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Life histories predict genetic diversity and population structure within three species of Octopus targeted by small-scale fisheries in Northwest Mexico

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The fishery for octopus in Northwest Mexico has increased to over 2,000 tons annually, but to date the specific composition of the catch has been ignored. With at least three main species with varying life histories targeted by artisanal fisheries in the region, lack of information about the distribution of each species and metapopulation size and structure could impede effective fisheries management to avoid overexploitation. Here we tested if different life histories in three species of octopus help to predict observed patterns of genetic diversity, population dynamics, structure and connectivity that could be relevant to the sustainable management of the fishery. We sequenced two mitochondrial genes and genotyped seven nuclear microsatellite loci to identify the distribution of each species in 20 locations from the Gulf of California and the Pacific coast of the Baja California peninsula. We tested four a priori hypothesis derived from population genetic theory based on differences in the fecundity and dispersal potential for each species. We found that the species with low fecundity and without a planktonic larval stage (*Octopus bimaculoides*) had lower average effective population size and genetic diversity, but higher levels of kinship, population structure, and richness of private alleles, suggesting limited dispersal and high local recruitment. In contrast, two species with higher fecundity and planktonic larvae (*O. bimaculatus*, *O. hubbsorum*) showed higher effective population size and genetic diversity, and overall lower kinship and population structure, supporting higher levels of gene flow over a larger geographical scale. Even among the latter, there were differences in the calculated parameters possibly associated with increased connectivity in the species with the longest planktonic larval duration (*O. bimaculatus*). We consider that *O. bimaculatus* could be more susceptible to over exploitation of small, isolated

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populations that could have longer recovery times, and suggest that management should take place within each local population. For the two species with pelagic larvae, management should consider metapopulation structure over larger geographic scales and the directionality and magnitude of larval dispersal between localities driven by ocean currents. The distribution of each species and variations in their reproductive timing should also be considered when establishing marine reserves or seasonal fishing closures.

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ABSTRACT

The fishery for octopus in Northwest Mexico has increased to over 2,000 tons annually, but to date the specific composition of the catch has been ignored. With at least three main species with varying life histories targeted by artisanal fisheries in the region, lack of information about the distribution of each species and metapopulation size and structure could impede effective fisheries management to avoid overexploitation. Here we tested if different life histories in three species of octopus help to predict observed patterns of genetic diversity, population dynamics, structure and connectivity that could be relevant to the sustainable management of the fishery. We sequenced two mitochondrial genes and genotyped seven nuclear microsatellite loci to identify the distribution of each species in 20 locations from the Gulf of California and the Pacific coast of the Baja California peninsula. We tested four a priori hypothesis derived from population genetic theory based on differences in the fecundity and dispersal potential for each species. We found that the species with low fecundity and without a planktonic larval stage (*Octopus bimaculoides*) had lower average effective population size and genetic diversity, but higher levels of kinship, population structure, and richness of private alleles, suggesting limited dispersal and high local recruitment. In contrast, two species with higher fecundity and planktonic larvae (*O. bimaculatus*, *O. hubbsorum*) showed higher effective population size and genetic diversity, and overall lower kinship and population structure, supporting higher levels of gene flow over a larger geographical scale. Even among the latter, there were differences in the calculated parameters possibly associated with increased connectivity in the species with the longest planktonic larval duration (*O. bimaculatus*). We consider that *O. bimaculatus* could be more susceptible to over exploitation of small, isolated populations that could have longer recovery times, and suggest that management should take place within each local population. For the two species with pelagic larvae, management should consider metapopulation structure over larger geographic scales and the directionality and magnitude of larval dispersal between localities driven by ocean currents. The distribution of each species and variations in their reproductive timing should also be considered when establishing marine reserves or seasonal fishing closures.

KEYWORDS: octopus, fecundity, planktonic larval duration, larval dispersal, marine connectivity, Gulf of California

INTRODUCTION

As fish catches are collapsing around the world, the focus of commercial fisheries has shifted to resources within lower trophic levels, but with similar or upper economic impact (Watson & Pauly 2001; Pauly et al. 2002; Sala et al. 2004). Some of the marine resources among lower trophic levels capable to support the substantial expansion of fisheries landings include cephalopods (Arkhipkin et al. 2015; Doubleday et al. 2016), for which fishing pressure is expected to increment as a response to growing demands of marine resources (Hunsicker et al. 2010). Cephalopods represent about 20% of the fisheries landing of the world, mainly represented by squids (FAO 2015). The octopus fisheries targeted by small-scale fisheries have incremented considerably since 1970 to date (from ~3,000 ton/year up to ~60,000 ton/year) and its value in the market is sometimes higher than squids (FAO 2015). From 2003 to 2013 most of the production has originated in Mexico (36%), Spain (17%), Portugal (15%), Italia (12%) and others (20%) (FAO 2015). In contrast to most countries where *Octopus vulgaris* is the main species targeted, in Mexico *O. maya* Voss and Solís-Ramírez, 1966 is the most important species along the Atlantic coast (NOM-008-PESC-1993; Jurado-Molina 2010).

In the Mexican pacific, there have been described at least 10 different *Octopus* species, including *Octopus bimaculatus* Verrill 1883, *Octopus chierchiae* Jatta 1889, *Octopus digueti* Perrier and Rocheburne 1894, *Octopus bimaculoides* Pickford and McConnaughey 1949, and Berry's (1953) octopuses: *Octopus alecto*, *Octopus fitchi*, *Octopus hubbsorum*, *Octopus veligero*, *Octopus rubescens* y *Octopus penicillifer* (Brusca 1980; Hochberg & Fields 1980; Roper et al. 1995; Gotshall 1998; Norman & Hochberg 2005). Recent studies indicate that probably three species contribute to the majority of the catch in the Pacific coast of Mexico, namely *O. hubbsorum* (López-Uriarte et al. 2005, Alejo-Plata et al. 2009, Domínguez-Contreras et al.

2013), *O. bimaculatus* (López-Rocha et al. 2012, Villegas et al. 2014) and *O. bimaculoides* (González-Meléndez 2012). In Northwest (NW) Mexico, the octopus fishery represents an important resource for small-scale fishers both in terms of local consumption and markets (Moreno-Báez et al. 2012; Finkbeiner 2015; Finkbeiner & Basurto 2015). However, it is unclear which species contribute to the catch in different localities, and even official fisheries statistics do not attempt to distinguish different species. During 2014, official reports indicate NW Mexico produced at least ~2,000 ton of octopus worth ~ 6 million Mexican pesos (~350,000 USD) (CONAPESCA 2014). Most of the capture for octopus in NW Mexico takes place in the Gulf of California year-round via hooka diving with an air compressor or using traps, and it has been suggested that the fishery might be targeting at least two different species (*O. bimaculatus* and *O. hubbsorum*) (Moreno-Báez et al. 2012). The lack of identification of octopus species in fisheries reports is due their dynamic behavior and ability to change color, pattern, texture and shape (Boyle & vonBoletzky 1996). Besides, their anatomy includes few hard structures that difficult their identification to the species level, especially in octopods (Hanlon 1988).

Ignoring which species are being fished and their geographic distribution could have serious detrimental consequences in the long term not only for local fisheries management but for the conservation of species (Garcia-Vazquez et al. 2012), including over or sub exploiting particular species in certain areas (Marko et al. 2004). The problem of not identifying different species could be particularly serious if they show contrasting life histories and population dynamics that may translate into distinct levels of maximum sustainable yield (MSY) and recovery times, requiring distinct management tools during different seasons and geographic scales. In NW Mexico, *Octopus bimaculatus* could potentially be sympatric with *O. bimaculoides* in the NW of the Baja California Peninsula (BCP), while *O. bimaculatus* could potentially overlap with *O. hubbsorum* within the Gulf of California (Table 1). The reproductive season is different for each species, and the three species differ in their fecundity, egg size and planktonic larval duration (PLD) (Table 1). *Octopus bimaculoides* lays hundreds of large eggs and lacks a paralarval stage and planktonic larval dispersal. *Octopus hubbsorum* lays thousands of smaller-sized eggs and a PLD probably similar to *Octopus vulgaris* based on the size of its eggs (~60 days, Iglesias et al. 2007). *Octopus bimaculatus* lays thousands of medium-sized eggs and shows a longer PLD (up to 90 days) (Table 1). All three species have similar short life spans between 1.5 and 2 years and size at sexual maturity is smaller for males than females (Table 1).

Our main hypothesis is that differences in the life history among three species of octopus from Northwestern Mexico could translate into distinct patterns of genetic diversity, population dynamics, structure and connectivity that could be relevant for sustainable fisheries management. To infer differences in population parameters and evolutionary processes that are important within species, we used two mitochondrial markers and seven nuclear microsatellite loci informative for the three species. We first established the geographic distribution of each species through genetic identification of tissue samples collected over the study region. We then tested four a priori hypotheses within each species derived from theoretical and empirical population genetic studies regarding expected effective population size, genetic diversity, genetic relatedness within populations (kinship) and population structure, based on the fecundity and potential for larval dispersal of each species reported in the scientific literature (Table 1). We discuss the implications of our results for the fisheries management of the three species.

MATERIALS & METHODS

Sample collection and DNA extraction

We obtained 316 samples of octopus (arm tissue) from 20 localities in both coasts of BCP, including the Gulf of California (Fig.1) and collected between 2008 and 2013. The sampling took place at fishing communities with help of small-scale fishers. Samples were collected at seven localities along the Eastern coast of BCP, (Ejido Erendira close to Ensenada B. C. down to El Conejo in Baja California Sur) and 13 sites from the central (Santa Rosalía) and northern Gulf of California (from the northern tip of Bahía de Los Angeles and Isla Tiburón up to Puerto Peñasco), including the Midriff islands. The Midriff islands include many islands and islets in the northern Gulf of California (Fig. 1). Some of these are very remote and access is difficult, which is reflected in smaller samples sizes, while others localities with low number of samples reflect the difficult of catch octopuses outside their reproductive season. We identified only three organisms based on morphology (one of each species). We distinguished between *O. bimaculatus* and *O. bimaculoides* using mature females from which distinctive characteristics of the gonads of each species have been described (Pickford & MacConnaughey 1949). For *O. hubbsorum* we followed morphological traits described previously by Domínguez-Contreras et al. (2013) and original descriptions of Berry (1953). Tissue samples were stored in 96% ethanol and in the lab they were preserved at -20 °C. We extracted DNA using the DNeasy blood and tissue kit (QIAGEN, Valencia, CA, U. S. A) following the manufacturer specifications.

Mitochondrial DNA sequencing

For a subset of the samples (97 individuals from 13 localities, including 8 samples from each locality except from Puerto Refugio where only one sample was analyzed), we amplified two fragments of the mitochondrial genome: the large ribosomal subunit rDNA (16S) employing primers L1987 5’-GCCTCGCCTGTTTACCAAAAAC-3’ and H2609 5’-CGGTCTGAACTCAGATCACGT-3’ (Palumbi et al. 1991) and the Cytochrome Oxidase subunit 1 (COI) with primers LCO 1490 5’-GGTCAAACAAATCATAAAGATATTGG-3’ and HCO2198 5’-TAAAATTCAGGGTGACCAAAAAATCA-3’ (Folmer et al. 1994), For both markers, we used 25µL volume PCRs with 15 - 40 ng genomic DNA, 1× PCR buffer, 0.2 mM each dNTP, 2 mM $MgCl_2$, 0.2% BSA, 1 U Taq DNA polymerase (Invitrogen) and 0.5 µM of each primer. PCR protocol consisted of denaturation at 94 °C for 2 min, 30 cycles of 94 °C for 1min, annealing at 51 °C (COI) or 45.5 °C (16s rDNA) for 1 min, and extension at 72 °C for 2 min, followed by a final extension of 72 °C for 7 min. PCR products were purified using ExoSAP (Affimetrix, INC). PCR products were sequenced from both strands on an Applied Biosystems 3730XL DNA Analyzer at the University of Arizona Genetics Core (UAGC).

Genotyping of microsatellites markers

We employed seven unlinked microsatellites (*Ocbi25*, *Ocbi35*, *Ocbi39*, *Ocbi41*, *Ocbi47*, *Ocbi48*, and *Ocbi50*) that were shared and proved informative among the three octopus species (Domínguez-Contreras et al. 2014). We genotyped the 316 samples following PCR methods previously described (Domínguez-Contreras et al. 2014). PCR products were sized on an Applied Biosystems 3730XL DNA Analyzer at the UAGC. Microsatellite electropherograms were scored using GeneMarker Version 2.6.0 (SoftGenetics LLC). Allele sizes were assigned bins using FLEXIBIN (Amos et al. 2007). Deviations from Hardy-Weinberg equilibrium (HWE) were estimated using GENEPOP 4.2 (Raymond & Rousset 1995). We used MICROCHECKER 2.2.3 to test for genotyping errors and presence of null alleles (Van Oosterhout et al. 2004).

Species assignment

We used the mitochondrial sequences and microsatellite genotypes to assign individuals to species using phylogenetic analyses of sequence data and Bayesian assignment analyses of microsatellite genotypes, respectively. The 16S rDNA and COI sequences were corrected by eye using Chromas Pro Version 1.6 and aligned using MUSCLE multiple alignment tools implemented in Mega6 (Tamura et al. 2013). We used JmodelTest 2 (Guindon & Gascuel 2003; Darriba et al. 2012) to select the best fit model of nucleotide substitution for phylogenetic analysis, according to Akaike and Bayesian information criteria. We applied the Jukes-Cantor (JC) model with 1,000 bootstraps to estimate genetic distances and constructed a Neighbor-joining (NJ) tree using 10,000 bootstraps replications in MEGA (Tamura et al. 2013).

We ran STRUCTURE version 2.3.4 (Pritchard et al. 2000) with the microsatellite genotypes using admixture and without prior location information, with allele frequencies correlated among populations. We used a length of the burning period of 1×10^6, a number of MCMC repeats after burning of 2×10^6, with 10 iterations for each number of genetic clusters (K), and K assumed to vary between 1 and 20. To determine the optimal number of K, we selected the number of cluster by looking at the highest likelihood values (mean of 10 iterations) as well as the highest ΔK value implemented in the online software CLUMPAK (Kopelman et al. 2015). We used both values because some evidence has suggested the likelihood method is not always accurate (Evanno et al. 2005). The value of ΔK is based on the rate of change in the log probability of data between successive K values, which provides a better estimate of the number of genetic clusters (Evanno et al. 2005).

Genetic diversity and effective population size within species

According to the neutral theory of molecular evolution (Kimura 1983), in a population of constant size genetic diversity should be proportional to the effective size of the population (N_e, or the size of an idealized population that would show the same amount of genetic diversity as a population of interest). This is because in an idealized, panmictic population the strength on the loss of neutral alleles via genetic drift is inversely proportional to the population size (Charlesworth 2009). Based on recent comparative studies, we expect that highly fecund species that release high numbers of small eggs into the environment (*O. bimaculatus* and *O. hubbsorum*) will show higher diversity and effective population size than low-fecundity species that produce a small number of relatively large offspring (*O. bimaculoides*) (Table1) (Romiguier et al. 2014; Ellegren & Galtier 2016). To evaluate genetic diversity from the microsatellite data, we calculated the number of alleles (N_A), effective number of alleles ($N_{E,}$ which takes into account different sample sizes among localities), expected heterozygosity (H_E) and observed heterozygosity (H_O) with GENALEX 6.501 (Peakall & Smouse 2012). Allelic richness (R_A) was estimated using HP-Rare to correct for differences in sample size among localities (Kalinowski 2005).

Private alleles, or alleles that are unique to one population, are expected to be more frequent in genetically isolated populations, while their frequency should be reduced in well connected sites (Beger et al. 2014; Munguía-Vega et al. 2015). If we extend this process to populations within each species, then populations of species with narrow opportunities for dispersal (direct developer, *O. bimaculoides*) should show higher frequency of private alleles than species with a pelagic larval stage (Table 1). Private allelic richness (R_{PA}) was estimated using HP-Rare to correct for different sample sizes. We estimated a global contemporary effective size (N_e) for each species via the linkage disequilibrium method with a bias correction and a lower allele frequency of 0.05 and 0.02, and with the molecular coancestry method as implemented in the software NE-ESTIMATOR V2 (Do et al. 2014).

Genetic structure within species

Species with a long PLD are expected to disperse further than species with short or absent PLD (e.g. direct developers) (Shanks 2009). Consequently, the species with direct development (PLD = 0, *O. bimaculoides*) should show higher genetic structure (e.g. global F_{ST}) (Riginos & Liggins 2013), than species with short PLD (*O. hubbsorum*) and particularly compared to species with long PLD (*O. bimaculatus*) (Table 1) (Selkoe & Toonen 2011; Selkoe et al. 2014). To estimate genetic structure, we conducted a hierarchical analysis of molecular of variance (AMOVA) using 999 permutations in GENALEX 6.501(Peakall & Smouse 2012) to estimate the genetic differences observed within and between populations. Both pairwise F_{ST} and F'_{ST} values were calculated using the software GENODIVE 2.0b24 (Meirmans & Van Tienderen 2004) as recommended to account for loci with high polymorphism such as microsatellites (Meirmans & Hedrick 2011). Additionally, we used FreeNA to measure the effect of null alleles on F_{ST} estimates of population structure, taking into account the frequency of null alleles estimated with the expectation maximization method (EM) (Chapuis & Estoup 2007).

Genetic relatedness within populations of each species

The magnitude of local larval retention, or the proportion of larvae produced within a site that remain in that site, is expected to increase the degree of genetic relatedness within populations (Christie et al. 2010; Burgess et al. 2014). We expect that species with direct development (PLD = 0, *O. bimaculoides*) should have a higher probability for individuals to remain near their natal site, and thus to show higher levels of genetic relatedness or kinship within populations than the other two species with a dispersive pelagic larval stage (Table 1). Since local retention is expected to decrease with increasing PLD (Byers & Pringle 2006), we expect that genetic relatedness within populations will be lower in the species with the longest PLD (*O. bimaculatus*). We calculated pairwise relatedness to describe the number of alleles shared between pairs of individuals using Queller & Goodnight (1989) relatedness metric and then calculated the average within each population as implemented in GenAlex 6.2 (Peakall & Smouse 2012). Statistical significance was assessed by 9,999 permutations and 10,000 bootstraps to estimate 95% confidence intervals around the hypothesis of random mating.

RESULTS

Species assignment

A total of 1054 bp were sequenced for each individual sample, including 473 bp from the 16S rDNA gene and 581 bp from the COI gene (GenBank Accession number KY985098 – KY985194 for 16S, and KY985005 – KY985097 for COI). The optimum model of substitution according to the Akaike and Bayesian criteria was JC for both 16S rDNA and COI. The resulting NJ trees showed the monophyletic status of the three species *O. bimaculatus*, *O. bimaculoides* and *O. hubbsorum* according to the topology of both 16S rDNA and COI trees (Fig 2 A). *O. bimaculoides* was present in locations from the Pacific coast of BCP (Ejido Erendira, San Quintin, and Bahía Magdalena), but absent in the Gulf of California. *O. bimaculatus* was present at only one locality from the Pacific coast of the BCP (Malarrimo) and in samples from the Northern Gulf of California including Puerto Peñasco, Puerto Refugio, Puerto Lobos, San Luis

Gonzaga, Bahía de los Ángeles and only one individual from Puerto Libertad for 16S rDNA, (no data was obtained for the COI sequence of this individual). *O. hubbsorum* was present in some localities from the Northern Gulf of California (Puerto Libertad, Isla San Lorenzo, and Bahía Kino) and also in the Central Gulf of California (Santa Rosalía) (Fig 2 A). Nucleotide divergence between the three species ranged from 3.3 – 7.1% for the 16S rDNA gene and from 6.3 – 10.4% for the COI gene (Table 2). *Octopus bimaculoides* showed less divergence with *O. bimaculatus* (3.3% and 6.3%, respectively) than with *O. hubbsorum* (6.3% and 10.0%, respectively), while the largest divergence was observed between *O. bimaculatus* and *O. hubbsorum* (7.1% and 10.4%, respectively).

We genotyped seven microsatellite loci in 316 samples from 20 localities and observed an average frequency of missing data of 3.75% (range 1.26 – 7.27) by locus, and 3.84% (range 0 – 28.5) by sample. Hardy-Weinberg tests suggested significant deviations at only 7 out of 140 unique loci/locality combinations tested without any clear pattern observed within localities or species (after Bonferroni correction P = 0.00036). Only *Ocbi39*, *Ocbi41* and *Ocbi50* were significant deviated in 1, 2 and 4 localities from the 20 tested, respectively (P = 0.00036). Two loci were monomorphic (*Ocbi41* and *Ocbi50*) in 1 and 6 localities, respectively (Table S1). Except for two loci (*Ocbi35* and *Ocbi41*), all other loci showed null alleles in at least one locality, with *Ocbi39* showing null alleles in 8 localities. The average frequency of null alleles among loci varied from 0.000 – 0.108 for *O. bimaculatus* 0.025, for *O. bimaculoides* 0.026, and for *O. hubbsorum* 0.041, according to EM method (Table S2).

The STRUCTURE analysis showed a modal frequency that supported the presence of at least two clusters or species (ΔK = 2, Fig. S1A) according to the ΔK method (Evanno et al. 2005). However the highest mean value of the ln probability of data for K = 2 (average ln [K] = -

8362.29, Fig. S1B) was very close to K = 3 (average ln [K] = -8086.16, Fig. S1B) in 10/10 repetitions, and in both cases the matrix of similarity scores produced by Clumpak between runs aligned were identical 0.999 (Fig. S1C). The STRUCTURE bar plots (Fig 2 B) showed that K = 3 clearly distinguished the three clusters or species previously identified in the phylogenetic analyses of the mitochondrial markers and corresponding to *O. bimaculoides*, *O. bimaculatus* and *O. hubbsorum* among the 20 localities from NW Mexico (Fig 2 B). All localities assigned to each species using 16S rDNA and COI sequences (Fig. 2 A) were correctly assigned using microsatellites (Fig. 2 B). Based on the STRUCTURE analysis, *O. bimaculoides* is only present in the Pacific coast of BCP, while *O. bimaculatus* and *O. hubbsorum* are present on both the Pacific coast of BCP and in the Gulf of California. On the Pacific coast of BCP, *O. bimaculoides* is present in Ejido Erendira, San Quintin and Bahía Magdalena; *O. bimaculatus* in La Bocana, Las Barrancas and Malarrimo, and *O. hubbsorum* in El Conejo. In the Gulf of California, *O. bimaculatus* is present in Puerto Peñasco, San Luis Gonzaga, Isla Smith, Bahía de Los Angeles and Puerto Lobos, while *O. hubbsorum* is present in Puerto Libertad, Isla San Lorenzo, Isla Tiburon, Bahía Kino and Santa Rosalía (Fig. 2 C). In some localities like Las Barrancas in the Pacific coast of BCP and Puerto Peñasco, Puerto Refugio and Isla Tiburón in the Northern Gulf of California STRUCTURE suggested the presence of individuals from both *O. bimaculatus* and *O. hubbsorum* (Fig. 2 B, C).

Genetic diversity and effective population size within species

The seven loci were polymorphic for the three species (Table 3). Results generally supported our prediction about higher allelic diversity and effective size in highly fecund species with small eggs (*O. bimaculatus* and *O. hubbsorum*) than in species that are less fecund and have larger eggs (*O. bimaculoides*). We observed lower average levels of allelic diversity in *O. bimaculoides* (N_E = 3.67 ± 0.47, R_A = 4.56 ± 0.45) than in *O. bimaculatus* (N_E = 5.93 ± 0.28, R_A = 5.05 ± 0.05), while results for *O. hubbsorum* were mixed and showed intermediate values for one metric (N_E = 4.75 ± 0.45), and similar values to *O. bimaculoides* in the other (R_A = 4.47 ± 0.28).

We observed that the species with direct development (*O. bimaculoides*) had the largest average frequency of private alleles (P_{AR} = 1.71 ± 0.43), compared to the species with a pelagic larval stage (Table 3). The lowest values were observed in *O. bimaculatus* (P_{AR} = 0.28 ± 0.05), while *O. hubbsorum* again showed intermediate values (P_{AR} = 0.49 ± 0.20).

The highest contemporary effective population size *Ne* was calculated for *Octopus bimaculatus* using both linkage disequilibrium and molecular ancestry methods (average LDNE = 261 – 265, M_C = 28), followed by *O. hubbsorum* (LDNE = 88 – 125, M_C = 23). *Octopus bimaculoides* had the lowest effective size according to the two methods (LDNE = 5 – 10, M_C = 11) (Table 4).

Genetic structure within species

After pooling sampling locations according to the results of the species assignment (Fig 1), we found that the AMOVA results for the microsatellite data supported the prediction that species with direct development (*O. bimaculoides*) show higher levels of genetic structure (F_{ST} = 0.19, P = 0.000), compared to species with pelagic larvae (Table 5). Also, we observed that the species with the longest PLD had overall lower genetic structure (*O. bimaculatus*, F_{ST} = 0.09, P = 0.000) compared to the species with shorter PLD (*O. hubbsorum*, F_{ST} = 0.15, P = 0.000).

The frequency of null alleles can affect the estimates of genetic differentiation, reducing the genetic diversity and overestimating the F_{ST} values (Chapuis & Estoup 2007). In the present study, the values of genetic differentiation with (Null F_{ST}) and without (F_{ST}) null alleles estimated with FREENA were very similar within each species: *O. bimaculoides* (Null F_{ST} = 0.020 and F_{ST} = 0.020), *O. bimaculatus* (Null F_{ST} = 0.091 and F_{ST} = 0.089) and *O. hubbsorum* (Null F_{ST} = 0.170 and F_{ST} = 0.163) (Table S3).

O. bimaculoides showed both higher and significant genetic differentiation between all population pairs (range of F_{ST} = 0.174 – 0.232; F'_{ST} = 0.481 – 0.653, Table S4), with respect to *O. hubbsorum* that showed only 60.7% of paired values that were moderated and significant (F_{ST} = 0.086 – 0.258; F'_{ST} = 0.216 – 0.751, Table S5), and *O. bimaculatus* with 69.5% of paired values that were significant and showed the lowest genetic differentiation (F_{ST} = 0.007 – 0.144; F'_{ST} = -0.165 – 0.668, Table S6). We observed both high and low values of genetic differentiation between localities from the Pacific coasts of BCP when compared to the Gulf of California for *O. hubbsorum* and *O. bimaculatus*, Tables S5, S6).

Genetic relatedness within populations of each species

The three species showed average levels of relatedness that were significantly greater than expectations based on random mating (all values p = 0.000, Fig. 3). We found that the direct developer (*O. bimaculoides*) had the highest average level of relatedness within populations (R = 0.244), followed by the species with the intermediate PLD (*O. hubbsorum,* R = 0.104), while the species with the longest PLD had the lowest levels (*O. bimaculatus,* R = 0.016).

DISCUSSION

Our study employed both slow evolving haploid markers (mitochondrial DNA) and fast-evolving and hypervariable nuclear markers (microsatellites) to establish the geographic distribution of three species of octopus among fishing localities from NW Mexico and corroborated that differences in the fecundity and potential for larval dispersal (or lack thereof) affect the levels of genetic diversity and structure found within each species.

A minimum of 3% genetic divergence in the COI gene is considered a threshold to differentiate different octopus species (Hebert et al. 2003). Our results showed a higher divergence among the three species (6% – 10%), suggesting they are reproductively isolated taxa. We observed a smaller nucleotide divergence between *O. bimaculoides* and *O. bimaculatus* probably due to their more recent origin from a common ancestor (Hebert et al. 2003). The three taxa studied are the most relevant species for small-scale fisheries from NW Mexico and our results showed that, although their ranges sometimes overlap, most of the surveyed localities had evidence for the presence of a single species, which seem to occur in different habitats. *Octopus bimaculoides* prefers habitats with low wave energy as enclosed bays and coastal lagoons, although it can also inhabit at 20 m depth over rocks and kelps forests (Forsythe & Hanlon 1988; Sinn 2008). In the Pacific coast of the BCP exist at least 16 coastal lagoons located between Ensenada BC and Bahía Magdalena BCS (Lankford 1977), which probably have been colonized by stepping-stone events during rafting behavior (Gillespie et al. 2012). Rafting has been documented for *O. bimaculoides* and *O. bimaculatus* on floating objects including macroalgae (Thiel & Gutow 2005) and besides larval dispersal could help explain colonization events and range expansions. Our study expanded the distribution of the three species in the Pacific coast of BCP with regard to published records: ~800 km to the south for *O. bimaculoides*, ~400 km to the south for *O. bimaculatus* and ~150 km to the north for *O. hubbsorum*. In the Gulf of California, *Octopus bimaculatus* was restricted to the northern Gulf of California where its distribution might be influenced by the geographic extent of a cyclonic (anti-clockwise) oceanographic gyre that transports larvae during its spawning period in summer (Castellanos-Martínez 2008; Marinone et al. 2008; Munguía-Vega et al. 2014). *O. bimaculatus* seems to show the pattern of disjunct distribution reported for several temperate species of fishes that are present in the Pacific coast of BCP, disappear in the Southern Gulf of California and reappear in the Northern Gulf of California (Bernadi et al. 2003). The distribution of *O. hubbsorum* was redefined to include the south of the Midriff Island region in the Gulf of California (López-Uriarte et al. 2005; Moreno-Báez et al. 2012).

The three species were sympatric in the Pacific coast of the BCP around the Bahia Magdalena region, while in the Gulf of California only *O. bimaculatus* and *O. hubbsorum* were sympatric around Midriff Island region. Both regions have been considered transition zones between template and tropical species (Briggs 1974; Brusca 2010; Briggs & Bowen 2012). In this sense, it is possible that *O. bimaculatus* and *O. hubbsorum* could be sharing the same shelters around the Midriff Islands region in different season along the year, with *O. bimaculatus* being more frequent during the cold-temperate seasons, while *O. hubbsorum* prefers warm-tropical water conditions. A pattern of alternate presence of the two species through the year could explain why the octopus fishery is carried out yearlong in the Northern Gulf of California (Moreno-Báez et al. 2012). Thus, at some localities in the Northern Gulf of California both species could be the main target of the fishery according to the time of the year, and at least in some localities where samples in our study were assigned to *O. bimaculatus* (e.g. Puerto Lobos) there have been recent field observations were only *O. hubbsorum* individuals were recorded (unpublish data J. F. D. C and A. M. V.), highlighting the need of a temporal sampling during different seasons to complement our understanding of the species being captured and their seasons, particularly near geographic transition zones.

The life history parameters differing among species played an important role on levels of genetic diversity and structure within species, suggesting that significant differences in population dynamics and connectivity are present. The direct developer *O. bimaculoides* had the lower levels of effective population size and genetic diversity and showed higher levels of relatedness within populations, more structure among populations and a higher proportion of private alleles, compared to the two species with a planktonic larval stage. These observations suggest that populations of *O. bimaculoide*s are comparatively smaller and structured at a local geographic scale, and are likely highly dependent upon local recruitment. In contrast, *O. hubbsorum* and *O. bimaculatus* have higher fecundity and a planktonic life phase that increase their dispersal potential and the opportunities for gene flow among populations (Villanueva et al. 2016), which is consistent with our hypotheses regarding a larger effective population size associated to higher levels of genetic diversity and lower levels of genetic relatedness within populations, less genetic structure among populations and fewer private alleles. These results suggest that *O. hubbsorum* and *O. bimaculatus* might depend less on local larval retention and more on larval dispersal among populations. However, *O. bimaculatus* had lower levels of genetic differentiation between populations, and lower frequency of private alleles and genetic relatedness within populations compared to *O. hubbsorum*. In addition, genetic diversity and effective population size for *O. hubbsorum* were lower compared to *O. bimaculatus*. Although no studies exist about the PLD of *O. hubbsorum*, our results are consistent with a shorter PLD and less potential for dispersal compared to *O. bimaculatus*. This is also in line with a recent study suggesting that for species with a planktonic stage, the duration of the planktonic phase increases with hatchling size (*O. hubbsorum* = 1.2 mm ML *O. bimaculatus* = 2.6 mm ML (Ambrose 1981; Alejo-Plata & Herrero-Alejo 2014; Villanueva et al. 2016).

An inability to properly identify biological species hampers any effort towards their management and conservation (Bickford et al. 2007). The distinct geographic and habitat distributions along with contrasting life history traits are expected to have strong direct effects over population parameters that are key for establishing the spatial scale, location and timing of management actions and rates of sustainable fishing for each species. Therefore, is not advisable to continue with the current management that does not differentiate among the three species. A species as *O. bimaculoides* with a lower effective population size, and with local populations that are mostly self-sustaining and partially isolated from other nearby populations could be susceptible to over exploitation, severe bottlenecks and long recovery times if fisheries management erroneously considers all populations as a single stock and ignores the importance of local population dynamics. We recommend that in *O. bimaculoides* management should take place at the level of local populations, for instance, to assign catch quotes per individual bay. For the species with higher fecundity and dispersal potential (*O. bimaculatus* and *O. hubbsorum*) the implementation of management tools should consider metapopulation dynamics on a larger geographic scale and the presence of larval dispersal among populations, identifying key larval sources and larval dispersal routes during the PLD, spawning and hatching seasons for each species.

An important consideration for management of the octopus fishery in the Northern Gulf of California is the differences in the spawning season between *O. hubbsorum* (spring and fall) and *O. bimaculatus* (summer) and its relationship to the direction of larval dispersal and its impact on source-sink metapopulation dynamics. Patterns of oceanographic currents in the

Northern Gulf of California are highly directional or asymmetric driven by a cyclonic (anti-clockwise) gyre during spring and summer (Marinone et al. 2008; Marinone 2012) when both *O. hubbsorum* and *O. bimaculatus* spawn. However, *O. hubbsorum* also spawns during Fall-winter (unpublish data J. F. D. C and A. M. V.), when the gyre reverses to an anti-cyclonic (clockwise) direction (Lavin & Marinone 2003; Marinone 2012), effectively transforming key larval sources during spring-summer into larval sinks during fall-winter. When implementing spatial management tools in systems with strong asymmetry in the direction of the currents, including marine reserves, it is advised that reserves are located upstream according to the main flow to protect the sources of larvae that support multiple downstream fishing sites (Beger et al. 2014; Munguía-Vega et al. 2014) These observations imply that the location of marine reserves for octopus in the northern Gulf of California will have to consider the cyclonic phase of the oceanographic gyre for both species in addition to the anti-cyclonic phase for *O. hubbsorum*. Also, temporal fishing closures based on the spawning period of a single species, like the one recently implemented in the northern Gulf of California based on *O. bimaculatus* (Opinión Técnica No. RJL/INAPESCA/DGAIPP/1065/2015; DOF. 2016, 01 junio), might be only partially effective for protecting the recruitment of the other species present in the same locations but with a different spawning season (e. g., *O. hubbsorum*, López-Uriarte et al. 2005; Moreno-Báez et al. 2012). Similarly, minimum sizes established based on size at sexual maturity for *O. bimaculatus* might overestimate the minimum size required for *O. hubbsorum* (Table 1). Our findings highlight that sustainable fisheries management will heavily depend upon establishing management tools that match the geographic and habitat distribution, life history and population dynamics of the biological entities targeted by multi-specific fisheries.

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Competing Interests

The authors declare there are no competing interests.

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Table 1(on next page)

Life history & Hypotheses

Life history and hypotheses regarding levels of genetic diversity and structure in three species of octopus from Northwest Mexico. BCP = Baja California Peninsula, ML = Mantle Length.

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Life history	*O. bimaculoides*	*O. hubbsorum*	*O. bimaculatus*	References
Geographic distribution	 From CA, USA to Bahia San Quintin in BC, Mexico.	 From Bahia Magdalena, BCS to Oaxaca, including the Gulf of California.	 From CA, USA to Bahia Vizcaino BCS, including the Gulf of California	(2, 3, 4, and 11)
Reproductive period	Santa Barbara, CA, USA (Dec-May) San Quintin, BCP, Mexico (Oct-Jan)	Pacific coast of BCP (May-Oct) Gulf of California (Mar, Sep-Dec)	Pacific coast of BCP (Jan-Jun) Gulf of California (Jun-Sep)	(1, 2, 3, 5, and 9)
***Fecundity**	Eggs laid in festoons 137 – 780	Clutch eggs 105,000 – 144,000 Ripe ovarian eggs 240, 050 (range 22,447 – 545,444)	Clutch eggs >20,000 Ripe ovarian eggs 91,407 ± 75,361 SD (range 11,618 – 372,269)	(1, 2, 6, 10, 13 and 19)
***Egg size (length) and ripe ovarian eggs size**	10 – 12 mm (range 9.5 – 16 mm)	1.66 ± 0.74 mm Ripe ovarian eggs 2.07 mm (range 0.7 – 3.7 mm)	4 –7 mm Ripe ovarian eggs (range 1.8 – 4 mm)	(1, 2, 3, 10, 13, and 19)
Planktonic larval duration	absent, direct development to	Present but the time is uncertain (Probably ~ 60	2 – 3 months (60 to 90 days)	(1, 2, 3, and 13)

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(paralarvae)	juvenile, benthic hatchlings	days)		
Size at sexual maturity	55 mm (ML) males 110 mm (ML) females	70 mm (ML) males 119.7 mm (ML) females	124.5 mm (ML) males 147.0 mm (ML) females	(2, 6, 9, and 19)
Lifespan (years)	Short (1.0 - 1.5)	Short (1.5)	Short (1.5 – 2.0)	(2, 3, and 6)
Hypotheses	***O. bimaculoides***	***O. hubbsorum***	***O. bimaculatus***	**References**
Effective population size (N_e)	Small	Medium	Large	(17 and 20)
Genetic diversity (allelic richness)	Low	Medium	High	(17 and 20)
Diversity of private alleles	High	Medium	Low	(14 and 18)
Genetic Structure	High	Medium	Low	(8, 12, and 15)
Genetic relatedness	High	Medium	Low	(7 and 16)

* = considering average, min and max reported value. (1) Ambrose (1981), (2) Forsythe & Hanlon (1988), (3) Ambrose (1990), (4) López-Uriarte et al. (2005), (5) Castellanos-Martínez (2008), (6) López-Uriarte & Rios-Jara (2009), (7) Christie et al. (2010), (8) Selkoe & Toonen (2011), (9) Domínguez-Contreras (2011), (10) Cardenas-Robles (2013), (11) Domínguez-Contreras et al. (2013), (12) Riginos & Liggins (2013), (13) Alejo-Plata & Herrero-Alejo (2014), (14) Beger et al. (2014), (15) Selkoe et al. (2014), (16) Burgess et al. (2014), (17) Romiguier et al. (2014), (18) Munguía-Vega et al. (2015) (19) Alejo-Plata & Gómez-Márquez (2015) and (20) Ellegren & Galtier (2016).

Table 2(on next page)

Nucleotide divergence of both: 16s rDNA gene and COI gene

Nucleotide divergence between species of octopus identified through the analysis of both the 16s rDNA gene (below the diagonal) and COI gene (above the diagonal). Standard error estimates are shown in parentheses.

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	O. bimaculoides	***O. bimaculatus***	***O. hubbsorum***
O. bimaculoides	-	0.0632 (±0.0104)	0.1005 (±0.0142)
O. bimaculatus	0.0328 (±0.0079)	-	0.1042 (±0.0139)
O. hubbsorum	0.0629 (±0.0113)	0.0708 (±0.123)	-

1

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Table 3(on next page)

Genetic variation within population

Genetic variation within populations of three species of octopus. Sample Size (N), Mean ± Standard Error (SE) of the number of alleles (N_A), effective alleles (N_E), and observed (H_O), expected (H_E) heterozygosities, allelic richness (R_A) and private allelic richness (P_{AR}).

Species	Population	N	N_A	N_E	H_O	H_E	R_A	P_{AR}
Octopus bimaculoides	Ejido Erendira	14	5.00 ± 0.93	3.22 ± 0.58	0.77 ± 0.09	0.62 ± 0.07	4.15 ± 0.68	1.09 ± 0.33
	San Quintín	9	6.14 ± 1.49	4.44 ± 1.18	0.52 ± 0.12	0.62 ± 0.11	5.46 ± 1.23	2.53 ± 1.23
	Bahía Magdalena	9	4.29 ± 0.71	3.34 ± 0.62	0.91 ± 0.05	0.65 ± 0.05	4.08 ± 0.65	1.50 ± 0.58
	Mean ± SE		**5.14 ± 0.62**	**3.67 ± 0.47**	**0.74 ± 0.06**	**0.63 ± 0.04**	**4.56 ± 0.45**	**1.71 ± 0.43**
Octopus hubbsorum	Puerto Libertad	14	8.86 ± 1.18	5.85 ± 1.39	0.70 ± 0.10	0.72 ± 0.08	5.47 ± 0.71	1.84 ± 0.40
	Isla San Lorenzo	22	7.71 ± 2.11	5.17 ± 1.55	0.57 ± 0.15	0.61 ± 0.13	4.44 ± 0.96	0.30 ± 0.14
	Isla Tiburón	31	10.0 ± 2.35	5.89 ± 1.47	0.53 ± 0.12	0.69 ± 0.10	4.94 ± 0.85	0.39 ± 0.10
	Isla el Dátil	3	4.00 ± 0.31	3.23 ± 0.39	0.76 ± 0.06	0.66 ± 0.04	4.00 ± 0.31	0.33 ± 0.28
	Bahía Kino	32	10.0 ± 2.86	6.32 ± 1.77	0.70 ± 0.14	0.66 ± 0.13	4.79 ± 1.00	0.29 ± 0.19
	I. San Pedro Mártir	3	2.86 ± 0.63	2.58 ± 0.56	0.41 ± 0.17	0.46 ± 0.13	2.86 ± 0.63	0.01 ± 0.01
	Santa Rosalía	8	6.57 ± 1.51	4.82 ± 1.14	0.75 ± 0.12	0.66 ± 0.11	5.00 ± 0.99	0.50 ± 0.20
	El Conejo	8	5.00 ± 1.31	4.09 ± 1.07	0.65 ± 0.12	0.60 ± 0.12	4.28 ± 0.98	0.27 ± 0.25
	Mean ± SE		**6.89 ± 0.67**	**4.75 ± 0.45**	**0.63 ± 0.05**	**0.63 ± 0.04**	**4.47 ± 0.28**	**0.49 ± 0.20**
Octopus bimaculatus	La Bocana	5	5.86 ± 0.51	4.73 ± 0.49	0.94 ± 0.06	0.77 ± 0.03	5.16 ± 0.42	0.06 ± 0.03
	Las Barrancas	5	5.43 ± 0.53	4.49 ± 0.61	0.72 ± 0.11	0.73 ± 0.07	5.09 ± 0.50	0.43 ± 0.24
	Malarrimo	32	11.71 ± 0.71	6.01 ± 0.79	0.79 ± 0.08	0.79 ± 0.06	4.90 ± 0.37	0.39 ± 0.16
	Puerto Peñasco	32	11.42 ± 0.87	7.29 ± 1.15	0.87 ± 0.06	0.81 ± 0.07	5.15 ± 0.48	0.34 ± 0.10
	San Luis Gonzaga	8	6.71 ± 1.02	5.21 ± 0.76	0.79 ± 0.14	0.71 ± 0.12	4.81 ± 0.66	0.10 ± 0.05
	Puerto Refugio	17	9.14 ± 1.20	6.11 ± 0.96	0.68 ± 0.11	0.77 ± 0.08	4.89 ± 0.52	0.25 ± 0.09
	Isla Smith	25	11.14 ± 1.24	6.76 ± 0.89	0.84 ± 0.06	0.81 ± 0.06	5.14 ± 0.41	0.39 ± 0.11
	B.de Los Ángeles	14	9.57 ± 0.75	6.20 ± 0.89	0.68 ± 0.10	0.78 ± 0.07	5.13 ± 0.44	0.19 ± 0.06
	Puerto Lobos	25	10.43 ± 0.75	6.66 ± 0.83	0.77 ± 0.08	0.82 ± 0.04	5.19 ± 0.34	0.39 ± 0.18
	Mean ± SE		**9.08 ± 0.40**	**5.93 ± 0.28**	**0.79 ± 0.03**	**0.78 ± 0.02**	**5.05 ± 0.05**	**0.28 ± 0.05**

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Table 4(on next page)

Contemporary effective population size

Average and 95% confidence intervals for the contemporary effective population size (N_e) for three species of octopus. Locations were pooled according to the results of the genetic assignment of species (Fig. 2). N_e was estimated with two methods, including linkage disequilibrium (LD; lowest allele frequency used 0.05 and 0.02 respectively) and Molecular coancestry (M_C).

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	LDNE 0.05	LDNE 0.02	Molecular coancestry
O. bimaculoides	5.4 (3.4 - 8.8)	10.2 (7.4 - 13.8)	11.2 (3.0 - 24.4)
O. hubbsorum	88.0 (63.8 - 129.9)	125.5 (94.7 - 177.4)	22.9 (1.7 -71.5)
O. bimaculatus	261.4 (173.6 - 472.9)	264.9 (194.7 - 395.8)	27.7 (13.3 - 47.4)

1

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Table 5(on next page)

Analysis of molecular varianc

Analysis of molecular variance (AMOVA) from microsatellite data within three species of octopus from Northwest México.

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Species	Source of Variation	Variance	*df*	Sum of squares	Means of squares	Estimated Variance	*P* Value
Octopus bimaculoides	Among Populations (F_{ST})	19%	2	28.865	14.432	0.592	0.000
	Among Indiv (F_{IS})	0%	29	61.401	2.117	0.000	0.995
	Within Indiv (F_{IT})	81%	32	81.500	2.547	2.547	0.001
	Total	100%	63	171.766		3.139	
Octopus hubbsorum	Among Populations (F_{ST})	15%	7	110.224	15.746	0.459	0.000
	Among Indiv (F_{IS})	13%	113	330.838	2.928	0.400	0.000
	Within Indiv (F_{IT})	71%	121	257.500	2.128	2.128	0.000
	Total	100%	241	698.562		2.987	
Octopus bimaculatus	Among Populations (F_{ST})	9%	8	103.068	12.884	0.283	0.000
	Among Indiv (F_{IS})	5%	154	467.367	3.035	0.162	0.000
	Within Indiv (F_{IT})	86%	163	442.000	2.712	2.712	0.000
	Total	100%	325	1012.436		3.156	

Figure 1(on next page)

Study area

Locations of 20 octopus populations sampled from Northwest Mexico. B.C = Baja California. B. C. S = Baja California Sur. NGC = Northern Gulf of California. The blue stars represent main fishing locations, and the red circle represents the Midriff Island region.

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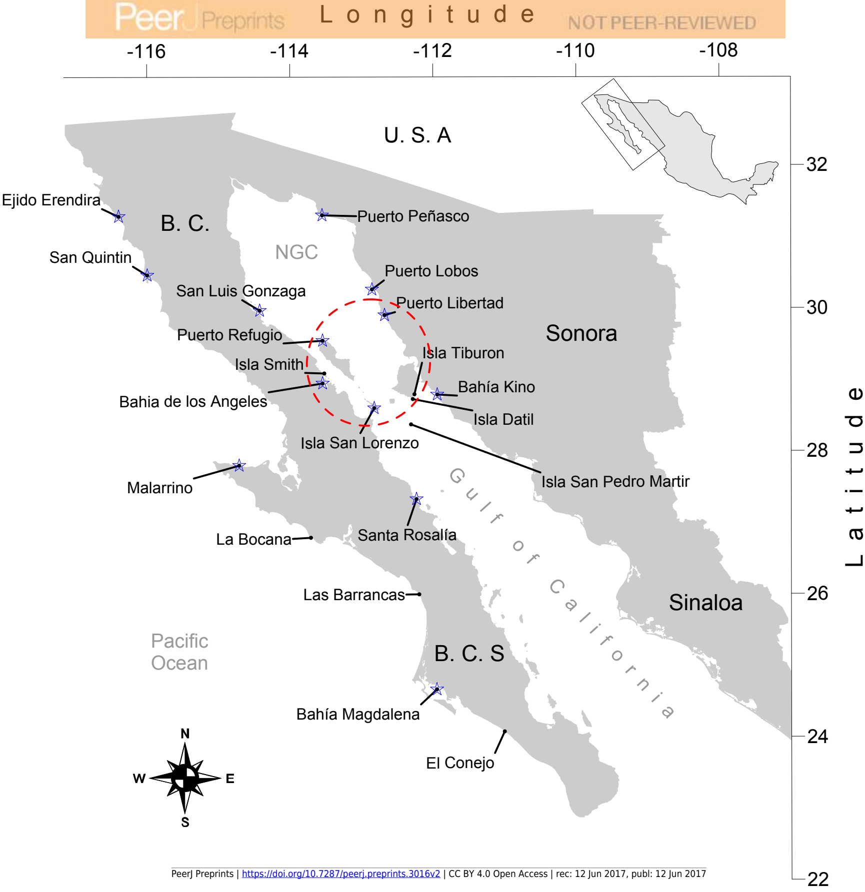

Longitude
-116
-114
-112
-110
-108
Latitude
32
30
28
26
24
22
U. S. A
B. C.
NGC
Sonora
Sinaloa
B. C. S
Gulf of California
Pacific Ocean
Ejido Erendira
San Quintin
San Luis Gonzaga
Puerto Peñasco
Puerto Lobos
Puerto Libertad
Puerto Refugio
Isla Smith
Isla Tiburon
Bahía Kino
Bahia de los Angeles
Isla Datil
Isla San Lorenzo
Isla San Pedro Martir
Malarrino
Santa Rosalía
La Bocana
Las Barrancas
Bahía Magdalena
El Conejo
N
W
E
S

Figure 2(on next page)

Genetic assignment of octopus samples in Northwest Mexico

Genetic assignment of octopus samples from fishery locations in Northwest Mexico to three species. Locations used for both 16s rDNA and COI are indicated with stars. All locations were used for microsatellites analysis. A) Neighbor-joining trees constructed with 97 haplotypes for both 16s rDNA and COI for *O. bimaculatus* (blue), *O. bimaculoides* (purple) and *O. hubbsorum* (orange). Bootstrap support >99% in 1000 replicates are shown for branches separating the three species. B) Bayesian cluster from STRUCTURE shows the probability of individual membership to three genetic clusters (K = 3, 316 individuals). C) Distribution of octopus species in 20 localities from Northwest Mexico according to phylogenetic and Bayesian analyses.

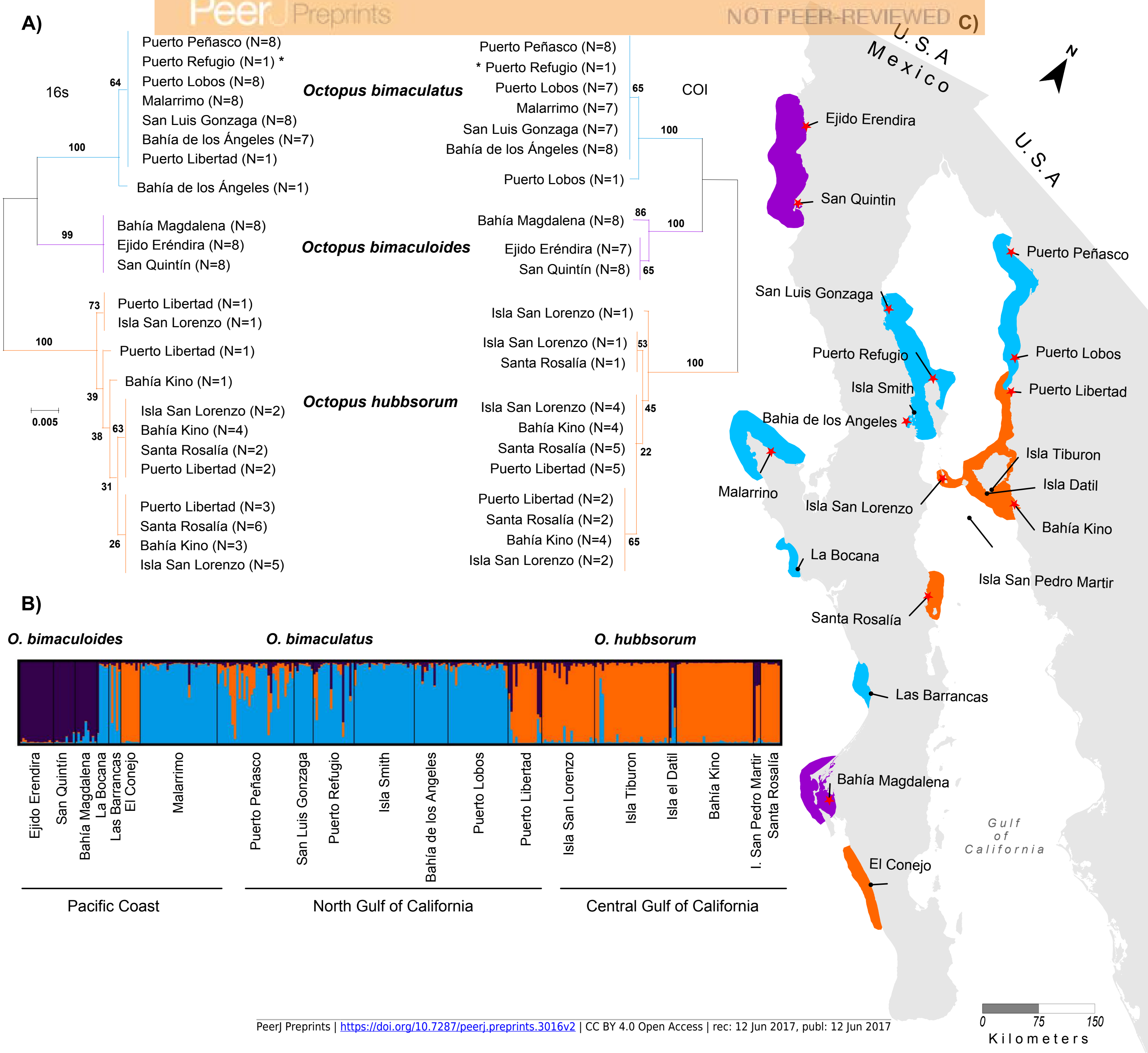
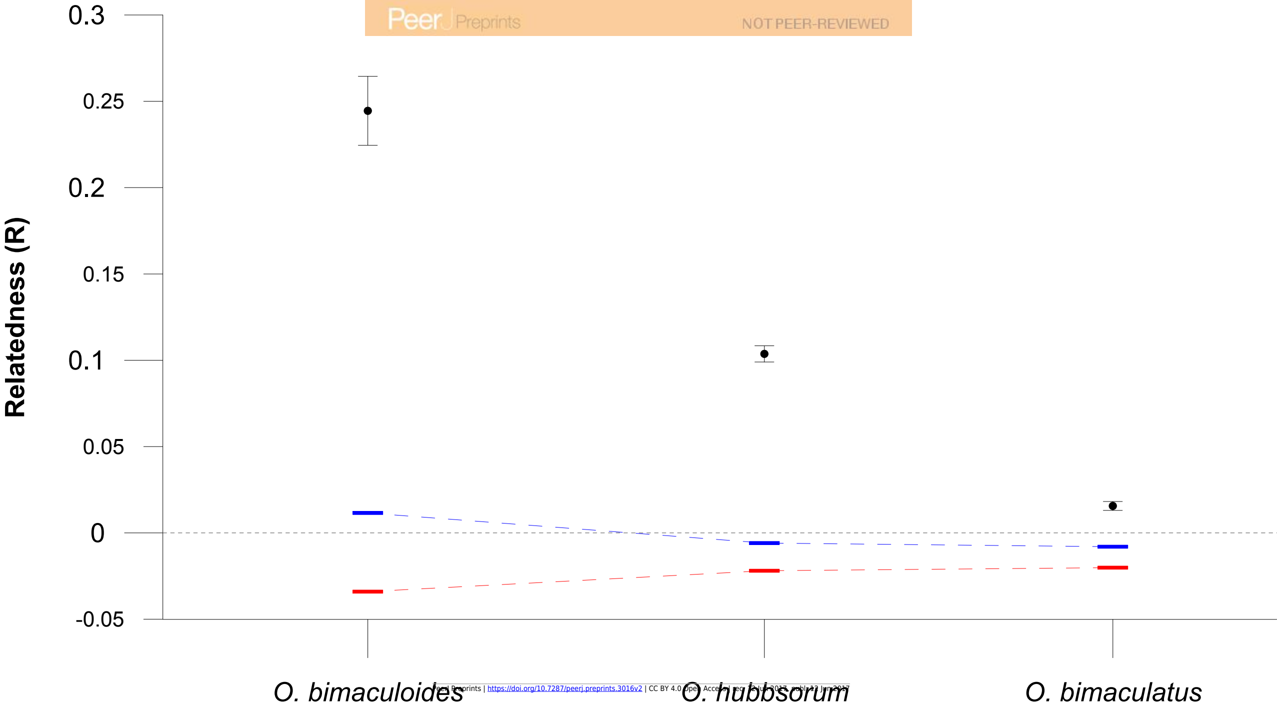

A)
16s
Puerto Peñasco (N=8)
Puerto Refugio (N=1) *
Puerto Lobos (N=8)
Malarrimo (N=8)
San Luis Gonzaga (N=8)
Bahía de los Ángeles (N=7)
Puerto Libertad (N=1)
Bahía de los Ángeles (N=1)
64
100
99
Bahía Magdalena (N=8)
Ejido Eréndira (N=8)
San Quintín (N=8)
73
Puerto Libertad (N=1)
Isla San Lorenzo (N=1)
100
Puerto Libertad (N=1)
Bahía Kino (N=1)
39
0.005
38
63
Isla San Lorenzo (N=2)
Bahía Kino (N=4)
Santa Rosalía (N=2)
Puerto Libertad (N=2)
31
Puerto Libertad (N=3)
Santa Rosalía (N=6)
26
Bahía Kino (N=3)
Isla San Lorenzo (N=5)
Octopus bimaculatus
Octopus bimaculoides
Octopus hubbsorum
COI
Puerto Peñasco (N=8)
* Puerto Refugio (N=1)
Puerto Lobos (N=7)
65
Malarrimo (N=7)
San Luis Gonzaga (N=7)
100
Bahía de los Ángeles (N=8)
Puerto Lobos (N=1)
86
Bahía Magdalena (N=8)
100
Ejido Eréndira (N=7)
San Quintín (N=8)
65
Isla San Lorenzo (N=1)
Isla San Lorenzo (N=1)
53
Santa Rosalía (N=1)
100
Isla San Lorenzo (N=4)
45
Bahía Kino (N=4)
Santa Rosalía (N=5)
22
Puerto Libertad (N=5)
Puerto Libertad (N=2)
Santa Rosalía (N=2)
Bahía Kino (N=4)
65
Isla San Lorenzo (N=2)
B)
O. bimaculoides
O. bimaculatus
O. hubbsorum
Ejido Erendira
San Quintín
Bahía Magdalena
La Bocana
Las Barrancas
El Conejo
Malarrimo
Puerto Peñasco
San Luis Gonzaga
Puerto Refugio
Isla Smith
Bahía de los Angeles
Puerto Lobos
Puerto Libertad
Isla San Lorenzo
Isla Tiburon
Isla el Datil
Bahía Kino
I. San Pedro Martir
Santa Rosalia
Pacific Coast
North Gulf of California
Central Gulf of California
C)
U. S. A
Mexico
N
U. S. A
Ejido Erendira
San Quintin
Puerto Peñasco
San Luis Gonzaga
Puerto Refugio
Puerto Lobos
Isla Smith
Puerto Libertad
Bahia de los Angeles
Malarrino
Isla Tiburon
Isla Datil
Isla San Lorenzo
Bahía Kino
La Bocana
Isla San Pedro Martir
Santa Rosalía
Las Barrancas
Bahía Magdalena
Gulf of California
El Conejo
0
75
150
Kilometers

Table 6(on next page)

Relatedness within three octopus species

Mean pairwise relatedness (R) values (±95% confidence intervals) within three octopus species, compared with bootstrapped upper (Blue) and lower (Red) 95% confidence intervals assuming random mating (10,000 bootstraps replicates).


0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
Relatedness (R)
O. bimaculoides
O. hubbsorum
O. bimaculatus