure XP (Beckman Coulter, CA, USA) was used for direct cycle sequencing with the ABI BigDye v.3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA). Sequences generated from the 3130 Genetic Analyzer (Applied Biosystems, CA, USA) (Accession Numbers: LC588356- LC588371, LC588373-LC588374) were edited and manually aligned using Chromas version 2.6.6 and the MEGA X [26] software, obtaining 1431 bp after truncation. The two specimens that failed to show the high-intensity PCR bands required for RFLP were directly sequenced. A Maximum Likelihood tree was constructed using the HKY+G+I model with *Stemonidium hypomelas* (NC013628) as an outgroup using MEGA X [26] with 1000 bootstrap probabilities for species identification of unknown RFLP patterns and confirmation of representative specimens with expected ones.

2.5 Mitochondrial DNA Analysis

All (72 individuals) *A. luzonensis* partial COI target fragment genes were directly sequenced using the designed primer 6126F3 (5'-VCCAGTCCTAGCTGCAGG-3') by ABI BigDye v.3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA) and were manually aligned using Chromas version 2.6.6 and the MEGA X [26] software, obtaining 663 bp after truncation. A model test was conducted using MEGA X [26] and the model with lowest Bayesian Information Criterion (BIC) score was selected. A Maximum Likelihood tree was constructed using T92 (lowest BIC score) with *A. japonica* (AB038556.2) as an outgroup using MEGA X [26] with 1000 bootstrap probabilities to examine the geographical similarities between individuals and the phylogenetic relationship.

2.6 Statistical Analysis

Genetic variability was characterized by the amount of nucleotide substitutions calculated by the ARLEQUIN

DOI: https://doi.org/10.30564/jfsr.v3i1.3010

3.5.2.2 software [27]. The genetic diversity between rivers was investigated using the fixation index (F_{ST}) for all pairwise comparison (10230 permutations) using the ARLEQUIN 3.5.2.2 software [27]. The Bato river was excluded in computations since only 1 *A. luzonensis* individual was identified there.

3. Results

3.1 Species Identification

Three patterns were observed among the expected PCR-RFLP patterns for six Anguillid species (lanes 1-3, Figure 2a). Lane 1 was similar to the *A. luzonensis* pattern, and the band sizes were 669, 367, 344, 171 and 110 bp. Lane 2 was similar to the *A. marmorata* pattern, and the band sizes were 837, 366, and 341 bp. Lane 3 had a similar pattern to A bicolor pacifica or *A. bicolor bicolor*, with band sizes of 824 and 681 bp. Lane 1 (Figure 2b) also showed a similar pattern to *A. bicolor pacifica*, and the band sizes of 854, 278, and 223 bp confirmed that we were able to identify almost all *A. bicolor pacifica* (lane 1, Figure 2b). There were 536 individuals identified by PCR-RFLP using *Msp* I. The 33 specimens distinguished as *A. bicolor pacifica* or *A. bicolor bicolor* by *Msp* I (lane 1; Figure 2a) were confirmed by *Dde* I as *A. bicolor pacifica* (lane 1, Figure 2b). There were 16 specimens showing one of the unknown RFLP patterns similar to those in lanes 4-8 (Figure 2a). No expected patterns were observed for *A. celebesensis* and *A. japonica*.

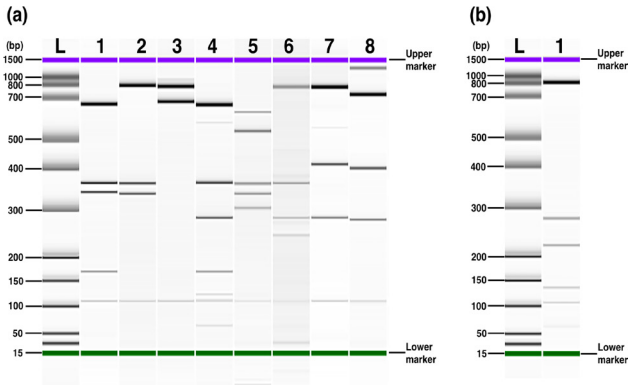

Figure 2. Restriction digestion by (a) *Msp* I for *A. luzonensis*, *A. marmorata*, *A. bicolor pacifica* or *A. bicolor bicolor* and unknown PCR-RFLP patterns of *A. luzonensis*, *A. marmorata*, *A. bicolor pacifica*, and *A. celebesensis*. L, Ladder; lane 1, *A. luzonensis*; 2, *A. marmorata*; 3, *A. bicolor pacifica* (expected). Lane 4, *A. luzonensis*; 5-6, *A. marmorata*; 7, *A. bicolor pacifica*; 8, *A. celebesensis*; confirmed by DNA sequencing analysis. (b) *Dde* I patterns for *A. bicolor pacifica*, lane 1; bp, base pairs

The phylogenetic tree (Figure 3) with *Stemonidium hypomelas* as an outgroup showed that DNA sequences with expected and unknown RFLP patterns were grouped under its specific reference sequences. Individuals with an expected RFLP pattern similar to *A. luzonensis* (lane 1, Figure 2a; LC588366-LC588367), the unknown pattern in lane 4 (Figure 2a; LC588368), and a directly sequenced specimen (LC588369) were grouped under the *A. luzonensis* clade (AB469437; Figure 3). The individuals with expected RFLP similar to *A. marmorata* (lane 2, Figure 2a; LC588356-LC588358, LC588363- LC588364), unknown (lanes 5-6, Figure 2a; LC588359-LC588362), and another individual directly sequenced pattern (LC588365) were grouped with the *A. marmorata* reference sequence (AP007242). Furthermore, individuals with a pattern similar to *A. bicolor pacifica* (lane 1, Figure 2b; LC588370-LC588371) and unknown (lane 7, Figure 2a; LC588373) were grouped under the clade of *A. bicolor pacifica* (AP007237). One of the sequenced unknowns (lane 8, Figure 2a; LC588374) belonged to the clade of *A. celebesensis* (AP007239).

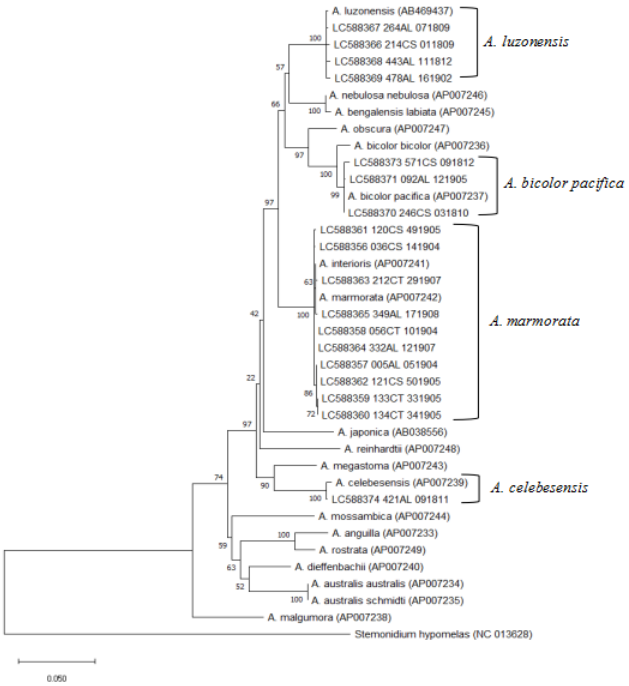

Figure 3. Maximum likelihood tree of the DNA sequences of specimens showing typical and unknown RFLP patterns.

3.2 Species Composition

Anguilla luzonensis was found to recruit in the Lagonoy Gulf, mainly in the Comun and Lagonoy rivers, in addition to *A. marmorata* and *A. bicolor pacifica* (Figure 4). Further, *A. luzonensis* was the second most abundant species in the Comun (9.5 %) and Lagonoy (22.4 %) riv-

 DOI: https://doi.org/10.30564/jfsr.v3i1.3010

ers, next to *A. marmorata*. *Anguilla marmorata* dominantly occur in the Comun (81.8 %), Lagonoy (71.1 %), and Bato (98.0 %) rivers, while *Anguilla* bicolor pacifica was the third most abundant species in the Comun (7.7 %) and Lagonoy (6.5 %) rivers. In the Bato river, *A. luzonensis* and *A. bicolor pacifica* (1.0 %) recruited at a considerably low percentage. In addition, a rare occurrence of *A. celebesensis* (0.9 %) was observed only in the Comun river.

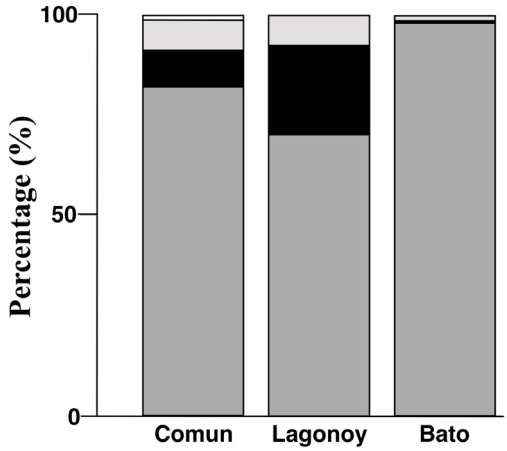

Figure 4. Percent (%) composition of freshwater eels recruiting in each of the rivers. ■, *A. luzonensis*; ▒, *A. marmorata*; ▒, *A. bicolor pacifica*; □, *A. celebesensis*

3.3 Population Structure of *A. luzonensis*

When examining the genetic variability among the 663 nucleotide sequence sites obtained from 72 individuals, a total of 24 variable positions and 20 haplotypes were observed. The pairwise comparison based on the partial COI sequences of *A. luzonensis* from Comun and Lagonoy rivers showed low genetic diversity with an F_{ST} value of 0.00825, which was not significantly different (P > 0.05) (Table 1).

Table 1. Genetic diversity based on the partial COI (663 bp) gene of *A. luzonensis* individuals collected from Comun and Lagonoy rivers.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage variation	Fixation index (F_{ST})
Among populations	1	1.234	0.00823	0.82	
Within populations	70	69.252	0.98932	99.18	0.00825

p-value>0.05; 10230 permutations

The phylogenetic tree with *A. japonica* as an outgroup (Figure 5) showed that almost all *A. luzonensis* individuals from Comun and Lagonoy and one individual from the Bato river formed a monophyly in one clade (B) with one individual diverged first (A) but node is only supported by 54 % bootstrap value which is weak. In addition, the two reference sequences downloaded from NCBI, which were found in the Cagayan river (*) also belonged to this large clade (B).

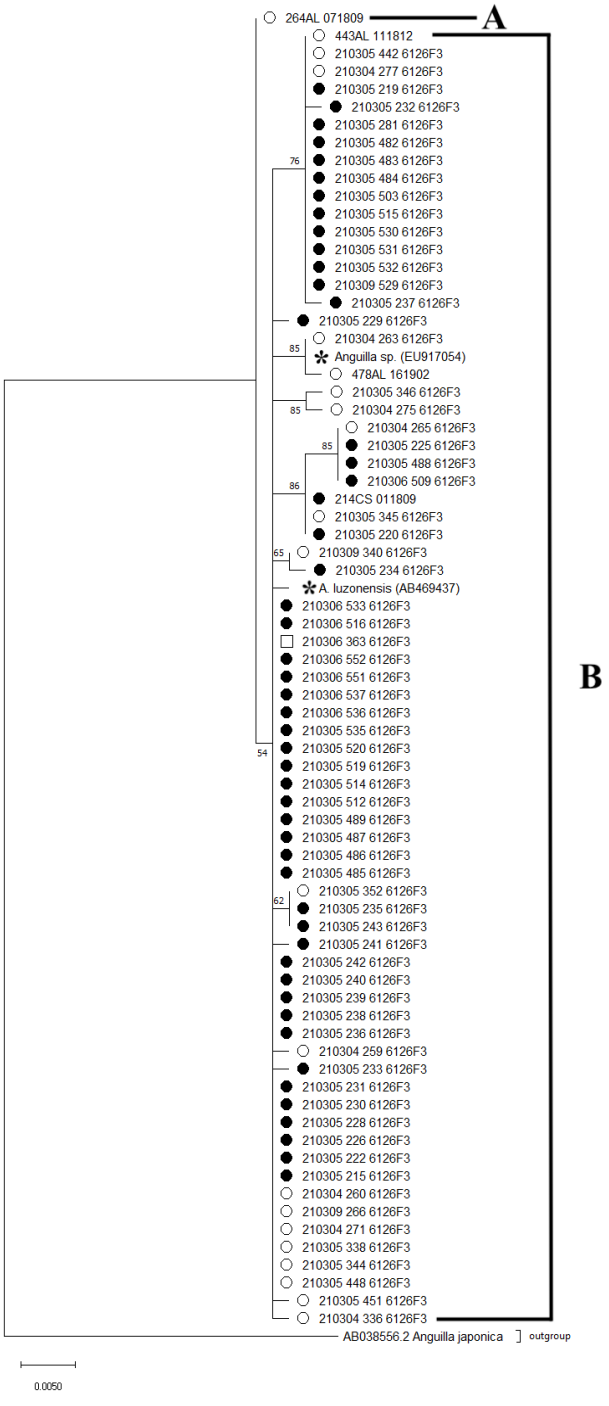

Figure 5. Maximum likelihood tree showing phylogenetic relationships between *A. luzonensis* collected mainly from Comun (○) and Lagonoy (●) and one individual from Bato (□) river. Reference sequences (AB469437, EU917054) of *A. luzonensis* from Cagayan (*) downloaded from NCBI was also included.

DOI: https://doi.org/10.30564/jfsr.v3i1.3010

4. Discussion

4.1 Species Identification

Anguilla luzonensis was found in the Lagonoy Gulf by molecular analysis. The unknown RFLP patterns showed that lane 4 (Figure 2a) had a largest band of around 671 bp, which was similar to a band detected in *A. luzonensis* (lane 1, Figure 2a). *A. luzonensis* individuals showing expected and unknown patterns were grouped in one distinct clade, implying that these are the same species supported by 100 % bootstrap value.

Anguilla marmorata was also identified. Lanes 6 had a largest band of more than 800 bp, similar to the largest band for both *A. marmorata* (lane 2) and *A. bicolor pacifica* or *A. bicolor bicolor* (lane 3) (Figure 2a), hence, could not be identified. Doublets between 300-400 bp observed in lane 5 were similar to the doublets of *A. luzonensis* (lane 1) and *A. marmorata* (lane 2) (Figure 2a). However, the sequences of *A. marmorata* individuals with expected and unknown patterns that were grouped into one clade were the same species (100 % bootstrap value).

In the case of *A. bicolor pacifica*, the use of *Dde* I in addition to *Msp* I was able to confirm that the 33 individuals identified as *A. bicolor pacifica* or *A. bicolor bicolor* were *A. bicolor pacifica* individuals, and not *A. bicolor bicolor* (Figure 2b). The largest band of lane 7 (more than 800 bp) is similar to the largest band for both *A. marmorata* (lane 2) and *A. bicolor pacifica* or *A. bicolor bicolor* (lane 3) (Figure 2a), however, the DNA sequence was grouped with the specimens having expected RFLP patterns and reference sequence indicated that these are identical species. It may also be noted that, all detected bands of the *Msp* I digests of the three species the and the *Dde* I digest of *A. bicolor pacifica* were ranged within ±10 % band sizing accuracy of the expected band sizes. Almost all individuals (536) were identified by PCR-RFLP using *Msp* I. Although we were not able to observe the expected pattern for *A. celebesensis*, we found an unknown individual (lane 8, Figure 2a; LC588374) was *A. celebesensis*, since it was under the clade of its reference sequence (AP007239; Figure 3).

PCR-RFLP using 16S rRNA have been reported appropriate for identification of the genus *Anguilla* [20, 22-23] before *A. luzonensis* was discovered as a new species in 2009 [9]. No available study used PCR-RFLP to identify *A. luzonensis*, hence, we simulated the restriction pattern for COI gene, in addition, to 16S rRNA for six target species and found that COI gene digested by *Msp* I and *Dde* I produced distinctive RFLP pattern for *A. luzonensis* and all the other five species. The combination of PCR-RFLP for COI gene by *Msp* I and *Dde* I and for unknown patterns using DNA sequencing analysis, finally, we were able to completely identify four Anguillid species in Lagonoy Gulf.

4.2 Species Composition

It is interesting to note that *A. luzonensis* was found to be the 2nd most abundant species in the Comun and Lagonoy rivers. *Anguilla luzonensis* abundance and its stable recruitment for two periods of study were reported in Cagayan [1] and its rare occurrence in Mindanao [6]. Our study and the references indicated the abundance of *A. luzonensis* in the northern and eastern part of the Philippines and its rarity in the south.

In the case of *A. marmorata*, decrease on the percentage of recruitment in Comun, Lagonoy and Bato rivers (87.5 %, 82.3 %, and 99.7 % respectively) reported by morphology [14] was due to the occurrence of *A. luzonensis* (9.5 %, 22.4 %, and 1 %, respectively). Similarly, *A. marmorata* has dominantly recruited in Cagayan [1]and Mindanao [6]. This means that *A. marmorata* is widely distributed in the Philippines.

For *A. bicolor pacifica*, molecular analysis found lower than 10 % recruitment in Comun (7.7 %) and Lagonoy (6.5 %), which is less than the occurrence reported by morphological identification (12.4 % and 17.5 %, respectively) [14] since we found that some *A. luzonensis* individuals were misidentified as *A. bicolor pacifica* by morphology. In Cagayan, an increase in the annual percentage of recruitment during two periods of their study was observed for *A. bicolor pacifica* [1]. *Anguilla bicolor pacifica* was the 2nd most abundant species occurred in Mindanao [6]. Based on our results and other studies, among *A. bicolor* subspecies, *A. bicolor pacifica* is abundant in the whole Philippines. The decrease in the percent composition reported by morphology for *A. marmorata* and *A. bicolor pacifica* was due to the occurrence of *A. luzonensis* in Lagonoy Gulf.

In the Bato river, the *A. luzonensis* and *A. bicolor pacifica* species with low percentage occurrence may recruit during months when no samples were collected, since these were found to recruit in the Comun and Lagonoy rivers. *A. celebesensis* was rarely observed in the Comun river only, which is similar in Cagayan wherein this species was extremely rare [1]. The use of morphology specific to pigmentation patterns was not able to distinguish *A. luzonensis* from *A. marmorata* [18]. Pigmentation patterns specific to *A. luzonensis*, *A marmorata*, and *A. celebesensis* are not yet established [7]. The molecular analysis we carried out affirmed that pigmentation pattern alone is not enough to distinguish *A. luzonensis* among the pigmented eels [28]. The species composition based on morphology can be confirmed and revised by molecular techniques. In

 DOI: https://doi.org/10.30564/jfsr.v3i1.3010

addition, we were also able to confirm that no *A. bicolor bicolor* and only *A. bicolor pacifica* recruit in the Lagonoy Gulf. Hence, molecular analysis has provided a more precise estimate of Anguillid species composition in the tributaries along Lagonoy Gulf.

4.3 Population Structure of *A. luzonensis*

Anguilla luzonensis was mostly found in two rivers, Comun and Lagonoy, along the Lagonoy Gulf. Individuals of *A. luzonensis* recruited in Comun and Lagonoy seem to have genetically variable sites and haplotypes. In addition, *A. luzonensis* between these two rivers were found to have low genetic diversity, which was not statistically significant (Table 1), possibly indicating a panmictic population. The dispersed *A. luzonensis* individuals from the Comun and Lagonoy rivers in clade B (Figure 5), including the two DNA sequences downloaded from NCBI, which were collected from Cagayan, could imply that these individuals share similar genetic materials. *Anguilla luzonensis* and *A. japonica* were presumed to have similar spawning area [3,15], experience similar oceanographic features [3], though distribution are different, wherein the latter was reported to have a single panmictic population in the East Asia [29]. Although our study was only in Lagonoy Gulf, *A. luzonensis* might have a similar case with *A. japonica* having panmictic population. Since this is the first investigation of the population structure of *A. luzonensis* recruited in tributaries along the Lagonoy Gulf and being the 2nd abundant species that may have high economic importance, this study could indicate important implications for resource management of this species. Furthermore, genetic differences and population structure of *A. luzonensis* from Luzon, Mindanao, Philippines, Taiwan, and Okinawa, Ryuku archipelago may be compared and studied in the future.

5. Conclusions

Our study using molecular analysis found *A. luzonensis* in the Lagonoy Gulf along the reported *A. marmorata* and *A. bicolor pacifica*. Interestingly, *A. luzonensis* was identified as the 2nd most abundant species in the Comun and Lagonoy rivers. Although genetic variability was found in *A. luzonensis* individuals, genetic diversity was very low and not significantly different, which was inferred from the partial COI gene fragment. It maybe noted that our current study was not able to find enough *A. luzonensis* individuals from Bato river but highly possible to occur during months with no samples were collected; therefore, this study will be further continued. With *A. luzonensis* classified as a vulnerable species, this study provides information that will be of help to effective management and utilization of freshwater eels.

Acknowledgments

This study was part of "The eel fishery in tributaries along Lagonoy Gulf: Implications for conservation and management" Project that was supported by the Department of Science and Technology-Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (DOST- PCAARRD). This study was also supported in part by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Japan. We are thankful for the assistance extended by Joshua M. Vacarizas and Sam Edward N. Manalili in the analysis of DNA sequences, and the fishermen during sample collection. We are also grateful for the support of the Department of Agriculture-Bureau of Fisheries and Aquatic Resources (DA-BFAR), and the Local Government Units (LGU) of Malinao, Albay; Lagonoy, Camarines Sur; and Bato, Catanduanes for their approval of the conduct of the study in the specified target sites. Collections of the samples were made with a permit from the Department of Agriculture (DA) Gratuitous Permit No. 0173-19.

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