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Integrating parasite assemblages and host genetics for population discrimination in the black grouper (*Mycteroperca bonaci*) in natural protected areas of the northern Yucatan peninsula

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Research Article

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Abstract

The population structure of the black grouper (*Mycteroperca bonaci*) from the northern Yucatan Peninsula was evaluated with a two-fold emphasis on the spatial scales (whether island or coastal localities), as well as the effects of protection based on three natural protected areas (NPA) with different categories. To this end, specimens were collected at each NPA: Celestun (Biosphere Reserve) and Dzilam (State NPA) which are coastal and Alacranes (National Park) which is an island. Population discrimination was carried out by means of intestinal helminth parasite infracommunities and the hosts' genetic similarities, highlighting the contradictions or coincidences between approaches. The intestinal parasitic fauna was examined in 161 specimens, of which 150 were genetically characterized using microsatellite DNA markers. Three distinct parasite communities were observed, in which taxa mainly responsible for the differences were the digeneans *Prosorhynchus atlanticus*, *Prosorhynchus* sp., *Lepidapedoides epinepheli* and *Hamacreadium mutabile* as well as the acanthocephalan *Gorgorhynchus* sp. The hosts' genotypes indicated three genetically separated subunits that deviated significantly from Hardy–Weinberg equilibrium, and genetic differences evidenced a structured population. Despite the expectation that island NPAs would be distinctive, the coastal locality of Dzilam was the most differentiated. The present recognition of population subunits would indicate the beneficial effects of preserving the gene pool variability of the coastal Dzilam subunit since it is, at present, the least restrictive NPA where unregulated fishing is still allowed. Thus, this study indicates fishing regulations should be strengthened (e.g. determine catch quotas or reduce fishing effort) to prevent diversity loss (whether biological or genetic) in these NPAs, particularly in Dzilam, which is probably the most threatened area.

Introduction

All around the world increased fishing pressure has resulted in overexploitation of populations with declines in overall abundance of populations and average fish size along with loss of genetic diversity (Marchal *et al.*, 2003; Froese, 2004; Pérez-Ruzafa *et al.*, 2006; Favoretto *et al.*, 2020). A major management strategy for fisheries and marine conservation has been the implementation of natural protected areas (NPAs), whose success is based on the connectivity between populations (Palumbi, 2003; Galindo *et al.*, 2006; Green *et al.*, 2015; Salles *et al.*, 2015), thus understanding the extent of connectivity between fragmented populations is integral to understanding the stability of the subpopulations and their patterns of dispersal (Cowen *et al.*, 2000, 2006; Christie *et al.*, 2010). Fisheries management tools can protect spawning biomass and intraspecific genetic diversity, thus maintaining population abundances and keeping fisheries healthy (Hellberg *et al.*, 2002; Jones, 2002; Ablan, 2006).

A population has been defined as a group of individuals of the same species that live together in an area of sufficient size to permit normal dispersal and/or migration behaviour and in which numerical changes are largely determined by birth and death processes (Berryman, 2002). In this regard, population structure has been acknowledged as essential to infer potential evolution and extinction risk derived from over-exploitation and inappropriate management of marine resources (Ihssen *et al.*, 1981; Cadrin *et al.*, 2014; Cadrin, 2020). Different techniques have been used for population discrimination (or stock identification) in fisheries, including the use of parasites (MacKenzie *et al.*, 2008; Lester & MacKenzie, 2009; Baldwin *et al.*, 2012; Vasconcelos *et al.*, 2017), comparison of patterns of morphometric variation in fish (Murta, 2000; Turan *et al.*, 2006); differences in the morphology (Tracey *et al.*, 2006; Stransky *et al.*, 2008*a*, 2008*b*) and chemical composition of otoliths (Campana, 1999; Bergenius *et al.*, 2005; Jónsdóttir *et al.*, 2006) and molecular analysis such as mitochondrial DNA or microsatellite DNA sequencing (Beacham *et al.*, 1999, 2000; Shaklee & Currens, 2003; Corander *et al.*, 2006). Despite progress in the different methodologies applicable to

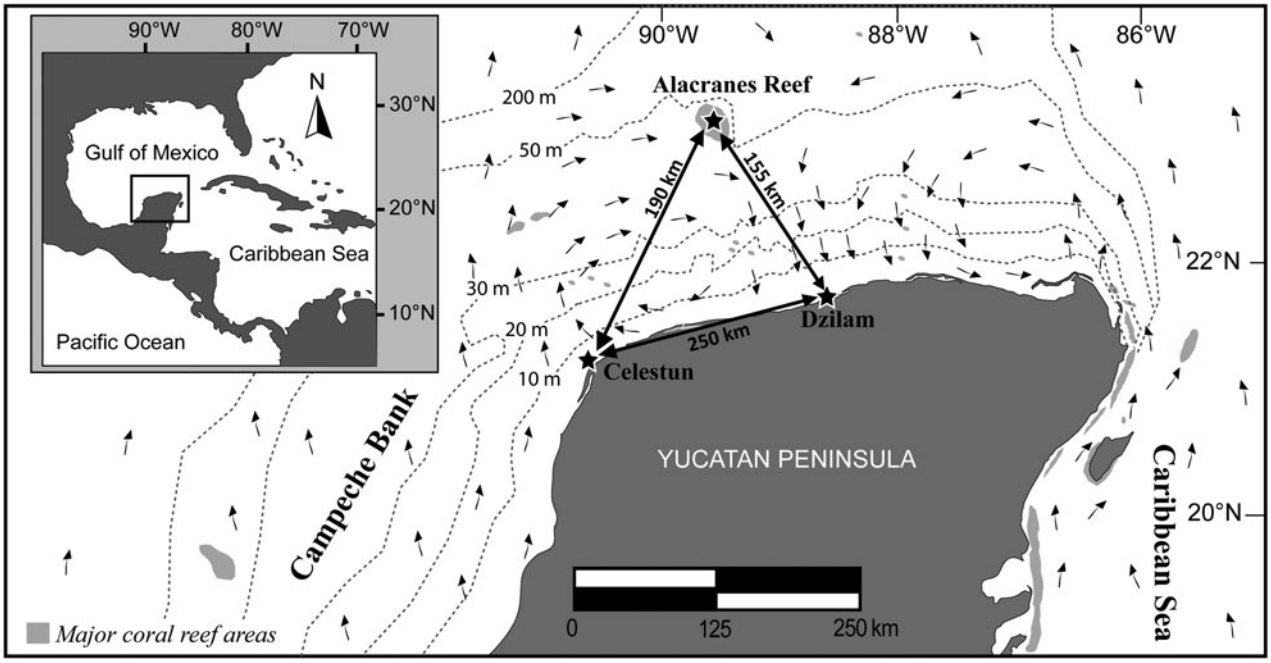

Fig. 1. Study area and location of three sampling sites of *M. bonaci* within each NPA in the northern coast of the Yucatan Peninsula. Alacranes is a reef island (National Park 130 km offshore), Dzilam (state NPA) and Celestun (Biosphere Reserve) are coastal locations. Dominant direction of ocean surface currents field in the Gulf of Mexico (determined with geostrophic currents by NOAA/AOML and NOAA/CoastWatch) are shown with dark arrows. Bathymetry isobaths (dashed lines) are shown (modified from the Mexican Navy Nautical Chart) at 10, 20, 30, 50 and 200 m of depth. Major coral reef areas (shaded light grey).

population identification, the fullest possible picture can be obtained in response to the ecological, evolutionary and operational requirements that the population structure may involve, thus a recommended protocol is the complementary application of genotypic, ecological and/or phenotypic-based approaches in a holistic manner to maximize the probability of defining population structure properly (Begg & Waldman, 1999; Abaunza *et al.*, 2008; Pita *et al.*, 2016).

Currently, the black grouper, *Mycteroperca bonaci*, is one of the species within the regional multispecies fishery of the groupers, but unfortunately remains as an unregulated resource in coastal waters of the northern coast of the Yucatan Peninsula (DOF, 2006). Although NPAs have been considered the most efficient means of maintaining any population (Jennings *et al.*, 1999; Palumbi, 2003) the mechanisms underlying the differentiation (ergo reduced connectivity) are still not well understood for the black grouper within the region, especially taking into account the poor vagility of the black grouper (Farmer, 2009). Thus, we evaluated population discrimination with special emphasis on the spatial scales (whether islands or coastal locations) because Alacranes is a reef island while Dzilam and Celestun are coastal locations, as well as the effects of protection from fishing on the population structure because despite the fact that all locations are NPAs each one has different fishing protection levels. Dzilam has a state NPA category where fishing is allowed, Celestun is a Biosphere Reserve where fishing is allowed under certain parameters, meanwhile Alacranes is a National Park where fishing of this species is not carried out on a regular basis.

Our research group of the marine biology laboratory (LABIOMA) from the University Autonomous of Yucatan (UADY) had previously used parasites as biological tags and DNA microsatellites separately through collecting specimens of *M. bonaci* among three separated (155–250 km) localities (Celestun, Dzilam and Alacranes Reef). First, the parasite communities of *M. bonaci* revealed the existence of two distinct metazoan parasite communities, Celestun and Alacranes (Espínola-Novelo *et al.*, 2015), however, the Dzilam sample had not been analysed until the present study. Then, by means of the hosts' genetic similarities those results were investigated at genotypic level in a recently published study to shed light on the population structure of this species (González-Salas *et al.*, 2020). However, the aim of the present study was a joint analysis of the parasite community and the molecular markers of their hosts, to facilitate population discrimination in the black grouper (*M. bonaci*) of the northern coast of the Yucatan peninsula focusing on the comparative discussion, highlighting the contradictions or coincidences between techniques to provide a final holistic overview. To this end, we combined the samples used for both approaches by including the parasites of the Dzilam sample (unpublished data) and by using the genotypic data of most of the hosts (150 out of 161 specimens) with similar size ranges.

Materials and methods

Study area and sampling

From July 2008 to February 2011, specimens of *M. bonaci* were collected at three geographically distinct locations in the northern coast of the Yucatan Peninsula (Figure 1). Celestun is located in the east region and Dzilam in the west region of the state of Yucatan; both areas are coastal and include two of the main ports of the state where fishing takes place by mostly artisanal fishermen. In contrast, Alacranes is a reef island located 130 km from the coast, where this species is usually caught by sportfishermen. A total of 161 individuals of *M. bonaci* were provided by the artisanal fishermen who caught them with hook and line or spear fishing on selected fishing grounds with depths from 1.5–20 m during the same period among the locations in order to avoid temporal bias. Immediately after capture, fish were transported on ice to the laboratory, where they were examined, measured (cm) and weighed (g).

Parasite analysis

Entire fish were examined fresh for endoparasites. Recovered parasites were counted, preserved in 70% alcohol and processed

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for subsequent identification by means of conventional helminthological methods (Lamothe-Argumedo, 1997). The overall black grouper sample (161 hosts) were distributed as follows: 63 at Alacranes (TL = 40.14 ± 8.25), 54 at Celestun (TL = 35.75 ± 7.52) and 44 at Dzilam (TL = 38.89 ± 6.27). The overall size of specimens used for the parasite approach ranged from 28.0–46 cm TL. According to Crabtree & Bullock (1998) two age classes might occur, i.e. <42 cm (<2-years) and >42 cm (>2-years) in *M. bonaci*, however, only five fish were greater than 42 cm TL, thus most of the sample consisted of specimens of about the same age class (<2-years). Moreover, in a preliminary analysis similar values of abundance and richness of parasite taxa were observed between hosts of these two age classes, therefore the effect of fish length and age on parasite communities were disregarded. The comparative analyses between the infracommunities registered in each locality were carried out considering only the intestinal helminths (actually from whole digestive tract including intestine and stomach), in which case ecological parameters such as prevalence, mean intensity and mean abundance values were obtained per locality, and parasite communities were described at the infracommunity level following Bush *et al.* (1997).

In order to show quantitative similarity between localities, resemblance matrices among samples (hosts per locality) were estimated using Bray–Curtis similarity with parasite abundances, previously transformed by fourth root to reduce the importance of the dominant taxa and allowing low abundance taxa to become more relevant and reduce dispersion data (Anderson, 2001, 2005). Then, using the PRIMER package (Clarke & Gorley, 2015) an analysis of similarity (ANOSIM) was used to determine if there was a significant difference in parasite assemblage structure between localities (overall and pairwise), in which case ANOSIM statistic (termed *R*) compares the mean of ranked dissimilarities between groups to the mean of ranked dissimilarities within groups, so that an *R* value close to '1' suggests dissimilarity between groups while an *R* value close to '0' suggests that dissimilarities are greater within groups than between groups (Clarke & Gorley, 2015). A non-metric multidimensional scaling (MDS) was used to 2-D visualize differences in the parasite assemblages between localities, and a canonical discriminant analysis of principal coordinates (sub-routine PERMANOVA CAP for Bray–Curtis similarity) was used to identify the percentage of correctly classified individuals per sampling locality. Finally, a similarity percentage (SIMPER) analysis was also performed to identify the taxa mainly responsible for similarities and differences in parasite assemblage structure between localities.

Genetic analysis

In order to avoid possible genetic structure bias, only specimens whose size ranged between 30.0 and 45.0 cm TL were sampled to ensure that those individuals were about the same age and equal size sample, that is to say 150 (50 per sampling locality) out of the 161 hosts whose size ranges were about the same (28.0–48 cm TL). The molecular procedures (DNA extraction, PCR reactions) are detailed in González-Salas *et al.* (2020), in which five microsatellite loci from the black grouper genome were amplified by PCR based on sequences in Zatcoff *et al.* (2002). Alleles were scored, and a matrix with the microsatellite genotype data was obtained with the number and size of alleles per locus by sampling sites.

As stated in González-Salas *et al.* (2020), different measures of genetic variability, such as the number of alleles (*Na*), number of genotypes (*Ng*), observed (*Ho*) and expected heterozygosity (*He*) were calculated either per locus and multilocus per locality. Wright's inbreeding coefficient (F_{IS}) was used to test whether the black grouper localities were in Hardy–Weinberg equilibrium, whereas Slatkin's linearized fixation index F_{ST} (Slatkin, 1995) was used as a measure of interpopulation variability. Analysis of molecular variance (AMOVA) was also used to assess the relative partitioning of genetic variation among localities both on a multilocus basis and locus by locus. Pairwise analyses of Nei's genetic distances (*Ds*) and Wright's gene flow (*Nm*) among localities were estimated. Additionally, the genetic structure was visualized in a multivariate space performing a Principal Coordinate Analysis (PCoA) on a matrix of Nei's individual pairwise genetic distances. Finally, a Bayesian clustering (STRUCTURE analysis) was estimated to identify any potential cryptic genetic variation within and among samples.

Results

Parasites of M. bonaci

A total of 21,632 individual intestinal parasites belonging to 15 taxa, 6 of which were identified to species level, were found in the 161 black groupers examined (Table 1). Thirteen taxa were found as adults and only 2 as larvae (*Tetraphyllidea* gen. sp. and *Pseudoterranova* sp.). Digeneans and nematodes were the groups with the highest number of taxa (6 each), followed with 2 acanthocephalans and only one taxon of cestodes. The greatest number of taxa were found at Celestun (12) followed by Alacranes (9) and Dzilam (9). Alacranes and Celestun, as well as Dzilam and Celestun, shared 47% (7 out of 15 taxa) of the parasites, while Alacranes and Dzilam shared 5 (33%) of the 15 taxa found in the intestine of this host species (Table 1).

Average richness and abundance of parasites per host were observed to be higher in Dzilam: 2.91 (±1.25) taxa and 349.32 (±620.86) total parasites in comparison with Celestun and Alacranes with lower values (Table 2). In Dzilam abundance was ranked first by *Prosorhynchus atlanticus* (digenean), secondly by *Prosorhynchus* sp. and thirdly by *Gorgorhynchus* sp. (acanthocephalan). In Alacranes, the dominant parasites were *P. atlanticus*, *Hamacreadium mutabile* and *Prosorhynchus* sp., all of them digeneans. *Prosorhynchus atlanticus* was also the most abundant taxa in Celestun, followed by *Lepidapedoides epinepheli* (digenean) and *Prosorhynchus* sp.

Significant differences in the infracommunity compositions were found, indicating that parasite assemblages were structured locally (ANOSIM, global $R = 0.289$, 0.1% significance or $P = 0.001$, Figure 2A). Pair-wise comparisons revealed significant differences between all localities (Figure 2A), but Alacranes–Dzilam were apparently more dissimilar to each other ($R = 0.447$ and 0.1% significance), followed by Celestun–Dzilam ($R = 0.398$ and 0.1% significance), whereas Celestun–Dzilam were the least dissimilar ones ($R = 0.094$ and 0.1% significance). Non-metric MDS evidenced this parasite assemblage structure, where Dzilam is clearly differentiated in the multivariate space (Figure 2B). In terms of composition, the SIMPER analysis revealed that among the parasites found in *M. bonaci*, the taxa mainly responsible for the differences among localities were the digeneans *Prosorhynchus atlanticus*, *Prosorhynchus* sp., *Lepidapedoides epinepheli* and *Hamacreadium mutabile* as well as the acanthocephalan *Gorgorhynchus* sp. The comparison of the assemblage structure between Alacranes and Dzilam found the highest average dissimilarity (84.61), mainly due to three taxa: *Prosorhynchus* sp., *Prosorhynchus atlanticus* and *Gorgorhynchus* sp. (Figure 2C). Celestun and Dzilam showed an estimated 79.8 average dissimilarity, in this case the same three taxa contributed most to this dissimilarity. In contrast, the assemblage structure between Celestun and Alacranes was observed with a lower average dissimilarity (74.52), but along with *Prosorhynchus atlanticus*, another two taxa (*Lepidapedoides epinepheli* and *Hamacreadium mutabile*)

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Table 1. Infection levels of the metazoan intestinal parasites, such as prevalence (*P*%), mean abundance (MA), and mean intensity (MI) of *M. bonaci* in three different localities of Yucatán, Mexico

	Celestun (N = 54)			Alacranes (N = 63)			Dzilam (N = 44)		
Parasites per locality	*P* (%)	MA	MI	*P* (%)	MA	MI	*P* (%)	MA	MI
Digenea									
Prosorhynchus atlanticus	70.4	27.20	38.5	51.6	50.3	96.1	63.6	176.70	170.7
Prosorhynchus sp.	11.1	5.22	47.0	12.9	0.81	12.0	79.5	163.90	199.4
Bucephalidae gen. sp.	0	0	0	6.3	0.47	7.5	0	0	0
Paracryptogonimus sp.	1.9	0.12	7.0	0	0	0	0	0	0
Lepidapedoides epinepheli	51.9	7.85	15.1	17.2	1.17	6.7	43.2	1.90	3.9
Hamacreadium mutabile	5.5	0.12	2.3	29.7	5.90	19.6	0	0	0
Cestoda									
Tetraphyllidea gen. sp. (larvae)	1.8	0.04	2.0	0	0	0	2.3	0.07	3.0
Acanthocephala									
Gorgorhynchus sp.	18.5	1.37	7.3	4.7	0.04	1.0	59.1	4.89	5.9
Serrasentis sp.	5.3	0.07	1.5	0	0	0	0	0	0
Nematoda									
Pseudoterranova sp. (larvae)	12.9	0.28	1.6	1.6	0.18	6.0	0	0	0
Hysterothylacium fortalezae	1.9	0.12	7.0	0	0	0	15.9	0.70	4.4
Hysterothylacium sp.	16.6	1.22	7.3	20.3	0.79	3.8	2.3	0.05	2.0
Cucullanus mycteropercae	0	0	0	0	0	0	20.5	1.07	5.2
Raphidascaris sp.	0	0	0	12.5	1.00	7.9	2.3	0.02	1.0
Dichelyne bonaci	1.9	0.12	7.0	0	0	0	0	0	0

N, number of hosts examined per locality.

Table 2. Average values of the parasite infracommunity descriptors from specimens of *M. bonaci* collected from Celestun, Alacranes and Dzilam, with standard deviations in parentheses. The rank of parasites with highest abundance per locality is also shown

Descriptor	Celestun	Alacranes	Dzilam
Species of intestinal parasites	12	9	9
Average richness	1.98 (±1.14)	1.52 (±1.13)	2.91 (±1.25)
Average abundance	43.46 (±85.15)	61.97 (±156.25)	349.32 (±620.86)
Rank of parasites with highest abundance	*Prosorhynchus atlanticus* (1)	*Prosorhynchus atlanticus* (1)	*Prosorhynchus atlanticus* (1)
	Lepidapedoides epinepheli (2)	*Hamacreadium mutabile* (2)	*Prosorhynchus* sp. (2)
	Prosorhynchus sp. (3)	*Prosorhynchus* sp. (3)	*Gorgorhynchus* sp. (3)

contributed to the abundance differences between these localities (Figure 2C).

Finally, discriminant analysis for Bray–Curtis similarity using the fourth root-transformed abundances showed significant differences between samples (tr = 0.7535, P <0.0001), with 76.40% of individuals correctly classified to their locality of origin (Table 3), in which 87.30% of individuals were correctly classified to Celestun, 62.96% in Alacranes and 77.27% to Dzilam.

Genetics of M. bonaci

A total of 35 alleles (varying from 80 to 262 base pairs long) and 69 genotypes were found across all five loci and localities (Table 4). The greatest allelic richness was observed in Dzilam (Na = 30), followed by Celestun and Alacranes Reef with 29 alleles in both (Figure 3A), whereas the genotypic richness was observed to be very similar among localities: Dzilam (Ng = 49), Alacranes Reef (Ng = 52) and Celestun (Ng = 53). Both values of observed (Ho) and expected heterozygosity (He) reflected the high rate of heterozygosity that occurs in five microsatellite loci (Table 4), all of them giving similar estimates for each locality. In a multilocus manner all three expected heterozygosity values (0.82–0.85) were lower than observed values ($Ho \approx 1.0$) (Table 4); however, it is worth mentioning that Dzilam showed the highest expected heterozygosity (0.85) in comparison with both Celestun and Alacranes (He = 0.82) (Figure 3A).

Multilocus structure analysis showed a significant excess of heterozygotes ($F_{IS} = -0.141$, $P < 0.001$) and a substructured population ($F_{ST} = 0.056$, $P < 0.0001$), which suggests that it may not be panmictic. The pairwise F_{IS} multilocus analysis showed that all three black grouper localities were significantly in Hardy–Weinberg disequilibrium ($F_{IS} = -0.1410$, $P < 0.001$), in which case on a pairwise basis the higher F_{IS} values were observed between Alacranes and Dzilam ($F_{IS} = -0.2007$, $P < 0.001$)

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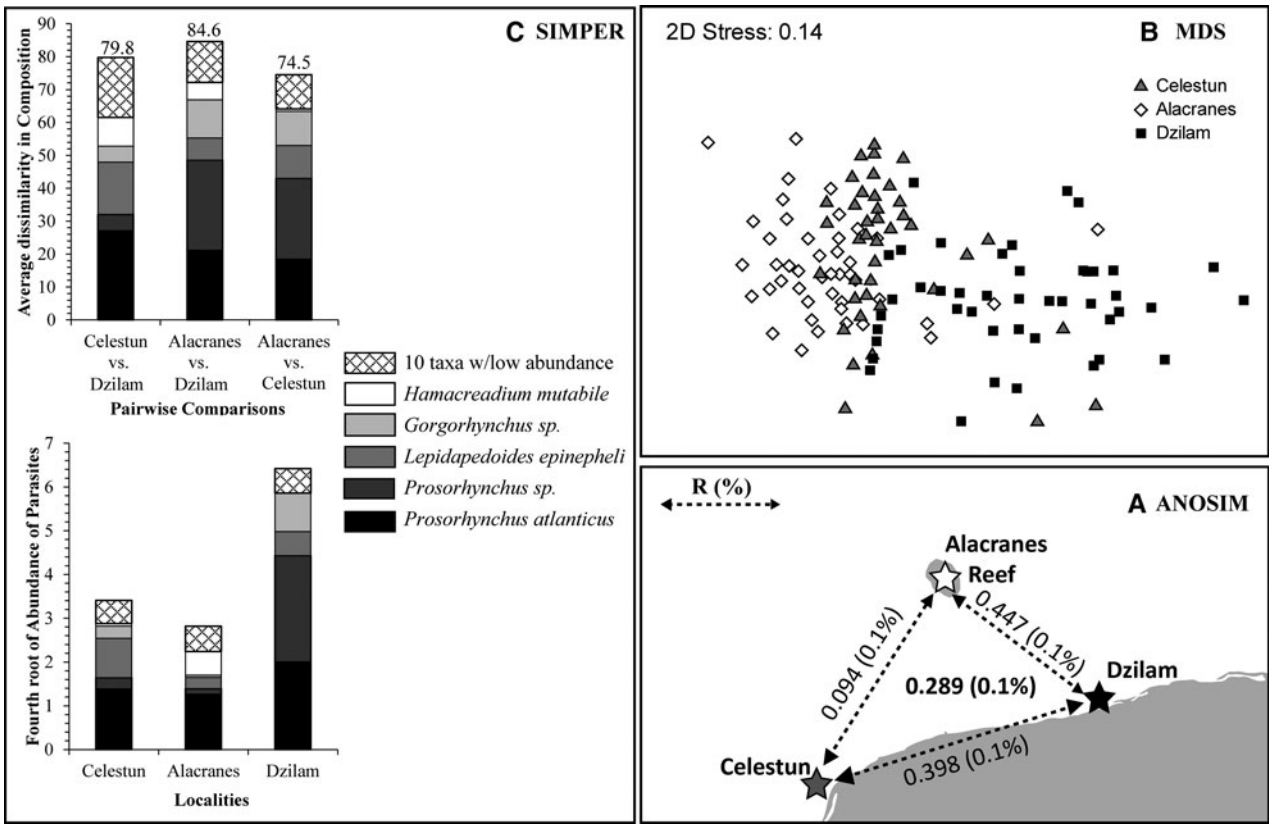

Fig. 2. Graphical abstract of population structure of *M. bonaci* using intestinal parasites as biological tags for differentiation among localities based on the global and pairwise ANOSIM (A) with R (significance in percentage), MDS plot (B), and SIMPER (C). Symbols indicate position of individual samples in the multivariate space in the MDS.

Table 3. Cross validation of the discriminant analysis of principal coordinates (sub-routine PERMANOVA CAP for Bray–Curtis similarity) with fourth root transformed abundances showing the number and percentage of hosts correctly classified to their locality of origin (Celestun, Alacranes and Dzilam) based on the parasite infracommunity

Group of origin	Celestun	Alacranes	Dzilam	Total	Hosts correctly classified
Celestun	55 (87.30%)	8 (12.70%)	0 (0.00%)	63	123 (76.40%)
Alacranes	18 (33.33%)	34 (62.96%)	2 (3.70%)	54	
Dzilam	5 (11.36%)	5 (11.36%)	34 (77.27%)	44	

and lower values observed between Alacranes and Celestun (F_{IS} = −0.1791, $P < 0.001$). Additionally, pairwise locality differentiation using the F_{ST} indices showed that in fact there is a significant ($P < 0.001$) structuring among all three localities (Figure 3B). AMOVA confirmed this later by identifying a significant genetic structure among localities ($F_{ST} = 0.056$, $P < 0.0001$) with 5.6% of the total genetic variation explained by the variation among localities. Nei's (1978) genetic distances (*Ds*) showed that Celestun and Dzilam were least similar localities to each other (*Ds* = 0.364) while the most similar ones were Celestun and Alacranes (*Ds* = 0.174) (Figure 3B). The lowest gene flow (number of effective migrants per generation) among pairs of localities was observed between Celestun and Dzilam (*Nm* = 4.29) and highest between Celestun and Alacranes (*Nm* = 8.42).

STRUCTURE analysis supported Dzilam as the most genetically distinct locality with most of its samples assigned around 80–90% to the same differentiated genetic cluster (Figure 3C), in contrast, all Celestun and Alacranes samples show an admixed genetic composition. PCoA confirmed the genetic structure in *M. bonaci* populations with the first two principal coordinates (Figure 3D) explaining most of the variation (14.78%). Dzilam samples stand out as the most differentiated, mainly along coordinate 1, while most Celestun and Alacranes samples overlap in the multivariate space.

Discussion

The present work represents the first study using both ecological and genotypic approaches used on the same specimens to explore the population structure of the black grouper *Mycteroperca bonaci* at a regional level in Gulf of Mexico, where three sampling sites (Celestun, Alacranes and Dzilam) were significantly different from each other.

The use of intestinal parasites as biological tags led to the first conclusion that a structured population of *M. bonaci* occurs in the northern coast of the Yucatan peninsula, since parasite infracommunities revealed the parasite assemblage structure between these distinct localities (Celestun, Alacranes and Dzilam), where the Dzilam sample was the most differentiated one, as observed altogether with the ANOSIM, MDS and SIMPER analyses. Intestinal helminth assemblages represent the available pool of parasite taxa a fish can acquire over time in a given location,

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Table 4. Resume of genetic characteristics of the five microsatellite loci used for *M. bonaci* such as range of alleles (base pairs), number of alleles (*Na*) and genotypic richness (*Ng*) per locus

Locus	Range of alleles	Na	Ng	Locality	Sample size (N)	He	Ho	F_{IS} per locality	F_{IS} overall	F_{ST} (*P*-value)
Mbo029	164–206	10	13	Celestun	50	0.87	0.96	−0.0870	−0.1672	0.0302 **(<0.0001)**
				Dzilam	50	0.75	1.00	−0.3183		
				Alacranes	50	0.84	0.94	−0.1145		
Mbo048	106–212	10	11	Celestun	50	0.84	1.00	−0.1849	−0.1929	0.0171 **(0.0004)**
				Dzilam	50	0.83	1.00	−0.1867		
				Alacranes	50	0.82	1.00	−0.2068		
Mbo066	104–262	10	14	Celestun	50	0.82	1.00	−0.2105	−0.2148	0.0535 **(<0.0001)**
				Dzilam	50	0.84	1.00	−0.1787		
				Alacranes	50	0.78	1.00	−0.2568		
Mbo088	090–138	14	13	Celestun	50	0.87	1.00	−0.1401	−0.1496	0.0279 **(<0.0001)**
				Dzilam	50	0.86	1.00	−0.1465		
				Alacranes	50	0.85	1.00	−0.1620		
Gag45	080–142	12	17	Celestun	50	0.82	1.00	−0.2015	−0.2148	0.0208 **(<0.0001)**
				Dzilam	50	0.83	1.00	−0.2090		
				Alacranes	50	0.80	1.00	−0.2337		
Multilocus	080–262	35	69	Celestun	250	0.82	1.00	−0.1632	−0.1874	0.0300 **(<0.0001)**
				Dzilam	250	0.85	0.99	−0.2060		
				Alacranes	250	0.82	0.99	−0.1955		

Observed heterozygosity (*Ho*), expected heterozygosity (*He*), inbreeding coefficient (F_{IS}) and fixation index (F_{ST} and its *P*-value) are also shown per locality and overall. All probability values indicated in bold were significant after false discovery rate. Multilocus estimates are also given for each locality.

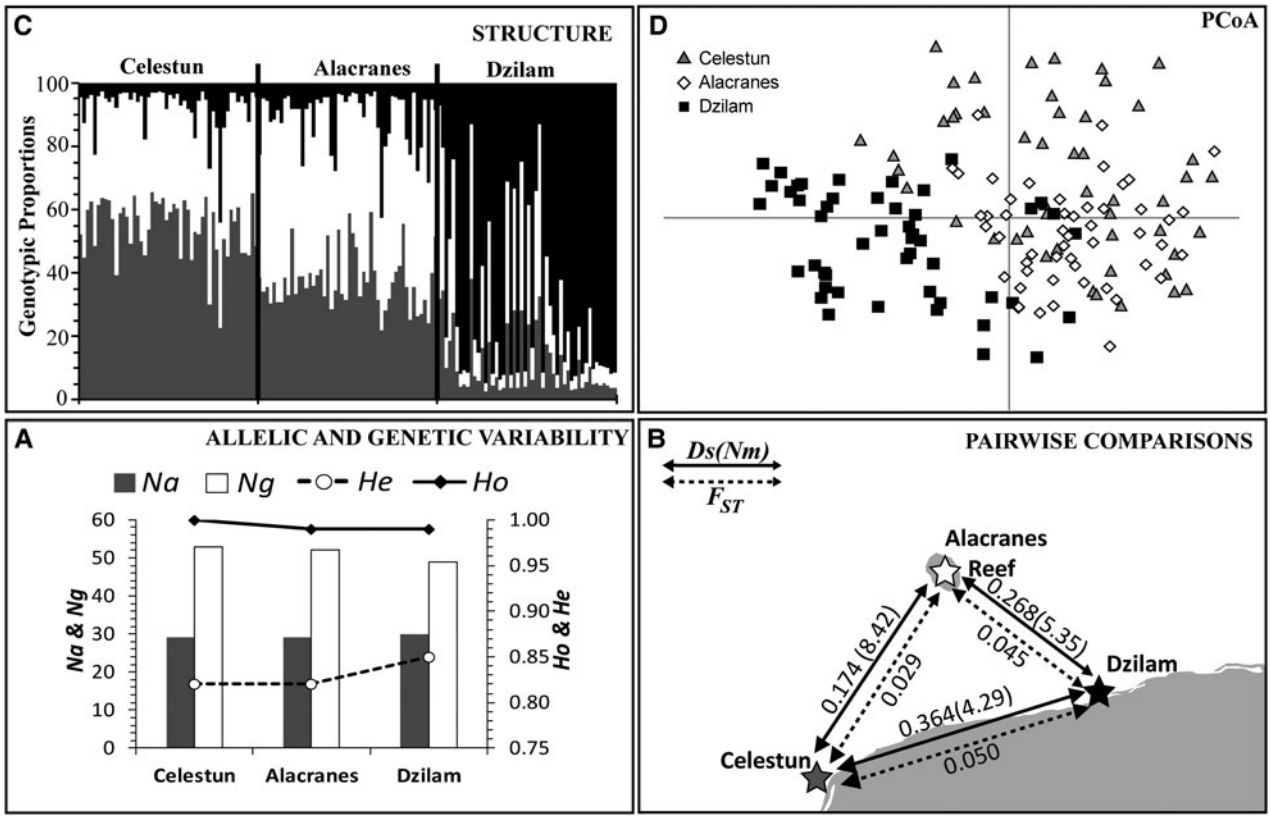

Fig. 3. Graphical abstract of population structure of *M. bonaci* using the genetic connectivity for differentiation among localities based on the genotypic estimates (A) of number of alleles (*Na*), genotypic richness (*Ng*), observed heterozygosity (*Ho*), and expected heterozygosity (*He*). The pairwise Nei's distances (*Ds*), gene flow (*Nm*), and the fixation index (F_{ST}) over loci at localities are also given (B), whose genotypic variations are also shown in the STRUCTURE (C) and the PCoA plots (D). Symbols indicate position of individual samples in the multivariate space in the PCoA.

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thus differences between localities may be related to specific characteristics of the host ontogeny, or to particular biotic and abiotic environmental conditions (Poulin & Mouillot, 2004; Quiroz-Martínez & Salgado-Maldonado, 2013). Therefore, hosts with high vagility might be expected to show high similarity values among infracommunities from different spatially separated locations, since high vagility may be an important determinant in the spread or exchange of parasites between host populations and thus determine similarity among parasite communities (Poulin, 2003). However, a reduced availability and abundance of suitable intermediate hosts to complete parasite life cycles might be key in the observed differences in the parasite infracommunity assemblages.

The parasite taxa mainly responsible for the differences among localities were the digeneans *Prosorhynchus atlanticus*, *Prosorhynchus* sp., *Lepidapedoides epinepheli* and *Hamacreadium mutabile* as well as the acanthocephalan *Gorgorhynchus* sp. These parasites may infect *M. bonaci* via the food chain, so differences between parasite assemblages may be explained by differences in the geographic distribution and/or abundance of organisms used as intermediate hosts by the parasites. We believe that the digeneans *P. atlanticus* and *L. epinepheli* may be short-lived intestinal parasites (probably between 10 and 18 months) that could be eliminated at the end of their lifespans. There is no clear relationship between the lifespan of the parasite and the characteristics of the host, but it seems that intestinal parasites from homeothermic hosts live longer than those from ectotherms (Kennedy, 2006). Regarding this issue, some parasites (acanthocephalans and cestodes) are known to have attachment structures such as a proboscis which may leave evidence of their presence even after their death and expulsion from the host (de Buron & Nickol, 1994). However, other helminths such as digeneans or nematodes do not have these kinds of structures and their detection within the host is determined only by their lifespan within it, which varies according to the characteristics of their life cycle, i.e. some intestinal parasites can live up to 2.5 years inside their bird host, but some live less as 2 months (Ellis & Williams, 1973). The latter period of permanence is shorter than those seen in the larval stages, which can remain in the host from host infection to its death (Williams *et al.*, 1992; Marcogliese, 2004). In addition, since fish length has been shown to influence the richness and abundance of parasite infracommunities (Díaz & George-Nascimento, 2002; Flores & George-Nascimento, 2009; Muñoz-Muga & Muñoz, 2010), in our study comparisons between localities were made using hosts of the same length, to reduce the impact that may have in the discrimination between populations using these parasite species as biological markers (MacKenzie & Abaunza, 1998; MacKenzie, 2002).

Parasite infracommunities have been used previously as tools for host population discrimination, however, these studies used predominantly long-lived larval parasites, such as digenean metacercariae, cestode plerocercoids and juvenile acanthocephalans (George-Nascimento, 2000; Sardella & Timi, 2004; Timi & Lanfranchi, 2009), since the residence time in the host has been considered the most important factor in the use of parasites as biological tags (MacKenzie, 2002). In this regard, although ectoparasites (such as copepods and isopods) were also observed in *M. bonaci* they were excluded because the intestine was the site of infection where the largest number of different parasite taxa was found, besides ectoparasites (especially copepods) did not show differences in prevalence among locations. In addition, the acknowledged criteria for the use of parasites as biological tags, such as the geographic variation in prevalence or abundance along with longevity of infection and absence of reproduction directly in the host (Williams *et al.*, 1992), encouraged us to use only intestinal helminth parasites excluding ectoparasites. Although the use of short-lived endoparasites (such as the tetraphyllideans) in population discrimination should be used with care (Lester & MacKenzie, 2009), when integrated with a complementary method, such as microsatellites or the chemical composition or the shape of otoliths, it can be a tool that reinforces the results (MacKenzie & Abaunza, 1998, 2014). For example, spatial discrimination of the deep-water redfish (*Sebastes mentella*) has been found within the Gulf of St. Lawrence and Newfoundland using fish molecular markers (Roques *et al.*, 2002), and then by examining parasite communities host populations were further subdivided into four smaller groups (Marcogliese *et al.*, 2003). In the latter study the authors only used three species, including an ectoparasite copepod (*Sphyrion lumpi*) along with the nematode larvae of *Anisakis simplex* and *Hysterothylacium aduncum*, since these fulfilled the criteria as biological tags. In this sense, in our study ectoparasites did not fulfil this criteria, nonetheless intestinal parasites did, and in fact detected similar spatial population discrimination, being that Celestun and Alacranes were the least dissimilar to each other, as also shown with the genetics of the hosts. As seen in our results, the lowest percentage of correct allocation of fish to their localities, based on their parasites, was recorded in Alacranes (62.96%), where 33.33% of the remaining host would be allocated instead in Celestun (Table 3). The genetic results prove that Alacranes, together with Celestun, were the localities with the highest allelic and genotypic richness as well as the pairwise highest gene flow and lowest genetic distance between them. It is likely that the connectivity between these two localities is driven by the marine currents of the Gulf of Mexico (Figure 1) enhancing therefore higher black grouper migration-emigration processes than those between the other two localities (Alacranes and Dzilam).

Population discrimination using parasites as biological tags represents a process in ecological time in all the necessary host and environmental conditions for parasite differences between localities, whereas differences at the host's genetic level would represent long-term changes in evolutionary time. Therefore, it seems that the environmental conditions and the absence/presence of the hosts necessary to complete the parasite life cycles, regulate their presence and therefore the composition of parasite communities present in fish from all locations (Thieltges *et al.*, 2010), as well as the poor vagility of the black grouper which could be influencing the composition of parasite communities (Farmer, 2009). Either way, the use of parasites confirmed the sensitivity of this technique in the detection of different hosts' population subunits, which would help to confirm the genotypic-based population discrimination of the black grouper by integrating our findings and reducing the type I error in identifying its subpopulations.

Similar to the parasitological results, the population discrimination based on the genotypic approach indicated that *M. bonaci* showed an excess of heterozygotes within each locality and the estimates of the F_{IS} (−0.141) and F_{ST} (0.056) suggested that all three localities deviated significantly from Hardy–Weinberg equilibrium and that there was a structuring within the study area, suggesting that the black grouper population cannot be considered a panmictic. In addition, heterozygote excess, usually revealed by negative inbreeding coefficient (F_{IS}) such as ours, has several potential causes. For instance it may result from small reproductive population size (Pudovkin *et al.*, 1996; Coscia *et al.*, 2016), small sexual or self-incompatible populations (Balloux *et al.*, 2004), negative assortative mating (also known as disassortative mating or heterogamy) between individuals carrying different alleles (Stoeckel *et al.*, 2006). That is to say individuals with dissimilar genotypes or phenotypes mate with one another more frequently than would be expected under random mating, which in turn would reduce the genetic similarities within the population.

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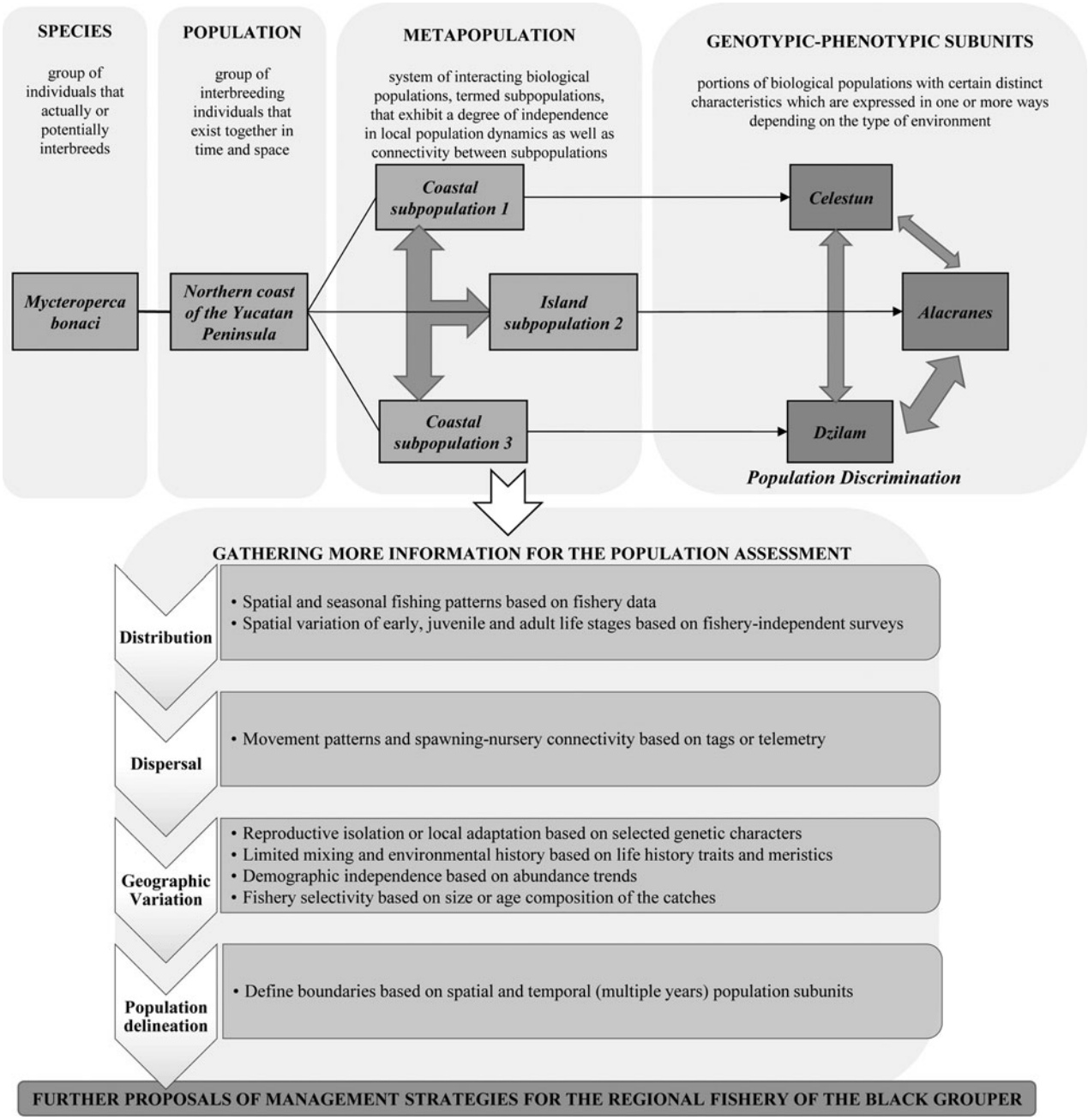

Fig. 4. Overall highlights of the exploratory population discrimination for *Mycteroperca bonaci* from the northern coast of the Yucatan peninsula in the present study using parasite assemblages and host genetics, and further challenges for the population assessment, in line with best practice suggested by Cadrin (2020), in a previous proposal for management strategies for this fishery.

Significant genetic differences among localities showed by the multilocus AMOVA, then supported by the PCoA and STRUCTURE analyses, highlighted a gradient-like arrangement of all the localities and a singular genetic distinctness of *M. bonaci* samples from Dzilam. Despite Dzilam and Alacranes being geographically the closest (~155 km) localities, genetically the least distant ones were Celestun and Alacranes, whose small genetic differentiation might be explained by the non-linear distribution (but triangle shaped) of the sampling sites as well as the northwestern flow (known as the Yucatan Current) on the western side of the Yucatan peninsula (Merino, 1997). The Yucatan Current flows into the Gulf of Mexico through the Yucatan Channel and it eventually separates from the Campeche Bank and becomes the Loop Current forced by the easterly winds (Figure 1), which primarily prevail throughout most of the year and the hydrodynamics (surface currents) of the shelf have been mostly associated with this easterly wind stress pattern commonly observed in the entire region with little seasonal and spatial (depth) variation (Molinari & Morrison, 1988; Enriquez *et al.*, 2010; Ruiz-Castillo *et al.*, 2016). Ocean currents are known to have a major influence on larval dispersion with genetic consequences in fish populations (Cowen *et al.*, 2000, 2006; Purcell *et al.*, 2006). In this regard, a recent study (Johnston & Bernard, 2017) showed that the larval dispersal of several species (lionfish, red grouper and marine species in general) decreases markedly from 89°W (close to Dzilam), seriously delaying the recruitment in the area between 91.1–91.5°W (close to Celestun), which would help explain our results in terms of genetic connectivity.

Deviations from Hardy–Weinberg equilibrium at each locality have been associated with inbreeding due to low larval dispersal in most marine species (Planes *et al.*, 1998; Taylor & Hellberg, 2003;

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Purcell *et al.*, 2006). Doherty *et al.* (1995), as well as Riginos & Victor (2001), found that fish species with long planktonic larval duration (> 40 days) usually have low levels of genetic differentiation among localities because their larvae have a greater chance of migration and gene flow, and therefore may contribute to the population gene pool. In this regard, the genus *Mycteroperca* normally presents an egg phase of 2 days, and a larval phase that lasts on average 43 days (Keener *et al.*, 1988), however, given the oceanographic characteristics found by Johnston & Bernard (2017) for the Yucatan coast, its dispersion can indeed be limited by the restricted dispersion reported in the area, limiting the connectivity between populations, which suggests that self-recruitment is responsible for maintaining this locality with a limited genetic flow. Thus, the population structure observed in this study could indeed be due to local larval retention and a limited gene flow between localities, especially for Dzilam.

Regional fisheries might threaten not only the targeted population, but also entire communities of organisms, including their parasites, by reducing host abundance and food web complexity, which affect their transmission efficiency, population density and assemblage taxa richness (Wood & Lafferty, 2015; Braicovich *et al.*, 2021). Furthermore, it is important to preserve black groupers from Dzilam since they may act as reservoirs for rare genotypes (7.2% were unique), which gives a certain degree of distinctness to this particular subunit. Fishing is known to be one of the causes of reduced heterozygosity (Bergh & Getz, 1989). For example, fishing authorities should determine catch quotas or even regulate the fishing effort. In addition, in order to define a definitive spatial structure for the further population assessment for the regional fishery of this species, as shown in Figure 4, more information related to its distribution, dispersal, geographic variations and boundaries, needs to be gathered in line with recommendations by Cadrin (2020), along with temporal stability of spatial differences over multiple years.

NPAs, where samples of the black grouper were collected, have distinctive features. For instance, Dzilam was acknowledged as a State NPA in January 1989 and controlled by a dependency of the State government called Secretary of Sustainable Development. In the management plan of this NPA, *M. bonaci* is listed as a commercial fish. In contrast, the NPA of Celestun is known as a Biosphere Reserve controlled since November 2000 by a dependency of the Federal government called CONANP (the acronym in Spanish of *Comisión Nacional de Áreas Naturales Protegidas*). Its management plan only mentions the presence of 140 species of fish in the NPA, in which the red grouper (*E. morio*) is mentioned as a commercially important species and heavily exploited, but *M. bonaci* is not mentioned at all. Finally, the NPA of Alacranes, known as a National Park also controlled by the CONANP since June 1994, is located 140 km from the northern coast of the Yucatan Peninsula, in which *M. bonaci* is one of the species that occurs in the area, but the management plant of this NPA does not provide fishing regulations specifically for the black grouper, instead the fishing regulations declared in the management plan are only oriented to limit the number of fishermen and boats to certain areas (core zone or no-take zone) where fishing is not allowed (in comparison with Celestun and Dzilam where there are no-take zones). However, due to its insular nature and distance from the coast, the fishing pressure even in the areas where the extraction of some organisms is allowed (buffer zone and zone of sustainable use of natural resources) has not been particularly high (except in the summer months when this activity is intensified mainly by sport fishing). In these latter areas, the fishing regulation is aimed only at the use of low-impact fishing gears (e.g. hooks, traps, or collection by free diving and spearfishing), in addition to respecting the provisions for the temporary ban on groupers, which have a closed season for fishing (from 15 February to 15 March) in the State of Yucatan but this is related to the main target species (red grouper *Epinephelus morio*) (DOF, 2014), and the spawning season for *M. bonaci* usually extends from December to March (Brulé *et al.*, 2003). The present recognition of population subunits would bring beneficial effects to preserve more important gene pool variability of the coastal Dzilam subunit where fishing regulations are the least restrictive compared with the other studied NPAs.

Regional currents are expected to allow dispersion between distant localities to form a single panmictic population, however, both approaches (parasites and genetics) used in our study were consistent in the overall population discrimination. Moreover, the present study shows that although island NPAs (such as Alacranes) are expected to have some degree of distinctness, either ecological (Moore *et al.*, 2003; Lester & Moore, 2015) or genetic (Ruzzante *et al.*, 1997; Hemmer-Hansen *et al.*, 2007), our results suggest that even though the black grouper population is spatially structured into at least three units (Celestun, Dzilam and Alacranes), in this case we identified the coastal locality of Dzilam as the more differentiated (genetically and lowest parasite diversity) probably caused by oceanographic-driven low levels of larval dispersal and adults' migration.

Data. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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