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Genetic diversity and population structure of the yellowfin tuna (*Thunnus albacares*) comparing samples collected from artisanal fisheries of Ecuador and Mexico using microsatellite markers

Proyecto de Investigación

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Ingeniería en Procesos Biotecnológicos

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“El caos es un orden por descifrar”

-José Saramago

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RESUMEN

El atún de aleta amarilla (*Thunnus albacares*) es una especie tropical pelágica de alto valor económico, ecológico y social al dar sustento a una de las mayores industrias pesqueras del mundo. Por ende, el adecuado manejo de stocks de esta especie es importante para mantener tanto una pesca sustentable como el intercambio comercial. Al ser un depredador tope, la conservación de *T. albacares* también es relevante para preservar ecosistemas marinos saludables y prevenir la pérdida de esta especie por fragmentación de sus poblaciones. Sin embargo, se han reportado discrepancias entre unidades biológicas y de manejo, poniendo en peligro una correcta administración de este recurso en el Océano Pacífico Este. Estudios previos han sugerido diferencias genéticas entre muestras del norte y el ecuador. En tal sentido, el objetivo de este estudio fue caracterizar la diversidad genética y estructura poblacional de *T. albacares* en una sección del Pacífico Este. Esto se llevó a cabo a través del análisis de 630 muestras colectadas de pesquerías artesanales en Ecuador y México contándose con réplicas temporales a lo largo de tres años. Las muestras fueron caracterizadas molecularmente usando 18 marcadores microsatélite. El índice de heterocigosidad esperada (H_E =0.85) junto con una riqueza alélica de 18.40 por locus mostraron una alta diversidad genética. No se encontró estructura poblacional, aunque si bajas diferencias genéticas significativas entre las muestras de Ecuador y México ($F_{ST} < 0.02$). Análisis adicionales sugieren flujo génico en la sección del Océano Pacífico Este analizada tales como la detección de migrantes de primera generación y bajos niveles de parentesco. Sin embargo, posibles subestimaciones de las distancias pareadas F_{ST} utilizando marcadores microsatélite ha sido sugerido en literatura reciente de atún de aleta amarilla y otras especies marinas. Se recomiendan estudios adicionales para continuar el monitoreo de *T. albacares* en el Océano Pacifico Este. La colección de muestras procedentes de mayores latitudes, arriba de la corriente fría de California y abajo de la corriente fría de Humboldt, pueden ilustrar mejor las diferencias genéticas reportadas anteriormente como respuesta a una divergencia dada por una barrera física. Así también, la implementación de nuevos enfoques como secuenciamiento de nueva generación para el análisis de polimorfismos de nucleótido simple atípicos pueden proporcionar nueva información que contribuya a mejores planes de manejo para *T. albacares* en el Pacífico Este.

Palabras clave: Diversidad genética, stock, marcadores microsatélite, manejo de pesquerías, atún de aleta amarilla.

ABSTRACT

The yellowfin tuna (*Thunnus albacares*) is a tropical pelagic marine species of high economic, ecologic, and social value as it supports one of the major fishing industries around the world. Thus, stock management of this species is important to maintain both sustainability and commercial trade. As an apex predator, conservation of *T. albacares* is relevant to preserve healthy marine ecosystems as well as to prevent diminishment of this species by the fragmentation of populations. However, cases have been reported of mismatches between biological and management units, endangering adequate management of this resource. In the Eastern Pacific Ocean, previous studies have suggested genetic differences between northern and equatorial individuals. To clarify the situation of *T. albacares* this study aimed at characterizing the species genetic diversity and population structure in a section of the Eastern Pacific. We collected 630 samples collected from artisanal fisheries from Ecuador and Mexico with temporal replicates across three years. The samples were molecularly characterized using 18 microsatellite markers. The expected heterozygosity index (H_E = 0.85), together with an allelic richness of 18.40 per locus, show a high genetic diversity. No population structure was found, although low, yet significant genetic differences were found between Ecuadorian and Mexican samples (F_{ST} <0.02). Further evidence suggests continuous gene flow in the section of the Eastern Pacific Ocean analyzed. First generation migrants were detected and low levels of relatedness were found. However, possible underestimations of F_{ST} values using microsatellite markers has been suggested by recent literature in the yellowfin tuna and other marine species. Further studies are recommended to maintain monitoring the situation of *T. albacares* in the Eastern Pacific Ocean. The collection of samples from higher latitudes outside the cold California or Humboldt currents could illustrate better the genetic differences previously reported as a result of a divergence due to a physical barrier. Moreover, the use of new approaches such as next generation sequencing for the analysis of outlier single nucleotide polymorphism loci could retrieve additional information to achieve a better management of *T. albacares* in the Eastern Pacific.

Keywords: Genetic diversity, stock, microsatellite markers, fisheries management, yellowfin tuna.

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1. INTRODUCTION

1.1. Fisheries

Fisheries play a fundamental role in human society as fish currently account to 17% of the total animal protein consumed worldwide. In 2015 alone, fish meat was consumed by 3.2 billion people as a part of their animal protein intake. Moreover, about 59.6 million people had a job directly linked to the primary sector of capture fisheries and aquaculture. Although fish meat production has seen a small decrease over the last years, developing countries located in tropical areas show a continuously rising trend indicating the relevance of this resource in the region (Food and Agriculture Organization FAO, 2018).

The importance of fisheries has increased the necessity of a population unit as a basis for the proper assessment and management of marine resources. Thus, the concept of 'stock' has become the foundation of fisheries science. However, the definition of stock has been widely discussed and not rigorously established. In general terms, a stock refers to a discrete population that inhabits one specific area at a particular time (Hawkins et al., 2016). There are different criteria to delimit a population: morphology, spawning area, satellite tag, biochemistry, or genetics. Between them, genetic tools have proved to be more sensitive and reliable (Grewe et al., 2015).

The actual state of marine fisheries shows a decline in the current marine fish stocks assessed, according to the last FAO 2018 report. Furthermore, Pauly & Zeller (2017) have commented that the decline of marine resources could be underestimated by the lack of reliable data. This decline in marine fish stocks are result of an overexploitation and incorrect stock management from the fisheries worldwide. A problem with actual stock assessment involves inconsistencies with the operational stock boundaries. Mismatches between biological and management units are a threat to global fishery sustainability. On one hand, a management unit that includes only a fraction of a

larger population implies problems with the understanding of true stock dynamics. On the other hand, a stock constituting of multiple biological populations leads to incorrect estimates of species-abundances and productivity, making it more vulnerable. Particularly, scombrids which are one of the more heavily exploited groups (Juan-Jordá et al., 2013), have been proved in the past o have one of these misalignments. Consequently, proper delimitation of stocks is essential in order to meet fishery sustainability goals (Grewe et al., 2015; Mullins et al. 2018).

1.2. Yellowfin Tuna (*Thunnus albacares*)

The Scombridae family consists of species of high ecologic, economic and social value, as they support the most important fisheries around the world. Scombrids are predators whose life cycle is confined to marine waters and are usually associated as highly migratory. These species (tunas, bonitos, and mackerels) play important roles in euphotic zone ecosystems of the oceanic waters, which represent 70% of the earth's surface (Miya et al., 2013). Furthermore, the scombrid species sustain diverse industries, ranging from small-scale artisanal fisheries to large industrial fisheries (Juan-Jordá et al. 2013). In this regard, the tuna market represents one of the most relevant and fully exploited. Tunas are an endothermic advanced group of scombrids, which have evolved a countercurrent heat exchanger system that allows them to maintain their body temperature above the surrounding water as well as high swimming speed and efficient oxygen uptake. Among them, the yellowfin tuna, *Thunnus albacares,* is highlighted as one of the scombrids with the fastest life histories in terms of its growth and reproduction patterns (Juan-Jordá et al. 2013). Additionally, the yellowfin tuna solely supports the second largest tuna fishery in the world (Pecoraro et al., 2017).

Thunnus albacares is currently catalogued as Nearly Threatened by the International Union for Conservation of Nature (IUCN) (Collete et al., 2011). The anatomical and

physiological features of the yellowfin tuna allow it a better performance in swimming-activities. However, these same features makes *T. albacares* sensitive to low O_2 concentrations (Bushnell et al., 1990) and metabolic-dependent to thermal conditions. For instance, decreases in sea temperature below 15 ^{o}C can cause a mortal drop in heart rate. The yellowfin tuna is distributed only in tropical and sub-tropical epipelagic waters (18-31 ^{o}C). This dependency on environmental factors such as temperature and O_2 concentration makes *T. albacares* only found at depths where there is enough oxygen available (Hoolihan et al., 2014; Schaefer et al., 2011). Additionally, a factor that influences habitat utilization is the presence of high prey density. The diet composition of the yellowfin tuna is size-dependent (Zudaire et al., 2015). As such, small individuals feed mainly on euphasiids and plankton while larger individuals have crustaceans, cephalopods and another fishes as prey (Pecoraro et al., 2017). In addition, recent reports of *Thunnus albacares* in the Eastern Pacific Ocean (EPO) tend to show a regional fidelity. These reports contradict previous descriptions of the species as highly migratory (Schaefer et al., 2014; Block et al., 2011). Additionally, geographic variation in phenotypic and genotypic features have suggested the possibility of various biological populations in the EPO (IATTC, 2018).

1.3. Yellowfin tuna fisheries in the Eastern Pacific Ocean (EPO)

On a global scale, the yellowfin tuna is divided into four distinct stocks located in one of each of the major ocean basins: the Indian (IO), Atlantic (AO) and Pacific Oceans (PO), subdividing the Pacific into the Western and Central Pacific Ocean (WCPO) and EPO. Recent genetic and genomic approaches have confirmed the genetic differences found among oceans between these current stocks (Pecoraro et al., 2016). However, the status of EPO stock has not been yet clarified. Both previous microsatellite (Díaz-Jaimes & Uribe-Alcocer, 2006) and whole genome sequencing studies (Barth et al, 2017;

Pecoraro et al. 2018) have been limited due to low sample sizes or a lack of sampling from southern regions of the EPO. The situation in the EPO is relevant as this ocean gathers important yellowfin tuna fishery industries such as the ones from the Ecuadorian and Mexican fleets.

1.3.1. Ecuador

Ecuador comprises one of the richest fish fauna in the world due to its unique geographical position (Reis et al., 2016). This diversity allows the existence of big pelagic fishes such as the yellowfin tuna in Ecuadorian waters (Worm et al. 2003). The Ecuadorian fishing fleet for tuna capture is one of the most important in the Eastern Pacific Ocean, registering during the period of 2010-2016 on average about 41.03% of the total captures in the Inter-American-Tropical-Tuna-Commission, followed by Mexico with 23.3%. Even more, in 2016 Ecuador was the second global exporter of canned tuna (Ministerio de Comercio Exterior, 2017). In addition, since the beginning of the century, *T. albacares* has been one of the three most of captured tuna species for this industry. In the last report of the Instituto Nacional de Pesca del Ecuador for 2017, yellowfin tuna represents the 18.2% of all tuna captured that year. *T. albacares* production is constituted of 56% captured in the Ecuadorian costal line, 21.6% in the surroundings of the Galapagos Marine Reserveand 16.4% in international waters (Instituto Nacional de Pesca, 2018). Two types of fisheries operate in these regions: large-scale industrial fisheries and artisanal fisheries (Martínez-Ortiz, Aires-da-Silva, Lennert-Cody, & Maunder, 2015).

The artisanal fisheries are relevant for both the economic and social aspects in Ecuador. An estimation made by Martínez-Ortiz et al. (2015) establishes that this market generates approximately 200 million dollars per year. The artisanal fisheries in Ecuador operate as far as 1400 nm from the coast to beyond the Galápagos Archipielago. The most dominant types of gears used have been the longline and the surface gillnets for large

pelagics (Abo-Tubikh, 2014). Nevertheless, yellowfin tuna catches represent only a small portion of the total catches of large pelagics; it is important to consider that there is an underestimation of current catch summaries due to the limited data available (Martínez-Ortíz et al., 2015).

1.3.2. Mexico

Mexico is the world's 16th largest fishing nation (FAO, 2018). Over the last two decades, Mexico fisheries yield has remained constant at approximately 1.6 million tons per year; its national production is strongly determined by their fishing activities in the Eastern Pacific Ocean (Chiappa-Carrara et al., 2019). For the yellowfin tuna, the most abundant tuna species in Mexico, the national production trend is to increase. As well as for Ecuador, the artisanal fisheries represent an important activity in Mexico. In 2004 artisanal fisheries production constituted about 40% of the national total capture. Despite the efforts to change to an aquaculture system, population reduction due to overexploitation, is expected to occur any time soon (Vásquez-Hurtado et al., 2010).

1.4. Genetic Markers

The relevance of fisheries in the Eastern Pacific Ocean calls for the employment of new approaches to accurately manage commercial species such as the yellowfin tuna. One approach is through the use of genetic markers (Cuélla-Pinzón et al., 2016). A genetic marker is defined as a locus, or its expression, that can be easily identified and assigned to a particular cell, individual or group that carries it (Semagn et al., 2006). In the field of aquaculture and fisheries, genetic markers have been used as informational tools to discern the genetic differences between individuals, species, and populations. In the particular case of stock structure analysis of tunas, four marker systems have been implemented predominantly: allozymes, mitochondrial DNA (mtDNA) assays (Li et al., 2015; Ely et al., 2005), simple sequence repeats (SSR) or microsatellites (Pecoraro et al.,

2016; Aguila, et al., 2015; Díaz-Jaimes Uribe-Alcocer, 2006), and single-nucleotide polymorphisms (SNPs) (Pecoraro et al., 2018; Barth et al., 2017; .The use of a particular method has depended on the state of the art at the moment as well as the advantages and limitations of each method (Kumar & Kocour, 2015). Microsatellites and SNPs have been the most popular molecular systems for molecular biology studies in the last decade (Grover & Sharma, 2016). Microsatellites are short DNA motifs (usually from one to six nucleotides) repeated in tandem and flanked by conserved sequences, which are located throughout the genome. They are characterized for exhibiting high mutation rates (between 10^{-3} and 10^{-4}) under the replication slippage model and for been neutral and codominant molecular markers. SSRs have specially been used in population genetic studies because of their cost effectiveness, as they are inexpensive compared to second-generation sequencing (SGS) techniques. Also, because they show extremely high levels of polymorphism and a relative abundance in the genome, granting high statistical power (Hodel et al., 2016).

Microsatellites have been previously used as molecular markers for yellowfin tuna genetic diversity and population structure studies (Pecoraro et al., 2016; Aguila et al. 2015; Díaz-Jaimes & Uribe-Alcocer, 2006; Appleyard et al., 2001). Such studies have revealed valuable data that has brought new insights to yellowfin tuna stock across the world. As an illustration, the detection of genetic differences in a region of the Western and Central Pacific Ocean suggests more than one population (Aguila et al., 2015). The genetic differences found between nearby locations as the Phillipines and Bismark have raised questions about the possibility of multiple populations within oceans. In the Eastern Pacifc Ocean, this possibility has been suggested by Díaz-Jaimes & Uribe-Alcocer (2006) study. The implementation of a molecular approach using microsatellite

markers could retrieve valuable information to resolve the still uncertain population structure of *T. albacares* in the Eastern Pacific Ocean.

The present study analyses the genetic diversity and population structure of *T. albacares* in a section of the Eastern Pacific Ocean through a molecular approach using microsatellite markers. The study comprises samples collected from artisanal fisheries located in Mexico and Ecuador, including a still not studied region of the ocean such as The Galapagos Marine Reserve. The genetic diversity of the yellowfin tuna is characterized in order to further understand the situation of the stock and the resilience of the population to environmental changes. Furthermore, population structure of *T. albacares* is evaluated in order to find whether or not genetic differences between individuals are present within the Eastern Pacific Ocean. The information obtained will be valuable for future management plans of *T. albacares* in favor of a sustainable fishery.

2. OBJECTIVES

2.1. General objective

- To characterize the genetic diversity and population structure of the yellowfin tuna based on a sample collected from artisanal fisheries in the mainland Ecuador, the Galapagos Marine Reserve and Mexico using microsatellite DNA.

2.2. Specific objectives

- To analyze the genetic diversity of yellowfin tuna in terms of its allelic diversity and heterozygosity in Mexican waters, the Ecuadorian continent and the Galapagos Marine Reserve through microsatellite loci.
- To determine the population structure of the yellowfin tuna in a section of the Eastern Pacific Ocean.
- To test by different analyses whether there is continuous gene flow between individuals from mainland Ecuador, the Galapagos Marine Reserve and Mexico.

3. JUSTIFICATION

The yellowfin tuna has been intensively studied throughout the world to secure a sustainable fishery for this marine resource (Pecoraro et al., 2017). Estimates have predicted that the erosion of genetic diversity could cause the complete collapse of the commercial species by 2048 (Worm et al., 2006). As a consequence, the maintenance of high genetic diversity is essential to meet sustainability. Studies have been focused on correctly matching biological populations with management units for a correct assessment and management of yellowfin tuna stocks (Mullins et al., 2018; Cuéllar-Pinzón et al., 2016). The current scientific criteria in this regard have been the use of genetic tools to adequately delineate stocks boundaries (Grewe et al., 2015). However, little information is available for the genetic diversity and population structure of the yellowfin tuna in the Eastern Pacific Ocean (Barth et al., 2017; Pecoraro et al., 2017). Recent evidence has suggested there is the possibility of multiple coexisting stocks of yellowfin tuna in the Eastern Pacific Ocean. The possibility of more than one yellowfin tuna stock is a problem as the Inter-American-Tropical-Tuna-Comission has stablished only one stock within this sea (Minte-Vera et al., 2018; Díaz-Jaimes & Uribe-Alcocer, 2006). Furthermore, previous mismatches between biological populations and management units have been reported in the past for yellowfin tuna (Mullins et al., 2018; Grewe et al., 2015). Thus, a genetic diversity study in this area is fundamental to properly characterizing and understanding the actual situation of yellowfin tuna in the Pacific Ocean. The information retrieved by the study will be valuable to make future management plans for the conservation of this species.

4. AREA OF STUDY

The area under study consists of s a section of the Eastern Pacific Ocean comprised by the oceanic artisanal fishery for large pelagic species in Ecuador and the artisanal fishery in Cabo San Lucas, La Paz, in Mexico. The first is located between 05º00'N and 15º00'S, and from the coastal line of continental Ecuador to the meridian of 100º00'W off the Galapagos Archipielago (Martinez-Ortiz et al., 2015). The second is located in La Paz bay in the southeast of the Baja California Sur state in Mexico between 24º07' and 24º21'N, and 110º17' and 110º40' W (González-Acosta et al., 2018).

Sample collection was performed in artisanal fishery Santa Rosa, Salinas, Ecuador; the Galápagos Marine Reserve (GMR) and in Cabo San Lucas, La Paz, Mexico. DNA extraction, amplification and other molecular as well as statistical analyses were conducted at the Laboratory of Plant Biotechnology of Universidad San Francisco de Quito (USFQ), Quito, Ecuador. However, marker genotyping was carried out at the Smithsonian Institute, Washington, United States of America.

5. MATERIALS

5.1. Muscle and fin tissue

- Yellowfin tuna tissue from muscle or fin collected from 336 yellowfin tuna individuals from different locations in the Eastern Pacific Ocean (Figure 1).

5.2. DNA extraction

- Extraction kit QUIAGEN for 200 samples.
- Eppendorf tubes of 1.5 ml.

5.3. DNA quantification and dilution

- UltraPure™ Distilled Water (GIBCO).
- NANODROP 1000 Spectrophotometer (Thermo Scientific).
- TE Buffer (Tris Base 10 mM, EDTA 1 mM, PH 8.0).

5.4. Microsatellite Marker Amplification

- 18 yellowfin tuna-specific Primer Pairs (Table 2).
- Taq Platinum DNA polymerase 5U/mL (Invitrogen).
- PCR Buffer 10X (Invitrogen).
- UltraPure™ Distilled Water (GIBCO).
- $MgCL_2$ 50 mM (Invitrogen).
- dNTPs 10 mM (Invitrogen).
- T-Personal Thermocycler (Biometra).
- T100 Termal Cycler (Bio-Rad).

5.5. Electrophoresis

- UltraPure Agarose (Invitrogen).
- BioRad Gel Doc XR Photo-documenter.

- SYBR Safe DNA Gel Stain (Invitrogen).
- TBE 1X Buffer (tris-Boric acid, EDTA).
- Blue Juice 10X Loading Buffer (Invitrogen).
- Ladder 100 bp (Invitrogen).
- MGU-502T Horizontal Midi-Gel Kit (C.B.S Scientific).
- EC360M Electrophoretic Gel System (Maxicell ®).
- Power source EPS-300 II (C.B.S Scientific).

5.6. Genotyping

- GeneMarker® (Softgenetics).

5.7. Data analysis

- R-studio software including the following packages:
    - adegenet v2.1.1 (Jombart, 2008).
    - ape (Paradis et al., 2004).
    - related v1.0. (Pew, Muir, Wang, & Frasier, 2014).
    - poppr v2.8.0 (Kamvar, Tabima, & Grünwald, 2013).
    - pegas v0.10 (Paradis, pegas: an R package for population genetics with an integrated–modular approach, 2010).
    - ade4 v1.7-11 (Dray & Dufour, 2007).
    - hierfstat v0.4-22 (Goudet, 2005).
    - ggplot2.
    - stats v3.5.0.
- Primer 3.0.
- ARLEQUIN v3.5.1.2 (Excoffier & Lischer, 2010).
- FreeNa software (Chapuis & Estoup, 2007).

- GENECLASS2 (Piry et al., 2004).
- STRUCTURE v2.3.4. Software (Pritchard et al., 2000).
- Structure Harvester (Earl D. , 2012).
- CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007).
- distruct v1.1 (Rosenberg, DISTRUCT: a program for the graphical display of population structure, 2004).
- Microsoft Excel ®.

6. METHODOLOGY

6.1. Sample collection

In the present study, 336 yellowfin tuna individuals were sampled. Specimens were obtained from three localities covering north and central eastern pacific: The Galápagos Marine Reserve (GAL), continental Ecuador (ECU) and Cabo San Lucas, La Paz, Mexico (MEX) (Figure 1). Samples were collected from February to October 2017. Additionally, samples from these same locations were obtained for years 2015 and 2016. A sample from Mexico in 2016 could not be obtained (Table 1). For mainland Ecuador, genotypic data were obtained from Muñoz (2016) samples while in the case of Mexico, samples were obtained from Felipe Galván, a researcher from the Centro Interdisciplinario de Ciencias Marinas (CICIMAR).

Only individuals from artisanal fisheries from which the approximate coordinate of capture was known were sampled. Coordinates were obtained from georeferenced information retrieved from the fishermen. This information assured that specimens came from the desired locations. An artisanal fishery was defined as the one that uses glass fibers of 10 to 12 meters long for captures and operates within an area of 200 nautical miles from the fishing port.

Samples consisted in 2 gr of muscle tissue or caudal fins. The samples were preserved in absolute ethanol and stored at -20 °C in Eppendorf © tubes 1.5 ml. Caudal fins were collected only in the cases where the fishermen did not allow cutting a piece of muscle. Additionally, for each specimen sampled, the individual's standard length was measured.

6.2. DNA extraction and quantification

Total genomic DNA extraction was carried out based on QIAGEN kit extraction protocol for 200 samples without modifications for either muscle tissue or caudal fin.

Caudal fins required a previous pulverization aided with liquid nitrogen. DNA concentration and quality were assessed with NANODROP 2000 (ThermoScientific). In addition, a visual inspection in agarose gels at 2% dyed with SYBR Safe (Invitrogen) to check if isolated DNA was not fragmented. Depending on the final DNA concentrations obtained, each sample was diluted to reach a standard concentration of 20 ng/l for posterior PCR amplification.

6.3. PCR amplification

A set of 18 microsatellite loci specific for *Thunnus albacares* (Antoni et al., 2014) were genotyped for all the samples. Each forward primer was marked with 6-FAM and HEX fluorophores at the end of the 5' –end. In order to optimize time and resources, the program Primer 3.0 was used to combine microsatellite loci into 8 multiplexed schemes (Table 2). Primer 3.0 grouped PCR reactions based on the annealing temperatures and size in bp. Each multiplex reaction consisted of 3 or 2 pairs of primers of two different colors. In cases in which primers had the same color in the multiplex scheme, the generated amplicons had to have different expected sizes.

PCR amplification conditions used were the ones described by Appleyard et al. (2001) with some modifications. The PCR master mix consisted of 20 ng of DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 100 µM dNTPs, 0.8 µM of each primer and 0.1 U of platinum taq polymerase (Invitrogen) in a total volume of 20 µl. The program parameters were carried out using 93 ° C for 10 minutes as initial denaturation temperature followed by 55 ° C for 15 sec. After that, 35 cycles of annealing temperature (Table 2) for 2 min and 93 ° C for 15 sec. The program ended with an extension at 72 ° C for 10 min.

To verify the successful amplification of the primers, electrophoresis was performed on agarose gels at 2%, which ran for 30 minutes at 100 volts. The amplification was further verified by visualizing that the sizes of base pairs in the agarose gels were within

the range of the expected size described by the author. The PCR products obtained were sent to the Smithsonian for genotyping. This genotyping was carried out in an automatic sequencer (ABI PRISM 310, Applied Biosystems).

6.4. Data Analysis

6.4.1. Data collection

The DNA sequences obtained were analyzed with the GeneMarker® software package (Softgenetics, Pennsylvania) for peak selection and consequent SSR marker scoring. Using as reference the alleles sizes described by Antoni et al. (2014) only the peaks that fitted the expected size ranges were selected. A label was assigned to each allele. The label consisted in the size of the allele in bp. This information was used to construct a codominant allelic matrix in Microsoft Office Excel v. 2016. The matrix was organized with the samples in each row and the loci in each column.

6.4.2. Data subdivision: analysis per year and per site.

An overall analysis was first performed to evaluate the quality of de data. Thus, levels of missing data, non-informative loci and private alleles were assessed with R package poppr. Null allele frequencies per locus were calculated using the method implemented in FreeNa software. As the data collected comprised individuals from different years and locations, only an overall analysis of the data could have not been interpreted directly. Hence, the data were subdivided in such a way that an analysis per year and per site could be performed.

As a consequence, the analysis was performed under three approaches: general, per year, and per site. General analysis involved all the samples constituted by individuals from all locations and years. The analyses per year compared individuals from different years within the same location. Thus, these analyses consisted in groups from GAL, ECU

and MEX subdivided in the three years studied. Meanwhile, the analyses per site compared individuals from the same year in the different locations sampled. Hence, the groups analyzed were the years 2015, 2016, and 2017 subdivided in the three different locations.

Inbreeding coefficients were calculated with the R packages adegenet and related. The estimator which better suits the data was defined as the one with the highest correlation between observed values, calculated form the data, and expected values, calculated from simulations. As a consequence, the dyadic likelihood estimator (Milligan, 2003) was chosen for the calculus of F with a correlation value of 0.9371722. This was done so following Wang & Jinliang (2011) guideline.

The detection of first generation migrants was performed for the comparison of different groups per site. It was carried out using the GENECLASS2 software. The program computes the probability of an individual of being a resident or a first generation migrant to a reference population. A first generation migrant is defined as an individual that migrated from a location A to a location B. Additionally, it can be seen as an individual born in a location B from a gravid female that migrated from the location A (Piry et al., 2004).

6.4.3. Genetic diversity

For each analysis, levels of genetic variation in microsatellite loci were determined in terms of number of alleles per locus (Na), allelic richness (Ra), observed heterozygosity (Ho) and expected heterozygocity (He) using R package adegenet. Analysis of Molecular Variance (AMOVA) was carried out using the program ARLEQUIN to calculate and understand the total variation within and between samples. Additionally, stats R package was implemented to test for significant differences between Ho and He according to Kamvar et al. (2017) guideline. In order to check for deviations

from Hardy-Weinberg equilibrium (HWE) in each locus the package pegas (Paradis, 2010) was used.

6.4.4. Genetic clustering

Genetic differentiation of the data was first estimated using Wright's F-statistics (Nei, 1977). Global and pairwise F_{ST} were calculated using the R package hierfstat. Pairwise F_{ST} analysis was carried out as an exploratory overview of the data as it is easy to implement and provides a description of the genetic distances among populations. However, global F_{ST} was implemented for each of the analyses per year and pear site.

Population structure was assessed using two approaches: Principal Components Analysis (PCA) and STRUCTURE, a model-based method, which uses a systematic Bayesian approach. Multivariate analysis approach effectively summarizes information from genetic markers into few synthetic variables or axis which display the largest variance in the data set (Zuur, Ieno, & Smith, 2007). The Bayesian approach provides *a posteriori* evidence of membership probabilities of individuals to clusters by applying *Markov Chain Monte Carlo* (MCMC) estimation (Porras-Hurtado et al., 2013).

The multivariate analysis of the data set was carried out implementing a PCA, which provides a visual display of the samples based on covariance coefficient. All multivariate analyses were done using the R package adegenet (Jombart, 2008) and following Jombart & Collins (2017) and Jombart (2016) guidelines. STRUCTURE analysis was run with 7 values of K (from K=1 to K=7) testing one to seven clusters under the admixture model. LOCPRIOR information was considered with correlated allele frequencies at set lambda equal to 1. For both burnin period and MCMC generations, a length of 1000000 steps was used. The admixture model assumes that data originates from the admixture of K putative parental populations from which individuals have

inherited some of its ancestry (Fracois & Durand, 2010). The Evanno method (Evanno, Regnaut, & Goudet, 2005) was implemented with the program Structure Harvester to infer the most suitable value of K (Earl & vonHoldt, 2012).

6.4.5. Relatedness coefficient

Relatedness coefficient was estimated using related package in R. Because there is no best estimator for this coefficient, comparisons between estimators were made to obtain the best one that fitted with the data such as with inbreeding. The Wang estimator obtained the highest values of correlation (0.9371112). Relatedness coefficient r was determined in order to summarize the average relationship between individuals from the different groups (Wang J. , 2014).

7. RESULTS

7.1. Overall analysis of the data

7.1.1. DNA extraction and PCR amplification

Successful total genomic DNA extraction and quantification were accomplished for 630 yellowfin tuna samples. DNA isolations showed both good quality (average 260/280 Index = 1.98) and quantity (range 0.26-320 ng/µl). In general, samples from Mexico 2017 presented lower quality (Table 3). A possible reason could have resulted from problems during shipping of the tissue samples to Ecuador, involving burns or rot. Nevertheless, mean missing data for all samples did not surpass 5%. Only one loci was found to be slightly above this threshold (Figure 2).

7.1.2. Genetic diversity

Thunnus albacares showed high genetic diversity per locus (Table 4) and per population (Table 5). All 18 SSR markers proved to be highly polymorphic, ranging number of alleles per loci from 8 to 56. Null alleles were found across all loci; however, the frequencies calculated were low (Table 6). The allelic richness, understood as the average allele count per locus, ranged from 3.75 to 34.7. The observed heterozygosity presented a range from 0.16 to 0.95 while the expected heterozygosity ranged from 0.35 to 0.95. Global observed and expected heterozygosity were 0.65 and 0.85, respectively. The observed heterozygosity in a vast majority of the loci was below the expected heterozygosity; therefore; a t-test was carried out to find if the difference was significant. In all populations, there was a significant difference between He and Ho ($P< 0.05$)

Overall results for AMOVA show a vast majority in the genetic variation occurring within individuals, with mean percentage in all analyses of 75.75%. Genetic variation among individuals within a same population follows this value with 23.03%. Only a small

percentage (1.22%) is due to variations among populations (Table 7). Hardy-Weinberg equilibrium was tested for all loci in each of the samples studied. The majority of loci in all the groups were not found to be in Hardy-Weinberg equilibrium (Figure 3).

7.2. Analyses per year

Analyses per year compare individuals from different years within the same location. The fixation index F_{IS} results per locus were significant for all the loci. Significance indicates that the values are statistically different from zero (Table 4). The mean value of inbreeding coefficient F for each year was low ($F<0.3$), with the highest value found in 2016 (Table 8). The proportion of individuals with higher values of F increased from 2015 to 2016 and 2017 (Figure 4). Average coefficient F in each location show an annual increase in inbreeding except for GAL, where the highest values of F is also found on 2016 (Table 5).

7.2.1. Genetic clustering

Genetic clustering was first assessed through Nei's F_{ST}. Genetic differentiation was found to be low ($F_{ST} < 0.02$) for the comparison of individuals from different years within the same location. Nevertheless, the years 2015 and 2017 from Mexico presented the highest genetic differences ($F_{ST} = 0.019646$) when compared to mainland and insular Ecuador (Table 9).

7.2.1.1. PCA

Principal Component Analyses for the comparisons per year for mainland Ecuador (Figure 5) and The Galapagos Marine Reserve (Figure 6) show that individuals from these localities are distributed uniformly without forming recognizable groups. In contrast, PCA for the comparisons per year in Mexico show a clear segregation of individuals from 2015 respect to individuals from 2017 (Figure 7). The two main principal components

shown in the axes of each graph explain only a small percentage of the total variance of the data. Hence, it is necessary to compare the present analysi to other types of evidence as not all the variability of the data is observed.

7.2.1.2. Model-based clustering

Model-based clustering analyses were carried out in STRUCTURE. The first approach was to determine clusters when comparing individuals from different years within the same location. The most suitable values of K were K=3 for the Galapagos Marine Reserve, K=3 for mainland Ecuador and K=2 for Mexico (Figure 8). Although population structure is not present, the pattern shows a distinctive genetic pool composition in each year. Locations sampled in three different years present a K=3. Meanwhile, in Mexico, a location sampled only in two years, showed a K=2. The differences seen between years in the same location could be due to migration of individuals in the studied zones.

7.2.2. Relatedness

Global relatedness values r per year are negative and close to 0 (Table 8). The values close to 0 mean that individuals from the same year share low ancestry between each other when compared to individuals from other years. Furthermore, negative values mean that there is a deficiency of ancestry from individuals within a same year. For instance, two individuals from 2015 could share less ancestry between each other than one from 2015 and other from 2016. These values imply that the individuals from the selected groups are as related as any two individuals from any population.

7.3. Analysis per site.

Analyses per site compare individuals from different locations within the same year. Inbreeding coefficient F was also calculated for these analyses. The mean value of F per location was low ($F < 0.3$) as in the analyses per year. The individuals at the site that

presented the highest value of F were the ones from Mexico (Table 8). Only in 2016 individuals from the Galapagos Marine Reserve presented the highest values. However, it is important to consider the absence of samples from Mexico in this year. It is possible that the high values of F in Mexico are a consequence of outlier individuals with an F coefficient unusually higher (F > 0.6) (Figure 9).

7.3.1. Genetic clustering

Pairwise F_{ST} shows low genetic differentiation between individuals from different sites within the same year ($F_{ST} < 0.02$). We find the same pattern as in the analyses per year. The individuals from Mexico presented the greater genetic differences when compared to the other locations in 2015 and 2017 (Table 9). In particular, individuals from Mexico in 2015 showed the highest degrees of differentiation found in this study.

7.3.1.1. PCA

In the year 2015, comparisons of individuals from different locations show a group of tuna samples from Mexico segregated from mainland Ecuador and the Galapagos samples (Figure 10). The PCA in 2016 shows one aggrupation with most individuals from Galapagos and Ecuador overlapping (Figure 11). For 2017, there is no particular aggrupation as samples from all the three localities overlap with each other (Figure 12). As with the analysis per year, the two principal components in each axe of the PCA analysis showed in these graphs explain only a small percentage of the total variance of the data. Thus, is important to consider that not all the variability of the data is shown and PCA should be complemented to other analyses.

7.3.1.2. Model-based clustering

Model-based clustering in STRUCTURE was also tested for individuals from different locations within the same year. The most suitable value of K for these analyses in the three locations during 2015 and 2017 was K=2. For the analysis per site in 2016,

the most suitable value of K was K=4 (Figure 13). No population structure was found for any of the analyzes. However, in 2015 and 2017 individuals from mainland Ecuador and the Galapagos showed a different genetic pool composition when compared to the one in Mexico. In contrast, in 2016 there is no clear structure nor distinction between lineages in each of the locations analyzed.

7.3.2. Relatedness

Global values of relatedness for the analyses per site show the same pattern seen with the analyses per year (Table 8). Results from the three years show that individuals are almost equally related to each other, independent to the sample to which they belong. The coefficient r values negative and close to 0 mean that two individuals from a same location share low ancestry between each other when compared with individuals from other years. As an illustration, two individuals from Galapagos could be equally or less related between each other than an individual from Galapagos and Mexico.

7.3.3. Detection of first generation migrants

The detection of first generation migrants was tested for all individuals from the three sites analyzed within the same year. Thus, the analysis was performed for each year. Twelve, 7 and 13 first generation migrants were found for the years 2015 (Table 10), 2016 (Table 11) and 2017 (Table 12) respectively. Individual migrants were detected from each of the locations: Ecuador, Galapagos and Mexico. The results show active migration of yellowfin tuna individuals from mainland and insular Ecuador to Mexico and vice versa. The presence of migrants in all the years analyzed reflects a continuous gene flow between the individuals from the studied locations.

8. DISCUSSION

8.1. Genetic diversity

In the present study, the genetic diversity and population structure of the yellowfin tuna was inferred using 18 microsatellite loci. . The average number of alleles per locus (Na) and allelic richness (Ra) was 19 and 18.40 respectively, indicating that all analyzed loci were polymorphic. We found a global observed heterozygosity (H_O) of 0.65 and a global expected heterozygosity (H_E) of 0.85. These values represent a high genetic diversity for yellowfin tuna in the Eastern Pacific Ocean (EPO). Similar H_E values have been reported in previous studies with the yellowfin tuna using microsatellite loci. For instance, Pecoraro et al. (2016) obtained a mean H_E of 0.88 with samples from the four major ocean basins. Meanwhile, studies from Antoniou et al. (2014), Dammannagoda et al. (2008) and Aguila et al. (2015) found mean H_E values of 0.87, 0.83 and 0.67 analyzing samples from the Atlantic Ocean (AO), Indic Ocean (IO) and Western and Central Pacific Ocean (WCPO). Lower values of H_E have also been reported: 0.62 by Appleyard et al. (2001) and 0.53 by Díaz-Jaimes & Uribe-Alcocer (2006). The H_E values reported for tunas and marine fishes in general is high. Genetic diversity studies for yellowfin tuna related species such as the Albacore tuna (*Thunnus alalunga*) and the Big Eye tuna (*Thunnus obesus*) have reported mean H_E values of 0.79 and 0.82 (Davies et al., 2011; Takagi et al., 2001). In a study that analyzed |1microsatellite variation across 78 fish species, De Woody & Avise (2000) found that marine fishes displayed higher genetic diversity (H_E = 0.79, Na = 20.6) in comparison to freshwater (H_E = 0.46, Na = 7.5) and andromous (H_E = 0.68, Na = 11.3) fishes. The higher genetic diversity in marine fish such as the yellow fin tuna is attributed to their large effective population sizes, migratory behavior and short life-history (De Woody & Avise, 2000; Mitton & Lewis, 1989).

The H_O was found to be statistically different from the H_E. These results could imply that the frequency of heterozygotes found in the present study was lower than the expected one under Hardy-Weinberg equilibrium allele frequencies. The same observation has been previously reported in yellowfin tuna and other marine fishes and has been attributed to the presence of null alleles as well as alleles in low frequency (Selwyn et al., 2016; Pusack et al., 2014; Dammannagoda et al., 2012; Hogan et al., 2010; Appleyard et al. 2001). Null alleles in microsatellite loci are non-amplifying alleles, which generate false homozygotes (Brookfield, 1996). Rare alleles are alleles found in low frequencies that can increase the H_E (Rosenberg & Jackobsson, 2008). Adittionally, they can be not informative for a correct assessment of the genetic diversity as the presence of rare alleles in an individual could be attributed to new mutations rather than heritage (Hale et al., 2012). According to the literature, we suggest that both factors could generate the difference observed between H_O and H_E as null alleles were found in low frequencies across all loci.

8.2. Population structure

Genetic differentiation was first addressed using genetic distances F_{ST}. Low genetic differentiation was found between all locations across the three years under study. However, the data showed small differences between Mexico and mainland Ecuador. The individuals from Mexico 2015 presented a higher degree of genetic differentiation. The same pattern was illustrated in the PCA analyses, where only individuals from Mexico in 2015 were found to be segregated from other samples. These partial results suggest a slight divergence between northern and southern Eastern Pacific Ocean yellowfin tuna individuals, just as has been suggested by a previous study by Díaz-Jaimes & Uribe-Alcocer (2006).

The slight genetic differentiation found between samples from Mexico and Ecuador could be due to temporal changes in allele frequencies or to the way samples were collected. Temporal changes in allele frequencies are caused by migration while the sample collection problem consist on the non-random sampling of individuals by artisanal fisheries. Both factors have already been suggested to cause low yet statistically significant genetic differentiation in the Atlantic cod and other marine species (Knutsen et al., 2011; Bohlmeyer, 1989). Kin-aggregation of marine species make them especially susceptible to non-random sampling in genetic studies (Hansen et al., 1997). Despite the effect of non-random sampling is small for heterozygosity inferences it is substantial in the case of number of alleles and polymorphic sites (Tajima, 1995). Therefore, non-random sampling could have a considerable effect in population structure inferences. Díaz-Jaimes & Uribe-Alcocer (2006) and Knutsen et al. (2011) suggested the addition of data with temporal variation as well as tag studies to achieve results that are more robust to these sources of bias. In the present study, analyses that compared individuals collected within same location in different years have shown genetic differences. Additionally, other reports with temporal replicates have presented similar observations between years in the Atlantic Bluefin tuna (*Thunnus thynnus*) (Antoniou et al., 2017; Knuten et al., 2011; Riccioni et al., 2010).

Model-based clustering carried out in STRUCTURE was another analysis that showed the slight genetic differentiation between Mexican and equatorial samples. Despite no population structure, the analyses per site presented a slight difference in the genetic pool composition of the samples from Mexico and Ecuador. However, it is important to consider that STRUCTURE models are prone to fail at the levels of genetic differentiation encountered in the present study (<0.02) (Putman & Carbone, 2014; Latch et al., 2006). Similar issues have been already reported for related species such as *T.*

thynnus (Antoniou et al., 2017; Riccioni et al., 2010). Simulations have suggested that assignments to the wrong population are prone to occur when there is weak genetic differentiation. In these scenarios, the model retrieves wrong estimates (Duchesne & Turgeon, 2012). Incorrect results from model-based clustering occur when individuals in the sample have an equal degree of admixture. As a result, the algorithm just chooses the number of ancestral populations that better explain the data. Therefore, model-based clustering results have to be compared with other types of evidence in order to interpret them correctly (Lawson et al., 2018). In the analyses per year, model-based clustering showed genetic differences between individuals from different years collected in a same location. The differences could be due to spatiotemporal variation of yellowfin tuna from year to year or bias caused by non-random sampling as mentioned above (Xu et al., 2019; Knutsen et al., 2011; Díaz-Jaimes & Uribe-Alcocer, 2006). Additionally, previous studies carried out in the Eastern Pacific Ocean have suggested one panmitic population that further contributes to the hypothesis of one single stock (Appleyard et al., 2001).

8.3. Genetic differentiation

Genetic differentiation between yellowfin tuna individuals has been previously reported within the Western and Central Pacific Ocean (Grewe et al., 2015; Aguila et al., 2015), Atlantic Ocean and Indic Ocean (Mullins et al., 2018; Barth et al., 2017). Reported mechanisms for genetic differentiation of *T. albacares* are the following: geographical barriers by cold currents, differences in spawning areas or the time of spawning (Barth et al., 2017) as well as the fragmentation of wild populations by industrial and artisanal fishery (Collins et al., 2010).

In the Eastern Pacific Ocean, two cold currents can act as natural barriers like the Benguela current in the Atlantic Ocean (Barth et al. 2017). The California current in the north and the Humbolt current in the south (Wyrtki, 1965). However, these currents

cannot function as barriers in the present study, as they are located outside the sampled areas in Mexico and Ecuador. In order to act as geographical barriers individuals from the two side of the barrier should be sampled and compared. As a consequence, is not possible to reject that genetic differences found in previous studies can be explained by one of these geographical barriers (Díaz-Jaimes & Uribe-Alcocer, 2006).

The spawning activity of the yellowfin tuna throughout the year has been reported in areas between 0º and 20º N. Therefore, there is no evidence to suggest an isolation by spawning at different times of the year. The report by Schaefer (1998) shows that spawning activity is present during all months disregarding the season. Reproductively active individuals have been found during summer and winter months.

Regarding the segmentation of *T. albacares* population due to exploitation, the last report from the Inter-American-Tropical-Tuna-Comission (IATTC) for the yellowfin tuna showed an above-average recruitment in 2015 and 2016. Furthermore, for 2017 and 2018 an increase of the spawning biomass ratio was estimated. Even environmental phenomena such as El Niño event of 2014-2016 coincided with these predictions. The estimations do not suggest a fragmented population. However, high uncertainty to the accuracy of the predictions is also mentioned due to possible bias (IATTC, 2018). As can be seen, there is an apparent absence of a clear mechanism for a genetic differentiation of *T. albacares* in the Eastern Pacific Ocean. Thus, suggesting that the differences found could be explained better due to the non-random sampling method used in this study.

8.4. Gene flow between the locations under study

The stock assessment of fishes depend greatly on gene flow. A population with no differentiation is characterized by random mating (panmixia) and extensive gene flow (Laikre et al., 2005). Therefore, distinct populations are those with few or no migrants between each other. In the present study the detection of first generation migrants suggest

continuous gene flow in the analyzed section of the Eastern Pacific Ocean. Tagging studies have also shown evidence of the migratory behavior of *T. albacares* even though recent literature point to regional fidelity (Pecoraro et al., 2017). Migration together with no isolation by spawning activity suggest continuous gene flow throughout the Eastern Pacific Ocean (Schaefer et al., 2014; Block et al., 2011; Schaefer et al., 1998). The lack of a mechanism for isolation together with low genetic differences are signs of a single population.

8.5. Implications for management

Although tunas are of great importance because of their ecological and economic value, their status is unknown or very poorly known. As a result, fisheries from different tuna species have experienced population declines (Antoniou et al., 2018; Craig et al., 2017; Nikolic et al., 2016). Currently is estimated that 43% of the global tuna stocks are overexploited (FAO, 2018). Thus, reliable data is necessary to design adequate management plans for these marine resources. In the case of yellowfin tuna, essential parameters such as the effective population size are still lacking (Pecoraro et al., 2017). Additionally, data from different approaches have to be taken into consideration: Reproductive patterns, tag studies and genetic population structure analysis. The molecular approach can avoid potential cases of localized over-fishing as well as lose of economic opportunities (Kolody et al., 2019). The present study has corroborated with a molecular approach the single management unit currently assessed by the IATTC. However, it has also showed slight genetic differences that suggest a complex population dynamic with spatial and temporal variations. Therefore, further studies have to be carried out to fully understand the situation of *T. albacares* in the Eastern Pacific Ocean in order to incorporate this information to management plans.

8.6. Limitations of the study

Recent literature has reported limited resolution of microsatellites to discriminate between different populations in marine fishes such as *T. albacares*. This limited resolution could be hiding larger values of genetic differentiation, even if apparent biological differences have been observed between individuals. The plausible lack of resolution to properly discriminate different populations from the genetic approach was suggested by Hauser & Ward (1998) and Waples (1998). Both studies reported separately that, due to large population sizes and high levels of gene flow, the signal from population differentiation was especially weak for pelagic marine species. This weak signal was argued to be caused by the high heterozygosity and allelic diversity inherent of these animals, thus increasing the incidence of allele homoplasy and underestimating genetic distances. Later on, DeWoody & Avise (2000) demonstrated this high variability in microsatellite loci in marine fish compared to other animals, even in freshwater and anadromous fishes. Moreover, in 2004, O'Reilly, Canino, Bailey & Bentzen demonstrated an inverse relationship between F_{ST} and microsatellite polymorphism in the walleye pollock (*Theragra chalcogramma)*.

However, the previous limitations described above could potentially lead to the incorrect conclusion that microsatellite loci cannot be used in population genetics of *T. albacares*. When large enough samples (≥ 50) and loci are included, microsatellites can be compared to the latest technologies (Hodel et al., 2016; Aguila et al., 2015; Díaz-Jaimes & Uribe-Alcocer, 2006). In addition, comparative studies between SSR and SNP performance have been carried out in different organisms. Phylogeographic and population genetic analyses have shown similar inferred structure and cluster identity of populations (Jeffries et al., 2016). On top of that, microsatellite have been proven to delimit better fine-scale population structuring (Tsykun, 2017; DeFaveri et al., 2013;

Hess, 2011) and to describe more efficiently diversity analysis (Singh, 2013). In pelagic fishes related to the yellowfin tuna as the Bluefin tuna (*Thunnus thynnus*), Antoniou et al. (2017) reached the same conclusions when using genome-wide SNPs and microsatellites; however, they pointed out that SSR results had to be interpreted with caution. Hence, genetic approaches when implemented correctly could still retrieve valuable information for stock delineation of the yellowfin tuna (Hodel et al., 2016).

9. CONCLUSIONS

- The study comprised the largest sample size up to date for yellowfin tuna. The 18 microsatellite loci analyzed where highly polymorphic and showed a high genetic diversity for the yellowfin tuna in the Eastern Pacific Ocean similar to previous reports on the species.
- Low, yet significant genetic distances were found across population samples between Mexico and Ecuador, especially for Mexico 2015, which showed the highest genetic distances. These results were further corroborated by PCA analyses that revealed the same pattern.
- Detection of first generation migrants in all the years studied suggest gene flow between the sampled locations.
- It is not possible to reject the hyptothesis that there is a single stock of yellowfin tuna in the Eatern Pacific Ocean.

10. RECOMENDATIONS

- Use next generation sequencing approaches, such as outlier SNPs, in future studies regarding genetic diversity and population structure of the yellowfin tuna in the Eastern Pacific Ocean, due to their better coverage of the genome.
- Perform a random sampling during different days throughout the year and by searching distinct fisheries in order to avoid bias in future studies.
- Analyze samples with temporal variation to further understand the population dynamics of the yellowfin tuna.
- Carry out a similar study using samples from higher latitudes at the north and south of the California and Humbolt currents to test if these currents act as geographical barriers.
- Perform new histological and biochemical analysis on the reproductive biology of the yellowfin tuna in the Eastern Pacific Ocean, especially regarding the spatiotemporal pattern of spawning activity, as the present information is outdated.

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12.TABLES

Table 1. Collection dates and sample sizes.

Location	**Time of collection (Year)**	**Sample Size (Number of individuals)**	**Sample label**
Continental Ecuador	2015	44	ECU 15
(Santa Rosa, Salinas)	2016	58	ECU 16
	2017	105	ECU 17
The Galapagos Marine	2015	191	GAL 15
Reserve	2016	143	GAL 16
(Santa Cruz)	2017	105	GAL 17
Mexico	2015	44	MEX 15
(Cabo San Lucas, La Paz)	2017	63	MEX 17

Table 2. Primers details (Antoni et al., 2014).

Multiplex scheme	Locus	Motif	Fluorescent dye	Allelic size-range* (pb)	Primer sequence (5' – 3') label	T_a (°C)
1	YT 84	(GATA)	HEX	228-320	F: TTACTTACTGCCGCCTACTGG R: AAGTTGGAACCAGAGAACCATT	62.5
	YT 95	(GA)	HEX	86-108	F: CATACGATTGGCTGCTGAAC R: CCGTCGTCTTTCGCTCTATC	
2	YT12	(CA)	6-FAM	317-368	F: GAGATGTGGCTTCCTCCAAA R: GGCGATTTATGGCATCAGTT	62
	YT24	(CA)	6-FAM	153-197	F: GCTCGAGCAGTTTCCAGTAG R: TGATCCACTACTCACAGTCAG	
	YT60	(GT)	HEX	180-222	F: TGTGCTGTGATGTCACCTTGT R: CCCGCCTACAGATCCCTAAT	
3	YT87	(CATA)	HEX	269-307	F: CGAGATACGCGAAGGACAA R: CAGTGGCATTCTTGGCAATAG	62.9
	YT94	(GA)	HEX	98-150	F: TCCATGGAGTTCCCTCTGAC R: GACATTAGTGCCTGGAGCTGA	
	YT121	(CA)	6-FAM	152-214	F: GAAGGCTCTTCAGCTGGTTG R: AGTTGTGGCACGATTGTCTG	
4	YT101	(CA)	6-FAM	265-367	F: ACCAGTTCAGGCCTCTGATG R: CCACTAACTTGCTGGCATGA	61.9
	YT103	(CA)	6-FAM	85-149	F: CCGAGTCTGACCGTTAATGC R: GCAGTTGTGATCACCGATTTT	
	YT111	(CA)	HEX	165-235	F: CGTCCAGTAGTGACGAGGAT R: GACTGTATCTGCTGAAACCAAA	
5	YT29	(CA)	HEX	166-220	F: TGCGTCTTTGAATGGCTAA R: GGTTGTCAGTCAGGACAAAGG	60.9
	YT43	(GT)	6-FAM	125-161	F: AAACGCCGTTGTGGATGT R: TTCCCATAAGCGTTACCATTG	
	YT92	(GA)	6-FAM	206-230	F: CCTCAGCCAAGGTGAGAAGA R: CGCTCGCTACTACTCACTCCA	
6	YT110	(CA)	6-FAM	148-156	F: TGACAAGTGCAGGGATTAAGG R: TGAGCCACGTCATCCAATAA	60.1
	YT112	(CA)	HEX	126-174	F: CAGCCTTGGCAGAATCCTAT R: ATTGAATGCACCAATGATCG	
7	YT107	(CA)	6-FAM	168-211	F: TCAAGCACATGGCTGTTGAC R: AAAGATGTGGCTGACAGATGG	63.4
8	YT122	(CA)	6-FAM	177-223	F: CATCTCCCACCAGGATGTTC R: CACCTGCTCAGCTGACTGTATC	63.6

*Data retrieved from Antoni et al. (2014); T_a Annealing temperature.

Table 3. Collections data summary: DNA concentration/quality ranges per population and number of alleles found in all loci.

Sample	Number of individuals collected	Number of individuals with successful DNA extraction	DNA concentration range (ng/μL)	260/280 quality index mean	Number of alleles per group	Private alleles
ECU 15*	44	44	-	-	320	12
ECU 16*	58	58	-	-	323	11
ECU 17	105	101	6.8-320	2.14	386	10
GAL 15*	91	91	-	-	387	8
GAL 16*	143	143	-	-	411	14
GAL 17	105	95	16.6-183.4	2.3	375	5
MEX 15	44	44	16-138.9	2.32	295	3
MEX 17	63	54	0.26-25	0.88	306	7

*From these samples, individuals already genotyped were obtained from Muñoz (2016) previous study.

Table 4. Global summary statistics per locus.

Locus	A	He	F_{ST}*	F_{IS}*
YT 84	56	0.95	0.0137	0.1242
YT 95	15	0.87	0.0114	0.1817
YT 12	31	0.92	0.0047	0.5056
YT 24	28	0.94	0.0043	0.2785
YT 60	32	0.94	0.0015	0.0897
YT 87	40	0.94	0.0030	0.1853
YT 94	27	0.72	0.0041	0.0646
YT 121	21	0.84	0.0134	0.3470
YT 101	35	0.93	0.0021	0.4479
YT 103	35	0.95	0.0057	0.1563
YT 111	41	0.95	0.0013	0.1767
YT 29	20	0.85	0.0211	0.1508
YT 43	14	0.77	0.0069	0.3409
YT 92	30	0.92	0.0006	0.1754
YT 110	8	0.43	0.0664	0.2481
YT 112	29	0.83	0.0265	0.2673
YT 107	24	0.94	0.0095	0.1421
YT 122	24	0.93	0.0199	0.3147
Mean	28.33	0.87	0.0120	0.2332

A Number of alleles per loci; *He* Expected heterozygosity; F_{ST} Weir and Cockerham' F-statistic; F_{IS} Endogamy coefficient. *All values are significant ($P < 0.05$).

Table 5. Average across loci summary statistics per population.

Sample	H_O	H_E	Na	Ra	F
ECU 15	0.661	0.869	17.78	16.35	0.243
ECU 16	0.626	0.839	17.94	16.79	0.27319
ECU 17	0.691	0.866	21.44	20.20	0.23736
GAL 15	0.704	0.860	21.50	20.19	0.18611
GAL 16	0.647	0.859	22.83	21.84	0.25001
GAL 17	0.656	0.856	20.83	19.74	0.23736
MEX 15	0.648	0.865	16.39	15.11	0.26099
MEX 17	0.606	0.843	16.44	15.68	0.29564

H_O Observed heterozygosity; H_E Expected heterozygosity; *Na* Number of alleles per locus; *Ra* Allelich richness corrected by rarefaction; *F* Inbreeding coefficient.

Table 6. Null allele frequencies per locus.

Locus	Observed frequency	Median frequency
YT 84	0.06969242	0.06906310
YT 95	0.09654749	0.09655035
YT 12	0.3192388	0.3192958
YT 24	0.1568567	0.1565494
YT 60	0.04548879	0.04538089
YT 121	0.09902702	0.09860877
YT 87	0.02864783	0.02891462
YT 94	0.1923189	0.1923282
YT 101	0.2755850	0.2740212
YT 103	0.08404521	0.08318880
YT 111	0.09421806	0.09326336
YT 43	0.08205400	0.08164904
YT 92	0.1751263	0.1739065
YT 29	0.09134488	0.09116136
YT 110	0.09235490	0.09167193
YT 112	0.1463752	0.1466572
YT 107	0.07705708	0.07579667
YT 122	0.1854869	0.1845804

Table 7. AMOVA results.

Analyses			
Source of variation	**Sum of squares**	**Variance Components**	**Variation Porcentaje (%)**
GAL per year			
Among populations	45.989	0.06326	0.81
Among individuals within populations	3078.246	1.73339	22.30
Within individuals	1966.000	5.97568	76.88
ECU per year			
Among populations	37.588	0.07422	0.96
Among individuals within populations	1878.927	1.71210	22.07
Within individuals	1212.000	5.97044	76.97
MEX per year			
Among populations	29.075	0.20085	2.57
Among individuals within populations	921.267	1.99724	25.61
Within individuals	549.000	5.60204	71.82
2015 per site			
Among populations	43.952	0.11341	1.45
Among individuals within populations	1650.050	1.64155	20.92
Within individuals	1090.500	6.09218	77.64
2016 per site			
Among populations	18.034	0.05147	0.67
Among individuals within populations	1898.269	1.89639	24.65
Within individuals	1155.000	5.74627	74.68
2017 per site			
Among populations	39.750	0.06468	0.84
Among individuals within populations	2330.120	1.75384	22.65
Within individuals	1481.500	5.92600	76.52

Table 8. Average relatedness (Wang, 2011) and inbreeding (Milligan, 2003) coefficient for each site and year.

Location	Relatedness (variance)	F (variance)	Year	Relatedness (variance)	F (variance)
GAL	-0.03466 (0.00499)	0.22722 (0.01299)	**2015**	-0.03545 (0.00545)	0.21415 (0.01547)
MEX	-0.04730 (0.00620)	0.26985 (0.01978)	**2016**	-0.03832 (0.00528)	0.25073 (0.01434)
ECU	-0.03533 (0.00535)	0.22757 (0.01543)	**2017**	-0.03728 (0.00543)	0.23075 (0.01545)

Table 9. Pairwise F_{ST} and associated P-values (above the diagonal). threshold set α = 0.05.

	GAL 2015	ECU 2015	GAL 2016	ECU 2016	ECU 2017	GAL 2017	MEX 2015	MEX 2017
GAL 2015	-	0.018	0.18	0.029	0.108	0.001	0.001	0.03
ECU 2015	**0.0069753**	-	0.072	0.001	0.013	0.027	0.001	0.001
GAL 2016	0.0046249	0.0059268	-	0.082	0.133	0.042	0.001	0.046
ECU 2016	**0.0068474**	**0.011908**	0.005886	-	0.002	0.001	0.001	0.001
ECU 2017	0.0055204	**0.0075461**	0.0053971	**0.0085412**	-	0.102	0.001	0.08
GAL 2017	**0.0097301**	**0.0070327**	**0.0068539**	**0.012541**	0.0063070	-	0.001	0.001
MEX 2015	**0.013757**	**0.016833**	**0.012760**	**0.022338**	**0.012766**	**0.12797**	-	0.001
MEX 2017	**0.0073865**	**0.011031**	**0.0070907**	**0.0097027**	0.0075490	**0.010932**	**0.019646**	-

Table 10. Detection of first generation migrants for 2015.

		Location found		
	2015	GAL	ECU	MEX
Presumed origin	GAL	-	3	1
	ECU	2	-	1
	MEX	4	1	-

Table 11. Detection of first generation migrants for 2016.

		Location found	
	2016	GAL	ECU
Presumed origin	GAL	-	2
	ECU	5	-

Table 12. Detection of first generation migrants for 2017.

		Location found		
	2017	GAL	ECU	MEX
Presumed origin	GAL	-	3	1
	ECU	2	-	3
	MEX	3	1	-

13. FIGURES

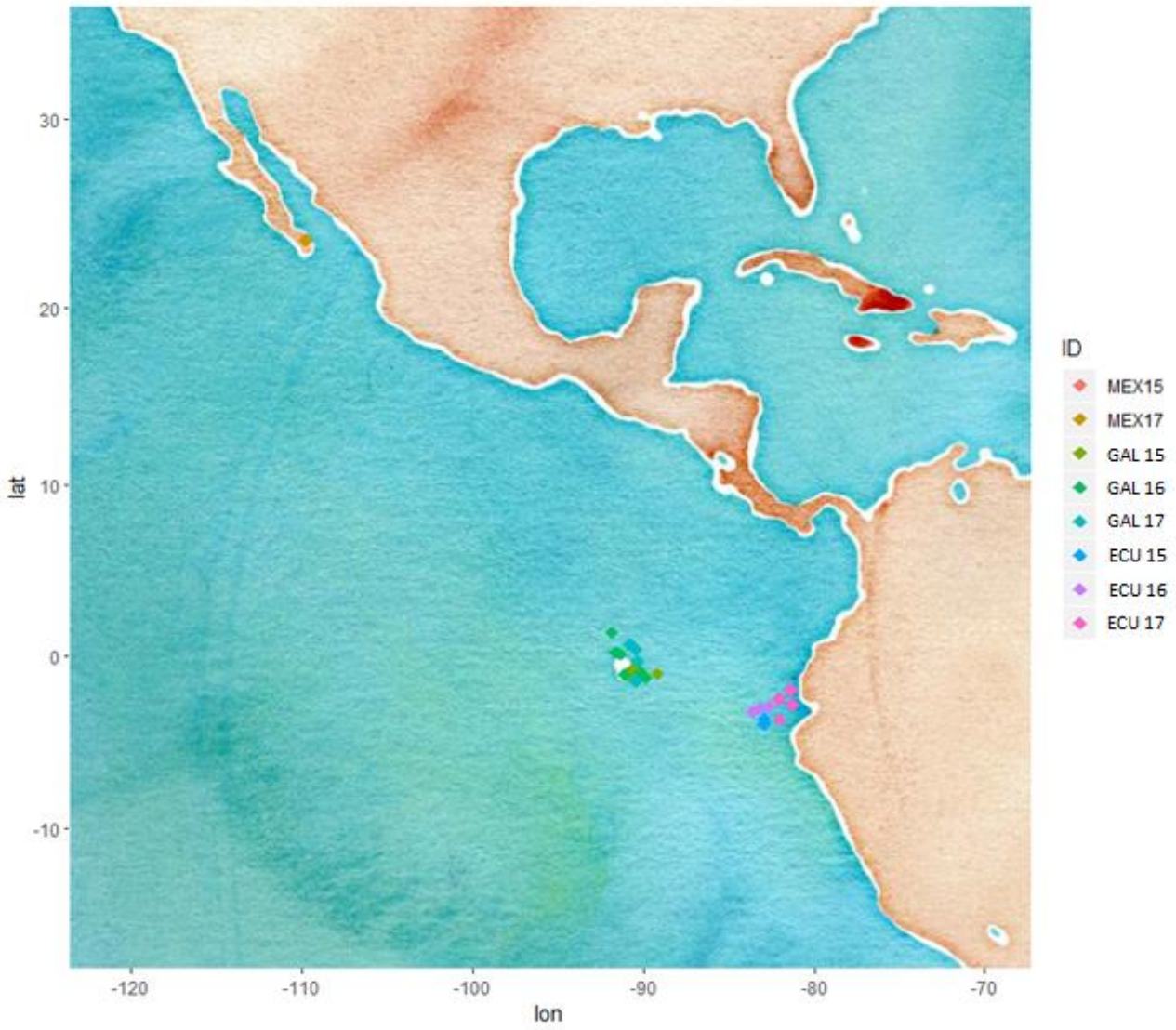

Figure 1. Sample locations.

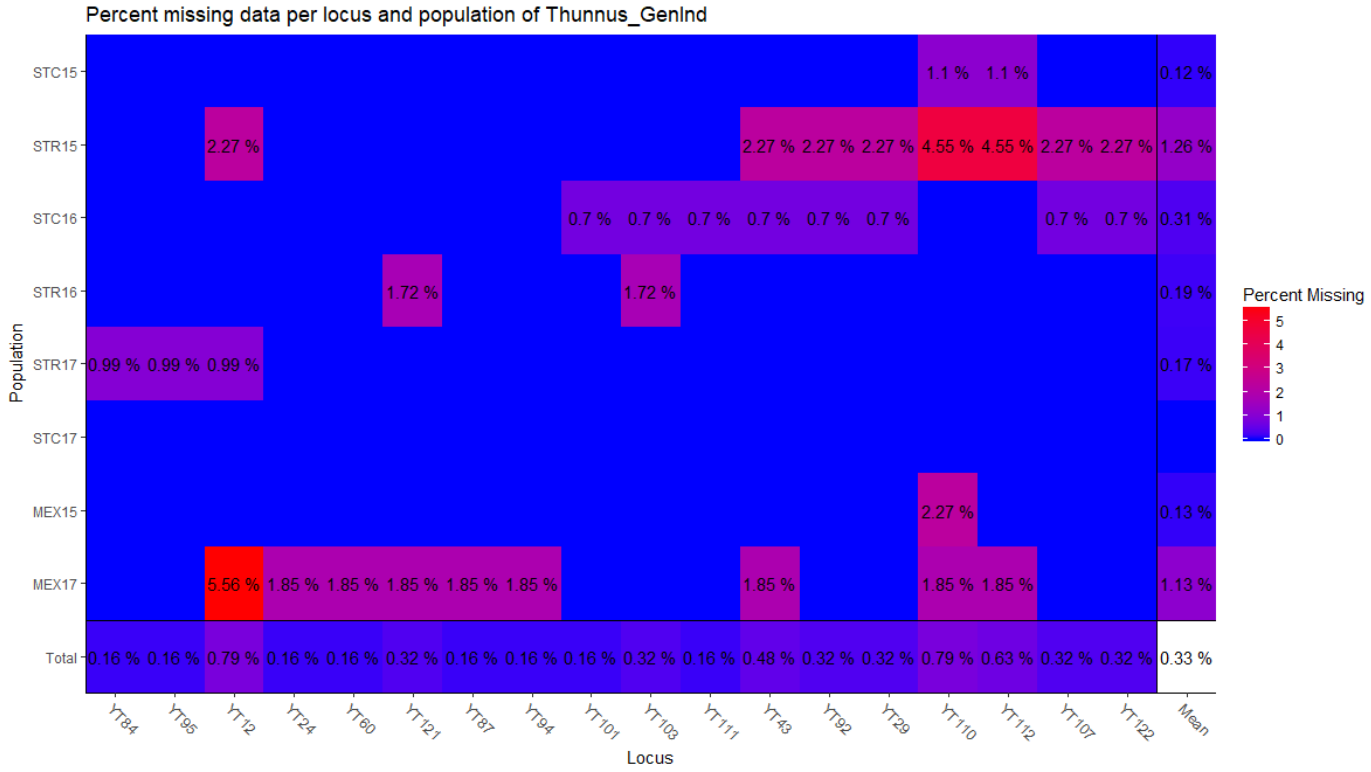

Figure 2. Heat map showing the percentage of missing data per locus and sample.

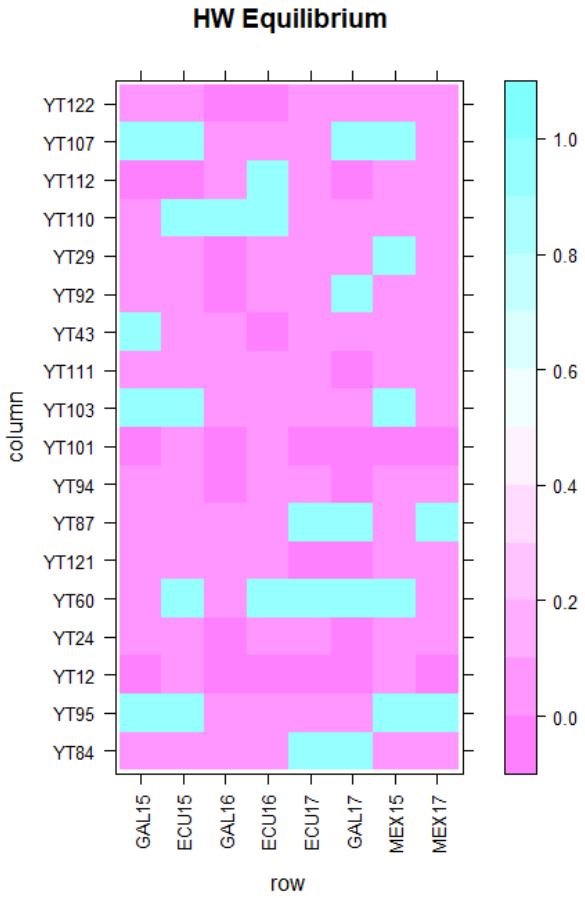

Figure 3. Heat map representing Hardy-Weinberg equilibrium deviations per locus and samples.

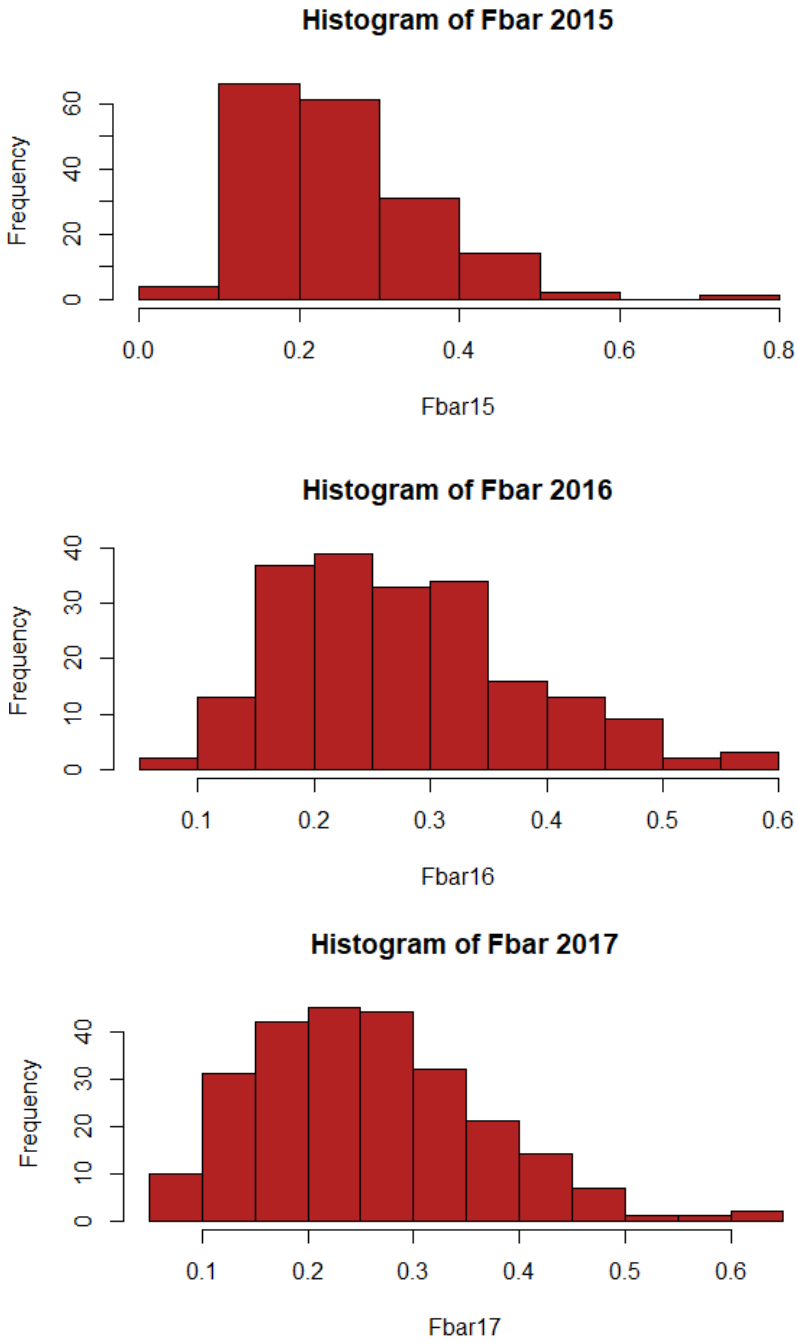

Figure 4. Histogram for F values of *Thunnus albacares* individuals from 2015 (above), 2016 (center) and 2017 (below).

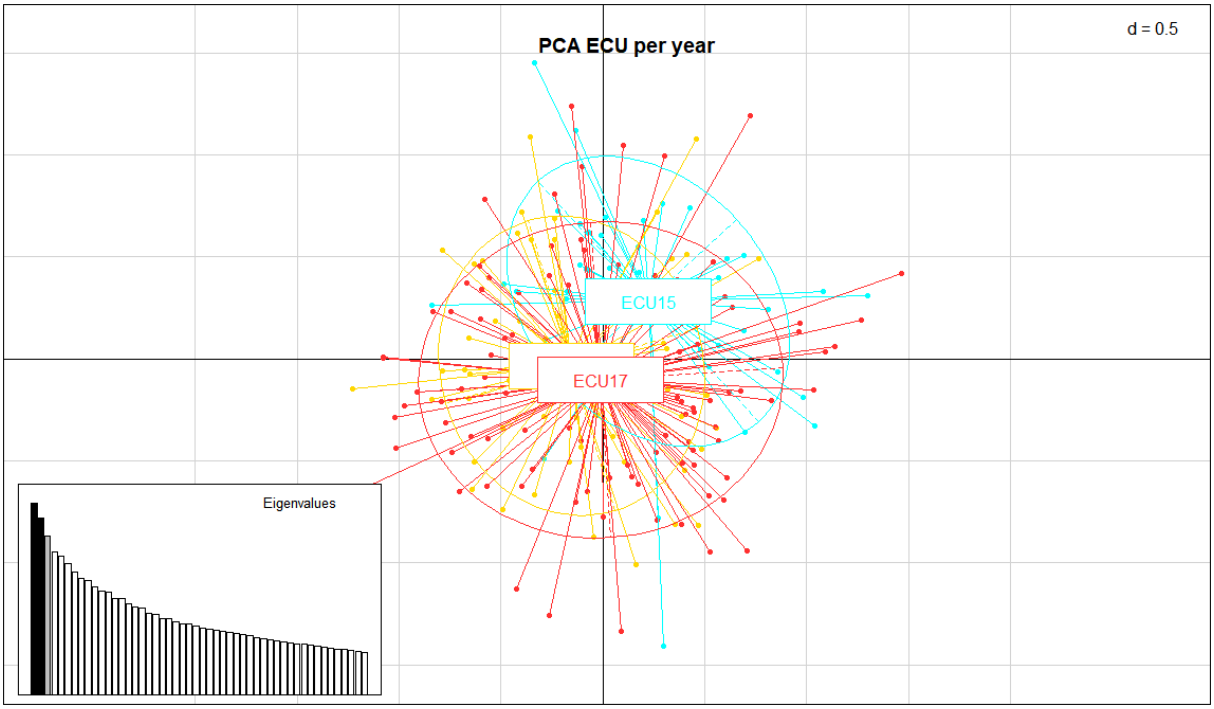

Figure 5. PCA for the analysis per year in continental Ecuador. The percentage of total variation explained by the two principal components shown is 5.99%.

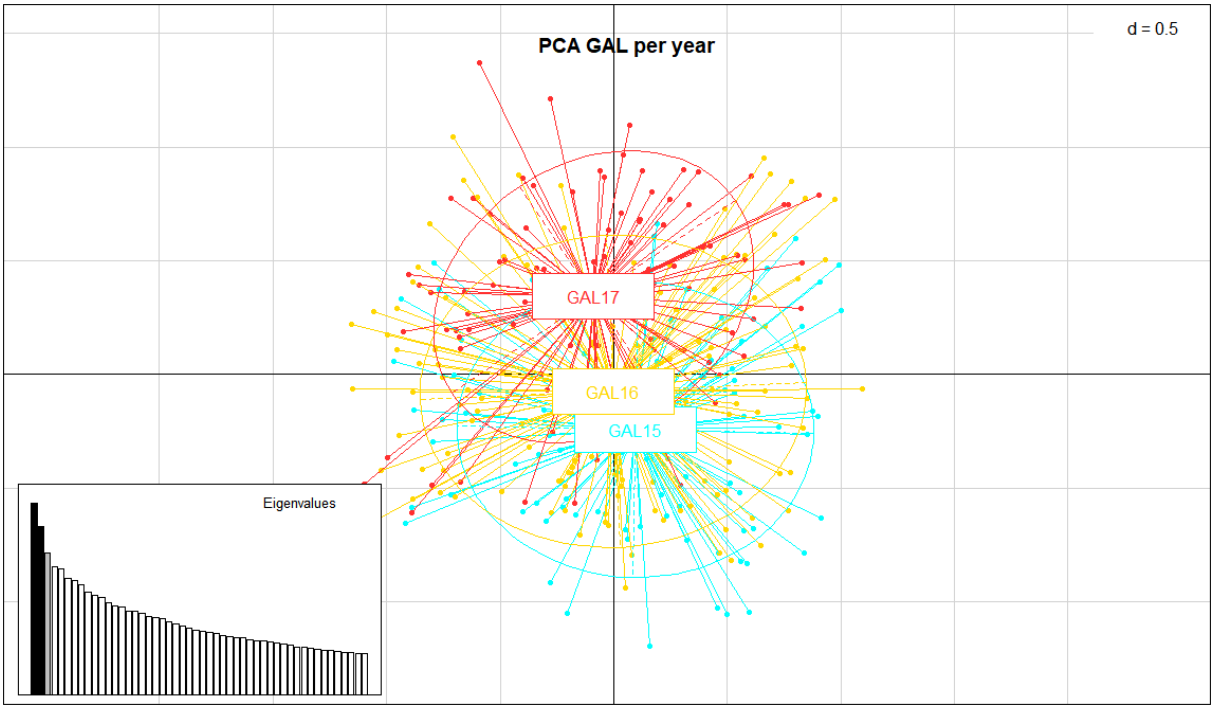

Figure 6. PCA for the analysis per year in The Galapagos Marine Reserve. The percentage of total variation explained by the two principal components shown is 5.56%.

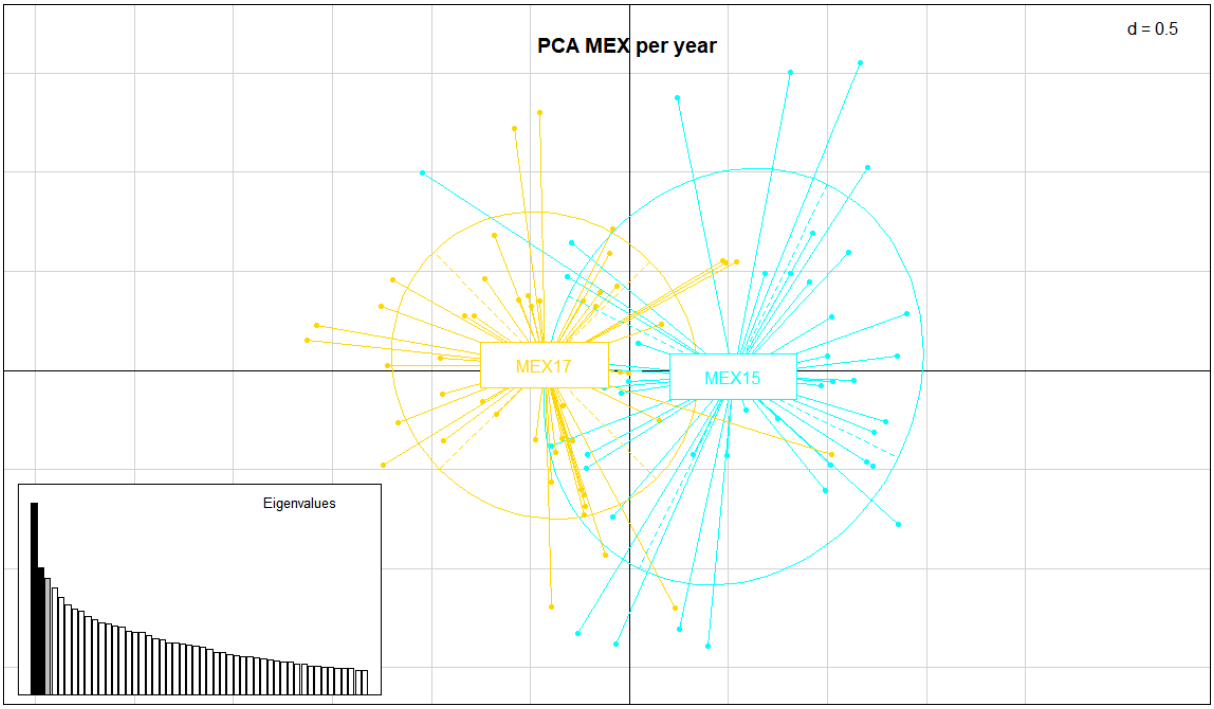

Figure 7. PCA for the analysis per year in Mexico. The percentage of total variation explained by the two principal components shown is 9.42%.

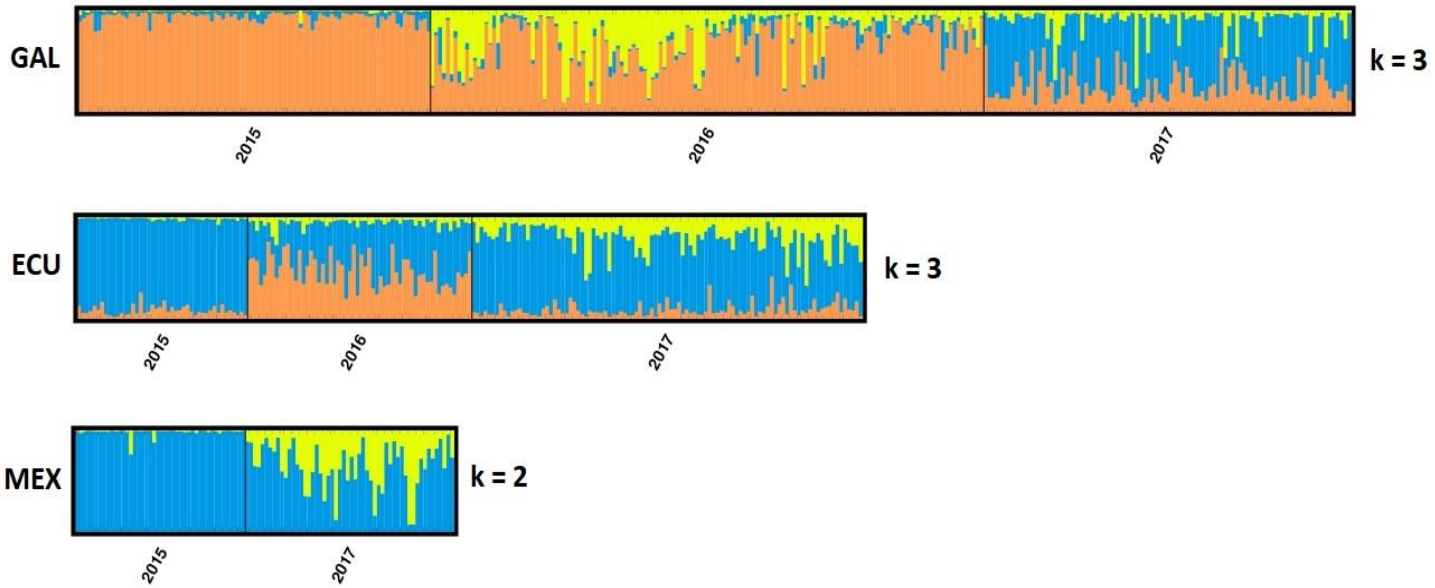

Figure 8. STRUCTURE analyses per year with their respective optimum value of K determined by the Evanno method. Distinct colors represent different genetic pool contribution for each sample.

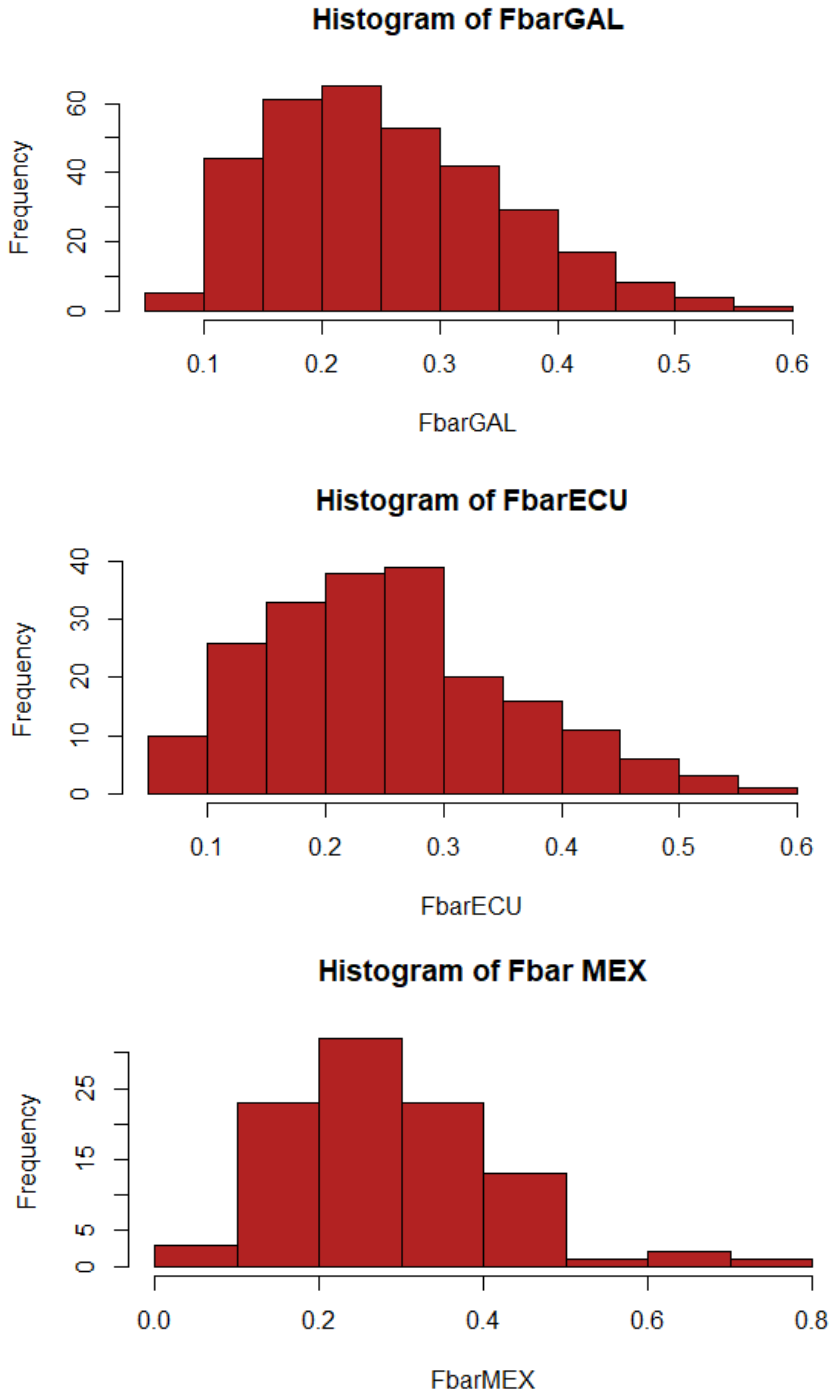

Figure 9. Histogram for F values of *Thunnus albacares* individuals from GAL (above), ECU (center) and MEX (below).

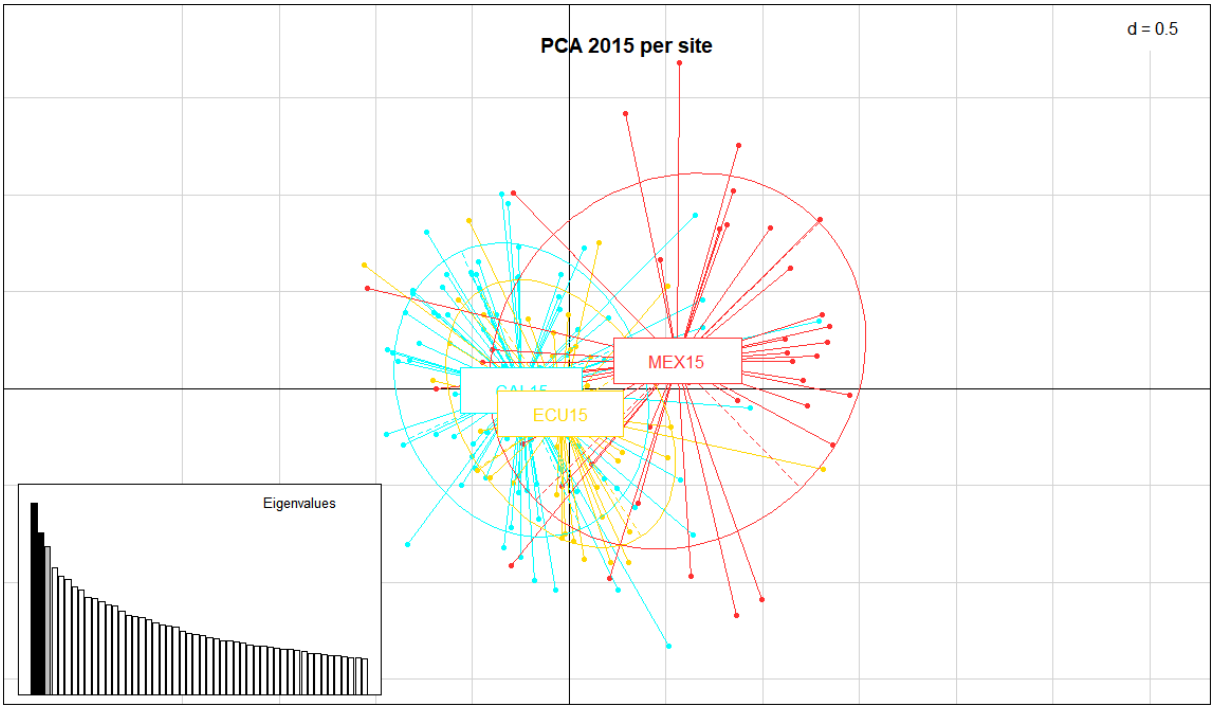

Figure 10. PCA for the analysis per site at 2015. The percentage of total variation explained by the two principal components shown is 6.5%.

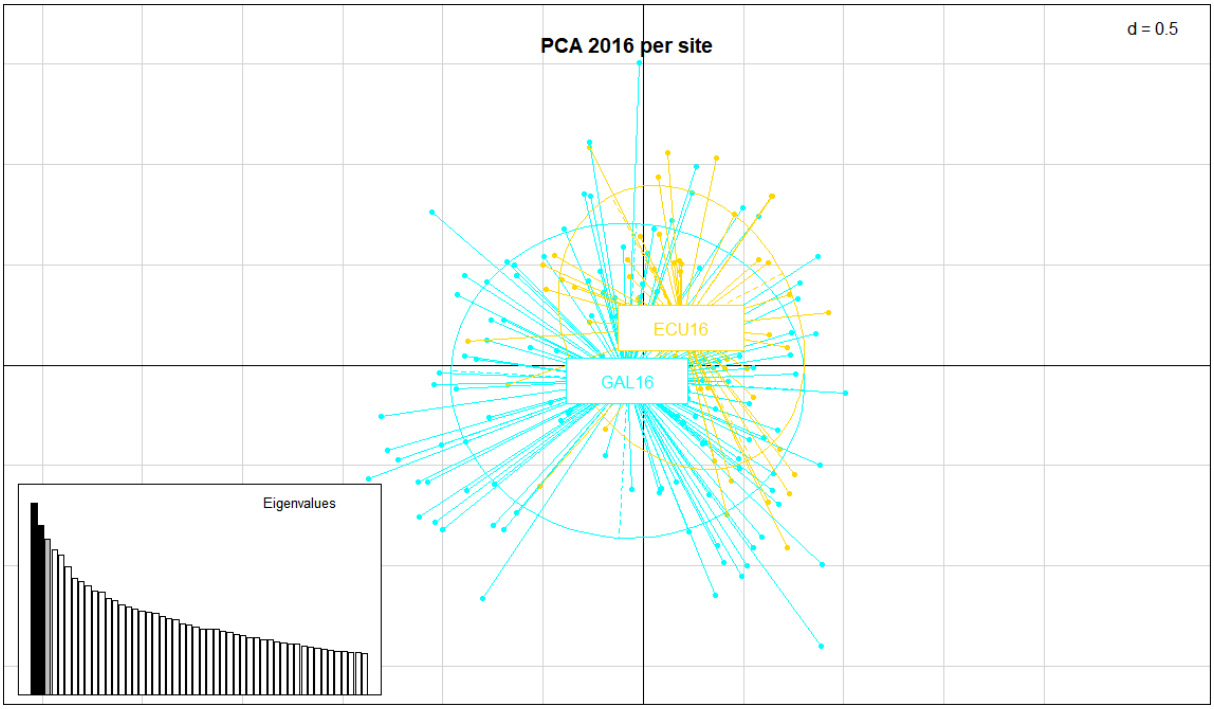

Figure 11. PCA for the analysis per site at 2016. The percentage of total variation explained by the two principal components shown is 5.99%.

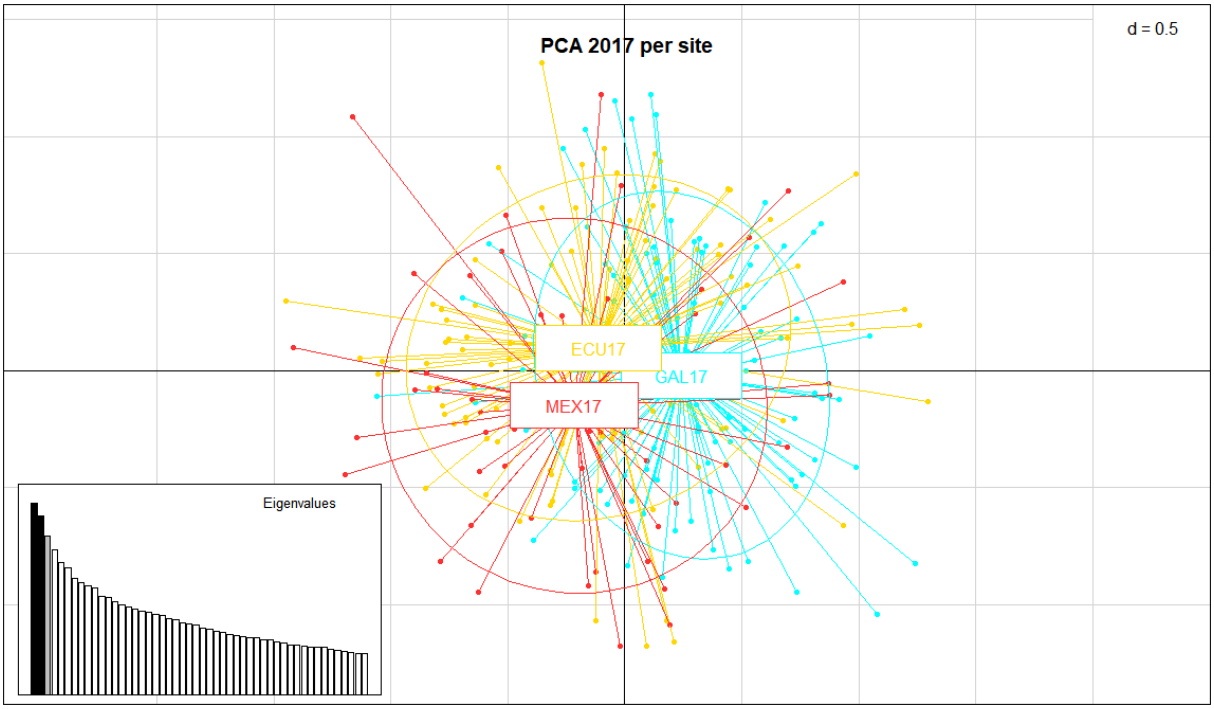

Figure 12. PCA for the analysis per site at 2017. The percentage of total variation explained by the two principal components shown is 5.92%.

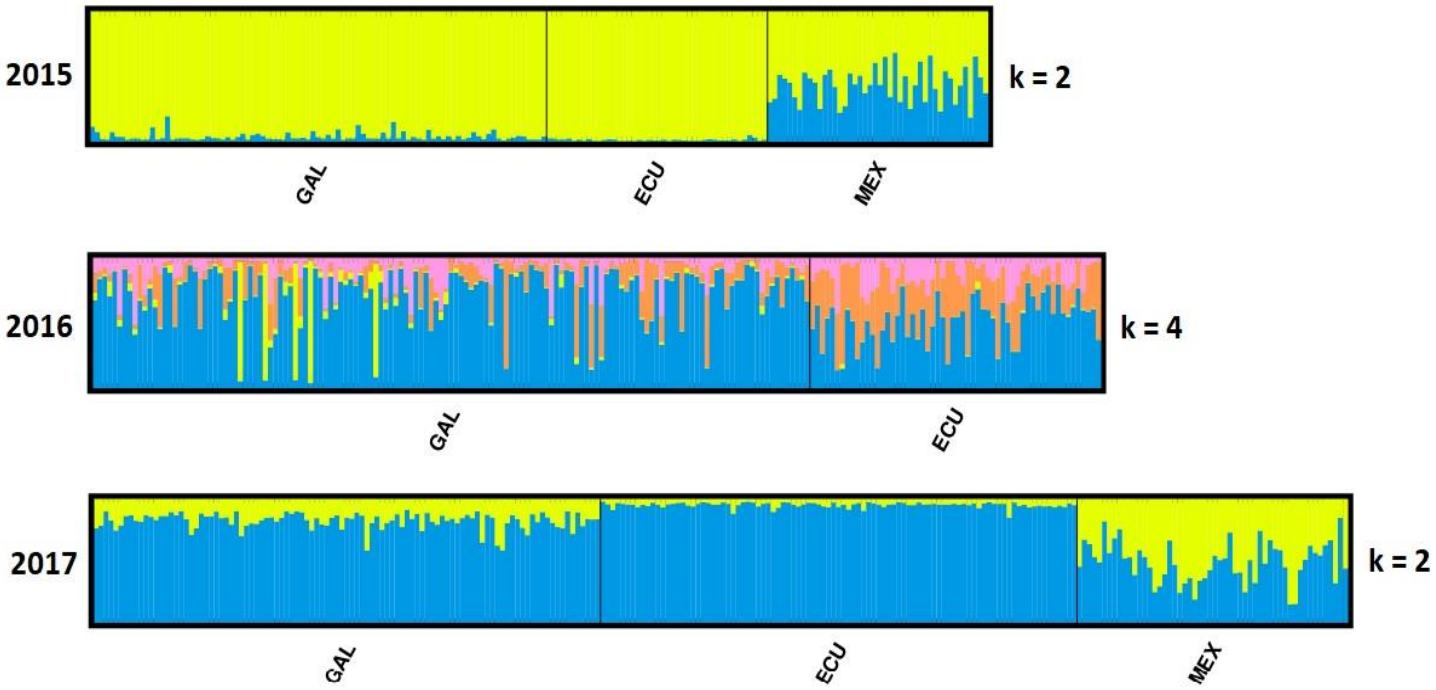

Figure 13. STRUCTURE analyses per site with their respective optimum value of K determined by the Evanno method. Distinct colors represent different genetic pool contribution for each sample.