UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO

PROGRAMA INSTITUCIONAL DE ME MAESTRÍA EN CIENCIAS BIOLÓGICAS

Historia evolutiva y diferenciación genética del pez halcón de coral *Cirrhitichthys oxycephalus* en el Pacífico Tropical

Que presenta

Rolando Quetzalcoatl Torres García

Tesis

Para obtener el grado de maestro en ciencias biológicas

Director de tesis:

Dr. Omar Domínguez Domínguez

Morelia, Mich., mayo 2019

Programa Institucional Maestría en Ciencias Biológicas

UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO
DIVISIÓN DE ESTUDIOS DE POSGRADO
Programa Institucional de Maestría en Ciencias Biológicas

DRA. LILIANA MÁRQUEZ BENAVIDES
COORDINADORA GENERAL DEL PROGRAMA INSTITUCIONAL DE MAESTRÍA EN CIENCIAS BIOLÓGICAS
P R E S E N T E

Por este conducto nos permitimos comunicarle que después de haber revisado el manuscrito final de la Tesis Titulada: "Historia Evolutiva y diferenciación genética del pez halcón de coral *Cirrhitichthys oxycephalus* en el Pacifico Tropical" presentado por el BIOL. ROLANDO QUERZALCÓATL TORRES GARCÍA con Número de Matrícula 0544469C, consideramos que reúne los requisitos suficientes para ser publicado y defendido en Examen de Grado de Maestro en Ciencias.

Sin otro particular por el momento, reiteramos a usted un cordial saludo.

A T E N T A M E N T E
Morelia, Michoacán, a 27 de mayo de 2019

MIEMBROS DE LA COMISIÓN REVISORA

Dr. Omar Domínguez Domínguez

Dr. Rodolfo Pérez Rodríguez

Dra. Juan Carlos Montero Castro

Dra. Sabina Irene Lara Cabrera

Dr. Antonio González Rodríguez

Índice

Resumen: ***1***

Abstract ***2***

Introduction ***3***

Materials and Methods ***5***

Sample collection **5**

DNA extraction, PCR sequencing **9**

Data analyses **10**

Phylogenetic analyses and haplotype network 10

Genetic structure and distances 11

Genetic diversity 11

Results ***12***

Phylogenetic analyses and haplotype networks 12

Genetic structure and distances 17

Discussion ***19***

Two main groups; the effects of the Eastern Pacific Barrier **20**

Tropical Indo West Pacific group **21**

Tropical Eastern Pacific group **22**

Conclusions ***23***

References ***24***

Resumen
El Pacífico tropical (PT) es un área extensa que cubre casí toda la superficie de la tierra con un alto número de endemismos, esta distribución de especies se ve afectada por las discontinuidades en el hábitat, así como por las corrientes oceánicas y los giros. El PT presenta tres barreras reconocidas; La Barrera del Mar Rojo, la Barrera Indo del Pacífico y la Barrera del Pacífico Oriental (BPO). Aquí analizamos si estas barreras biogeográficas tienen un efecto sobre la estructura genética de las poblaciones de *Cirrhitichthys oxycephalus*, un pez críptico que se distribuye a lo largo del PT, que tiene una fuerte asociación con las formaciones de coral. Aplicamos el ADN mitocondrial citocromo b (*Cytb*) y los genes nucleares S7 y RAG1 a las muestras recolectadas en todo el rango de la especie. Usando *Cytb* de ADN mitocondrial, las poblaciones de *C. oxycephalus* muestran una estructura genética alta (ΦST = 0.488, 0.604 y 0.63), y también distancias genéticas altas (Cytb 1.4%, 2.6% y 2.7%) entre los tres haplogrupos encontrados. Indica que BPO tiene un efecto, que separa las poblaciones en dos grupos: un grupo distribuido en el Océano Índico y el Pacífico Central y otro grupo distribuido en el Pacífico Oriental Tropical (POT). Dentro de POT se encontró una segregación de las muestras del atolón de Clipperton, lo que sugiere que esta región se puede aislar, tal vez influenciada por las corrientes de eddy que retienen la larva.

Palabras clave: Pacífico tropical, barreras biogeográficas, crípticas, haplogrupos, y la estructura genética.

Evolutive history and genetic differences of the coral hawkfish *Cirrhitichthys oxycephalus* in the Tropical Pacific.

Abstract

Tropical Pacific (TP) is an extensive area that almost cover all the surface of the earth with a high number of endemism's, this distribution of species are affected by the discontinuities in the habitat, as well as ocean currents and gyres. The TP present three recognize barriers; the Red Sea Barrier, Indo Pacific Barrier and the Eastern Pacific Barrier (EPB). Here we analyze if this biogeographic barriers have an effect in genetic structure of populations of *Cirrhitichthys oxycephalus,* a cryptic fish that is distributed along the TP, which has a strong association with coral formations. We applied mtDNA cytochrome b (*Cytb*) and the nuclear genes S7 and RAG1 to samples collected across the entire range of the species. Using mtDNA *Cytb,* populations of *C. oxycephalus* show high genetic structure (Φ_{ST}= 0.488, 0.604 and 0.63), and also high genetic distances (*Cytb* 1.4%, 2.6% and 2.7%) between the three haplogroups found. Indicating that EPB has an effect, which separates populations in two groups: a group distributed in the Indian Ocean and Central Pacific and another group distributed in the Tropical Eastern Pacific (TEP). Within TEP a segregation of Clipperton atoll samples was found, suggesting that this region may be isolated maybe influenced by eddy currents that retain larva.

Keywords: Tropical Pacific, biogeographic barriers, cryptic, haplogroups, and genetic structure.

Introduction

Examining variations among populations can reveal the genetic structuring processes and their historical associations, thereby infer the evolutionary processes that affect natural populations (Kirchman et al., 2000). Recently, evaluations of the phylogeographic structure of the Tropical Pacific species have been carried out using molecular markers, in which it has been observed that the fish diversity is being underestimated due to the presence of species complexes in widely distributed groups. The reefs of the Tropical Pacific contain thousands of marine species, making this ecosystem one of the most diverse in the world (Palumbi, 1996). The distribution of tropical fishes and their association with reef-buildings corals has long been of interest in biodiversity science (Cowman et al. 2017).

Dispersal is fundamental in the degree of genetic structure in natural populations, and the wide range of dispersal strategies found among marine species is presumed to have deep influence in micro- and macro-evolutionary process (Jablonksi, 1986, Bohanak, 1999, Kinlan & Gaines 2003, Bullock et al. 2006). The pelagic stage of most marine organisms facilitates dispersal (Riginos et al., 2014), and many species have the capacity to delay metamorphosis for long periods of time (Lessios et al., 2001). This dispersal strategy makes barriers to gene flow within the world's oceans intriguing, since they are all physically connected. The isolation of some marine organisms, including fishes, must therefore mean that the sea indeed contains present and past barriers to gene flow (Lessios et al., 2001). Analysis of such regional schemes can be used to inform an understanding of the historical processes that have shaped past- and present-day biodiversity patterns (Renema et al., 2008).

Using genetic markers it has been demonstrated that many marine populations exhibit similar patterns of barriers to gene flow that reflect the geographical or oceanographic history of the region (Sandoval-Castillo et al., 2004, Sandoval-Huerta et al., 2019). The delimitation of genetic barriers and boundaries for marine

assemblages are important steps in evaluating conservation priorities in marine environments (Whiting & Lawler, 2000; Olson et al., 2001; Carpenter et al, 2011; Toonen et al. 2011). Over time, these geographic barriers that limit the movement of individuals between populations can lead to the evolution of discrete phylogenetic lineages (Taylor & Hellberg, 2006).

Within the Tropical Pacific (TP) there are three major biogeographical barriers that can shape the species distributions of tropical species. The first is the Red Sea Barrier, represented by the shallow Strait of Bab al Mandab which reduces water exchange between the Red Sea and the Indian Ocean during glacial maxima (Rohling et al., 1998; Siddall et al., 2003; Bailey, 2009). Indo-Pacific Barrier (IPB), that is a recognized partition between the Central Pacific and Indian Ocean provinces (Briggs, 1974; Gaither et al. 2010), this has been associated to the repeated episodes of lowered sea level in the Plio-Pleistocene glaciations, causing the two oceans to be separated by the emergence of the Sunda Shelf (Voris, 2000; Reid et al. 2006). Whereas the Eastern Pacific Barrier (EPB), the world´s largest barrier of deep water, separates the Central Pacific from the Tropical Eastern Pacific (Robertson, 2004)(Fig. 1). According with this barriers the Tropical Pacific have been divided by tropical regions and provinces limited by barriers, the region known as Tropical Indo-West Pacific (TIWP) that includes the Red Sea, the Western Indian Ocean, the Indo-Polynesian, the Hawaiian, the Marquesas and the Easter Islands provinces. Whereas Tropical Eastern Pacific region (TEP) include the Cortes, Panamic and Oceanic Island provinces (Robertson and Cramer, 2009; Briggs & Bowen, 2012). The environmental heterogeneity within the Tropical Pacific has allowed the development of unique evolutionary processes, which have generated diverse patterns of richness and abundance in fish species, which can be reflected in the genetic differences between the species widely distributed in the Tropical Pacific (Hastings 2000, Mora & Robertson 2005).

One of the widely distributed fish species is *Cirrhitichthys oxycephalus*, the coral hawkfish, inhabiting from the Red Sea, East Africa through Melanesia, Micronesia,

French Polynesia (Marquesas Islands), Australia, throughout the region of eastern India north of Japan and in the Tropical Eastern Pacific from the Gulf of California to the coasts of Ecuador, including all the oceanic islands (Allen & Erdmann, 2012). In general, this species is found at depths less than 40 meters (Lieske & Myers, 1994), presents sexual dimorphism in body size, in which males have a longer body length than females, have a pelagic spawning, resulting in pelagic eggs and larvae (Donaldson, 1988). It is strongly associated with corals and is organized in social groups or harems in which there is a dominant male and one or more females of different sizes (Donaldson, 1989).

Because the widely distribution of *C. oxycephalus* along the Tropical Pacific and the biogeographic particularities of this region, we analyze the genetic differentiation of population throughout the Tropical Pacific of the coral hawkfish in order to a) examine the genetic connectivity or fragmentation along its distribution range, b) understand the influence of habitat discontinuities and oceanographic processes affecting the genealogical lineage distribution.

Materials and Methods

Sample collection

A total of 167 individuals of *C. oxycephalus* were collected from 46 localities in all biogeographic regions and provinces from the TP (Fig 1 and Table 1). Coral hawkfishes were collected by SCUBA diving and using clove oil and a suction tool (Piñeros et al. 2019). Tissue samples were preserved in 96% ethanol and stored at -76 ºC. The specimens were deposited in the Universidad Michoacana de San Nicolás de Hidalgo, in Mexico (CPUM-PEC-227-07-09), University of Central Florida, National Museum of Natural History of the Smithsonian Institute, King Abdullah University of Science and Technology, University of California Santa Barbara and Ho Chi Minh City University of Science.

Table 1. Sample localities and associated information. UMSNH; Universidad Michoacana de San Nicolás de Hidalgo, UCF; University of Central Florida, KAUST; King Abdullah University of Science and Technology, UCSB; University of California Santa Barbara and HCMCUS; Ho Chi Minh City University of Science.

Country	Locality	State/Island	Coordenates	Collection	no. organism
Australia	Great barrier reef	Cairns	16º 51´0" S 145º 46´12" E	Smithsonian	1
		Christmas Island	10º 26´22.96" S 105º 38´9.41" E	KAUST	3
Colombia	Piedra Wacha	Choco	5º 36´19.7" N 77º 29´52.2" W	UMSNH	2
Costa Rica	Puntarenas	Coco island	5º 30´36" N 87º 3´36" W	UMSNH	2
	Punta María	Coco island	5º 32´7.3" N 87º 5´12.5" W	UMSNH	3
	Barco hundido, Wafer bay	Coco island	5º 30´36" N 87º 3´36" W	Smithsonian	1
	Cabo Blanco Island	Puntarenas	9º 32´26.21" N 85º 6´40.35" W	UMSNH	1
	Manuelita	Coco island	5o 33´37.67" N 87º 2´48.73" W	UMSNH	4
Ecuador	Ureles	Manabí	1º 15´32.62" N 81º 4´20.62" W	UMSNH	7
Ecuador	North of Rocas Daphne	Galapagos Archipelago	0º 25´27.71" N. 90º 21´30.4" W	UMSNH	3
	Barco Hundido Karagua	Galapagos Archipelago	0º 53´40.2" N 89º 37´2.19" W	UMSNH	2
	Islote Mosquera, between Baltra and Seymour	Galapagos Archipelago	0º 24´17.1" N. 90º 16´32.4" W	UMSNH	2
	León Dormido	Galapagos Archipelago	0º 46´42.76" N 89º 31´18.3" W	UMSNH	2

	Roca Ballena	Galapagos Archipelago	0º 56´48.01" N 89º 34´52.1" W	UMSNH	2
France		Clipperton	10º 18´14.4" N 109º 13´4.8" W	Smithsonian	15
		Clipperton		UCSB	4
French Polynesia	Ua-huka	Marquesas	8º 54´0" S 139º 33´0" E	Smithsonian	2
Kiribati		Kiritimati	1º 52´ 12" N 157º 18´36" W	Smithsonian	1
		Kiritimati	1º 57´ 0.238" N 157º 31´19.07" W	UCF	9
Mexico	North beach bay , Socorro Island	Revillagigedo Archipelago	18º 51´36.66´´N 110º 59´4.82"W	UMSNH	6
	North beach bay, Socorro Island	Revillagigedo Archipelago	18º 51´36.66´´N 110º 59´4.82"W	UMSNH	5
	North beach bay, Socorro Island	Revillagigedo Archipelago	18º 51´36.66´´N 110º 59´4.82"W	UMSNH	10
Mexico	Concepción bay	Baja California Sur	26º 53´ 39.2" N 111º 49´ 25.6"W	UMSNH	1
	Bahía de los Sueños	Baja California Sur	23º 59´37.1" N 109º 49´32.5"W	UMSNH	2
	Turtle Island	Baja California Sur	27º 25´50.3" N 111º 51´58.3" W	UMSNH	2
	La Ballena, Espíritu Santo Island	Baja California Sur	24º 29´9.78" N 110º 24´2.03" W	UMSNH	2
	Punta Pulpito	Baja California Sur	26º 30´52.3" N 111º 26´ 35.2" W	UMSNH	4
Mexico	Punta Carrizales	Colima	19º 5´47.8" N 104º 26´20.56" W	UMSNH	4
Mexico	Morros del Potosí	Guerrero	17º 32´2.8" N 101º 29´50.4" W	UMSNH	1

	Zacatoso, Ixtapa	Guerrero	17º 39´ 14.33" N 101º 37´ 20.53" W	UMSNH	1
	Zihuatanejo de Azueta	Guerrero	17º 37´ 20.4" N 101º 31´ 19.89" W	UMSNH	2
Mexico	Punta Chahue, Santa María	Oaxaca	15º 45´9.97" N 96º 7´27.39" W	UMSNH	3
	Punta Maguey	Oaxaca	15º 43´45.14" N 96º 8´44.38" W	UMSNH	4
Panama	Los Frailes	Darien, Panamá	10o 17´24" N 80º 2´59.99"W	Smithsonian	2
Panama	Los Frailes	Provincia los Santos	7º 20´51.6" N 80º 8´11.5" W	UMSNH	3
Reunion			21º 7´58.8" S 55º 32´16.799"E	Smithsonian	3
Salvador	El Acuario, Los Cobanos	Sonsonate	13º 31´51.2" N 89º 50´48.1" W	UMSNH	2
	Las Cruces, Los Cobanos	Sonsonate	13º 31´51.2" N 89º 50´48.1" W	UMSNH	1
	La Pared	Sonsonate		UMSNH	2
Saudi Arabia	Saudi Arabia	Farasan Banks (Atlantis Shoal)	18º 5´52.31" N 41º 18´38.35" E	KAUST	5
South Africa	Sodwana	KwaZulu-Natal	27º 33´0" S 32º 41´23.999" E	Smithsonian	2
	Durban	KwaZulu-Natal	29º 51´36" S 31º 3´35.999" E	Smithsonian	2
Taiwan	Dongsha Atoll	Dongsha Atoll	20º 42´9.72" N 116º 0´0" E	UCF	19
United Kingdom	Chagos Archipelago	Egmont Atoll	6º 41´26.18" S 71º 50´12.67"E	UCF / KAUST	2
	Chagos Archipelago	Salomon Atoll	5º 13´57.16" S 72º 22´25.71"E		5
	Chagos Archipelago	Great Chagos Bank	6º 41´26.18" S 71º 50´12.67"E		3
Vietnam				HCMCUS	3
				Total	167

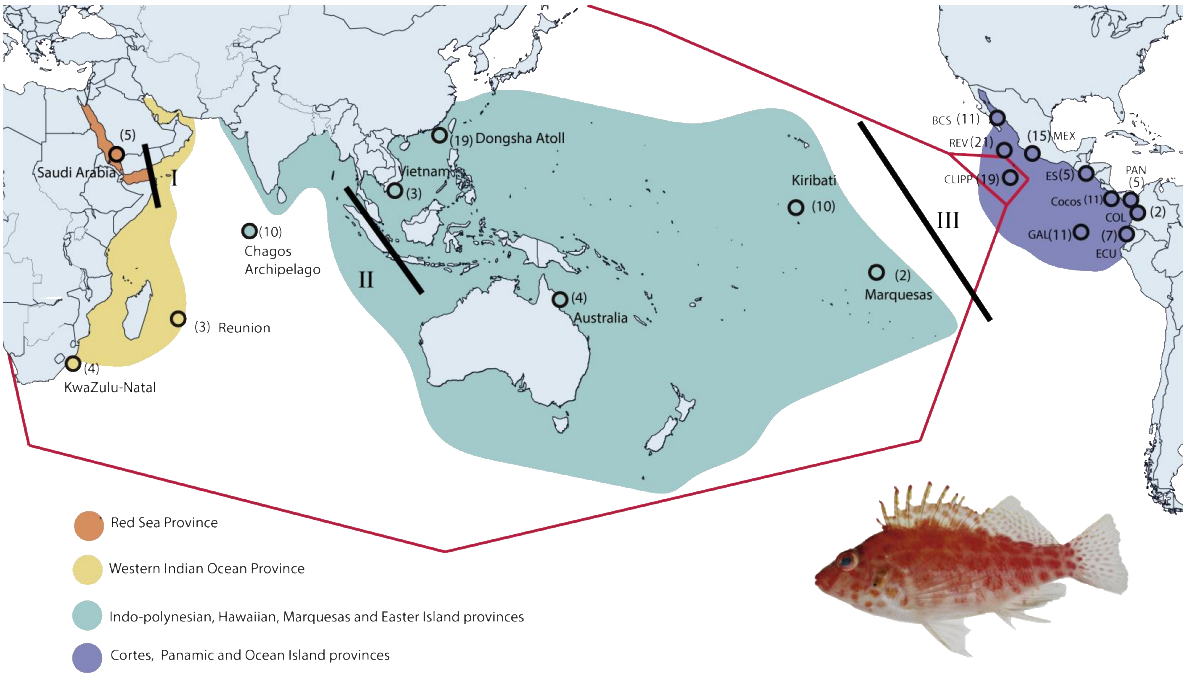

Figure 1 Collecting sites along the Tropical Pacific. Biogeographical provinces and regions are shown in colors. Numbers in parentheses are samples sizes for each collecting site. The red lines represent the hypothetic barriers of mitochondrial gene shown by the K=3 with SAMOVA. Lines in black represent the position of the biogeographic barriers: I=Red sea barrier, II=Indo Pacific barrier, III=Eastern Pacific barrier. BCS; Baja California Sur, REV; Revillagigedo Islands, MEX; Mexico, CLIPP; Clipperton, ES; El Salvador, PAN; Panama, COL; Colombia, GAL; Galapagos, ECU; Ecuador.

DNA extraction, PCR sequencing

The DNA was extracted from tissue samples using phenol-chloroform protocol (Sambrook et al., 1989). We amplified a 654 bp fragment of Cytochrome b, using primers GluDG (forward) and H16460 (reverse)(Perdices & Doadrio, 2001). For some samples we design specific primers 1F31 ACGGCTGACTAATCCGTA (forward) and 1R61 AATTAGGGATGCGACTTGTCC (reverse). For the nuclear genes, the recombination-activating gene 1 (*RAG1*) 819 bp was sequenced using primers Rag 1F (forward) and Rag 9R (reverse) (Quenouile, 2004) and for *S7* intron ribosomal protein 432 bp the primers S7-1F (forward) and S7-R (reverse)(Chow &

Hazama, 1998). All genes were amplified via polymerase chain reaction (PCR) and consisted of a total volume of 12.5-16.5 µL with 1 µL 50-100 ng DNA template 0.25 µM of each primer, 0.2-0.5µM MgCl2, 0.25-0.9 µL of dNTP,0.0625-0.088 µL of Taq, 1.25-1.5 µL of buffer and deionized sterile water to volume. PCRs utilized the following cycling parameters: initial denaturation at 94ºC (3 min) and final extension at 72ºC (10 min), with an intervening 35 cycles of 30s at 94ºC, 45 s at the annealing temperature (51ºC for cyt b; 58ºC for S7; 59ºC for RAG1), and 1 min at 72ºC. The PCR products were visualized electrophoretically on 1.5% agarose gels and sequencing was conducted by MACROGEN Inc. Korea.

Sequences were manually edited and aligned using MEGA 7.0 (Tamura et al., 2011). For the *S7* and *RAG1* sequences, heterozygotic individuals were identified through point mutation, and the alleles were separated using the PHASE algorithm with the software DNAsp v5.0 (Librado & Rozas, 2009). Analyze of recombination was generated using Split Tree 4 (Huson & Bryant, 2018) for nuclear genes S7 and RAG1.

Data analyses

Phylogenetic analyses and haplotype network

The evolutionary substitution model for mitochondrial (*Cytb*) and nuclear genes (S7 and RAG1) was obtained based on Alkaike information criteria (AIC) and optimal partition setting analysis using PartitionFinder v.1.1.0 (Lanfear et al., 2012). Phylogenetic reconstructions were assessed using two building algorithms. Maximum likelihood (ML) in RAxMLGUI v1.3.1 (Silvestro & Michalak, 2012; Stamatakis. 2014) using the GTR+Gamma+I substitution model, and 10,000 bootstrap replicates. A Bayesian inference (BI) reconstruction was conducted with the software MrBayes v3.2.1 (Ronquist et al., 2012), for 50 million generations, with two independent runs implementing four Markov Chain Monte Carlo (MCMC) processes and sampling every 1000 generations. The substitution model use was

the GTR+ Gamma + 1 and Chain convergence was evaluated with the log-likelihood (-InL) values of two independent runs on Tracer v1.5 (Rambaut & Drummond, 2007), discarding the initial 10% of the generations (burn-in) to construct the consensus tree (σ = 0.0002). In accordance with previous phylogenetic analyses, *Cirrhitichthys aprinus* and *Cirhitichthys falco* were used as the outgroup in both ML and BI analyses.

To determine the geographic distribution of haplotypes for the populations of *C. oxycephalus*, a statistical parsimony network was constructed for each molecular marker (*Cytb, S7* and *RAG1*) using the software PopArt v.1.7 (http://popart.otago.ac.nz) with the Median-Joining algorithm.

Genetic structure and distances

Genetic difference among groups found in the haplotype network of *C. oxycephalus* were estimated with paired test fixation indices (Φst) for the mithocondrial gene *Cytb*. Genetic distances between the provinces and regions were obtained for *Cytb* in MEGA 7.0 (Tamura et al., 2011). Population structure was assessed with a spatial analysis of molecular variance (Samova 1.0) (Dupanloup et al, 2002). Samova was run with values of K=2 to 10 to identify the most likely number of populations (Eble et al., 2011).

Genetic diversity

Haplotype diversity (*h*), haplotype number (*hn*), nucleotide diversity (π) and segregating sites (*SS*) were estimated using the software DnaSP v.5.0 (Librado & Rozas, 2009).

Results

A total of 167 sequences were obtained of 654 base pairs (bp) of length for the *Cytb* gene with 108 haplotypes. For the nuclear genes we obtained a total of 96 sequences with 819 bp for RAG1 and 114 sequences with 432 bp for S7. The nuclear genes not reveal significant evidence of recombination (S7 =0.4823 and RAG1 =0.05213; p>0.05).

Phylogenetic analyses and haplotype networks

For *Cytb* gene, the phylogenetic analysis show a basal politomy for all the samples of the provinces within the TIWP samples. Whereas the Tropical Eastern Pacific samples were grouped in one clade with two subclades, one representing mainly samples from Clipperton and other including the samples from the rest of the Tropical Eastern Pacific (Fig.2). The phylogenetic analyses for both nuclear genes not resolve relationships, showing a basal politomy without resolution (not show).

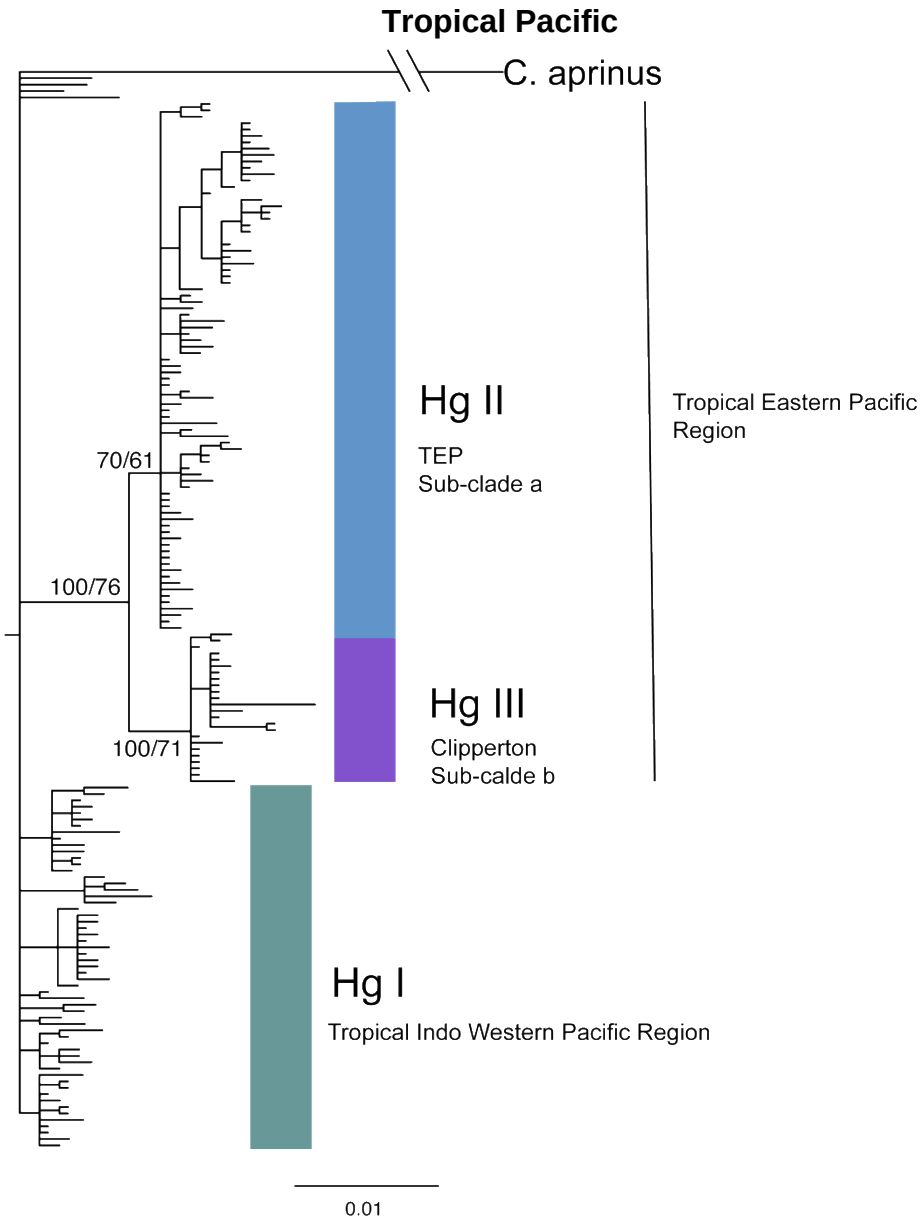

Figure 2. Phylogenetic tree estimated in RAxML and Mr Bayes, based on *Cytb* gene. Bootstrap/posterior probability supports are shown between principal groups. Colors represent the different haplogroups (Hg) found in the haplotype network (Fig. 3).

The haplotype networks for the *Cytb* show the same general pattern of the phylogenetic analyses. We observed three groups, two inside TEP, one including all the samples from Clipperton Atoll, four samples from Revillagigedo Archipelago, one

from Colima and one from Oaxaca separated by six mutation steps for the mainland TEP samples, the rest of the Revillagigedo Archipelago and one from Clipperton Atoll. This last haplogroup is separated by eight mutational steps from the haplogroup that is represented by the samples from the Tropical Indo-West Pacific region, within this haplogroup a mixture of haplotypes from the different provinces was found (Fig. 3). As in the phylogenetic analyses, the haplotype networks for the nuclear genes show a mixture of haplotypes for all regions and provinces, without segregation or formation of groups (Figs. 4 and 5). Due the lack of structure and the mix of haplotypes for all the samples in the nuclear markers, all the subsequent analyses were conducted only with the *Cytb* gene.

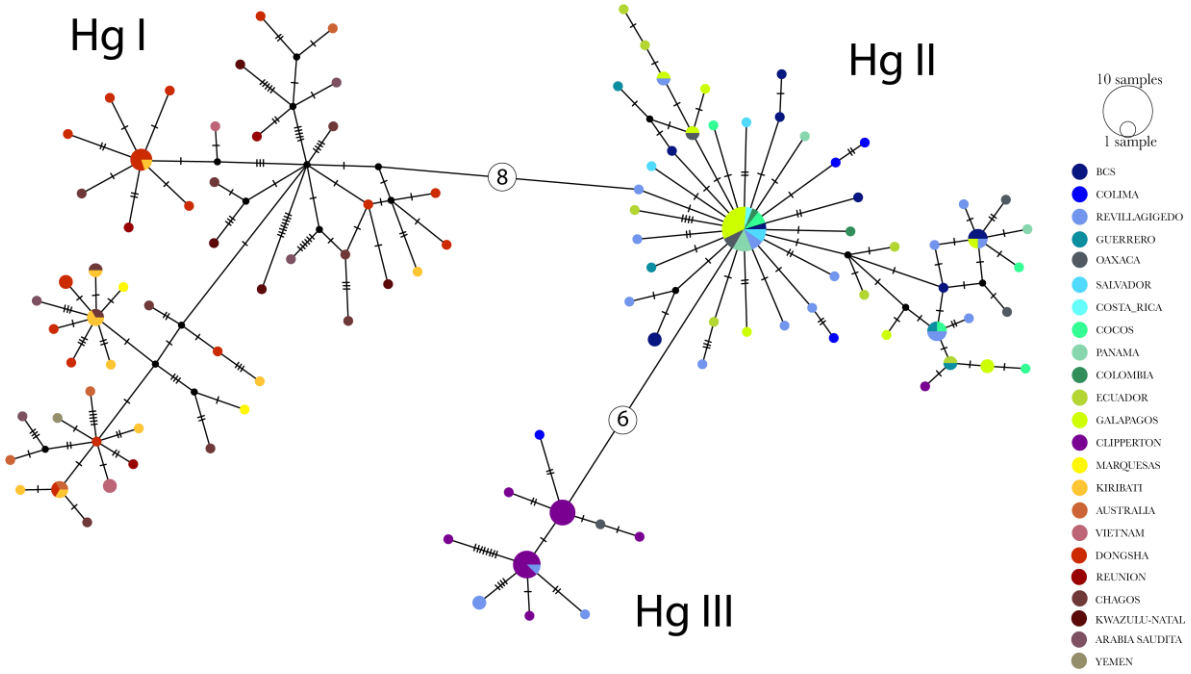

Figure 3. Median-Joining networks for *Cytb*. Size of the circles indicate the frequency of the haplotype according with the legend. Colors correspond to the regions were the samples belong as show in the box. Each line represent number of mutation steps, and numbers in the white circle represent mutation steps.

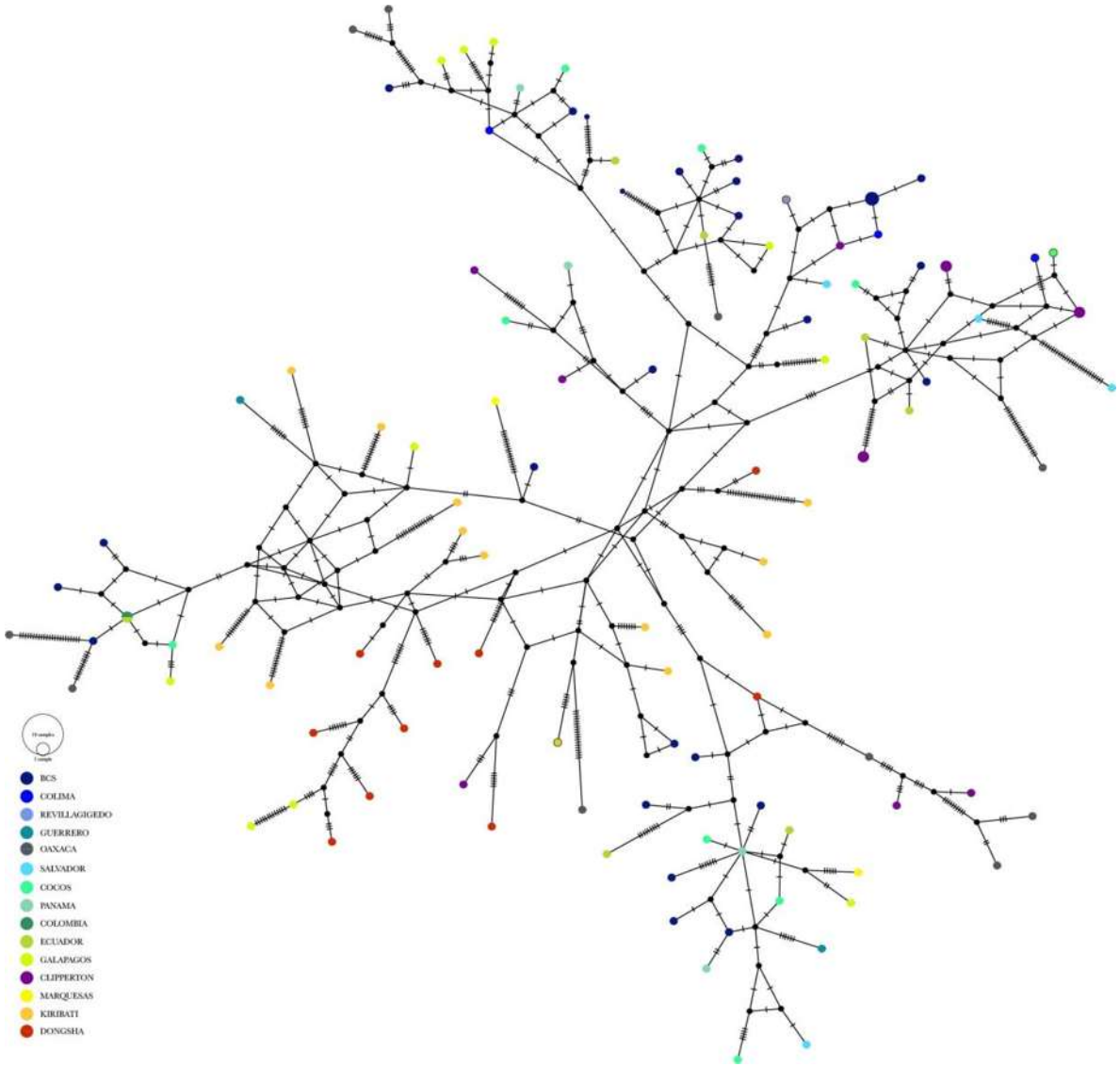

Figure 4. Median-Joining networks for S7 gene. Size of the circles indicate the frequency of the haplotype according with the legend. Colors correspond to the regions were the samples belong as show in the box.

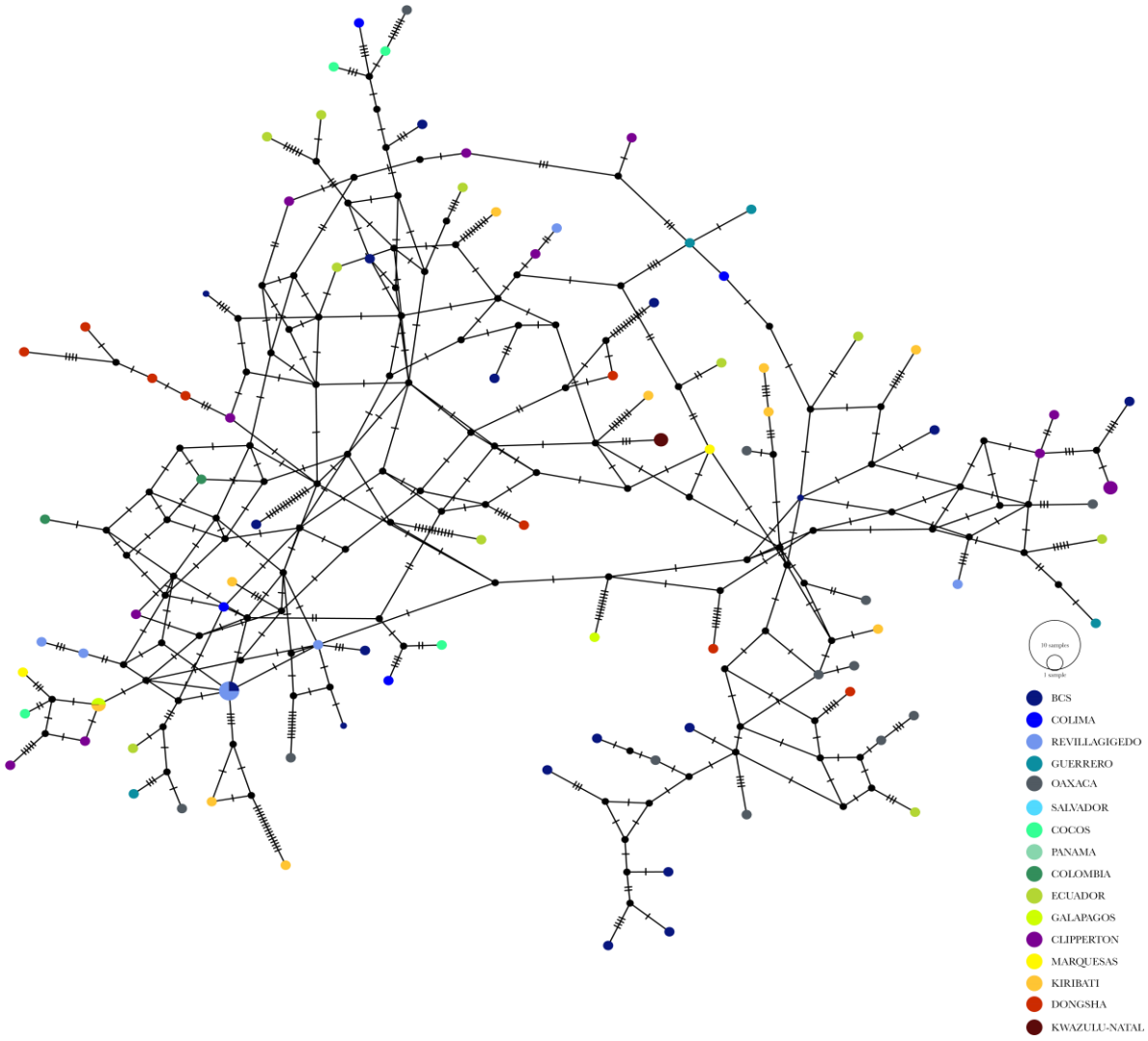

Figure 5. Median-Joining networks RAG1 gene. Size of the circles indicate the frequency of the haplotype according with the legend. Colors correspond to the regions were the samples belong as show in the box.

Genetic structure and distances

We obtained high and significant levels of genetic differences between the haplogroups. The highest and significate values was obtained between Tropical Indo-West Pacific *vs* Clipperton (CLIPP) (Φ_{ST} =0.63084), and the lowest between the rest of the Tropical Eastern Pacific R-TEP and CLIPP (Φ_{ST} = 0.48872) (Table 2). Genetic distances (D_p) between the three haplogroups were high (>1.3%). The highest was when TIWP *vs* CLIPP samples were compared (2.7%), and the lower when R-TEP *vs* CLIPP were compared (1.4%) (Table 2.).

Table 2. Above diagonal pairwise population comparison (Φ_{ST}), using *Cytb* gene. Below diagonal; mean genetic distances (D_p) in percentage. * Indicate significant values. RS: Red Sea, CP: Central Pacific, IP: Indo Pacific, TEP: Tropical Eastern Pacific, CLIPP: Clipperton.

%Divergence mtDNA	**TIWP**	**R-TEP**	**CLIPP**
TIWP	-	0.60452*	0.63084*
R-TEP	2.6*	-	0.48872*
CLIPP	2.7*	1.4*	-

The K=3 arrangement maximized the differences between groups in the SAMOVA analyses, grouping the populations into 1) Tropical Eastern Pacific, 2) Clipperton and 3) TIWP, with the high and significant variation percentage value of 59.43 % and Φct (0.594). These results are congruent with the haplotype network (Fig. 1 and Table 3).

Table 3. SAMOVA of mitochondrial gene *Cytb*. * Indicate significant values.

Analysis	Molecular marker	K	Groups	Φst	Φct	Φsc	Within populations	Among groups	Among populations within groups
SAMOVA	*Cytb*	2	**R-TEP,CLIPP/TIWP**	0.643	0.565*	0.178	35.7	56.57	7.73
		3	**R-TEP/CLIPP/TIWP**	0.611	0.594*	0.043	38.81	59.43	1.76

Genetic diversity

For *Cytb* gene, the lowest nucleotide diversity was found in Clipperton (∏ 0.00477) whereas the other regions the values ranged from 0.00823 to 0.01247. The haplotype diversity (ℏ) was high, with values with a range from ℏ 0.754 to 1 in all the regions (Table 4). Haplotypic diversity was relatively high (≥0.5) and nucleotide diversity was low (<0.03)(Table 4).

Table 4. Diversity indices of *C. oxycephalus*. *N*: number of individuals per Haplogroup, *ℏn*: number of haplotypes, SS: segregating sites, *ℏ*: haplotype diversity, *π*: Nucleotidic diversity.

Groups	*N*	*ℏn*	*SS*	*ℏ*	*π*
TIWP	60	49	93	0.989 +/- 0.0064	0.012382 +/- 0.006464
RTEP	88	53	91	0.9329 +/- 0.0217	0.008481 +/- 0.004558
CLIPP	19	7	25	0.7544 +/- 0.0710	0.004668 +/- 0.00284
General	167	108	163	0.9766 +/- 0.0066	0.017711 +/- 0.008927

Discussion

The analyses presented herein for *Cytb* gene of the coral Hawkfish *Cirrhitichthys oxycephalus* reveals the existence of divergent groups, one distributed in the Clipperton Atoll, other in the rest of the TEP, and a group distributed in the TIWP (Fig. 3). Nuclear genes S7 and RAG1 reveal discordance with mithochondrial gene *Cytb*, since nuclear genes not show structure and all the haplotypes mix in spite of the geographic origin and even with high number of mutation steps between them. The discordance between the mitochondrial and nuclear genomes is a prevalent phenom that occurs in many cases with animals, with a high frequency in mammals and fish compared with other groups (Toews & Brelsford, 2012). According with Toews & Brelsford (2012) this cases of mito-nuclear discordance could be related with mitochondrial introgression, hibridization and incomplete lineage sorting. In our case we have several possibilities; 1) a process of incomplete lineage sorting, but the high number of mutation steps between haplotypes (as much as 25 for S7 and 15 for RAG1) seems to no support this interpretation; 2) a possible hybridization process, but in this case the absence of mixed mitochondrial haplotypes between TEP and TIWP reveal that the Eastern Pacific barrier is highly effective to prevent the migration of this species, the only possibility to support this interpretation is the reproductive behavior in the species, organized in social groups or harems in which there is a dominant male and one or more females of different sizes (Donaldson, 1989), and even had been seen that some males have a distribution pattern named male territory visiting (MTV) polygamy (Kuwamura 1996, 1997) in which males moved between coral heads, and used them for feeding sites, refuge, and courtship sites with resident females (Donaldson, 1990). This behavior can promote the movement of nuclear genes via male movements but no for mitochondrial *Cytb* marker, this may happened because hawkfish females apparently, always stay in the same coral heads and only move for feeding activities (Kadota & Sakai, 2016). In such case could be a possibility of a male biased dispersal, but the existence of mixed mitochondrial haplotypes in two genetically divergent groups (RTEP *vs* CLIP) seems to not support the idea of male biased dispersal; and 3) although with our result we cannot state the origin of the *C. oxycephalus*, according with the number

of *Cirrhitichthys* species in the TIWP and the diversity indices, the TEP population seems to be originated in the TIWP, in such case the origin of the TEP population could be related with an historical hybridization process of *C. oxycephalus* with other species of *Cirrhitichthys* from the TIWP that could explain the high number of mutation steps and the mixture of haplotypes with a lack of geographic arrangement along the distribution range of the species. Hybridization have been reported between *C. oxycephalus* and *C. calliurus* in the Socotra Archipelago (DiBattista *et al*., 2015), and also males of *C. oxycephalus* have been register interacting with harems of *C. aprinus* and *C. falco* at Miyake-Jima island, and even male of *C. aprinus* regularly attempted courtship with a female of *C. falco* when conspecific females were absent (Donaldson, 1986). Although this is the most plausible scenario, more *Cirrhitichthys* species must to be added in order to corroborate the hypothesis of hybrid origin of the nuclear genome of *C. oxycephalus,* our results are disconcerting and we not have certainty that discordance in mito-nuclear genes it is due to lineage sorting, hybridization, biological or ecological conditions of the specie.

Two main groups; the effects of the Eastern Pacific Barrier

The results presented herein show that *C. oxycephalus* have sorted out into main separate evolutionary units with the mDNA (Fig 3, Table 2 and Table 3), one distributed in the TIWP, and the other restricted to the TEP, separated by eight mutation steps. This results are in accordance with the previous results, suggesting that the two populations from the two regions are relicts of an old separation with no subsequent gene flow since *ca.* 700 000 years ago (Lessios & Robertson, 2006). The Eastern pacific barrier (EPB) is a 4000-7000km extension of deep water that has been there for about 65 Myr (Griggs & Hey, 1992; Lessios & Robertson, 2006). Darwin (1872) recognized it as impassable barrier due the long deep sea distance between islands. As the results presented herein, recent studies found that EPB is a barrier that isolate populations of *Panulirus penticiallatus, Conus ebraeus* and *Doryhamphus excisus* (Lessios & Robertson, 2006; Duda & Lessios, 2009; Lacchei *et al.* 2016) in both sides of the EPB barrier. *Cirritichthys oxycephalus* is a benthic carnivorous organism that is found in a depth range of one to 40 m but usually found in a range of 10 to 25 meters, and is strongly associated with corals (Baensch &

Debelius, 1997), this shallow corals reliance could be a factor for which the deep sea EPB is and effective dispersal barrier for this species.

The results presented herein also have taxonomic implications. The formation of the two haplogroups show in all the analyses, one including the TIWP and the second including the TEP samples, and the high genetic distances (D_p >2.6%), a value that is above of the mean cut-off value for recognition of species with the *Cytb* gene (Avise et al., 1998), indicate that *C. oxycephalus* could be represented by a species complex, and even the population from the TEP must to be resurrected as *Cirrhitichthys corallicola* Tee-Van, 1940, which type locality registered in Gorgona island in Colombia. Other fish species distributed in the TEP have its sister species in the TIWP, some of them showing minor morphological differences between closely related but distinct species (Rosenblat & Waples, 1986).

Tropical Indo West Pacific group

Even though that many cases of reef fishes of the provinces of the TIWP region had highest levels of endemism, like in the Marquesas Islands and the Red Sea, the result presented herein not shows evidence of genetic structure of *C. oxycephlaus*, and even show a mixture of haplotypes between provinces that populations distributed along the TIWP region, the Red Sea, the Western Indian Ocean, the Indo-Polynesian, the Hawaiian, the Marquesas and the Easter Islands provinces not show, even though they are so distant in , a region that extends over approximately half of the circumference of the globe (Briggs & Bowen,2012; Toonen et al., 2016),.

The TIWP region had been limited by two barriers, the Indo Pacific Barrier that is considered as a ´hard barrier´ formed by a group of Islands with large land areas that can prevent dispersal (Craig et al. 2007) and the Red Sea barrier that is considered as a ´soft barrier´ due it is the unusual environmental conditions and narrow entrance (Di Battista et al. 2016). Some marine species that are found in the TIWP region have genetic population partitions that geographically coincide with a barrier somewhere between the Red Sea, the Western Indian Ocean, the Indo-Polynesian, the Hawaiian, the Marquesas and the Easter Islands provinces (Wiilaims and Benzi, 1998; Lessios et al .2001). But this biogeographic barriers does

not affect the dispersion of *C. oxycephalus,* species that apparently have a high dispersal capacity. There are more cases of genetic studies that not match with this barriers, *Upeneus moluccensis* (Tikochinski et al., 2013) *Naso brevirostris*, *Naso unicornis* and *Naso vlamingii* (Horne et al. 2008), that no showed sequence differences along the TIWP populations with a high inter-oceanic gene flow occurred on a relatively recent evolutionary time. Another case is *Pristipomoides filamentosus* is a highly dispersive specie with gene flow across deep water within TIWP region and only show differentiation for the Hawaiian islands (Gaither et al. 2011).

Tropical Eastern Pacific group

We found unexpected results within the TEP, reveling two divergent groups, one with private haplotypes in Clipperton and the other for the rest of the TEP, with some haplotypes close related with Clipperton, mixture that we interpret as recent migrants from Clipperton to the rest of the TEP. The two groups are well segregated by six mutation steps (Fig. 3), show significant values in the Φ_{ST} and SAMOVA analyses and D_p = 1.4%. Clipperton is considered the most isolated Shoaling reef in the TEP (<950 km from the nereast coast)(Allen & Robertson, 1997), moreover the small current vectors surrounding the island indicate that larvae are not dispersed long distances from the island and alternatively, eddy currents in the island may return larvae to their natal site (Craig et al., 2006). This mesoscale eddies have the potential to significantly impact the dispersal of larvae, this can affect evolution and maintenance of endemic island species (Adams & Glenn, 2010). The eddy currents transport biological material, including eggs that can be carried into entrapment centers and retained long enough to mature, subsequently they can be returned to the same reef (Lobel & Robinson, 1986). Retention can be as high to retain as many as 20-40% of the particles, including eggs and larvae, if the size of the reefs and currents are suitable, so the mesoscale eddies and the high cover of corals in Clipperton seems to promote larval retention (Robertson & Allen, 1996). *Cyrrithychthys oxycephlaus* is strongly associated with corals and Clipperton atoll is surrounded by well-cemented coral reefs that extend beyond a depth of 40 m and are composed predominantly of corals of the genera *Porties*, *Pavona* and *Pocillopora* (Glynn et al., 1996; Carricart-Ganivet & Reyes-Bonilla 1999; Reyes-

Bonilla et al., 2014), Clipperton endemic fishes can be considered to be “live-coral dependent”, but despite the fact that they have evolved in an environment dominated by live corals, only two of Clippertons fishes (*Cirrhitichthys oxycephalus* and *Arothron meleagris*) could be regarded as being live-coral dependent(Robertson & Allen, 1996). There have been previous genetic studies at Clipperton atoll in which they have found differentiation in population of *Symbiodinium,* even considering that Clipperton population appear to be endemic to the atoll (Pinzón, 2017). Other examples is *Epinephelus clippertonensis* that have genetic differences between Clipperton and the rest of TEP (Craig et al., 2006). These studies have attributed the isolation of Clipperton from the rest of TEP with the combination of special conditions within the island and oceanographic currents.

Conclusions

This work revealed a pattern of population subdivisions with mitochondrial *Cytb* marker in *C. oxycephalus,* demonstrating structure in tree groups across the Tropical Pacific. Also our results agree with previous study that suggest difference between the Central Pacific and the Tropical Eastern Pacific, demonstrating that the Eastern Pacific Barrier has strong influence on the *C. oxycephalus* populations. For the group distributed along the TIWP provinces shown that the Red Sea barrier and Indo-Pacific barrier are not limiting the dispersal of *C. oxycephalus.* While in the Eastern Pacific region had divided in two groups, one mainly distributed in Clipperton atoll and the other in the rest of the TEP, the distinctiveness of the Clipperton population could be related to the eddy currents and high coral cover around the Atoll.

References

- Allen G.R., Steene R, Humann P, DeLoach N (2003) Reef fish identification: tropical Pacific. New World Publications Jacksonville, FL.
- Allen, G. R. 2008. Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. Aquat. Conserv. Mar. Freshwater Ecosyst. 18:541–556.
- Allen, G. R.; Erdmann, M. V. (2012). Reef fishes of the East Indies. *Perth, Australia: Universitiy of Hawai'i Press, Volumes I-III. Tropical Reef Research.*
- Avise, J.C., Walker, D. & Johns, G.C. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc. Biol. Sci. 265, 1707–1712.
- Bailey, R. (2009) Ecosystem geography: from ecoregions to sites, 2nd edn. Springer Publishing, New York, USA.
- Baensch, H.A. and H. Debelius, 1997. Meerwasser atlas. Mergus Verlag GmbH, Postfach 86, 49302, Melle, Germany. 1216 p. 3rd edition.
- BLACK, K. P., S. L. GAY, AND J. C. ANDREWS. 1990. Resident times of neutrally buoyant matter such as larvae, sewage or nutrients on coral reefs. Coral Reefs 9: 105–114.
- Briggs, J.C. (1974) Marine zoogeography. McGraw-Hill, New York.
- Cowman P.F., Valeriano P., Michel K. & Serigio R. Floeter. (2017) The biogeography of tropical reef fishes :endemism and provinciality through time. Biological reviews Vol.92 pp. 2112-2130.
- Craig MT, Eble JA, Bowen BW, Robertson DR (2007) High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). Marine Ecology-Progress Series, 334, 245–254.
- Darren J. Coker, Andre S. Hoey, Shaun K. Wilson etc… (2015) Habitat selectivity and Reliance on live corals for Indo-Pacific Hawkfishes (Family: Cirrhitidae).
- Donaldson, T.J. 1986a. Courtship and spawning of the hawkfish *Cirrhitichthys falco* at Miyake-jima, Japan. J. Ichthyol., 33 (3): 329-333.

- Donaldson, T.J. 1989. Facultative monogamy in obligate coral-dwelling hawkfishes (Cirrhitidae). Env. Biol. Fish., 26: 295-302.
- Donaldson T. J. (1990) Reproductive behavior an social organization of some pacific hawkfishes (Cirrhitidae). Japanese Journal of Ichthyology Vol. 36, No.4
- Duda TF, Lessios HA (2009) Connectivity of populations within and between major biogeographic regions of the tropical Pacific in Conus ebraeus, a widespread marine gastropod. Coral Reefs, 28, 651–659.
- Dupanloup, S. Schneider, and L. Excoffier, “A simulated annealing approach to define the genetic structure of populations,”Molecular Ecology, vol. 11, no. 12, pp. 2571–2581.
- Gaither M.R, Brian W. B., Luis A. R. & John C. B.(2016) Fishes that rule the world:circumtropical distributions revisted. Fish and Fisheries, 2016, 17, 664-679.
- Glynn, P. W., Veron, J. E. N. & Wellington, G. M. (1996) Clipperton Atoll (eastern Pacific): oceanography, geomorphology, reef-building coral ecology and biogeography. Coral Reefs 15, 71–99
- Hastings P. A. 2000. Biogeography of the Tropical Eastern Pacific: distribution and phylogeny of chaenopsid fishes. *Zoological Journal of the Linnean Society* 128: 319-335.
- Horne J. B., Lynne van H., J. Howard C. & D.R. Robertson (2008). High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners, Molecular Phylogenetics and Evolution 49, 629-638.
- J. B. Horne, L.V. Herwerden, J.H. Choat, D.R. Robertson 2008, High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in the three reef fish congeners. Molecular Phylogenetics and Evolution 49 (2008) 629-638.
- Kinlan B. P. & S. D. Gaines (2003) Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology, 84(8), pp. 2007-2020.

- Kuwamura T (1996) An introduction to reproductive strategies of fishes. In: Kuwamura T, Nakashima Y (eds) Reproductive strategies in fishes, vol 1. Kaiyusha, Tokyo, pp. 1–41
- Kuwamura T (1997) The evolution of parental care and mating systems among Tanganyikan cichlids. In: Kawanabe H, Hori M, Nagoshi M (eds) Fish communities in lake Tanganyika. Kyoto University press, Kyoto, pp. 57–86.
- Leis (1991) The pelagic phase of coral reef fishes: larval biology of coral reef fishes. In: Sale PF (ed) The ecology of fishes on coral reefs. Academic Press, San Diego, p 183–230.
- Lessios, H. A., Kessing, B. D. & Pearse, J. S. 2001 Population structure and speciation in tropical seas: global phylogeography of the sea urchin Diadema. Evolution 55, 955–975.
- Lieske E., and Myers R., 1994, Collins pocket guide. Coral reef fishes. Indo-Pacific and caribean including the red Sea. Harper Collins Publishers, 400p.
- Liu L, Edwards SV (2009) Phylogenetic analysis in the anomaly zone. Systematic Biology, 58, 452–460.
- Rigions C., Y. M. Buclkey, S. P. Blomberg & Eric A. Treml (2014) Dispersal Capacity predicts both population genetic structure and species Richness in reef fishes. The American Naturalist Vol 184, No1.
- Rohling, E.J., Fenton, M., Jorissen, F.J., Bertrand, P., Gnassen, G. & Caulet, J.P. (1998) Magnitudes of sea-level low stands of the past 500,000 years. Nature, 394, 162–165.
- Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. Annu. Rev. Ecol. Syst. 25:547-572.
- Palumbi S. R. (1996). What can molecular genetics contribute to marine biogeography? An urchin´s tale.
- Renema, W., Bellwood, D. R., Braga, J. C., Bromfield, K., Hall, R., Johnson, K. G., Lunt, P., Meyer, C. P., McMonagle, L. B., Morley, R. J., O'Dea, A., Todd, J. A., Wesselingh, F. P., Wilson, M. E. J. & Pandolfi, J. M. (2008). Hopping hotspots: global shifts in marine biodiversity. *Science* **321**, 654–657.

- Robertson, D.R., Grove, J.S. & McCosker, J.E. (2004) Tropical transpacific shorefishes. Pacific Science, 58, 507–565.
- Rosenblatt, R.H. & R.S. Waples (1986) A genetic Comparison of allopatric populations of shore fish species from the Eastern and Central Pacific ocean: Dispersal of Vicariance? Copeia, pp. 275-284.
- Russell J. Schmitt, Sally J. Holbrook, etc.. (2009) Intraguild predation in a structured hábitat: distinguishing multiple-predator effects from competitor effects. Ecology 90(9) pp. 2434-2443.
- Theisen, T.C., Bowen, B.W., Lanier, W. & Baldwin, J.D. (2008) High connectivity on a global scale in the pelagic wahoo, *Acanthacybrium solandri* (tuna family Scombridae). Molecular Ecology, 17, 4233–4247.
- Sale PF (1978) Reef fishes and other vertebrates: a comparison of social structures. In: Reese ES, Lighter FJ (eds) Contrasts in behavior. John Wiley & Sons, New York, p 313-346.
- Sambrook, J., Fritschi, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
- Voris, H.K. (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. Journal of Biogeography, 27, 1153–1167.
- Weersing_K,_ Toonen RJ(2009) Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393: 112
- Wenink P. W., Allan J. B., Hans-Ulrich R. & Marcel G. T. T. (1996) Global mitocondrial DNA phylogeography of holarctic breeding dunlins (Calidris alpina). Evolution 50(1), pp. 318-330.

Agradecimientos

Agradezco a mi director de tesis Dr. Omar Domínguez Domínguez por el apoyo, el conocimiento brindado y permitirme ser parte de su equipo de trabajo.

A la Dra. Sabina Lara, Dr. Rodolfo Pérez Rodríguez, Dr. Antonio Gonzales y al Dr. Juan Carlos Montero, por sus comentarios, apoyo y críticas constructivas para realizar un buen trabajo en la tesis.

A la Dra. Michelle por recibirme en su laboratorio para realizar mi estancia, su amabilidad, el conocimiento brindado, el apoyo para conseguir muestras y a todo su equipo de laboratorio.

A mi papás y mi hermano por el gran apoyo que me han brindado siempre para lograr mis metas, a pesar de los tropiezos y caídas, siempre han estado ahí para ayudarme a levantarme y sacar lo mejor de mi. Los quiero muchísimo.

A todos mis amigos del laboratorio Mayra Gómez, Paola Palmerin, Gabriela Beltrán, Eloísa Torres, Yareli López, Berenice García, Francisco Martínez, Francisco Loera, Moisés , Isaí Betancur, Victor Piñeiros, Fredy López, Alex Lievanos Edgar Acevedo Luis Fernando Martínez y Rodrigo Jaramillo por el apoyo brindado y el conocimiento transmitido, además de los momentos divertidos y experiencias únicas a su lado.

A todas las instituciones que nos apoyaron con muestras: University of Central Florida, National Museum of Natural History of the Smithsonian Institute, King Abdullah University of Science and Technology, University of California Santa Barbara and Ho Chi Minh City University of Science

Al Consejo Nacional de Ciencia y Tecnología (CONACYT) por la beca otorgada para mi estudio de maestría.