International Journal of Agricultural Technology 2018 Vol. 14(7): 2051-2066
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

DNA barcoding of two commercially important fish families (Carangidae and Lutjanidae) collected from Cuyo, Palawan, Philippines

Templonuevo, R. M., Alcantara, S., Juanico, C. S. and Yambot, A.*

Molecular Biology and Biotechnology Laboratory, College of Fisheries, Central Luzon State University, Science City of Munoz, Nueva Ecija 3120, Philippines.

Templonuevo, R. M., Alcantara, S., Juanico, C. S. and Yambot, A. (2018). DNA barcoding of two commercially important fish families (Carangidae and Lutjanidae) collected from Cuyo, Palawan, Philippines. International Journal of Agricultural Technology 14(7): 2051-2066.

Abstract DNA barcoding has been proven as an effective and accurate tool for species identification all throughout the world. In this study, the technique was used to molecularly identify different species of the two major commercially important fish families (Carangidae and Lutjanidae) collected in Cuyo Island, Palawan. The island is a part of Coral triangle which is well recognized as a global apogee of marine biodiversity. It is also a major source of commercially important fishes being shipped to other neighboring provinces including Metro Manila. An estimated >600 base pair region of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified using four sets of universal primers. Fourteen high quality COI sequences were amplified and submitted to GenBank and IBOL database representing 7 species of carangids and 7 species of snappers. The generated sequences were compared to the published COI sequences in public databases and revealed 99-100% identity. Genetic distances were also calculated using Kimura-two parameter (K2P) model along with the construction of Neighbour-joining tree to support the identification. Overall, K2P distance was 20.3% with 22.5% and 20.5% genetic distances corresponding to interfamily and interorder divergences. A strong, cohesive and distinct clusters representing the taxonomic status of each of the species was also evident. Among the molecularly identified fishes in the island, one species of trevally, *Caranx sexfasciatus* was tagged with "decreasing population" by International Union for the Conservation of Nature (IUCN). The inventory of these commercially important aquatic species in Cuyo Island, Palawan is important to identify their sources for export, domestic consumption and aquaculture purposes. It is also vital in implementing efficient assessment and management of the stocks. With the baseline information that is generated by the study, the fisherfolks will know the richness of their resources.

Keywords: molecular species identification, cytochrome c oxidase subunit 1, genetic divergence

Introduction

Species identification and classification are done by taxonomists to classify each organism and to provide information for biological studies. Indeed, our society nowadays has to resolve many vital biological issues, among which

* **Coressponding Author:** Apolinario V. Yambot; **Email:** polyambot@yahoo.com

are the need to maintain genetic biodiversity, to ensure bio-security, to protect species and to avoid pandemics. It is vital to discover the world's diversity because it gives us the information whether the species belongs to a same family or not (Dayrat, 2005). The identification of species depends on the knowledge and facts held by taxonomists whose work cannot cover all taxon identification requested by non specialists.

Cuyo is a municipality and was once a capital of the province of Palawan (Dangan, 2006). It is located approximately 110 nautical miles from Puerto Princesa City and 30 nautical miles from Panay Island. It is the center of trade and commerce between the other municipalities near its location which includes Magsaysay and Agutaya. It is a part of the coral triangle (BFAR, 2012) which holds the world's highest diversity of coral (Veron *et al.,* 2011). It is tagged as the epicentre for the biodiversity of not only corals and fish, but many other marine organisms as it encloses 76% of reef-building coral species, and 37% of coral reef fish species (Hoegh-Guldberg *et al.,* 2009). The municipality includes 13 barangays and almost 98% of people's livelihood activities are on fishing. Both live and fresh fish are traded (BFAR, 2012). In 2017, the total landed/out-going fresh fishes were 937.12 metric tonnes while there were a total of 69.81 metric tonnes for live fish and 19.15 metric tonnes for dried fishes and other molluscs (BFAR, 2017).

However, despite of the well-known importance of Cuyo in terms of the richness of its fishery resource, there is limited information on the existing species within the area except for the information given the by Bureau of Fisheries and Aquatic Resources, Philippines which monitored the abundance of aquatic species in the municipality based on the morphological and meristic characteristics alone. Since fish identification is a tough and crucial work because of the large number of intraspesific variants or interspecific overlappings (Zhang and Hanner, 2011), taxonomists are now harnessing the power of molecular approaches in species identification (Steninke *et al.,* 2009). This approach is helpful in fish identification which is generally challenging because of their unpronounced and overlapping morphological characters (Alcantara and Yambot, 2016). Moreover, accurate characterization of the fishes must be established especially those that have deficiencies in existing taxonomic keys to deal with morphologically indistinct immature life stages which are termed as cryptic species (Armstrong and Bold, 2005). This information is needed so that the state and condition of the existing species of fishes in the island which might be threatened due to overexploitation and habitat destruction. In this study, fishes from two major commercially important families, Carangidae and Lutjanidae were identified accurately using the cytochrome *c* oxidase subunit I (COI). DNA barcoding is a solution for

faster and more accessible species identification (Hebert *et al.,* 2003a; Hebert *et al.,* 2009). All organisms can be universally identified by even a short stretch of DNA (Blaxter 2003; Hebert *et al.,* 2003a, 2003b; Kress *et al.,* 2005; Saunders, 2005; Calmin *et al.,* 2007). The differences and similarities between stocks and individuals, and the population of origin of single fish can be determined by these molecular markers combined with new statistical developments resulting in numerous new researches and applications in practical fisheries and aquaculture stock management (Okumu and Çiftci, 2004). COI is a protein that is found in mitochondria that is often used in DNA barcoding. It has been widely employed in phylogenetic studies of animals because it evolves much more rapidly than nuclear DNA, resulting to the accumulation of difference between closely related species (Moore, 1995). Evaluating the COI sequences provides the information that would help unlock the identification of many other animal species (Hebert *et al.,* 2003b). The utility of COI gene in species level has been widely demonstrated in an array of fishes.

DNA barcoding promises to improve the capacity to identify, monitor, and manage biodiversity, with profound societal and economic benefits (Vernooy *et al.,* 2010). This genomics-based methodology of specimen identification has taken the limelight of scientific realm in the year 2003 after Dr. Paul D. Hebert and his colleagues at the University of Guelph, Ontario, Canada used sequence diversity in the mitochondrial gene cytochrome c oxidase I (COI) to identify a wide array of arthropods, chordates, echinoderms, mollusks (both cephalopods and pulmunates), nematodes, platyhelminthes and eight insect orders. From the generated DNA sequences, the team created a comprehensive COI barcode profiles (about 650 base pairs) to attain species-level assignments of the analyzed specimens. After the success of the team's research findings, COI gene has been widely used around the globe as the gold standard for global bioidentification system for animals including several group of marine fishes around the globe (Alcantara and Yambot, 2016; Bingpeng *et al.,* 2018; Costa *et al.,* 2012; Ma and Craig, 2018), freshwater fishes (Hubert *et al.,* 2008; De Carvalho *et al.,* 2011; Iyiola *et al.,* 2018; Qayoom *et al.,* 2018; Pereira *et al.,* 2013; Bhattacharjee *et al.,* 2012) and ornamental fish (Hebert *et al.,* 2003a; Kadarusman, 2012; Liu *et al.,* 2013).

Species identification is essential in addressing economic fraud issues and aiding in food-borne illness outbreak investigations and is improved by an accurate barcoding method. A systematized library of barcodes will enable more people to identify species — whether abundant or rare, native or invasive — producing appreciation of biodiversity locally and globally. This is essential for developing countries and regions which tend to have a lot of species but less

taxonomic resources (Munch *et al.,* 2008). This study applied DNA barcoding to properly identify snappers, trevallies and scads collected from Cuyo, Palawan to evaluate stock assessment and set baselines for the fisher folks in catching these fishes.

Materials and Methods

Sample collection

Fishes were collected from three major fish landing sites of Cuyo (10° 51′ 01.06" N 121° 01′ 00.27" E), Palawan specifically in the localities of Cabigsing, Suba and Tenga-tenga where fishes are mostly caught using gill nets, seine nets, spears, hand lines and cast nets respectively. Some fish species that were obtained in these fish landing sites are determined based on the list given by the Bureau of Fisheries and Aquatic Resources. Fish samples were collected from October to December 2012.

Tissue collection

Fish muscles were dissected within the day of acquisition to obtain the tissues for DNA extraction. About 2 g of fish tissue was dissected in the lateral part of the fish. The collected tissue samples were preserved individually in 1.5 mL centrifuge tubes with 95% ethanol, transported to the Molecular Biology and Biotechnology Laboratory of the College of Fisheries, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines and stored at –80^{o}C until further use. Digital images of the collected fishes were also taken before dissection.

DNA Extraction from the collected tissues

Extraction of genomic DNA was accomplished using DNeasy blood and tissue kit (QIAGEN® Group, Hilden Germany). Initially, the homogenized tissue was added with 180 µl Buffer ATL and 20 µl proteinase K before incubating to 56℃. After the incubation, the working solution was vortexed after adding 200 µl Buffer AL and 200 µl ethanol (95%). The sample was then pipetted to the DNEasy Mini Spin column and was centrifuged at 8000 rpm for one min. The supernatant was then discarded and the DNEasy Mini Spin column was transferred to a new two mL tube. 500 µl of Buffer AW1 was then dispensed to the sample and was centrifuged for 3 min. The flow-through was again discarded and the DNEasy Mini Spin column was finally placed in a new 1.5 mL tube. Buffer AE was directly pipetted to the DNEasy Mini Spin column membrane before subjecting to final centrifugation.

Polymerase Chain Reaction (PCR) Assay

Mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified using four universal primers set (Table 1) for fish (Ward *et al.*, 2005 and Ivanova *et al.*, 2007). Initially, components of the PCR mix (DNA template, primer, TAQ master mix and nuclease free water) were thawed by placing them in a room temperature for 5-10 min. To ensure recovery of all the contents, the vials were vortexed and centrifuged for 10 s. Components were mixed in a 1.5 µl micro centrifuge tube placed on ice by pipetting up and down. The final volume of the PCR mix was 50 µl with following components: 25 µl of TAQ Master Mix (Vivantis Technologies, Malaysia), one µl of forward primer, one µl of reverse primer, three µl of nuclease free water and 20 µl of DNA template. The PCR assay was performed using a programmable thermal cycler (Flex Cycler, Analytic Jena AG, Germany) with the following thermal conditions: initial denaturation of 94^{o}C for 4 min followed by 40 cycles of denaturation at 94^{o}C for 30 se, annealing at 60.1^{o}C for1 min; elongation at 72^{o}C for 30 s and an additional extension step of 7 min at 72 ℃.

Table 1. Universal fish primer sets for cytochrome *c* oxidase subunit 1 (CO1) gene used in the study

Name	Primer sequence 5'-3'	Reference
Fish Primer 1 F1	TCA-ACC-AAC-CAC-AAA-GAC-ATT-GGG-AC	Ward *et al.* (2005)
Fish Primer 2 R1	TAG-ACT-TCT-GGG-TGG-CCA-AAG-AAT-CA	Ward *et al.* (2005)
F/R F1	TCG-ACT-AAT-CAT-AAA-GAT-ATC-GGC-AC	Ward *et al.* (2005)
F/R R1	ACT-TCA-GGG-TGA-CCG-AAG-AATCAG-AA	Ward *et al.* (2005)
FF2D F1	TTC-TCC-ACC-AAC-CAC-AAR-GAY-ATY-GG	Ivanova *et al.*
FF2D R1	CAC-CTC-AGG-GTG-TCC-GAA-RAA-YCA-RAA	(2007) Ivanova *et al.*
VF1 F1	TGT-AAA-ACG-ACG-GCC-AGT-TCT-CAA-CCA-ACC-ACA-AAG-ACA-TTG-G	(2007)
VF1 R1	CAG-GAA-ACA-GCT-ATG-ACT-AGA-CTT-CTG-GGT-GGC-CAA-AGA-ATC-A	Ivanova *et al.* (2007) Ivanova *et al.* (2007)

Gel electrophoresis and UV visualization

PCR products were visualized on 1% agarose gel. 10 µl of the PCR product was loaded into agarose gel and electrophoresed at 150 volts for 45 min. The size of the amplicons was determined using 100 base pair molecular weight

ladder (Hoffman-La Roche Ltd., Switzerland). The electrophoresed gel was stained using GelRed™ Nucleic Acid Gel Stain (Life Technologies, India) for 30 min and was visualized using Alphadigidoc Pro Imaging System.

DNA sequencing

PCR products were sent to AIT Biotech Laboratories, Singapore for bidirectional sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit and ABI PRISM 3730xl DNA Analyzer (Applied Biosystems, USA) following manufacturer's protocol. Concentrations of the PCR products in ng/µl were also measured before sending the samples to Singapore.

Sequence assembly and molecular identification

The forward and reverse sequences of the samples were assembled using Sequence Scanner (Applied Biosystems, USA). After the consensus editing, the generated sequences were pasted and matched to the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) to facilitate molecular identification.

Phylogenetic Analysis

The sequences were aligned using ClustalX software and were determined to be suitable for neighbor-joining trees (Saitou & Nei 1987). The Kimura-two parameter distance model (Kimura 1980) was used as a substitution model. All genetic distance analyses were established using Molecular Evolutionary Genetics Analysis (MEGA7) software (Kumar et al., 2016). To facilitate sequence comparison, the following sequences were acquired from the GenBank: EF609402.1 and EU600101.1 for *L. vitta*; JF493822.1 and GU805107.1 for L. bohar; JF952789.1 and EF609399.1 for *L. quinquelineatu;* ; JN182927.1 and EU502685.1 for *L. argentimaculatus;* JN021227.1 and EU595217.1 for *L. russellii*; FJ237569.1 and FJ237568.1 for *P. multidens*; EF609403.1 and FJ583629.1 for *M. macularis;* for the Family Lutjanidae. JF493022.1 and JF493021.1 for *C. coeruleopinnatus;* JX261305.1 and JX261275.1 for *C. hedlandensis;* JX261557.1 and HQ561020.1 for *Atule mate;* HQ560947.1 and HQ560966.1 for *C. sexfasciatus;* FJ347936.1 and FJ347905.1 for *C. ignobilis;* HQ560948.1 and JX261134.1 for *D. macrosoma;* HQ560967.1 and HQ561001.1 for *S. Crumenophthalmus* for the Family Carangidae.

Results

A total of 14 COI sequences representing 14 species belonging to 8 genera from the 2 major commercially important families of fishes namely Lutjanidae and Carangidae were generated from the three fish landing sites of Cuyo. After the sequence assembly and consensus editing, sequence read an average length of 573 base pair long. No insertions, deletions or stop codons were observed among sequences indicating the absence of nuclear mitochondrial pseudogenes (NUMTs).

Sequences obtained were compared on the data of on GenBank for its identification. The database revealed maximum identity matches of 99-100% for all the sequences of the 3 families. Kimura-two parameter (K2P) model was used to calculate sequence divergence and the Neighbour-joining (NJ) trees of K2P distances were created to provide a graphic representation and separation of species based on the 'Sequence Analysis' module of BOLD. The average overall K2P distance was 20.3% with 22.5% and 20.5% corresponding to, interfamily and interorder K2P genetic distances respectively. Bootstrapping was completed using 2000 pseudoreplications for more accurate results. Average nucleotide frequencies are as follows: G: 18.4%, C: 27.6%, T: 29.5% and A: 24.5%. Overall GC content is 46.14%. The sequences were submitted to GenBank and accession numbers for each species were acquired (Table 2).

Table 2. Fish species identified with their corresponding Families and GenBank acquired sequences

Family	Species	Genbank Accession No.
Lutjanidae	*Lutjanus vitta*	KC970486
	Lutjanus bohar	KC970483
	Lutjanus quinquelineatus	KC970484
	Lutjanus argentimaculatus	KC970482
	Lutjanus russellii	KC970485
	Pristipomoides multidens	KC970498
	Macolor macularis	KC970487
Carangidae	*Carangoides coeruleopinnatus*	KC970454
	Carangoides hedlandensis	KC970455
	Atule mate	KC970450
	Caranx sexfasciatus	KC970458
	Caranx ignobilis	KC970457
	Decapterus macrosoma	KC970467
	Selar crumenophthalmus	KC970506

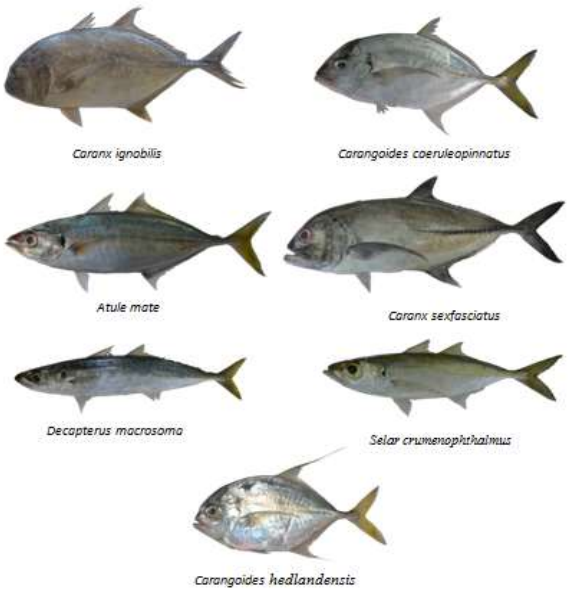

Figure 1. Molecularly identified species of Family Carangidae collected from Cuyo, Palawan, Philippines

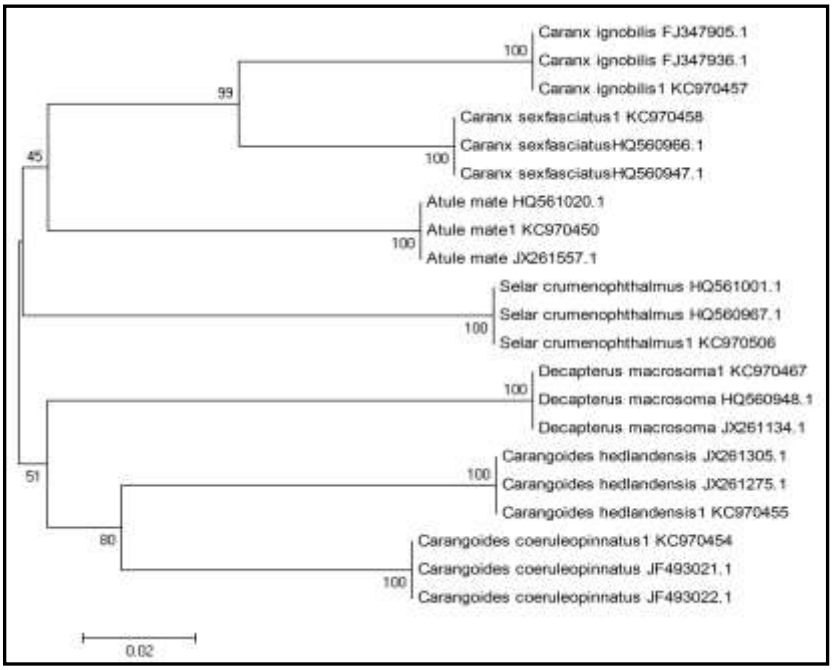

Figure 2. Neighbour- joining tree of cytochrome oxidase subunit 1 (CO1) gene sequences derived from Family Carangidae compared to the sequences obtained from Genbank

Carangids

Caranx, Carangoides, Decapterus, Selar and *Atule* were the genus of the collected seven species from Family Carangidae (Figure 1). The study revealed that genetic distance within species is also 0.000% whereas 15.90% is the distance between species. Total mean distance among species is 14%. Samples were clustered per species with a bootstrap value of 100% (Figure 2). Average nucleotide frequencies are as follows: G: 18.7%, C: 26.8%, T: 30.1% and A: 24.3% with an average GC content of 45.59%.

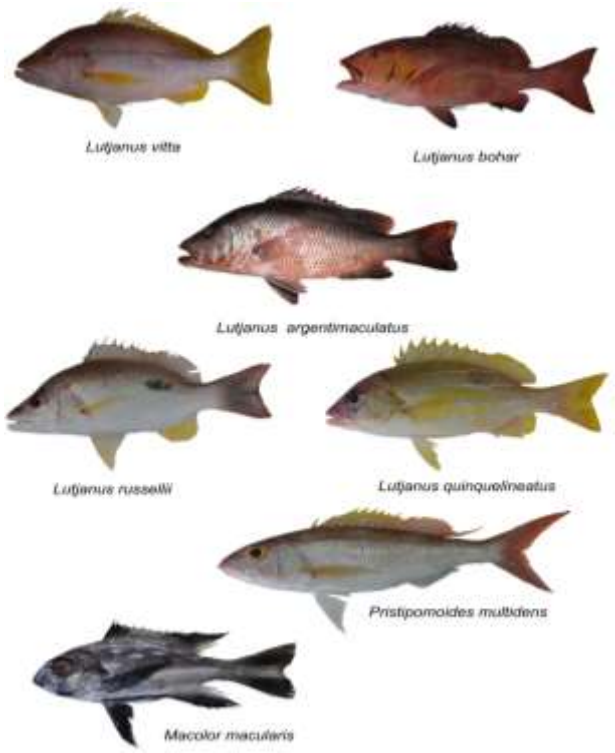

Figure 3. Molecularly identified Species of Family Lutjanidae collected from Cuyo, Palawan

Snappers

There were seven species collected from the family Lutjanidae (Figure 3) which belongs to genus *Lutjanus*, *Macolor* and *Pristipomoides*. The average genetic distance within was calculated with a value of 0.000% while distance between species is 15.56%. Overall mean distance among species is 14.8%. Average nucleotide frequencies were tallied as G: 18.1%, C: 28.3%, T: 28.8%

and A: 24.7% with an average GC content of 46.69%. Distinct clusters according to species were observed in the NJ tree (Figure 4) with a bootstrap value of 100%. One of the species, *Macolor macularis* has clustered to the other species of genus *Lutjanus*.

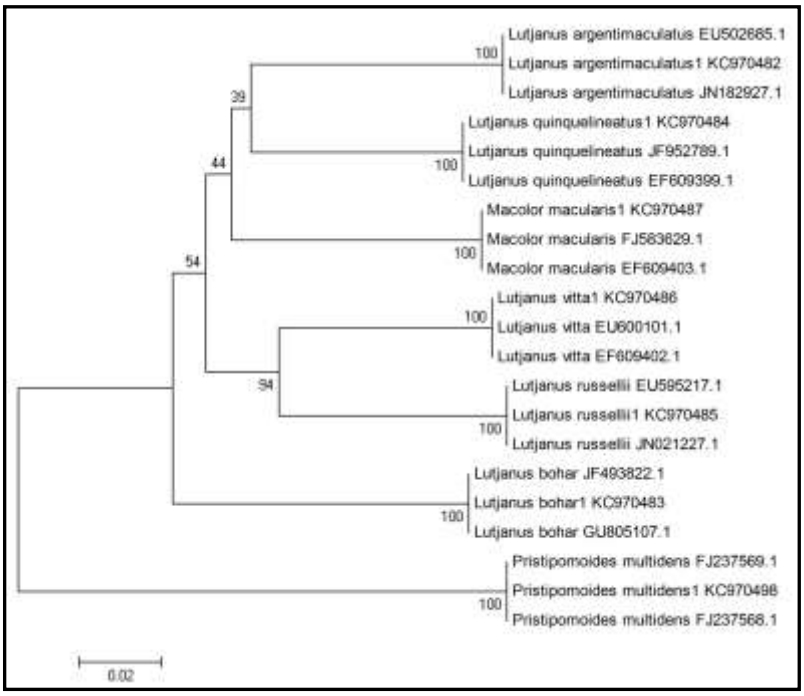

Figure 4. Neighbour- joining tree of cytochrome oxidase subunit 1 (CO1) gene sequences of Family Lutjanidae compared to the sequences acquired from GenBank

Discussion

After the COI sequence generation and BLAST identification, this study formally established the molecular inventory of 2 major commercially important families of fishes in the Philippines particularly in Cuyo, Palawan. Efficacy of DNA barcoding through the COI gene was achieved after generating 14 COI sequences which embodies 14 species from family Carangidae and Lutjanidae in Cuyo within the three-month sampling period. No insertions, deletions or stop codons were observed among sequences indicating the absence of nuclear mitochondrial pseudogenes (NUMTs). These pseudogenes are problematic when coamplified in DNA barcoding as it can lead to misidentification of species (Moulton *et al.*, 2010).

Identification of species through molecular approach depends on the intraspecific homogeneity and interspecific heterogeneity of species being examined (Leivens *et al.,* 2001; Zhang and Hebert, 2011). In this study, average K2P distances were analyzed by observing the Neighbour-joining (NJ) trees. All species resemble very low K2P value of 0.000% (Table 2) after comparing sequences to GenBank. This means that there is no confusion in taxonomic assignments as a result of inter-specific hybridization (Verspoor and Hammar, 1991). It also connotes that no major genetic variation occurred regardless of location of the sampling area that has been proven earlier in the study of Ward *et al.,* 2008 in which they found out that thirteen species which are shared between North Atlantic and Australasian southern waters exhibit no significant evidence of spatial genetic differentiation. Higher genetic divergences lead to doubtful taxonomic classification and may result to identification of new species (Avise, 2000).

Significance of the branching order of trees was estimated by bootstrapping (Collins *et al.,* 1994). Bootstrap values were very high for each cluster of species, and the value is constant at 100%. It implies that each species represents a distinct group in the tree (Suau *et al.,* 1999). Species with the same genus and family usually clustered together in most cases (Lakra *et al.,* 2011).

Average GC content was computed with a value of 46.14% which is a little lower from the value acquired in the barcoding of Lakra *et al.,* (2011) of marine fishes in India. GC content per family was also computed. Species from family Lutjanidae has higher GC content of 46.69% and Carangidae has 45.59%. These values are high enough and close also to the GC content obtained from species of Osteichthyes in Australia which is 47.1% (Ward *et al.,* 2005).

Identification based on morphological characteristics using conventional taxonomy is usually laborious, may create ambiguity and in some cases, impossible to accomplish. With this, molecular identification based on COI sequences proved to be an effective tool for a more reliable identification of high-valued aquatic species from Cuyo, Palawan. In a global perspective, DNA barcoding has been proven to be a fast and accurate tool for standardized molecular identification system (Lakra *et al.,* 2011, Frézal and Leblois, 2008; Hebert *et al.,* 2003a). This exceptionally distinct methodology of species classification has emerged as one of the leading tools in taxonomy, molecular phylogenetics and population genetics worldwide (Hajibabaei *et al.,* 2007). In several countries worldwide, DNA barcoding has already exhibited its noble purpose of DNA- based species identification after successfully barcoded 115 species of Indian marine fishes (Lakra *et al.,* 2011), 121 species of marine fishes in China (Zhang, 2011) and 207 Australian fish species (Ward *et al.,*

2005). Moreover, DNA barcoding has been used for the conservation and management of Amazonian commercial fishes (Ardura *et al.,* 2010) and plays a very crucial role in correctly identifying species.

DNA barcoding as a tool promises to rapidly resolve almost all of the identification questions in the Taxonomy world (Victor *et al.,* 2008). Moreover, one of the noble features of barcoding is that it permits the separation of rare from common species. In the study of Victor *et al.,* (2008), two species of *Lutjanus* were molecularly identified because of their deep genetic divergence in their early life stages while in their appearance in their adult phase converge. There are many reef-fish genera which includes abundant species with close relatives that can be rare, or even undescribed (Victor, 2007).

DNA barcoding has also proved its noble purpose and function in biodiversity. Zemlack *et al.,* (2009) estimated that the future DNA barcoding studies will reveal deep genetic divergences of species. It is expected that further revelations of overlooked diversity will be answered by barcode studies assessing the genetic divergences in fish species currently thought to span other abyssal divides.

Since Cuyo, Palawan is a part of Coral triangle which is well recognized as the global apogee of marine biodiversity, with species richness incrementally decreasing from this region eastward across the Pacific Ocean and westward across the Indian Ocean (Veron *et al.,* 2009 and Hoeksema, 2007). It is important therefore to know the species present through its vicinity for sustainable balance of ecosystem and to avoid extinction. Moreover, correct identification of species in a particular area is vital in implementing efficient assessment and management of the stocks, thereby assuring their availability for domestic consumption and exports.

With regard to stock assessment and management, this molecular identification study has revealed the inventory of the most vulnerable fish species prone to human exploitation. One species of trevally, *Caranx sexfasciatus*, is listed under "decreasing population". In Cuyo, Palawan, the bigeye trevally is one of the high-valued fishes which have a greater demand in their market. In general, all the molecularly identified species is vulnerable to exploitation because of the heavy fishing efforts in the area. Commercially important fishes decrease because of large number of consuming population, demands on marine protein sources, pollution, medicinal value of some marine aquatic animals and also demands of fishery trade (Holmlund and Hammer, 1999).

The diversity of fish species especially in a place where there is a rich fish variety is important in informing people properly the practices that should be done or avoided in order to maintain the abundance of the nature and

environment. The sequences of the species collected closely resembled that of GenBank's in terms of the percentage identity registered at NBCI. DNA barcoding is proven to be an effective and accurate tool for proper species identification. Hence, further study is needed to determine the biodiversity of fishes in Cuyo.

Acknowledgement

The researchers would like to extend its deepest gratitude to the Bureau of Fisheries and Aquatic Resources—Cuyo, Palawan particularly to Mr. Rey R. Templonuevo for giving all the data needed about Cuyo and its fishing industry and to Mr. Sherwin L. Orilla for helping in collecting the fish samples from the fish landing sites.

The researchers would also like to thank the Department of Agriculture- Biotech for granting funds for the research.

References

Alcantara, S. G. and Yambot, A. P. (2016). DNA barcoding of commercially important Grouper species (Perciformes, Serranidae) in the Philippines. National Center for Biotechnology Information. 6:3837-3845.

Ardura, A., Linde, A. R., Moreira, J. C. and Garcia-Vazquez, E. (2010). DNA barcoding for conservation and management of Amazonian commercial fish. Biological Conservation. 143:1438-1443.

Armstrong, K. F. and Ball, S. L. (2005). DNA barcodes for biosecurity: invasive species identification. Philosophical Transactions of the Royal Society B. 360:1813-1823.

Avise, J. C. (2001). Phylogeography: The history and formation of species. American Zoology. 41:133-135.

Bingpeng, X., Heshan L., Zhilan, Z., Chunguang, W., Yanguo, W. and Jianjun, W. (2018). DNA barcoding for identification of fish species in the Taiwan Strait. PLoS ONE. 13:e0198109.

BFAR (2012). Status and Facts of Municipal Fishing in Cuyo Island. Bureau of Fisheries and Aquatic Resources. Cuyo, Palawan, Philippines. pp.3.

BFAR (2017). Monthly Consolidated for Landed Fishery Products for the Month of January-December 2017. Bureau of Fisheries and Aquatic Resources. Cuyo, Palawan, Philippines. pp.12.

Bhattacharjee, M. J., Laskar, B. A., Dhar, B. and Ghosh, S. K. (2012). Identification and re-evaluation of freshwater catfishes through DNA barcocing. PLoS One. 7:e49950.

Blaxter, M. (2003). Molecular systematics — counting angels with DNA. Nature. 421:122-124.

Calmin, G., Belbahri, L. and Lefort, F. (2007). Direct PCR for DNA barcoding in the genera *Phytophopthora* and *Pythium*. Biotechnoly and Biotechnological Equipment. 21:40-42.

Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. and Farrow, J. A. E. (1994). The phylogeny of the genus *Clostridium*: Proposal of five new genera and eleven new species combinations. International Journal of Systematic and Evolutionary Microbiology.44:812-826.

Costa, F. O., Landi, M., Martins, R., Costa, M. H., Costa, M. E., Carneiro, M., Alves, M. J., Steinke, D. and Carvalho, G. R. (2012). A ranking system for reference libraries of DNA barcodes: application to marine fish species from Portugal. PLoS One. 7:e35858.

Dangan, F. V. (2006). Contrastive Analysis of True and False Cognates in Filipino and Cuyonon. Tenth International Conference on Austronesian Linguistics, Puerto Princesa City, Palawan, Philippines. pp.10.

Dayrat, B. (2005). Towards integrative taxonomy. Biological Journal of the Linnean Society. 8:407-415.

De Carvalho, D. C., Oliveira, D. A. A., Pompeu, P. S., Leal, C. G., Oliveira, C. and Hanner, R. (2011). Deep barcode divergence in Brazilian freshwater fishes: the case of Sao Francisco River Basin. Mitochondrial DNA. 22:80-86.

Frézal, L. and Leblois, R. (2008). Four years of DNA barcoding: current advances and prospects. Infection Genetics and Evolution. 8:727-36.

Hajibabaei, M., Singer, G. A. C., Hebert, P. D. N. and Hickey, D. A. (2007). DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Elsevier. 24:167-172.

Hebert, P. D. N., Cywinska, A., Ball, S. L. and de Waard, J. R. (2003a). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B. 270:313-321.

Hebert, P. D. N., Ratnasingham, S. and deWaard, J. R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B. 270:96-99.

Hebert, P. D. N., deWaard, J. R. and Landry, J. F. (2009). DNA barcodes for 1/1000 of the animal kingdom. Biology Letters. 6:359-362.

Holmlund, C. M. and Hammer, M. (1999). Ecosystem services generated by fish populations. Ecological Economics. 29:253-268.

Hoegh-Guldberg, O., Hoegh-Guldberg, H., Veron, J. E. N., Green, A., Gomez, E. D., Lough, J., King, M., Ambariyanto, Hansen, L., Cinner, J., Dews, G., Russ, G., Schuttenberg, H. Z., Peñafl or, E. L., Eakin, C. M., Christensen, T. R. L., Abbey, M., Areki, F., Kosaka, R. A., Tewfik, A. and Oliver, J. (2009). The Coral Triangle and Climate Change: Ecosystems, People and Societies at Risk. WWF Australia, Brisbane. pp. 276.

Hubert, N., Hanner, R., Holm, E., Mandrak, N. E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, A. and Bernatchez, L. (2008). Identifying Canadian freshwater fishes through DNA barcodes. PLoS One. 3:e2490.

Ivanova, N. V., Zemlak, T. S., Hanner, R. H. and Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes. 7:544-548.

Iyiola, O. A., Nneji, L. M., Mustapha, M. K., Nzeh, C. G., Oladipo, S. O., Nneji, I. C., Okeyoyin, A. O., Nwani, C. D., Ugwumba, O. A., Ugwumba, A. A. A., Faturoti, E. O., Wang, Y., Chen, J., Wang, W. Z. and Adeola, A. C. (2018). DNA barcoding of economically important freshwater fish species from north- central Nigeria uncovers cryptic diversity. Ecology and Evolution. 8:6932-6951.

Kadarusman, Hubert, N., Hadiaty, R. K., Sudarto, Paradis E. and Pouyaud, L. (2012). Cryptic Diversity in Indo-Australian Rainbowfishes Revealed by DNA Barcoding:Implications for Conservation in a Biodiversity Hotspot Candidate. PLoS ONE. 7: e40627.

Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A. and Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences of the United States of America.102:8369-8374.

Lakra, W. S., Verma, M. S., Goswami, M., Lal, K. K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K. V., Ward, R. D. and Hebert, P. N. D. (2011). DNA barcoding Indian marine fishes. Molecular Ecology Resources. 10:60-71.

Lievens, S., Goormachtig, S. and Holsters, M. (2001). A critical evaluation of differential display as a tool to identify genes involved in legume nodulation: looking back and looking forward. Nucleic Acids Research. 29:3459-3468.

Liu, S. Y. V., Ho, H. C. H. and Feng-Dai, C. (2013). A new species of Pomacentrus (Actinopterygii: Pomacentridae) from Micronesia, with comments on its phylogenetic relationships. Zoological Studies. 52:1-6.

Ma, K. Y. and Craig, M. T. (2018). An Inconvenient Monophyly: An Update on the Taxonomy of the Groupers (Epinephelidae). The American Society of Ichthyologists and Herpetologists. 106:443-456.

Moore, W. S. (1995). Inferring phylogenetics from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution. 49:718-726.

Moulton, M. J., Song, H. and Whiting, M. F. (2010). Assessing the effects of primer specificity on eliminating numt coamplification in DNA barcoding: a case study from Orthoptera (Arthropoda: Insecta). Molecular Ecology Resources. 10:615-627.

Munch, K., Boomsma, W., Willerslev, E. and Nielsen, R. (2008). Fast phylogenetic DNA barcoding. Philosophical Transactions of the Royal Society. 363:3997-4002.

Okumu, I. and Çiftci, Y. (2004). Fish population genetics and molecular markers: II-Molecular markers and their applications in fisheries and aquaculture. Turkish Journal of Fisheries and Aquatic Sciences. 3:51-79.

Pereira, L. H., Hanner, R., Foresti, F. and Oliveira, C. (2013). Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? .BMC Genetics. 20:1-14.

Qayoom, U., Pawar, R. A., Mohite, S. A., Sawant, M. S., Nirmale, V. H. Pawar, S. P., Goswami, M. and Lakra, W. S. (2018). DNA barcoding of some commonly exploited fishes from the northern Western Ghats, India. Indian Journal of Animal Sciences. 88:245-250.

Saitou, N. and Masatoshi, N. (1987). The neighbor-joining method—a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4:406-425.

Saunders, G. W. (2005). Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. Philosophical Transactions of the Royal Society B. 360:1879-1888.

Suau, A., Bonnet, R., Sutren, M., Godon, J. J., Gibson, G. R., Collins, M. D. and Doré, J. (1999). Direct Analysis of Genes Encoding 16S rRNA from Complex Communities Reveals Many Novel Molecular Species within the Human Gut. American Society for Microbiology. 65:4799-4807.

Steinke, D., Vences, M., Salzburger, W. and Meyer, A. (2005). TaxI: a software tool for DNA barcoding using distance methods. Philosophical Transactions of the Royal Society B. 360:1847-1857.

Verspoor, E. and Hammar, J. (1991). Introgressive hybridization in fishes: the biochemical evidence. Journal of Fish Biology. 39:309-334.

Vernooy, R., Haribabu, E., Muller, M. R., Vogel, J. H., Hebert, P. D. N., Schindel, D. E., Shimura, J. and Singer, G. A. C. (2010). Barcoding life to conserve biological diversity: beyond the taxonomic imperative. PLoS Biology. 8:417-1317.

Veron, J. E. N., DeVantier, L. M. and Turak, E. (2009). Delineating the coral triangle. Galaxea. 11:91-100.

Veron, J. E. N., DeVantier, L. M., Turak, E., Green, A. L., Kininmonth, S., Stafford-Smith, M. and Peterson, N. (2011). The Coral Triangle. In: Dubinsky Z, Stambler N ed. Coral Reefs: An Ecosystem in Transition, Dordrecht, The Netherlands: Springer. pp. 47-55.

Victor, B. C. (2007). *Coryphopterus kuna*, a new goby (Perciformes: Gobiidae: Gobiinae) from the western Caribbean, with the identification of the late larval stage and an estimate of the pelagic larval duration. Zootaxa. 1526:1-61.

Victor, B. C., Hanner, R., Shivji, M., Hyde, J. and Caldow, C. (2009). Identification of the larval and juvenile stages of the Cubera Snapper, *Lutjanus cyanopterus*, using DNA barcoding. Zootaxa. 2215:24-36.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. and Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. Proceedings of the Royal Society of London B. 360:1847-1857.

Ward, R. D., Costa, F. O., Holmes, B. H. and Steinke, D. (2008). DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence for most taxa, but *Zeus faber* and *Lepidopus caudatus* each probably constitute two species. Aquatic Biology. 3:71-78.

Zemlak, T. S., Ward, R. D., Connell, A. D., Holmes, B. H. and Hebert, P. D. N. (2009). DNA barcoding reveals overlooked marine fishes. Molecular Ecology Resources. 1:237-242.

Zhang, J. (2011). Species identification of marine fishes in China with DNA barcoding. Evidence Based Complementary and Alternative Medicine. 10:1-10.

Zhang, J. and Hanner, R. (2011). DNA barcoding is a useful tool for the identification of marine fishes from Japan. Biochemical and Systematics Ecology. 39:31-42.

(Received: 15 September 2018, accepted: 31 October 2018)