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NOTE

Two distinct, geographically overlapping lineages of the corallimorpharian *Ricordea florida* (Cnidaria: Hexacorallia: Ricordeidae)

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Abstract We examined the genetic variation of the corallimorpharian *Ricordea florida;* it is distributed throughout the Caribbean region and is heavily harvested for the marine aquarium trade. Eighty-four distinct individuals of *R. florida* were sequenced from four geographically distant Caribbean locations (Curaçao, Florida, Guadeloupe, and Puerto Rico). Analysis of the ribosomal nuclear region (ITS1, 5.8S, ITS2) uncovered two geographically partially overlapping genetic lineages in *R. florida*, probably representing two cryptic species. Lineage 1 was found in Florida and Puerto Rico, and Lineage 2 was found in Florida, Puerto Rico, Guadeloupe, and Curaçao. Because of the multi-allelic nature of the ITS region, four individuals from Lineage 1 and six from Lineage 2 were cloned to evaluate the levels of hidden intra-individual variability. Pairwise genetic comparisons indicated that the levels of intra-individual and intra-lineage variability (<1%) were approximately an order of magnitude lower than the divergence (~9%) observed between the two lineages. The fishery regulations of the aquarium trade regard *R. florida* as one species. More refined regulations should take into account the presence of two genetic lineages, and they should be managed separately in order to preserve the long-term evolutionary potential of this corallimorpharian. The discovery of two distinct lineages in *R. florida* illustrates the importance of evaluating genetic variability in harvested species prior to the implementation of management policies.

Keywords *Ricordea florida* · Corallimorpharia · Cryptic species · Aquarium trade

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Introduction

Corallimorpharians are distributed throughout the tropical reefs worldwide (Chadwick-Furman and Spiegel 2000) and can be a dominant component of tropical shallow water ecosystems (Chen et al. 1996). This group of corals lacks calcareous skeleton (den Hartog 1980; Daly et al. 2003, 2007; Fautin et al. 2007) and can be found either as solitary polyps (den Hartog 1980) or as colonies that cover extensive sections of a reef (Gerald and Roger 1994; Muhando et al. 2002). According to Daly et al. (2007), the order Corallimorpharia includes four families (Corallimorphidae, Discosomatidae, Ricordeidae, and Sideractiidae), nine genera

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(12 genera according to Fautin et al. 2007), and at least 24 valid species.

Corallimorpharians in the Caribbean benthic communities are represented by at least six recognized species (*Corynactis parvula*, *Pseudocorynactis caribberum*, *Discosoma sanctithomae*, *D. carlgreni*, *D. neglecta*, and *Ricordea florida*) that represent the families Corallimorphidae, Discosomatidae, and Ricordeidae (den Hartog 1980). Probably the most abundant Caribbean corallimorpharian is *Ricordea florida* Duchassaing and Michelotti 1860, which is distributed throughout the region. The ecological importance of *R. florida* has not been well studied, but it is highly preyed upon by the hawksbill turtles in SW Dominican Republic (León and Bjorndal 2002) where *R. florida* constituted up to 59% of stomach contents. In contrast, the commercial importance of *R. florida* is increasing because the species is among the most heavily harvested invertebrates in the marine aquarium trade.

The change in marine aquarium keeping, from fish tanks to reef mesocosms, has created a high demand for corallimorpharians and other "soft corals" (Rhyne et al. 2009). *Ricordea* is supplied to the aquarium trade both from a domestic fishery (Florida, USA) (Fig. 1) and from imported specimens originating from Haiti. The recent spike in domestic collection is disconcerting when one takes into account changes in the management of the Florida marine life fishery, which took effect in July 2009 (FWC 2009), effectively reversing a previous ban of live rock collections. Fishers are now allowed to legally harvest small amounts of substrata, with polyps of *R. florida* and other corallimorpharians attached to these substrata. These changes along with higher demand have increased the fishing pressure for a species for which we have very little knowledge (e.g., reproductive biology, distribution, dispersal, or genetic structure).

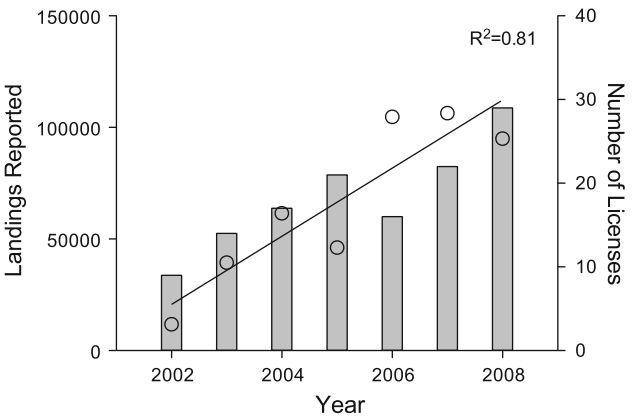

Fig. 1 Reported landings and number of licenses for the *Ricordea florida* fishery from 2002 to 2008. *Bars* are number of licenses reporting landings, and *circles* are number of landings reported. *Solid line* represents the linear regression *line* of landings over time. Data from the Florida Fish and Wildlife Commission Trip Ticket Program

The present study investigated the genetic variability of the internally transcribed spacer region (including ITS1, 5.8S, and ITS2) in *R. florida* to identify possible genetic boundaries among Caribbean populations.

Materials and methods

Samples were collected from four locations: Puerto Rico ($n = 46$), Florida ($n = 28$), Curaçao ($n = 7$), and Guadeloupe ($n = 3$) during 2006-07. Hereafter, samples from each location will be considered as different populations. Total genomic DNA was extracted using a DNA Easy kit (Qiagen). The amplification of the ITS region was performed with the primers A18S (GATCGAACGGTTTAG TGAGG; Takabayashi et al. 1998) and ITS-4 (TCCTCC GCTTATTGATATGC; Meroz-Fine et al. 2003). The amplification profile began with a denaturation step at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 45°C for 30 s, and 72°C for 1 min, ending with an extension step at 72°C for 5 min. The ITS region is one of the most widely used markers in molecular studies in corals (e.g., Vollmer and Palumbi 2004). However, molecular cloning is necessary to understand the intra-genomic variation of the ITS region. PCR products from 10 randomly selected individuals were ligated into the pGEM-T Easy Vector (Promega). Up to 14 clones from each of the 10 individuals were sequenced. Four of these 10 *R. florida* were from Lineage 1 and six from Lineage 2. All amplified templates were sequenced in both directions. The haplotypic (h) and genetic diversity (π) were estimated in Arlequin (Excoffier et al. 2005). Multi-allelic states in direct sequences were treated as degenerative sites, and gaps were regarded as a 5th character. Statistical parsimony networks were built in TCS (Clement et al. 2000) with the connection limit set at 95%.

Results

Eighty-four direct sequences of the ITS region from four populations of *Ricordea florida* were obtained and were collapsed into seven haplotypes (Table 1). Nucleotide sites with multiple base signals (e.g., *Y*) were considered identical to sequences with one base signal (either a clear *C* or *T*). The identity of sequences as corallimorpharian was verified against data published by Chen et al. (1995). The amplified region contained a portion of the 3′ end of 18S (20 bp), the ITS1 region (244–255 bp), all of 5.8S (164–167 bp), the ITS2 region (196–241 bp), and a portion of the 5′ end of 28S (15 bp). The sequenced region varied

Table 1 Locations, sampling size, and accession numbers of the ITS region of *Ricordea florida* obtained by direct sequencing

	Locations	Sampling size	GenBank accession numbers
Haplotype 1	Florida Bay, Florida	17	GQ465046–GQ465062
	La Parguera, Puerto Rico	41	GQ465063–GQ465079
			GQ465082–GQ465103
			GQ465105–GQ465106
Haplotype 2	La Parguera, Puerto Rico	1	GQ465080
Haplotype 3	La Parguera, Puerto Rico	1	GQ465081
Haplotype 4	La Parguera, Puerto Rico	1	GQ465104
Haplotype 5	La Parguera, Puerto Rico	2	GQ465107–GQ465108
	Curaçao	7	GQ465109–GQ465115
	Guadeloupe	2	GQ465116–GQ465117
	Hawk Channel, Florida	6	GQ465120–GQ465122
			GQ465124–GQ465126
Haplotype 6	Guadeloupe	1	GQ465118
Haplotype 7	Hawk Channel, Florida	5	GQ465119, GQ465123
			GQ465127—GQ465129

in length because of indels. The Florida population had three haplotypes ($H_d = 0.5741 \pm 0.0785$, $\pi = 0.3815 \pm 0.0192$), Puerto Rico had five haplotypes ($H_d = 0.2068 \pm 0.0791$, $\pi = 0.0068 \pm 0.0038$), Guadeloupe had two haplotypes ($H_d = 0.6667 \pm 0.3143$, $\pi = 0$), and Curaçao had one haplotype (Table 1).

Statistical parsimony analysis of seven haplotypes of the ITS region resulted in two disconnected networks (Fig. 2), representing hereafter Lineage 1 (L1) and Lineage 2 (L2). L1 was detected in Florida and Puerto Rico, and L2 was detected in Florida, Puerto Rico, Guadeloupe, and Curaçao (Fig. 2). The ITS region of L2 was longer compared to L1, mostly attributed to the length differences in ITS2, 237–243 bp (L2) vs. 196–197 bp (L1). The nucleotide ($\pi = 0.0256 \pm 0.0133$) and haplotype diversity ($H_d = 0.4229 \pm 0.1041$) of L2 ($N = 23$) showed higher values than L1 ($N = 61$; 0.0002 ± 0.0003; 0.0967 ± 0.0518, respectively), when using direct sequences. Cloning of the ITS region revealed some of the genetic diversity harbored within individuals and resulted in higher levels of divergence within both lineages compared to estimates of divergence resulted from direct sequences alone (Fig. 3). All of the 10 *R. florida* we cloned harbored multiple haplotypes (GenBank Accession Numbers: GQ465130–GQ465201). There were no individuals that harbored haplotypes from both L1 and L2. The pairwise distance between lineages (8.8–9.1%) was much higher than between individuals within each lineage (0.2–0.9%), when using either the cloned sequences alone (Fig. 3) or direct sequencing. The intra-genomic variability ranged from 0.6 to 0.7% (L1) and 0.2–0.9% (L2) in the cloning experiment, a fraction of the inter-lineage divergence values. The reported divergence values were not likely an artifact of Taq cloning errors since the Taq error estimates (about 0.01%; Eckert and Kunkel 1990) are much lower than our reported values. The within-individual pairwise sequence divergence ranged from 0.5 to 2% in ITS1, 0.3–1.3% in 5.8, and 0.5–1.3% in ITS2. The levels of variation among lineages were 7.5–10% in ITS1, 5.3–9.3% in 5.8, and 6.9–9.3% in ITS2.

Discussion

The genealogical analysis of the ITS region uncovered the presence of two distinct lineages (inter-lineage divergence 8.8–9.1%) in *Ricordea florida* with a partially overlapping distribution (Fig. 2). The ~9% divergence observed between the two lineages of *R. florida* resembles the typical differences found between anthozoan species. For comparison, divergence values of the ITS region between congeneric corallimorpharians ranged from 2.9% between *Rhodactis hoswesii* and *Rhodactis mussoides* to 19.2% between *Actinodiscus unguja* and *Actinodiscus nummiformis* (Chen et al. 1996). In other anthozoans, the ITS divergence between *Platygyra sinensis* and *P. pini* is ~14.3% (Lam and Morton 2003), and the inter-specific divergence among the Caribbean acroporids is about 13% (Vollmer and Palumbi 2004). As coalescence and homogenization time of ITS variants varies from species to species, the ITS

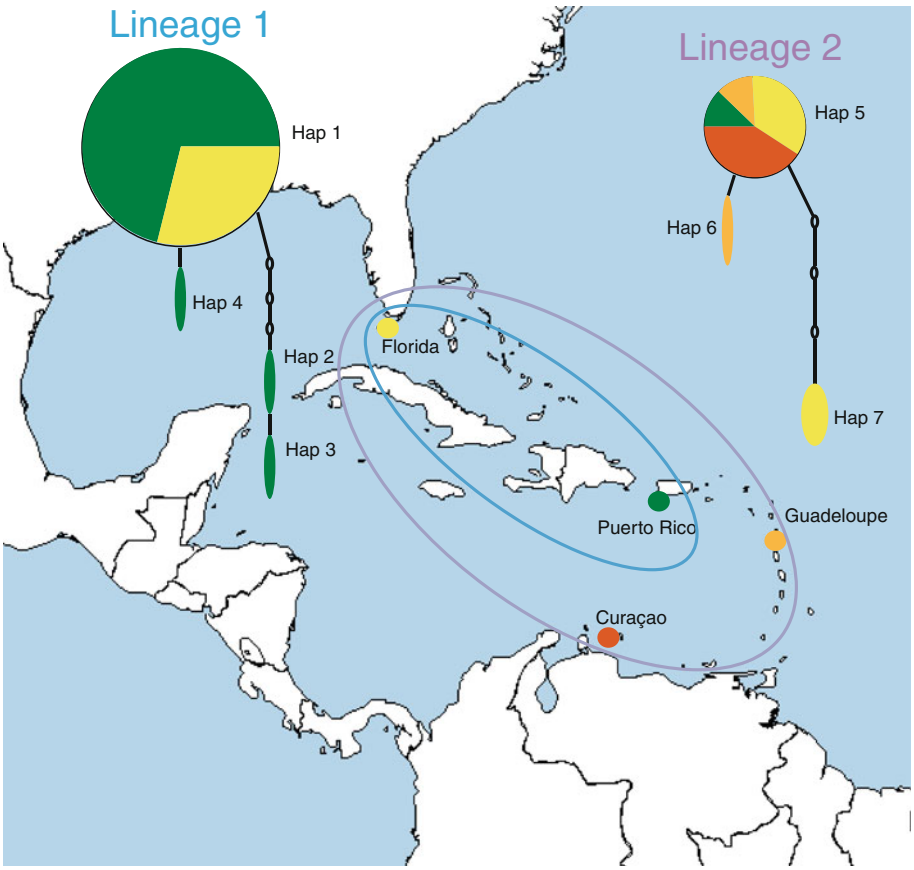

Fig. 2 Haplotype networks of the two lineages of *Ricordea florida* based on the ITS region. Unique haplotypes are shown as different ovals, and the size of the shape is proportional to the number of individuals sharing this haplotype. The small *open circles* between haplotypes represent mutational steps between alleles. Sampled locations are denoted by small *colored circles*, and geographic ranges of the two lineages are loosely depicted by the large *colored ovals*, corresponding to each lineage

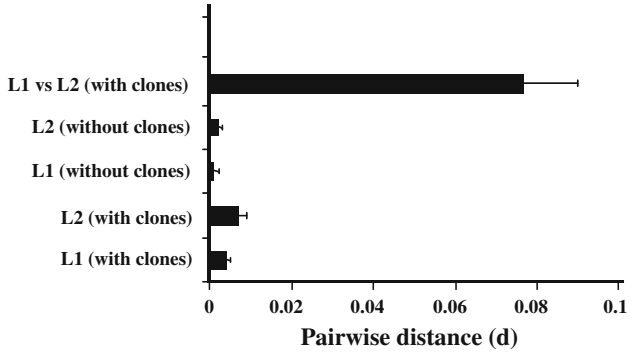

Fig. 3 Average pairwise distances within and between lineages, with and without cloning. The genetic distances have been corrected by the Tamura-Nei model of nucleotide substitution. *Error bars* denote 1 standard deviation

divergence among anthozoans will also be variable. The ~9% divergence between L1 and L2 falls between the intra-individual (7.6%) and intra-specific 10% rDNA variation found within *Acropora palmata* (Vollmer and Palumbi 2004); however, the Caribbean acroporids harbor unusual amounts of ITS variation (Chen et al. 2004).

Ricordea florida usually form colonies, consisted of 10–100 s of polyps in shallow waters. Specimens from deeper waters are either solitary polyps or in relative proximity to each other, but not in colonies (den Hartog 1980). All of the sampled *R. florida* in the present study were collected from shallow depths (3–8 m), where we did not detect an obvious depth difference between solitary polyps and colonial forms (Schizas, pers. obs). However, at 20–25 m depth in La Parguera, Puerto Rico, we observed only solitary polyps (Lado-Insua, pers. obs.). There was no relationship between the solitary and colonial forms and genetic lineage as each lineage was recovered from both forms. No obvious morphological differences were detected between lineages, except in coloration; however, a detailed morphometric study was not conducted. *Ricordea florida* of L2 exhibited khaki-colors rather than the bright colors (e.g., orange, red, bright green) usually seen in *R. florida.*

Additional sampling in these regions and other parts of the Caribbean and the use of other molecular markers might lead to the discovery of more lineages. Other relevant ecological data (e.g., depth, exposure, salinity, temperature) and morphometric characters (number, size and color of polyps, number and size of marginal and discal tentacles, etc.) should be recorded to test for possible ecological specialization to different habitats (Knowlton and Jackson 1994; Carlon et al. 2002; Prada et al. 2008) and sympatric divergence. Disruption of gene flow in proximate populations and

sympatric or parapatric speciation by ecological differentiation (Doebeli and Dieckmann 2003) is possible in the marine environment where very steep environmental gradients exist (e.g., depth, light, salinity).

Ecological implications and the use of *Ricordea florida* in the marine ornamental trade

If a goal of conservation biology is to preserve the evolutionary potential of species, then knowledge of basic biology and quantification of the genetic diversity is integral to any informed management policy. A large increase in fishing pressure has occurred for *R. florida* (Fig. 1), yet there is little information available for managers to determine acceptable harvest limits. While Florida has a management plan that includes a license cap and bag limits, landings of most species are well below the total allowable catch (Rhyne et al. 2009). In Haiti, the second most important source of *Ricordea* for the aquatic trade, fisheries are largely unregulated and harvesting of corallimorpharians will probably continue unchecked. *Ricordea florida* provides an example of a species in which a bag limit was recently established, but for which the number of license reporting landings is far below the capped limit. This could result in a regulated, yet uncontrolled fishery (Rhyne et al. 2009).

Are the two distinct lineages of *Ricordea florida* biologically meaningful for conservation? Genetic diversity has been overlooked in international conservation policy implementation (Laikre 2010). Conservation strategies now acknowledge not only the importance of conserving biodiversity at the species level but also the importance of maintaining the genetic diversity within these species (e.g., distinct genetic lineages or subdivided populations) (Moritz 2002; Caballero et al. 2010). There are at least two lineages in *R. florida,* and in addition, the absence of *R. florida* possessing sequence copies of both Lineages in sympatry provides strong evidence for reproductive isolation. Even if the two lineages do not represent different species, they are biologically meaningful and deserve separate management regulations by the marine aquarium trade. *Ricordea florida* is sought after because of the brilliant coloration of polyps; therefore, the brightly colored L1 will be heavily preferred over the relatively dull colored L2. The perceived unevenness in fishing effort may have adverse effects in the brightly colored L1, especially since the population size of each lineage is unknown.

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References

Caballero A, Rodríguez-Ramilo ST, Ávila V, Fernández J (2010) Management of genetic diversity of subdivided populations in conservation programmes. Conserv Genet 11:409–419

Carlon DB, Budd AF, Bonhomme F (2002) Incipient speciation across a depth gradient in a scleractinian coral? Evolution 56:2227–2242

Chadwick-Furman NE, Spiegel M (2000) Abundance and clonal replication in the tropical corallimorpharian *Rhodactis rhodostoma*. Invertebr Biol 119:351–360

Chen CA, Odorico DM, ten Lohuis M, Veron JE, Miller DJ (1995) Systematic relationships within the Anthozoa (Cnidaria: Anthozoa) using the 5′-end of the 28S rDNA. Mol Phylogenet Evol 4:175–183

Chen CA, Willis BL, Miller DJ (1996) Systematic relationships between tropical corallimorpharians (Cnidaria: Anthozoa: Corallimorpharia): Utility of the 5.8S and internal transcribed spacer (ITS) regions of the rRNA transcription unit. Bull Mar Sci 59:196–208

Chen CA, Chang CC, Wei NW, Chen CH, Lein YT, Lin HE, Dai CF, Wallace CC (2004) Secondary structure and phylogenetic utility of the ribosomal internal transcribed spacer 2 (ITS2) in scleractinian corals. Zool Stud 43:759–760

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1660

Daly M, Fautin DG, Cappola VA (2003) Systematics of the Hexacorallia (Cnidaria: Anthozoa). Zool J Linn Soc 139: 419–437

Daly M, Brugler MR, Cartwright P, Collins AG, Dawson MN, Fautin DG, France SC, McFadden CS, Opresko DM, Rodriguez E, Romano SL, Stake JL (2007) The phylum Cnidaria: a review of phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa 1668:127–182

den Hartog JC (1980) Caribbean shallow water Corallimorpharia. Zool Verhand Leiden 176:1–82

Doebeli M, Dieckmann U (2003) Speciation along environmental gradients. Nature 421:259–264

Eckert KA, Kunkel TA (1990) High fidelity DNA synthesis by the *Thermus aquaticus* DNA polymerase. Nucleic Acids Res 18:3739–3744

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinf Online 1:47–50

Fautin DG, Zelenchuk T, Raveendra D (2007) Genera of orders Actiniaria and Corallimorpharia (Cnidaria, Anthozoa, Hexacorallia), and their type species. Zootaxa 1668:183–244

FWC (2009) New rules approved for harvest of aquarium species. Florida Fish and Wildlife Conservation Commission http://myfwc.com/Newsroom/09/statewide/News_09_X_MarineLife.htm

Gerald RA, Roger S (1994) Indo-Pacific coral reef field guide tropical reef research. Calendar Print Pte Ltd, Singapore

Knowlton N, Jackson JBC (1994) New taxonomy and niche partitioning on coral reefs: Jack of all trades or master of some? Trends Ecol Evol 9:7–9

Laikre L (2010) Genetic diversity is overlooked in international conservation policy implementation. Conserv Genet 11: 349–354

Lam K, Morton B (2003) Morphological and ITS1, 5.8S, and partial ITS2 ribosomal DNA sequence distinctions between two species of *Platygyra* (Cnidaria: Scleractinia) from Hong Kong. Mar Biotech 5:555–567

León Y, Bjorndal KA (2002) Selective feeding in the hawksbill turtle, an important predator in coral reef ecosystems. Mar Ecol Prog Ser 245:249–258

Meroz-Fine E, Brickner I, Loya Y, Ilan M (2003) The hydrozoan coral *Millepora dichotoma*: speciation or phenotypic plasticity? Mar Biol 143:1175–1183

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst Biol 51:238–254

Muhando CA, Kuguru BL, Wagner GM, Mbije NE, Öhman MC (2002) Environmental effects on the distribution of corallimorpharians in Tanzania. Ambio 31:558–561

Prada C, Schizas NV, Yoshioka PM (2008) Phenotypic plasticity or speciation? A case from a clonal marine organism. BMC Evol Biol 8:47

Rhyne AL, Rotjan R, Bruckner A, Tlusty M (2009) Crawling to collapse: ecologically unsound ornamental invertebrate fisheries. Plos ONE 4:e8413. doi:10.1371/journal.pone.0008413

Takabayashi M, Carter DA, Loh WKW, Hoegh-Guldberg O (1998) A coral specific primer for PCR amplification of the internal transcribed spacer region in ribosomal DNA. Mol Ecol 7:925–931

Vollmer SV, Palumbi SR (2004) Testing the utility of internally transcribed spacer sequences in coral phylogenetics. Mol Ecol 13:2763–2772