

High levels of gene flow in phenotypically divergent populations of a coral reef fish (*Sparisoma rubripinne*)

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Abstract:

*The high biodiversity of tropical seas offers a challenge for establishing the most common mechanism for evolutionary diversification. Because requirements for allopatric models to operate in marine ecosystems are often not fully met, it has been recently proposed that ecologically based genetic divergence may be the most common mechanism leading to diversification in the marine realm. The abiotic characteristics of the environment play a role in determining the type and amount of resource availability for primary consumers. Hence, different niches confront primary consumers to differential selective pressures that might trigger ecologically-based divergent selection. To further explain the patterns in which ecologically based diversification occur in tropical seas, we tested for mtDNA and morphologic differentiation in populations of a parrotfish (*Sparisoma rubripinne*) species distributed in waters with varying structural complexity of the resource. We found significant morphologic and environmental differences and some degree of reproductive isolation between two populations despite high levels of gene flow. Because our patterns may not be explained by geographic distance, we suggest that specialization to different habitat types can result in divergence of key phenotypic traits even in the presence of gene flow. Our results can be explained both in a context of divergence with gene flow or secondary contact after allopatric differentiation.*

Introduction:

Arguably, the most critical stage in speciation is the development of reproductive isolation between populations (Coyne 1992). Geographical isolation and ecological adaptation play a role in the early development of reproductive isolation (Coyne and Orr 2004). Geographical isolation is a consequence of a physical reproductive barrier that separates members of different populations. This geographic isolation, in concert with natural selection or drift causes genetic divergence. After sufficient time, reproductive isolation evolves as a byproduct of this genetic differentiation. (Mayr 1963; Bush 1975). Because of divergent selection, local adaptations to different ecological regimes can also cause reproductive isolation (pre-mating: mechanical isolation, assortative mating; post-mating: hybrid inviability and sterility) even in the absence of physical barriers (Orr and Smith 1998; Schluter 2001; Rundle and Nosil 2005).

Allopatry is widely accepted as the most common mechanism generating genetic divergence between populations in terrestrial environments (Mayr 1942; Dobzhansky 1964). Alternatively, ecological adaptation may be an important source of genetic differentiation in the absence of physical barriers to gene flow. It is now widely recognized that natural selection can override the homogenizing action of gene flow and can drive genetic differentiation between populations in alternative

environments at adjacent locations (Orr and Smith 1998; Schluter 2001; Rundle and Nosil 2005; Pinho and Hey 2010). Ecologically-based divergent selection has received in recent years wide recognition. It has been shown that, even in the presence of gene flow, ecologically-based divergent natural selection can drive populations along separate evolutionary pathways in terrestrial (Smith 1997; Ogden and Thorpe 2002; Chavez et al. 2011) and marine (Rocha et al. 2005; Schluter and Conte 2009) habitats.

Because early models have shown that gene flow necessarily impedes population differentiation (Maynard-Smith 1966), sympatric speciation was long dismissed as theoretically implausible (Felsenstein 1981; Coyne 1992) and empirically unsubstantiated (Mayr 1963). However, more recent empirical (see Via 2001 for a review) and theoretical advances (Turelli et al. 2001) have shown scenarios in which speciation can proceed in sympatry (Coyne and Orr 2004; Bolnick and Fitzpatrick 2007). The effect of natural selection on individuals occupying alternative ecological environments (e.g., stability of alternative habitats, productivity, distribution of potential breeding sites, competition and predation risk; Schluter 1996; Schluter & Rambaut 1996; Rolán-Alvarez et al. 1997; Skulason et al. 1999; Doucette et al. 2004; Snorrason & Skulason 2004) can lead to genetic divergence and speciation of sympatric populations: either through disruptive selection directly on habitat choice (Thoday and Gibson 1962; Rice 1984) or as a pleiotropic effect of disruptive or divergent selection on other traits (Kondrashov 1983; 1986; Kondrashov and Mina 1986; Rice 1987; Rice and Salt 1990). Additionally, the absence of intermediate environmental zones and habitat fidelity can lead to selection against hybrids and may facilitate speciation by promoting assortative mating (Tauber and Tauber 1977; Bush 1994). Savolainen *et al* (2006) revealed that the absence of intermediate zones could have played a role in palm speciation on an oceanic island. They emphasized that speciation of this palms was likely to have proceeded in sympatry by flowering time-differences that evolved as a result of substrate-induced physiological changes. Via (2000) evidenced that gene exchange between host-associated pea aphid populations is restricted to habitat choice behavior. She showed that the genetic correlation between host preference and performance is a likely cause of assortative mating between groups that prefer different hosts.

Given the circumglobal nature of marine environments, there are few opportunities for geographical barriers to gene flow (Mayr 1954; Palumbi and Lessios 2005). In addition, most marine organisms have a pelagic larval stage with high dispersal potential (Palumbi 1994; Mora and Sale 2002). However, even when these factors should prevent the occurrence of genetic divergence between populations and thus speciation, tropical seas host some of the most species-rich habitats in the world: the coral reefs (Kay and Palumbi 1987; Spalding and Grenfell 1997). Therefore, species associated to coral reefs provide an excellent opportunity to test for natural occurring examples of ecologically-based divergent selection and may provide a framework to understand how genetic divergence occurs and ultimately how evolution works in marine environments.

However, researchers who have studied tropical reef fish have been reluctant to propose ecological adaptation as a likely source of genetic divergence in this group. Because tropical reef fish span to a variety of territories, then, migrant larvae are unable to predict the ecological characteristics of the waters in which they will develop. As a consequence, such studies fail to propose ecological adaptation because of the premise that a highly dispersive larvae impedes the evolution of local adaptations and thus plasticity is thought to be a better fit to explain morphological differences in reef fishes (Warner 1997). Novel theoretical arguments propose that local adaptation through developmental plasticity may result in reproductive isolation of populations in different environments (West-Eberhard 2003; Pigliucci 2006). Moreover, recent observations have shown local retention of reef fish larvae (Jones et al. 1999; Swearer et al. 2002), active habitat choice by larvae (Bierne et al. 2003) and reduced gene flow over short geographical distances (Taylor and Hellberg 2003). This evidence allows for the possibility of ecological partitions driving divergence in tropical reef fish and helps explain the biodiversity patterns of tropical seas.

The ecological characteristics of primary consumers could facilitate divergent evolutionary processes between populations at habitats with varying structural complexity. Primary consumers occupy intermediate levels within food chains and, thus, must often trade-off between gaining access to resources and minimizing predation risks (Fortin et al. 2005; Creel and Christianson 2008). While seeking for food, primary consumers take the risk of being preyed upon. This trade-off is expected to lead to habitat-specific adaptive evolution in morphological and behavioral traits to escape predation while being successful at reaching and using food sources (Brown and Kotler 2004). Structurally complex habitats are often more favorable than open habitats in terms of resource availability (Candolin and Voigt 2003; Candolin and Selin 2012). Since resource availability is negatively correlated with the time spent in foraging activities (Walters and Juanes 1993), the trade-off between foraging and predatory risks seems to vary as a function of habitat resource availability (Doucette et al. 2004). Consequently, compared to primary producers and top predators (which may not be as influenced to trade-offs between direct predation and risk effects, e.g., predatory risks; Creel et al. 2007; Heithaus et al. 2008), primary consumers may be mostly influenced by natural selection and thus, evolutionary divergence between populations in environments with contrasting resource availability is more likely to arise.

Parrotfish are among the most common and colorful fishes inhabiting Caribbean coral reefs. They are commonly classified as herbivores that scrape algae from the surface of hard substrates. As herbivore foragers, parrotfish may have to trade-off optimal foraging and predation risks. Because alternative foraging choices vary depending on the risk and reward, resource availability in different habitats might prompt alternative morphological and behavioral mechanisms to resolve this trade-off. Accordingly, it has been proposed that ecomorphological differences could have played a significant role in the initial divergence of parrotfish lineage evolution (Streelman et al. 2002).

In Colombia, parrotfish are distributed along most of the continental coast and in oceanic islands overlapping the distribution of coral reef and seagrass beds. In general, there is higher coral diversity and structural complexity around oceanic islands than in continental reefs (De'ath and Fabricius 2010; Sanciangco et al. 2013). Accordingly, the reefs around offshore islands on Colombia's continental shelf (e.g., Bolivar; Rosario Islands and Baru) and oceanic islands (e.g., San Andres and Providencia) show greater development, species richness, and structural complexity overall (Pfaff 1969; Geister 1973; 1992; Sánchez et al. 1998; Ordoñez 2004), than the ones in the continental coast (Antonius 1972). Structural complexity dictates energetic benefits and constraints of organisms (MacArthur and Pianka 1966). Structurally complex habitats are often more favorable than open habitats, because they provide more resources and better refuges against predators (Candolin and Voigt 2003; Candolin and Selin 2012). Therefore, structural complex habitats are often associated with more diverse and abundant species assemblages in both aquatic (Luckhurst and Luckhurst 1978; Diehl 1992; Chase and Leibold 2002) and terrestrial ecosystems (Purvis and Hector 2000). However, complex habitats can also harbor more predators and competitors than open habitats, which could result in a more costly situation for herbivores (Sih 1997). Thus, by altering resource availability and predations risk (Hixon and Menge 1991; Beukers and Jones 1998; Finke and Denno 2002; Warfe and Barmuta 2004; Humphries et al. 2011), the habitat structure has the ability to shape selective forces acting on herbivores. Hence, the Colombian Caribbean coast is an ecological mosaic, in which alternative habitats with different selective pressures are encountered and thus parrotfish in Colombian habitats are likely to be subject to different selective pressures that might trigger evolutionary divergence.

The aim of this work was to test for ecologically based intraspecific differentiation in the widely distributed redfin parrotfish (*Sparisoma rubripinne*) inhabiting two adjacent coastal areas, an archipelago close to the Caribbean coast and an oceanic island which have varying structural complexity in order to understand if habitats with different selective pressures can result in evolutionary divergence of marine populations. We tested for morphological divergence between populations of the redfin parrotfish in traits that have a close relation to feeding ecology and fitness and searched for genetic divergence on a neutral marker (Control Region-CR). Specifically, we predicted that: if differences in structural complexity of habitats promote distinct ecological adaptations and facilitate intraspecific divergence in morphologic and genetic traits, then genetic and morphologic differences will partition according to habitat distribution rather than to geographic or oceanographic barriers.

Methodology:

Study site selection:

It has been recognized that reefs around oceanic islands and archipelagoes have a higher structural complexity than reefs in the continental shore (De'ath and Fabricius 2010; Sanciangco et al. 2013). Therefore we chose two localities in which reefs are formed around oceanic islands and archipelagoes, and two localities close to the continental shore, that differ in the structural complexity of primary producers. Our

more structurally complex habitats were in Bolivar and San Andres. Bolivar province sampling site comprises a series of tiny islands of coral Archipelago that are formed close to the continental shore, whereas San Andres is the largest of a group of coral islands in the Caribbean Sea, located 750 km Northwest off the Colombian coast. The submarine environment around these islands forms an intricate mosaic, in which coral patches, fringing reefs, barrier reefs, seagrass meadows and mangroves co-occur (Díaz and Acero 2003; Garzon-Ferreira and Díaz 2003), making these much more complex than any other submarine environment in Colombia (Cendales et al. 2002). In contrast, our low structurally complex sites were in Magdalena and Guajira Baja. These localities lie southwest of the Peninsula de La Guajira at the foot of the Sierra Nevada de Santa Marta, the world's highest coastal mountain ridge. The submarine ecosystem in these localities is comprised mostly of small fringing reefs and patch reefs, scattered in sheltered bays, in which the resource is more patchily distributed and less structurally complex than in those reefs at San Andres and Bolivar (Antonius 1972; Cendales et al. 2002; Díaz and Acero 2003).

Specimen collection and DNA extraction:

We collected a total of 84 tissue samples of *Sparisoma rubripinne* individuals in the four localities studied; in Magdalena: Santa Marta (n=10), Taganga (n=10), Bahia Chengue (n=10), Nequange (n=10), Cabo San Juan (n=10); in Guajira: Dibulla (n=4); in San Andres: Bahia el Cove (n= 14), Sound Bay (n=1) and in Bolivar: Baru (n=15). DNA extraction was performed using the DNeasy kit from Quiagen® (instructions according to manufacturer).

Morphological measures

We measured body and fin morphology, which can give us a picture of the selective environmental pressures that an individual may deal within each locality. Differences in structural complexity of sites can result in morphological variation of fish individuals (Sibbing and Nagelkerke 2000; Fulton et al. 2001; Holzman et al. 2012). As we have stated above, structural complexity dictates energetic benefits and constraints of organisms by altering resource availability and predations risk (Hixon and Menge 1991; Beukers and Jones 1998; Finke and Denno 2002; Warfe and Barmuta 2004; Humphries et al. 2011). Hence, differences in body and fin morphology between sites can be used to predict the level by which structural complexity and predatory risk could act as important selective forces acting on the fitness of an specific individual (Wood and Bain 1995; Arbour et al. 2011). For each individual we measured its total length, head length, head depth, body depth, tail length and aspect ratio of the pectoral fin (the proportion between the square of the leading edge length and the projected area of the pectoral fin).

Increased body size affect fish foraging rate, vulnerability to predators and fecundity (Werner 1988; Persson and Diehl 1990; Godin 1999; Karpouzi and Stergiou 2003). For example, bigger herbivorous fish tend to have lower vulnerability to predators, higher fecundity and foraging rates and are more common in structurally complex habitats (Vergés et al. 2011). The trade-off between steady and unsteady swimming is useful to understand how body shape relates to various selective agents (e.g., foraging

mode, predators and abiotic factors; Schoener 1971; Webb 1984). This trade-off bases its premise in that traits that increase performance in one swimming mode necessarily decrease performance in the other (Weihs and Webb 1983; Videler 1993; Reidy et al. 2000; Domenici 2003). Steady swimming refers to constant speed locomotion in a straight line whereas unsteady swimming relates to a more complicated locomotor pattern in which changes in velocity or direction occur, such as fast starts and rapid turns (Bandyopadhyay 2002). Each type of swimming mode is preferable in different ecological situations and thus polymorphisms on specific morphological traits can be informative about the level of the habitat's selective pressures (Webb 1982; 1984; Godin 1999; Langerhans and Reznick 2010).

Based on swimming kinematics (Bandyopadhyay 2002), it has been shown that to maximize swimming performance, unsteady swimmers need a smaller aspect ratio of the pectoral fins (maneuverability), deeper/larger caudal (tail) fin length, shallower/smaller heads, smaller body depth to reduce drag during fast starts, and in general a smaller depth in anterior body tapering to a deeper caudal region, whereas maximal steady swimming performance involves the opposite characteristics (Wu 1971; Wainwright et al. 2002; Walker and Westneat 2002; Fulton et al. 2005; Fisher and Hogan 2007; Domenici et al. 2008; Langerhans and Reznick 2010). Unsteady swimming in the wild has been associated to structurally complex habitats and as a result of abundant predator-prey interactions (Langerhans 2009; Langerhans and Reznick 2010). Steady swimming is favored in nature during competition for limited resources in habitats with low structural complexity, such as searching food (which is often patchily distributed) and seeking favorable abiotic conditions in a low predator habitat (Plaut 2001; Blake 2004).

PCR and sequencing:

Polymerase chain reaction was carried out to amplify a 680 base pair section of the mitochondrial control region. We used the primer pair *ScarusDloopF*: 5' AATTCTCACCCCTAGCTCCCAAAG 3' and *ScarusDloopR*: 5' CCTGAAGTAGGAACCAGATG 3' (Lee et al. 1995) to perform PCR with the following thermal cycle: 1 cycle at 94°C (3 min); 33 cycles at 94°C (30 s), 50°C (30 s) and 72°C (1 min), followed by 1 cycle at 72°C (5 min), before cooling to 12°C. We used between 50 and 200 ng of template DNA in a 50 µL volumen reaction. Each 50 µL volume reaction contained 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 µM of each primer, 1 unit of Taq polymerase (Invitrogen) and approximately 100 ng of genomic DNA. PCR products were run on a 1% agarose gel to check yielding and were sequenced in both forward and reverse directions at Macrogen inc. services-Korea.

Morphologic analyses

All morphologic measures were converted into a single variable withholding the largest possible variance via a principal components analysis (PCA). We tested for morphological differentiation between populations using an ANOVA with the scores of the first principal component (PC1) taking as groups each of the four sampling

localities. Additionally, we used the scores of the PC1 to construct a dendrogram in order to graphically analyze the results. Prior to analysis we allometrically scaled the morphological data by doing a regression between each trait measured with the total length and working with the residuals of such model. All analyses were performed after removing outliers and testing for normality in the data. All variables were normally distributed (Shapiro-wilk > 0.05) except for morphological measurements of individuals of Guajira Baja. However after analyzing the distribution we found that non-normality is caused by skewness in the data set and not by outliers in which case the resultant significant test is still reliable (Tabachnick and Fidell 2012).

Genetic analysis:

To identify the number of genetically isolated populations that were represented by our gene sequences, we traced the intra-specific gene phylogeny of our samples. This is useful to assess whether there is a bipartition that generates monophyletic patterns among genes collected in different geographic sites. Knowing that we used genetic data that evolves rapidly and in a neutral fashion, this bipartition will give us insight into patterns of the degree of reproductive isolation among the samples.

We used the BEAST package (Drummond and Rambaut 2007) to reconstruct a phylogenetic tree in order to determine the way in which individuals were clustered. BEAST uses molecular sequences to infer the relationships between different individuals. In doing so, it uses MCMC to draw a posterior distribution of a set of evolutionary parameters. Multiple sequence alignment was performed using the Clustal W algorithm implemented in SEAVIEW (Gouy et al. 2010) and checked by eye afterwards. Using JMODELTEST (Posada 2008) we computed likelihood scores for 88 models of sequence evolution and determined the evolutionary model that best fitted our data using the Akaike information criteria (AIC). The model selected was the GTR with gamma-distributed rates. Afterwards, we ran BEAST using as tree prior a coalescent one that allows a parametric demographic function of population size through time (Constant size) and estimated branch lengths using a strict clock. We ran a first MCMC for 10 million generations sampling every 1000 steps using the evolutionary model that best fitted our data with four gamma categories. Afterwards we used Tracer V 1.4 (Rambaut and Drummond 2007) to summarize the distributions of the parameters. This information was used to re-set the parameters in the prior. Once we had these more feasible priors we performed a second run for 50 million generations sampling every 5000 steps. MCMC generation times and sampling effort was determined by assessing convergence under the basis of the Effective Sample Size (ESS) after a 10% Burn-in using Tracer Version 1.4. Only after achieving an ESS score of > 200 the sampling effort was accepted. The trees retrieved under the coalescent constant size tree prior were summarized in a target tree by the Tree Annotator program included in the BEAST package.

To compute estimates of evolutionary divergence over sequence pairs, we assigned individuals to populations as grouped by the geographic location of the sample. Following, we used MEGA5 (Tamura et al. 2011) to calculate two statistics that

estimate divergence between populations: D_{xy} and D_a . D_{xy} calculates the average pairwise number of nucleotide differences per site between populations and D_a corrects D_{xy} by subtracting the average genetic diversity within populations (Nei 1987). Standard error estimates were obtained by a bootstrap procedure (10000 replicates).

We used a coalescent approach using the Bayesian implementation available in the program MIGRATE-N (Beerli and Felsenstein 2001; Beerli 2006) to assess migration rates and to test hypothesis of population differentiation. We defined three models that could explain the genetic patterns in our samples: **I**) individuals belong to one panmictic population **II**) individuals are subdivided in two populations according to the differences in structural complexity between localities (San andres+ Bolivar and Guajira+Magdalena) **III**) individuals are separated into four populations according to the sampling localities. These analyses are useful to test our working hypothesis. Expecting the species to have high migration rates due to its biology, model **I** addresses the possibility that ecological barriers do not impose a restriction to gene flow and so sets the scenario with the least amount of parameters. On the contrary, model **III** evaluates a scenario with the highest number of parameters and states that each sampling site is genetically differentiated from the rest. Finally, a third possibility (model **II**) evaluates our working hypothesis and predicts a positive correlation between adaptation to different environments and neutral genetic differentiation (isolation by adaptation; Nosil *et al.* 2008).

For each model we used migrate-n to calculate migration rates (models **II** and **III**) and approximated marginal likelihood using thermodynamic integration with a Bezier curve (Beerli and Palczewski 2010). Two identical Bayesian analyses were run for each of the above, with different randomly selected starting seeds, to assess whether the parameter space was properly sampled. Parameters used for these analysis included 1 long chain with an increment of 150, a sampling of 1×10^6 , a burn-in of 1.5×10^7 , the default heating scheme and the F84 model of nucleotide substitution. We compared models using Bayes Factors and calculated each model's probability to establish which of the three models was more likely given the data.

Finally, we tested for isolation by distance by plotting the genetic similarity among population pairs as a function of the geographic distance between those pairs (Slatkin 1993; Hutchison and Templeton 1999), using the software in Isolation By distance, web service, Version 3.23 (Jensen *et al.* 2005). Statistical significance was assessed using a Mantel test.

Results:

Morphological analysis:

A PCA separates the samples into two distinct groups. The dendrogram constructed using the PC1 (27.8 % of the variation) of the morphological variables separates the individuals into two main clusters. One was composed mainly of individuals from San Andres and Bolivar (86 %) and another one containing individuals from Magdalena

and Guajira Baja (85 %; Fig 1, Supplementary Figure 1). The ANOVA used to compare the means of the PC1 scores from individuals in each of the localities showed that there were significant differences in morphological traits ($p < 0.0001$). However, a Tukey test revealed that neither the mean of Guajira Baja is significantly different from Magdalena nor that of San Andres is different from Bolivar. More specifically, the mean of Guajira Baja + Magdalena is significantly higher than that of San Andres + Bolivar. The attributes which most contributed to the PC1 were the body depth and the head length, which were directly associated, and the tail length which was negatively associated to this axis. This means that Magdalena + Guajira individuals have a larger head, greater anterior body depth and a smaller tail (characteristic of steady swimmers), whereas San Andres + Bolivar have the opposite characteristics (usually found in unsteady swimmers).

Genetic analysis

Genetic analyses partially agree with the morphological separation reported above. The Bayesian tree shows a separation between two well-supported groups (PP= 1 for each of this clades; Fig 2). Individuals from all four localities compose one of the clades. However, only San Andres and Bolivar individuals fall into the other clade (approximately 50 % of all individuals of San Andres + Bolivar). Bayes factor testing with Migrate-n shows that a two-population model partitioned according to the morphological clusters is more likely than the other two models tested (Table 1). Finally, pairwise genetic differences computed in MEGA5 show a similar pattern of differentiation. San Andres/Bolivar pair has a net divergence of 0, whereas Guajira Baja/Magdalena pair has a net divergence of 0.001 (or basically 0). In contrast, comparisons between these pairs (e.g., San Andres and Magdalena) are all > 0.007 (Table 2). In fact, San Andres differs by net divergences of 0.016 and 0.025 from Guajira and Magdalena. Thus, molecular data supports San Andres and Bolivar as one population, and Guajira and Magdalena as a second population, even though this divergence is small.

Using Migrate-n we tested for evolutionary gene flow between populations separated geographically into two and four populations. Disregarding of the way the samples were grouped, results show that there are high levels of genetic interaction between populations ($Nm > 1$). Together, these data show that some individuals are highly divergent morphologically despite high evolutionary gene flow. In cases where disruptive selection may be acting, a finding of even a small amount of gene flow and between individuals that differ phenotypically is interesting. For example, it has been shown that intense directional selection on morphologic traits in the presence of gene flow often results in prezygotic isolation (Via 2009). In fact, migration was always higher from Magdalena and Guajira populations to San Andres and Bolivar than in the opposite direction (Table 2). Among our results, the most extreme case shows 31 individuals migrating from Guajira to San Andres but only 7 from San Andres to Guajira. This decrease in migration may promote departures from random mating, which is a crucial component in most models of ecologically-based divergent natural selection.

Finally, geographic distance was a poor predictor of the genetic differences between populations ($R^2 = 0.0815$), and the correlation between these two variables was not significant ($P=0.43$), indicating that a model of Isolation By Distance does not explain our patterns.

Discussion:

Disentangling the role of genetics and environment promoting intraspecific phenotypic variation in coral reef fish is key to understand the factors that promote or constrain evolutionary diversification in marine biodiversity hotspots. Our analyses show that isolation by distance does not explain morphological differentiation. Bolivar can maintain genetic and morphologic connectivity across hundreds of kilometers of open-ocean with San Andres, and yet be capable of developing morphological discontinuity between much shorter distances across the continental coastline with Magdalena and Guajira Baja ($P < 0.0001$, Fig 1).

The most striking intraspecific morphological divergence between populations was found in characters associated to body shape and tail length. Phenotypic variation on these traits has been proven to be important for swimming performance (steady vs unsteady swimming) and likely reflect adaptation to different foraging environments (i.e., Habitat structural complexity; Langerhans 2008). Moreover, polymorphisms on these traits in the wild are commonly related to feeding ecology and fitness (McGurk 1986; Brönmark and Miner 1992; Sogard 1997; Nilsson and Brönmark 1999; Bellwood and Wainwright 2001; Fulton et al. 2001; Fulton and Bellwood 2002; Nakazawa et al. 2007) and have been shown to be maintained under common-garden rearing in the laboratory (Lavin and McPhail 1993; Hendry et al. 2002; Sharpe et al. 2008), indicating a genetic basis. In fact, genetic information in this study indicates some degree of reproductive isolation between two populations (1=San Andres+Bolivar, 2=Magdalena+Guajira; Fig 2, Tables 1 and 2), despite high levels of evolutionary gene flow (Table 2). Moreover, there are differences in the structural complexity of the habitat between San Andres plus Bolivar and Magdalena plus Guajira. Thus genetic and morphologic differences vary according to the habitat distribution rather to geographic or oceanographic barriers. However, confirmation of this latest hypothesis awaits a detailed examination of differences in the structural complexity and distribution of parrotfish resources between habitats.

Together, these intraspecific results support growing evidence suggesting that morphological divergence among populations of coral reef fishes are usually the result of ecologically based selection pressures (see review in Rocha and Bowen 2008) either in sympatry (Jones *et al.* 2003; Rocha *et al.* 2005; Puebla 2009) or allopatry (Avice 2000; Taylor and Hellberg 2005; Leray et al. 2010). In fact, similar to our results, most studies of processes of marine diversification have demonstrated that neutral genetic divergence is primarily partitioned by habitats, with little geographic structure (Fukami et al. 2004; Rocha et al. 2005; Nielsen et al. 2009; Bird et al. 2011; Prada and Hellberg 2013). In addition, our results provide evidence to suggest that despite high levels of gene flow between populations, strong natural selection

apparently generates (sympatry or parapatry) or maintains (allopatry following secondary contact) population-level adaptive divergence. In the case of parrotfish, Streelman *et al* (2002) showed that divergent natural selection due to ecological adaptation can preclude the homogenizing action of gene flow, and may well play a role in explaining the patterns of diversification of parrotfish. In comparison to the study of Streelman *et al* (2002), in which interspecific divergence of sympatric species was analyzed, our results are salient because demonstrate that studies of intraspecific variation can be useful for better understanding the processes that lead to diversification in this clade.

Several studies have reported high gene flow in the face of adaptive divergence (Smith 1997; Hendry and Taylor 2004; Smith *et al.* 2005; Crispo *et al.* 2006; Yatabe *et al.* 2007). Nonetheless, a question that inevitably arises is the timing of such gene flow (Nosil 2008). On one extreme, gene flow might have emerged upon secondary contact, after a period of allopatric divergence that was not long enough for the populations to develop full reproductive isolation. At the other extreme, adaptive divergence could be developing despite ongoing gene flow (Nosil 2008; Niemiller *et al.* 2008; Via and West 2008; Via 2009; Pinho and Hey 2010). Models of divergence with gene flow predict heterogeneous patterns of genomic divergence (Wu 2001; Nosil *et al.* 2009), with selection maintaining “islands” of differentiation around the presumably small number of loci responsible for adaptive differences between divergent populations (Via 2001; Via and West 2008; Feder *et al.* 2012; Nosil and Feder 2012; Via 2012). However, recognizing between secondary contact zones from ongoing primary differentiation has long been recognized as difficult (Endler 1977). This is because, during secondary contact, neutral markers converge to an equilibrium and thus reveal the history of geographic isolation only transiently, which is the same as in divergence with ongoing gene flow (Bierne *et al.* 2013).

Discriminating primary from secondary contact was not in the scope of this study. Arguing for ecologically based selection pressures promoting diversification at the population level certainly was. If primary contact were the case, then some mechanism, such as divergent selection must also occur frequently to counteract the homogenizing effects of gene flow. The other possibility is that at some point in time populations were allopatrically isolated, in which case a physical barrier will simultaneously block gene flow across the entire genome. In this case, any mechanism of speciation would cause genetic and likely phenotypic divergence. If divergence was caused by drift alone, loci would start to segregate from one population to another in contact zones and will ultimately homogenize both populations. This will cause a loss of neutral genetic and phenotypic divergence. Both a neutral locus gene tree and a morphological dendrogram will show individuals scattered randomly throughout the tree. However, if adaptation to different ecological environments, and not drift, is responsible for divergence in allopatry, the clustering of adaptive mutations may be maintained following secondary contact (Feder *et al.* 2012). The latter is more consistent with our findings. The dendrogram constructed with the PC1 of the morphological characters show two clearly differentiated clusters (Fig 1). In contrast, the gene tree shows weaker associations between individuals with distinct

morphologies (Fig 2). This is because following secondary contact, neutral markers can easily and quickly lose most of their associations with selected loci, and hence with the environment (Bierne *et al.* 2013).

Ecology may contribute to many mechanisms of speciation (Rundle and Nosil 2005), and thus we have shown that it may have influenced intraspecific divergence in this parrotfish species. However, whether the contribution of ecology was fundamental during the divergence process (primary contact), or if it has contributed to perpetuate differences built up in allopatry (secondary contact), we do not know. The coupling of phylogeographic methods based on the coalescent (Lemey *et al.* 2009) and paleo-distribution modeling should elucidate the geographic history of divergence and formation of such intergradation (Carstens and Richards 2007; Kozak *et al.* 2008; Pettengill and Moeller 2012). Moreover, because loci under selection have reduced gene flow, quantification of gene flow in loci responsible for the morphological divergence between populations would be a critical test for our hypothesis of ecological adaptation and may well serve as a good attempt to distinguish secondary from primary contact. Finally, plasticity cannot be ruled out completely as a causative agent. However, all of the traits measured are highly heritable in fish (Dickerson *et al.* 2005; Carlson and Seamons 2008; Varian and Nichols 2010) and frequently correlated with performance and fitness (Langerhans 2009; Langerhans and Reznick 2010). Even though it is possible that some morphological variation arises due to plasticity, the fact that the molecular data supports the morphological separation substantiates our assertion that at least some of this variation has a genetic basis.

Figure legends:

Figure 1. Dendrogram showing the morphological relationships of individuals constructed with the first principal component scores of all morphological measurements. Each individual is labeled according to the geographical location from which it was sampled.

Figure 2. Phylogenetic tree inferred with a Markov Chain Monte Carlo method implemented in BEAST. Posterior probabilities of > 0.8 are shown for major clades

only. Each individual is labeled according to the geographical location from which it was sampled. A map showing the geographical distribution of the sampling localities is shown.

Supplementary Figure 1. PCA based on morphological variables between environmentally differentiated populations.

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