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DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port

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Abstract. *Prehadi, Sembiring A, Kurniasih EM, Arafat D, Subhan B, Madduppa HH. 2015. DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port. Biodiversitas 16: 55-61.* Sharks are one of main fisheries commodity that are currently exploited on a large scale because of their high economic value. The identification of sharks has been a difficult one due to the specimen's similarity in morphology and mostly have had key diagnostic features removed. This study aimed to identify and to review the status of sharks, and also to reconstruct the shark species that were landed at South Java fishing port using molecular approaches. The DNA amplification was using cytochrome oxidase I mitochondrial of locus and 600-700 basepairs. A total of seven species from 59 individuals was identified including *Alopias pelagicus*, *Carcharhinus falciformis, C. sorrah, C. amblyrhynchos, Galeocerdo cuvier, Atelomycterus marmoratus,* and *Spyrna lewini*. The diversity of shark species landed in Muncar during the last 2 years has been decreased. The identified sharks species in this study sites were about 18% of all Indonesian sharks. The result of this study is expected help the Government to manage shark fisheries in Indonesia.

Keywords: DNA barcoding, Muncar, phylogenetic, sharks, southern Java.

INTRODUCTION

Sharks has been greatly exploited in various countries across the globe, including Indonesia, which ranked first in the list of the 20 biggest shark fishing countries above India, Spain, Taiwan and Argentina between 2002-2011 (Mundy et al. 2013). During that period, Indonesia has been exporting 10,762 tons of shark fins or 13% of the total world exports (Mundy et al. 2013).

The biological characters of shark are making them vulnerable to exploitation. In their natural habitat, sharks are known to be slow in reaching maturity stage (8-13 years) and also low reproduction level (Camhi et al. 1998). The effect from shark fisheries is damaging and hence the fast decreasing population of shark in the wild as proved by a number of studies in Northwest Atlantic, Southeast Australia, Gulf of Mexico, South Africa and Australian Great Barrier Reef (Holden 1973; Casey and Myers 1998; Graham et al. 2001; Baum and Myers 2004; Baum et al. 2005; Dudley and Simpfendorfer 2006; Robbins et al. 2006; Burgess et al. 2005). Population decline occurred as an effect from intensive and continuous fishing due to unmanaged and unregulated fisheries (Dharmadi and Fahmi 2005). The main problem is that the data given from the port is only the volume of total production without describing the shark species and the total individuals caught, for example in Muncar fish landing site (UPPPP Muncar 2013). Muncar is one of the ports that plays an important role in shark fishing in Southern Java, aside from Cilacap of Central Java and Palabuhanratu in West Java (White et al. 2006).

The identification of sharks has been a difficult one due to the specimen's similarity in morphology and mostly have had key diagnostic features removed. In recent years, molecular approach has been able to give solutions in identifying shark species, especially when the taxonomy method is impossible to conduct due to insufficient morphological information such as ones that has been implemented on shark fin and fillet (Smith et al. 2001). Hebert et al (2003) introduced DNA barcode method with mitochondrial marker for all animal species, for one chain of gene is claimed to be enough for distinguishing one species to another. DNA barcoding method uses primer in PCR (Polymerase Chain Reaction) process to amplify the DNA until around 600-700 bp on cytochrome oxidase I (COI) locus of mitochondrial. DNA barcoding method has been used to identify over 207 species of fish in Australia including 143 species of teleostean, 61 species of shark and stingrays, and 3 species of chimaerid (Ward et al. 2008).

There are around 35 species of sharks that has been fished in Indonesia based on DNA barcoding analysis (Sembiring et al. 2014). As many as 10 species of shark has been recorded in Cilacap and 6 others in Palabuhanratu through DNA barcoding method (Sembiring et al. 2014). Phylogenetic is a description of relationship based on DNA sequence composition or protein which resembles to that of a tree to estimate the past evolution process (Baldauf 2003). Phylogenetic reconstruction is able to analyze the

gene distance through DNA variation with Neighbor Joining Tree method (Saitou and Nei 1987) that is capable on calculating the distance which is shown with bootstrap value.

The current study was conducted to identify shark species landed in Muncar, Banyuwangi, East Java in 2013, and to reconstruct phylogenetic tree between species found in Muncar compared with Cilacap and Palabuhanratu which previously has been analyzed through DNA barcoding (Kurniasih 2013; Rahmad 2013).

MATERIALS AND METHODS

Sample collection

The tissue sample of shark were collected from three fisheries landing site in Java Island (Indonesia), i.e.: Muncar, Palabuhanratu and Cilacap (Figure 1). A total of 59 samples were collected in Muncar in July 2013. The tissue sample was stored in 95% ethanol. In 2012, samplings were done in 2 days covering all warehouses in Muncar, while the recent research was done within 30 days when all the catch is landed from ships.

PCR extraction, amplification, and sequencing

DNA was extracted using 10% chelex (Walsh 1991). The DNA extracts were stored at -30°C until required for laboratory analyses. The mitochondrial cytochrome oxidase I (COI) gene was amplified by polymerase chain reaction (PCR) using the forward primer fish-BCL (5' TCA ACY AAT CAY AAA GAT ATY GGC AC '3) and reverse primer fish-BCH (5'ACT TCY GGG TGR CCR AAR AAT CA '3) (Baldwin et al. 2009). PCR amplifications were performed in 26 µL reaction mixture containing 2 µL 25 mM MgCl2, 2.5 µL 8 M dNTPs ; 1.25 µL each primer pair 10 mM; 0,125 µL Taq DNA polymerase, 2.5 µL 10xPCR Buffer, 2 µL DNA template, 14.5 µL deionize water (ddH2O). Cycling parameters were an initial denaturation at 95°C for 2 min followed by 35 cycles of denaturation (94°C for 30 s), annealing (50 °C for 30 s), and extension (72°C for 45 s) with the final extension step at 72°C for 2 min. PCR-amplified DNAs were visualized on 1% agarose gels. Prior to sequencing, excess dNTPs and oligonucleotides were eliminated from the PCR product using shrimp alkaline phosphatase and exonuclease I (Exo-SAP-IT kit; Affymetrix, Santa Clara CA, U.S.A.) following the manufacturer's protocol. Sequence reactions were performed in both directions using the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems), 8-10 L purified PCR product, and 4-5 L of either primer (3 M) per reaction. Sequence-reaction products were loaded into an ABI 3130xl automated sequencer (Applied Biosystems) at the Berkeley Sequencing Facility located in the United States (Sanger et al. 1997).

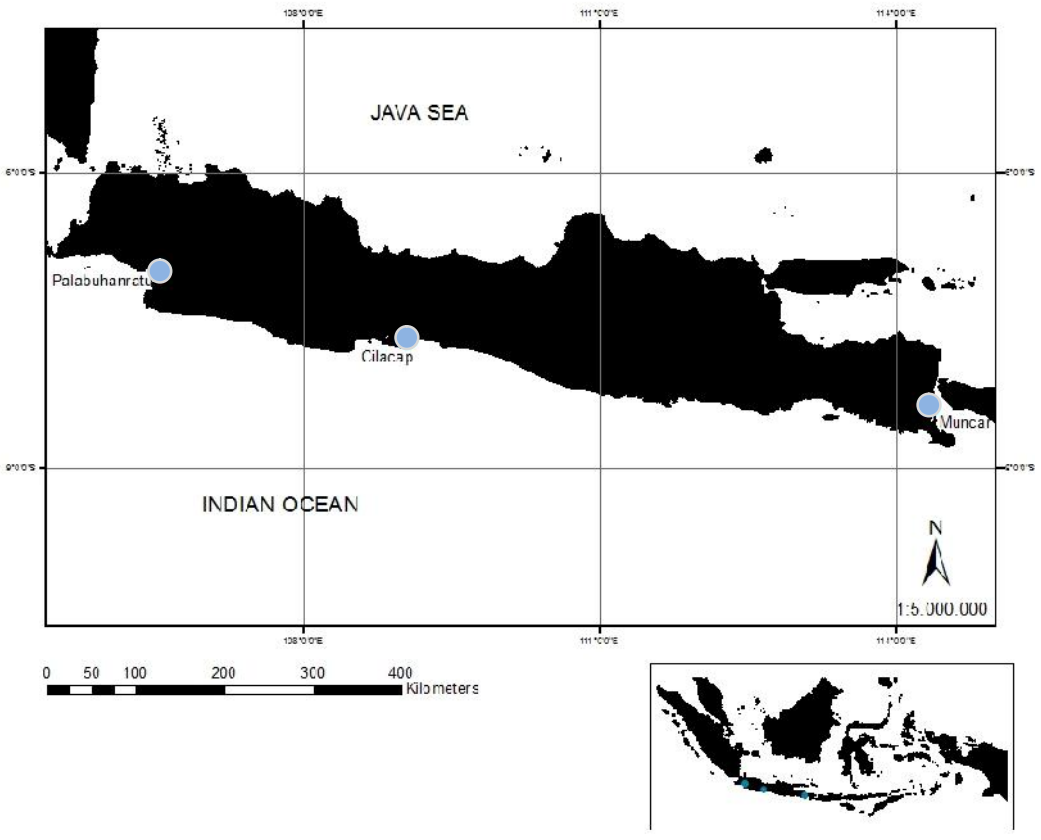

Figure 1. Sampling location in three main fisheries landing site in Indonesia (Muncar, Palabuhanratu, and Cilacap), located in Jawa Island (blue circle).

Data analysis

Forward and reverse sequences were proofread, aligned and edited using MEGA 5.2 (Tamura et al. 2011). Edited sequences were deposited in GENBANK (http://www.ncbi.nlm.nih.gov/). The nucleotide sequence data will be matched with the data contained in GenBank at the NCBI (National Center for Biotechnology Information) for species identification by using BLAST (Basic Local Alignment Search Tool) in website address http://blast.ncbi.nlm.nih.gov/Blast.cgi. A neighbor joining phylogenetic trees was reconstructed in MEGA 5.2 (Tamura et al. 2011) using Kimura-2-parameter models (Kimura 1980) with 1000 of bootstrap value. Samples that have been analyzed is then compared with samples from Palabuhanratu and Cilacap (39 and 35 samples, respectively). Furthermore, the data from July 2013 from Muncar will be compared to catch from August 2012. A review of the conservation status was determine with IUCN (2014), and the trade status was determine in CITES (2014). The near threatened species are the population that is in danger of declining in the near future if nothing can be done to prevent it. Vulnerable species are those who have a high risk of extinction in the wild (IUCN-SSC 2001).

RESULTS AND DICUSSION

DNA barcoding, conservation status, and trading status

A total of seven species from 59 tissue samples belongs to four different families (Alopiidae, Carcharhinidae, Scyliorhinidae, and Sphyrnidae) has been successfully identified (Table 1). Family of Carcharhinidae is the most common family found in Muncar with four species with a total of 46 individuals. 41 of those belong to species of *Carcharhinus falciformis* (Silky shark). Carcharhinidae Family is the most commonly caught might be due to their diversified habitat or fishing gear used by fishermen. The number of Sphyrnidae Family found in Muncar is 11 individuals which consist of a single species, *Sphyrna lewini* or scalloped hammerhead.

All sharks identified in Muncar have been assessed by IUCN: one species is categorized as Threatened (*Sphyrna lewini*), five species categorized as near threatened (*Carcharhinus, C. amblyrhynchos, C. sorrah, Galeocerdo cuvier,* and *Atelomycterus marmoratus*) and 1 other species is categorized as vulnerable (*Alopias pelagicus*). Most species are not evaluated by CITES, but *Sphyrna lewini* is included Appendix II.

Shark species landed in Muncar in 2012 and 2013

Table 2 describes sharks species landed in Muncar for the last 2 years and were identified using DNA barcoding. *C. falciformis* was the most collected species within two sampling period. There was a slight different in species identified between two period of sampling (2012 and 2013). Low number of species was recorded in 2013 (7 species) compared with in 2012 (11 species), even though sampling duration was longer in 2013 (30 days) than in 2012 (2 days).

The huge differences of duration of the sampling and the number of species observed might be indication in declining shark population in the wild. However, many factors could influence on status of exploited species, such as type of gear, location fished, biological characters of shark as migratory species (Lynch et al. 2011).

The number of shark species being exploited in Muncar reached 14 species or around 18% of the entire shark species found in Indonesia (White et al. 2006). That number can be considered as huge in shark fishing, as sharks greatly varied in terms of maturing and young being produced (Castro and Mejuto 1995). Based on these facts, it is inevitable that proper shark fishing management is urgent to be implemented in Muncar, Banyuwangi, East Java.

Table 2. The number of individual of identified shark species landed in Muncar between two period sampling (2012 and 2013) through DNA barcoding

Species	2012	2013
Carcharhinus falciformis	6	41
Carcharhinus brevipinna	5	-
Carcharhinus limbatus	1	-
Carcharhinus sorrah	2	2
Galeocerdo cuvier	1	2
Hemitriakis indroyonoi	4	-
Mustelus lenticulatus	6	-
Alopias pelagicus	3	1
Alopias superciliosus	1	-
Isurus oxyrinchus	1	-
Prionace glauca	1	-
Carcharhinus amblyrhynchos	-	1
Sphyrna lewini	-	11
Atelomycterus marmoratus	-	1

Table 1. Identified shark species landed in Muncar using BLAST program, along with IUCN and CITES status

Family	BLAST analysis	Common name	Max. Similarity (%)	No. of individual	Red List Status (2014)	CITES status (2014)
Alopiidae	*Alopias pelagicus*	Pelagic Thresher	100	1	Vulnerable	Not evaluated
Carcharhinidae	*Carcharhinus falciformis*	Silky Shark	100	41	Near threatened	Not evaluated
	Carcharhinus amblyrhynchos	Grey Reef Shark	99	1	Near threatened	Not evaluated
	Carcharhinus sorrah	Spot-tail Shark	100	2	Near threatened	Not evaluated
	Galeocerdo cuvier	Tiger shark	100	2	Near threatened	Not evaluated
Scyliorhinidae	*Atelomycterus marmoratus*	Coral catshark	99	1	Near threatened	Not evaluated
Sphyrnidae	*Sphyrna lewini*	Scalloped Hammerhead	100	11	Endangered	Appendix II

Phylogenetic reconstruction

A total of seven clades with a bootstrap value of 100 which means they possess close kinship between individuals (Figure 2). The large group consists of species such as *Carcharhinus falciformis, C. sorrah, C. amblyrhynchos, Galeocerdo cuvier, Alopias pelagicus, Sphyrna lewini,* and *Atelomycterus marmoratus*. Cladogram could be used to show that in every family there are species which is located very close. This indicates that the study on a species of one particular family could be described through phylogenetic reconstruction (Zuazo and Agnarson 2010).

Figure 3 describes phylogenetic reconstruction of *C. falciformis* from 3 locations and formed 2 different clades with both bootstrap value of 65 therefore not really strong to hold the position if added by another individuals, but these two clades will still be different nevertheless. There is one clade which the individual is a *C. falciformis* from Palabuhanratu, but on the other side there is one other clade. It indicates that there are two different genetic groups of *C. falciformis* in Palabuhanratu, which means that they come from two different populations (Zhao et al. 2013) in Southern Java and proved how wide the distribution of this species, from Southern Java to Bali Sea (White et al. 2006).

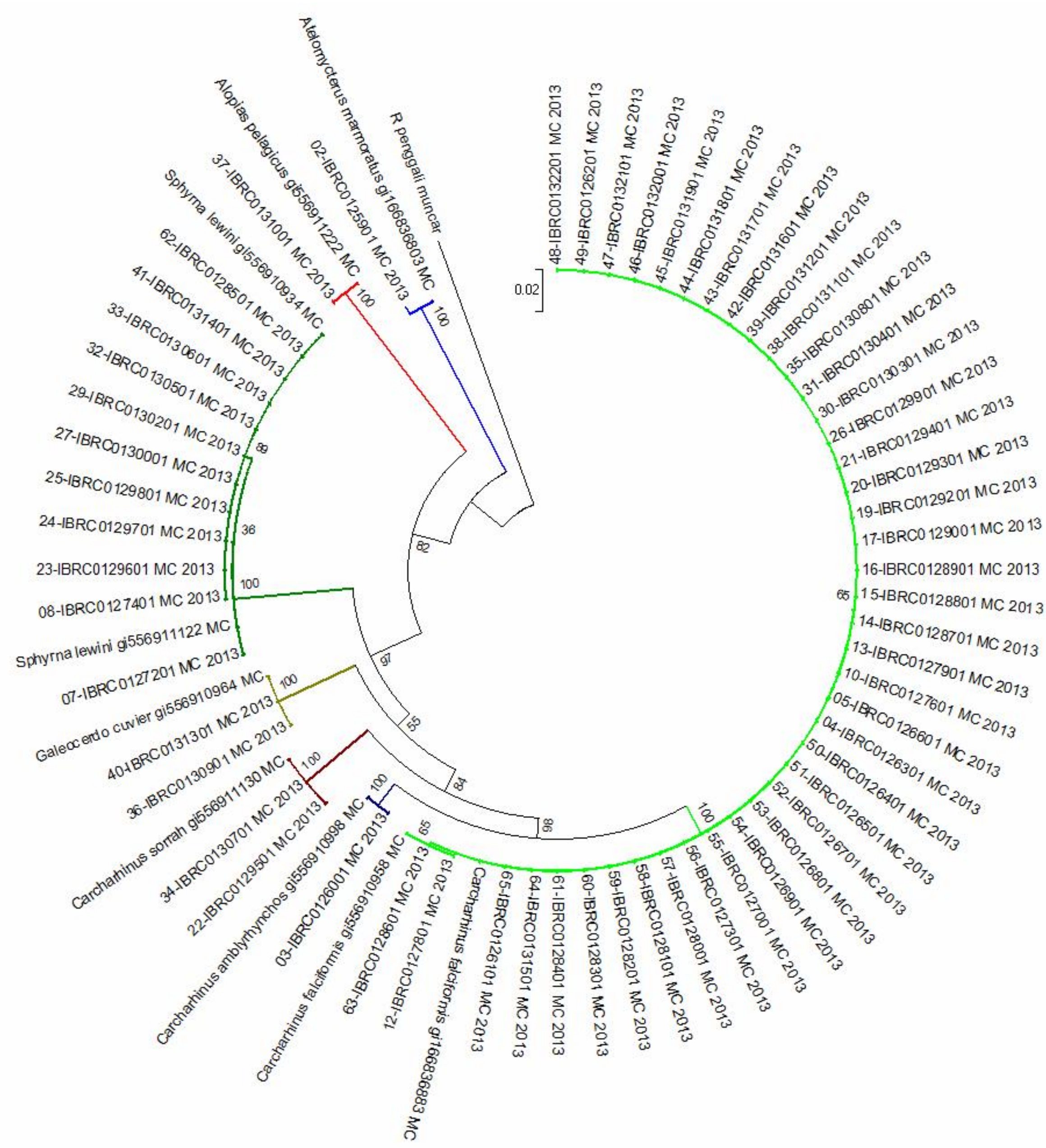

Figure 2. The Neighbour-joining tree based on COI sequence data using Kimura-2-parameter substitution model with 1000 bootstrap, from shark species landed in Muncar in 2013.

Phylogenetic reconstruction of *Carcharhinus brevipinna* landed in Muncar was divided into two clades with bootstrap value of 88-89 (Figure 4.A). Differences between these two branches are pretty strong (100% bootstrap value) and indicate these individuals might come from different population. The distribution of this species covers the central sea areas of Indonesia (White et al. 2006). The species of *Carcharhinus limbatus* consists of two different clades between from data pooled of Palabuhanratu and Muncar (Figure 4.B). It could be seen from the cladogarm that each of species grouped according to the locations respectively. Based on White experiment on 2006, *C. limbatus* can be found throughout Southern Java Sea until South China Sea. *Sphyrna lewini* was divided into two different clades between Muncar and Cilacap (Figure 4.C). This indicates that the aggregate location of this species is separated between Southern Java and Northern Java, because according to their behavior, this species is likely to seek the same shelter with their own groups, usually in underwater valley basin of sea mountains (Klimley et al. 1983). *S. lewini* is a species that could be found widely distributed throughout Indonesian Seas. However, it was unclear where and how fishermen catch this species.

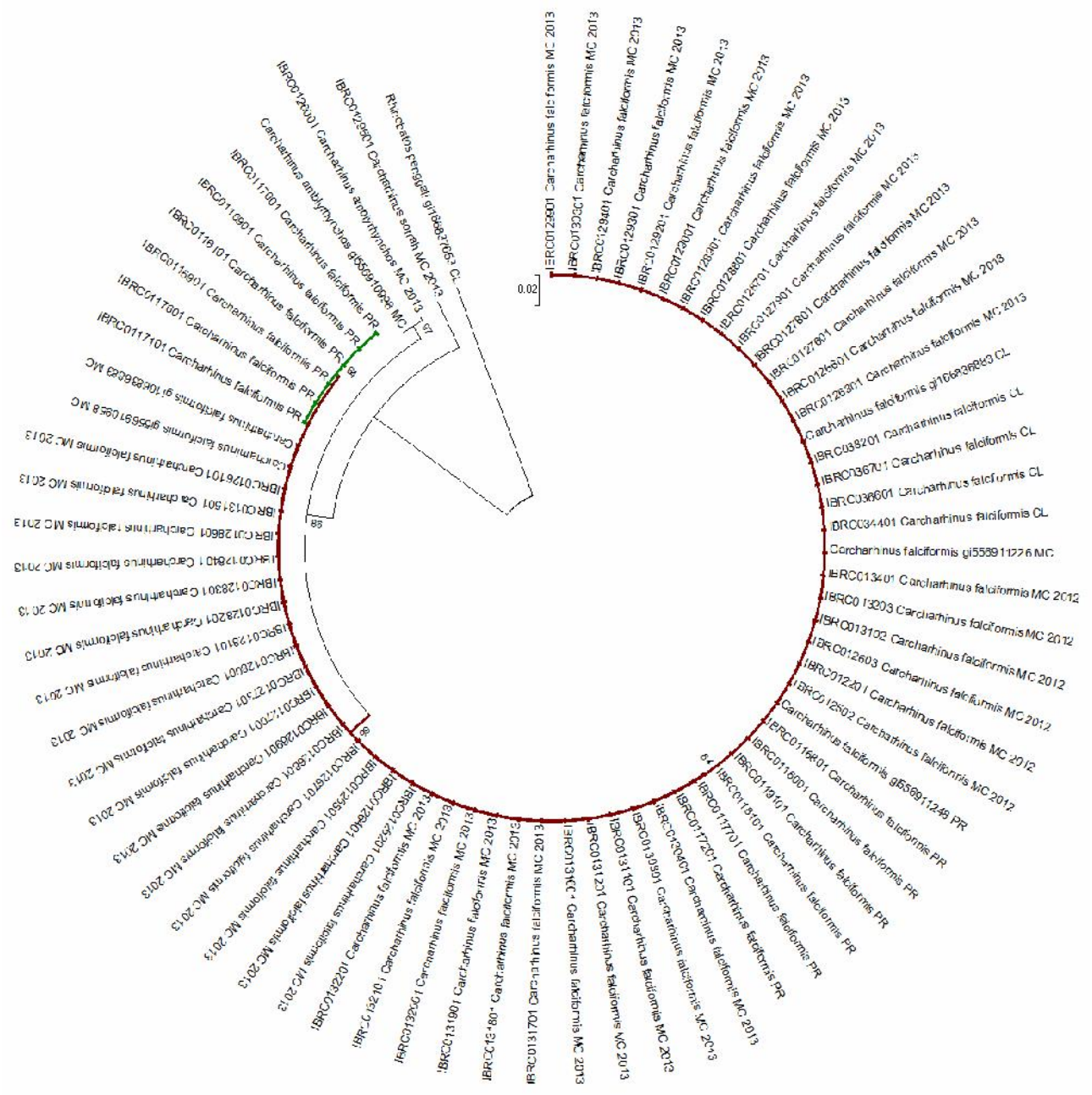

Figure 3. Phylogenetic reconstruction of *Carcharhinus falciformis* landed in landed in Ports in Southern Java for location, using the Neighbour-joining tree based on COI sequence data using Kimura-2-parameter substitution model with 1000 bootstrap.

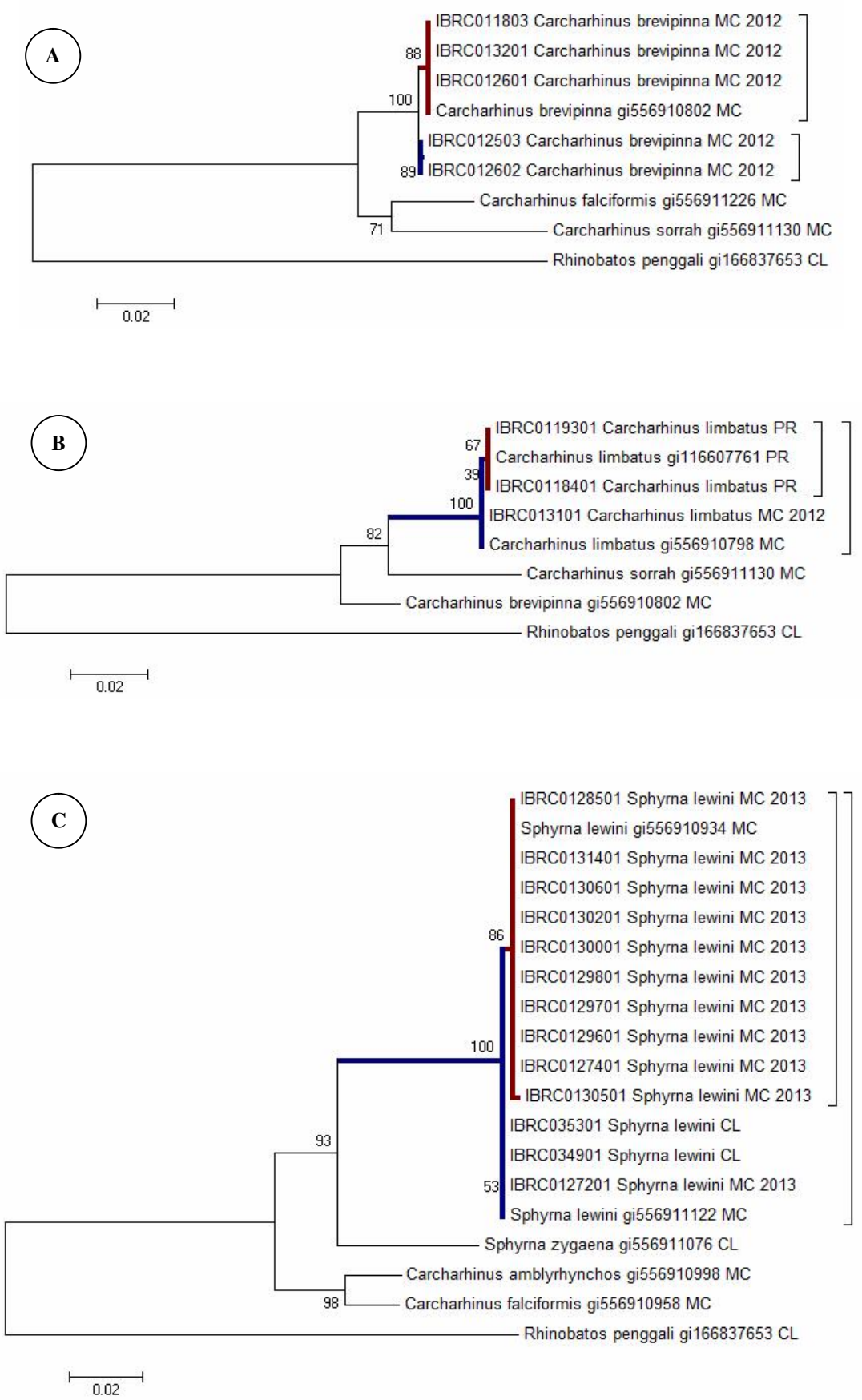

Figure 4. Phylogenetic reconstruction of pooled data of *Carcharhinus brevipinna* (A), *Carcharhinus limbatus* (B), and *Carcharhinus limbatus* (C) landed in three ports in Southern Java.

The diversity of shark species landed in Muncar during the last two years has been decreased, even though sampling efforts was prolonged in 2013. The identified sharks species in this study sites were about 18% of all Indonesian sharks. The phylogenetic reconstruction of shark catchment in Southern Java Fishing Port indicated that sharks might be coming from different population. The result of this study is expected help the Government of Indonesia to manage shark fisheries in Indonesia.

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