GENETIC POPULATION STRUCTURE OF TWO SPECIES OF BRITTLE STARS WITH CONTRASTING LIFE HISTORIES

By

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ABSTRACT

Echinoderms display a wide array of life histories, which can have a profound effect in the dispersal potential and population structure of species. The brittle stars *Ophiocoma echinata* and *Amphipholis squamata* are commonly found in the shallow waters of the Caribbean Sea. The two species exhibit differing modes of development: *O. echinata* is a spawning species having asynchronous breeding cycles and *A. squamata* is a brooding species, viviparous and self-fertilizing hermaphrodite. Their overlapping geographic range offers the opportunity to compare their genetic population structure as brooders are expected to exhibit higher population subdivision than spawners. Mitochondrial (16S) and ribosomal nuclear DNA sequences (ITS-1) were recovered from 16 populations of *O. echinata* and five populations of *A. squamata* around the Caribbean and Western Atlantic. Results indicate that the spawning *O. echinata* harbors moderate levels of genetic variability in the 16S gene (163 specimens, 66 haplotypes). There is no significant population structure (16S; F_{ST} = 0.00236; P = 0.38319) in this wide geographic area. This pattern is further supported by the ITS-1 gene. *A. squamata* however, harbors high levels of genetic variability (63 specimens, 45 haplotypes) with two well supported lineages. One of these is a newly discovered lineage of *A. squamata* around the world. Significant population structure (16S; F_{ST} = 0.63867; P = 0.0000) was observed between Puerto Rico and Florida, a result supported by the ITS-1 data. At a finer geographic scale in southwestern Puerto Rico, population differentiation was observed in *A. squamata* (F_{ST} = 0.14845; p<0.01369). These data provide compelling evidence that life history traits influence the connectivity of geographically distant populations. The spawner *O. echinata* showed no population structure from samples as far as Bermuda, Curaçao, Panama and Belize whereas the brooder *A. squamata* is potentially a complex of at least 2 cryptic species with partially overlapping geographic distributions.

Keywords: Population structure, life history, ophiuroids, Caribbean

RESUMEN

Los echinodermos poseen una amplia variabilidad de historias de vida, lo cual pueden tener un efecto profundo en el potencial de dispersión y estructura poblacional de las especies. Las estrellas quebradizas *Ophiocoma echinata* y *Amphipholis squamata* son comúnmente encontradas en aguas someras del mar Caribe. Las dos especies exhiben diferentes modos de desarrollo: *O. echinata* es una especie que desova y posee ciclos asíncronos y *A. squamata* es una especie que incuba, vivípara y hermafrodita que se fertiliza a si misma. Debido a que coinciden en su rango geográfico esto ofrece la oportunidad de comparar su estructura poblacional, se espera que la especie que incuba exhiba más alta subdivisión poblacional que la especie que desova. Secuencias de ADN mitocondrial (16S) y ribosomal nuclear (ITS-1) fueron obtenidas de 16 poblaciones de *O. echinata* y cinco poblaciones de *A. squamata* alrededor del Caribe y el Atlántico oeste. Los resultados indican que la especie que desova *O. echinata* posee niveles moderados de variabilidad genética en el gen 16S (163 especímenes, 66 haplotipos). No existe estructura poblacional significativa (16S; F_{ST} = 0.00236; P = 0.38319) en esta amplia región geográfica. Este comportamiento es apoyado por el gen ITS-1. Al contrario, *A. squamata* posee altos niveles de variabilidad genética (63 especímenes, 45 haplotipos) con dos linajes bien soportado. Uno de los linajes es un nuevo reporte para *A. squamata* alrededor del mundo. Estructura poblacional significativa (16S; F_{ST} = 0.63867; P = 0.0000) fue observada entre Puerto Rico y Florida, resultados son soportados con los datos del gen ITS-1. En una escala geográfica fina en el sudoeste de Puerto Rico se observó una diferenciación poblacional en *A. squamata* (16S; F_{ST} = 0.14845; p<0.01369). Estos datos proveen evidencia convincente de que los rasgos de historia de vida influencias las tasas de conectividad de las poblaciones geográficamente. *Ophiocoma echinata* la especie que desova, no exhibe estructura poblacional incluso muestras tan distantes como Bermuda, Curaçao, Panamá y Belize donde *A. squamata* la especie incubadora muestra un complejo de por lo menos 2 especies cripticas con una coincidencia parcial en la distribución geográfica.

Palabras Clave: Estructura poblacional, historia de vida, ophiuroideos, Caribe

To my Family…..

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1. INTRODUCTION

The Caribbean region is one of the major biogeographic provinces of the tropical Atlantic (Spalding et al. 2007). The region is inhabited by >40 million people in 29 countries and its natural resources are under intense use by the increased human population in near shore cities and the subsequent increase in tourism and fisheries.

The deterioration of shallow-water marine habitats have rendered ecological and biodiversity studies more important than ever (Rivera-Monroy et al. 2004; Sullivan & Bustamante 1999). Marine organisms are under increasing stress directly or indirectly from anthropogenic activities such as overfishing, alteration and/or loss of habitat, pollution, disease outbreaks, and climate change due to global warming (Jackson et al. 2001; Harvell et al. 2002; 2007; Weil 2004).

Knowledge of the genetic population structure of species is required for sustainable management plans, since changes in population dynamics and demographics have direct consequences on a species' exploitability and sustainability. Maintenance of genetic variability in managed populations at historical levels has been promoted as one of the most significant attributes for the long term sustainability of marine protected areas (Palumbi 2003).

Studies of genetic structure reveal a great deal about the history of gene flow patterns and population subdivision, past demographic events (population expansion or decline, bottlenecks), past effective population sizes and possible genetic interaction with other species through hybridization. The patterns of genetic variation can also elucidate selection events (e.g. hitchhiking, negative selection, balancing selection) of the past that have shaped the species' genome. Studies of genetic structure of marine taxa have been changing our view of marine biodiversity by revealing unexpected cryptic species and high genetic diversity (Palumbi 1992; Knowlton 1993; Taylor & Hellberg 2003). In recent years, population genetics and phylogeographic studies in the Caribbean have become increasingly valuable tools for inferring historical and present day genetic patterns within marine species. The genetic structure of Caribbean species such as the scleractinian corals *Acropora palmata* (Baums et al. 2005) and *A. cervicornis* (Vollmer & Palumbi 2006), the sea urchin *Diadema antillarum* (Lessios et al. 2001), several benthic fish (e.g. Taylor & Hellberg 2003; Purcell et al. 2006) and the ophiurids

Ophiocomella ophiactodes and *Ophiocoma pumila* (Mladenov & Emson 1990) are some of the examples of recent Caribbean studies. There is an unexpected level of population differentiation in most Caribbean species, except e.g. *Diadema* (Lessios et al. 2001) and *Montastraea cavernosa* (Nunes et al. 2009) indicating that larval dispersal potential is not the only determinant of population structure. Recent studies demonstrated the importance of genetic data in determining local population structure and conditional dispersal distances for marine organisms (Collin 2001; Richards et al. 2007). Understanding patterns of genetic variation is becoming increasingly important for conservation, management and remediation efforts of marine communities because the level of biodiversity refers to the total of genetic characteristics in the structure of the species and it is distinguished from genetic variability.

Efforts to protect reefs are usually based on information of one target species (e.g. the endangered scleractinian coral *Acropora palmata*) with no consideration of other key species important in the function of the reef ecosystem. Realistically, the target species is used as proxy to preserve the whole reef ecosystem. Each reef is inhabited by 1000's of species displaying a bewildering variation of life histories. Life history characteristics may determine the ability of species to disperse and the long-term population structure (Riginos & Victor 2001; Galarza et al. 2009), therefore, the effects of life history strategies on the differentiation and speciation in marine organisms should be considered.

One of the most informative comparative studies of population structure is the inclusion of related species with contrasting life histories and modes of development (Mladenov & Emson 1990; Hellberg 1996; Arndt & Smith 1998). This approach reduces the effects of accumulated divergence of many traits among unrelated species, and provides a clear evolutionary outline for understanding the genetic structure of particular species (Johnson & Black 2006). Marine species display many reproductive patterns and modes of development with potential active/passive dispersal over short and long distances. These include gonochoric and hermaphroditic species, brooders (Sponer & Roy 2002), species with sexually produced planktonic larvae (Hunt 1993) or asexually produced larvae by fission (Uthicke et al. 1998), broadcast spawning of sexually produced gametes (Richards et al. 2007) and species which combine different patterns of reproduction and modes of development and dispersal. One of the most recent work in the influence of life history on the genetic structure was published by Teske et al. (2007), who

compared the genetic structure, demographic histories and levels of gene flow of regional lineages of southern African coastal invertebrates exhibiting planktonic, abbreviated and direct development. They concluded that the amount of genetic structure within marine biogeographic regions strongly depended on the presence or absence of free swimming larvae.

Echinoderms are an important component in the marine environment and can exert a considerable influence on the ecosystem (Hendler et al. 1995) and display a wide range of reproductive strategies. Studies have shown that echinoderms with longer pelagic larval duration often show less population differentiation than those with abbreviated larval development (Arndt & Smith 1998). Among the echinoderm classes, the reproductive habits of brittle stars (Class Ophiuroidea) are remarkably diverse and display a wide variety of life histories. The Ophiuroidea is the most diverse class of echinoderms with numerous representatives found in all habitats (sea grass, coral reef, mangrove, sandy bottom) of the shallow waters of the Caribbean Sea. Ophiurids without a larval dispersal stage display higher population subdivision than those with planktotrophic larvae (Johnson & Black 2006). Today there are many studies focusing on the population structure of marine invertebrates but only a subset of them takes into consideration the differing modes of development (e.g. Collin 2001; Johnson & Black 2006; Richards et al. 2007). It is only lately that attention has been placed on the larval dispersal potential and its effect on the genetic population structure (e.g. Hunt 1993; Arndt & Smith 1998; Uthicke et al. 1998).

In this study, a comparative approach is used to test the effects of life history traits or modes of development (spawning vs. brooding) on the population genetic structure of two species of ophiuroids to test the hypothesis of there are significant differences in the genetic population structure between the two brittle stars with contrasting life histories. *Ophiocoma echinata* (Lamarck, 1816) is a spawning species having asynchronous annual reproductive cycle with a prolonged breeding season. *Ophiocoma echinata* spawn ophiopluteus larvae which have grown to a six-armed stage supported by skeletal spicules and bordered externally by a ciliated band. Metamorphosis to the juvenile stage commences while the larvae are still planktonic (Hendler 1975; 1991). Ophioplutei generally have a planktonic existence of several weeks to months (Hendler 1975; 1991). *Amphipholis squamata* (Chiaje, 1829) on the other hand is a brooding, viviparous, outcrossing and self-fertilizing (selfing) hermaphrodite (Hendler et al. 1995).

Amphipholis squamata broods its larvae 2–4 months to a crawl-away juvenile stage, and therefore does not have free-living larvae. (Sponer & Roy 2002). Juveniles of *O. echinata* occur in filamentous algae with *A. squamata* (Hendler et al. 1995). The brittle stars *O. echinata* and *A. squamata* are abundant and commonly found in the Caribbean and having similar geographic range offering the potential for a comparative study of their genetic population structure.

1.1. LITERATURE REVIEW

Brittle stars are a diverse and successful lineage of marine benthic echinoderms, with approximately 2,000 described species around the world (Hendler et al. 1995), presenting a variety of feeding behaviors and reproductive strategies. Ecologically, brittle stars play an important role in marine communities like preventing the growth of algal mats on coral reefs and as a diet of many reef fishes. In the Caribbean, brittle stars can be found on coral reefs and soft sediments in large densities (Hendler et al. 1995). Two of the more abundant brittle stars in this region are *Ophiocoma echinata* and *Amphipholis squamata.* The ophiuroid *Ophiocoma echinata* (Lamarck, 1816) (Fig. 1) is a suspension and deposit feeder in the family Ophiocomidae. *Ophiocoma echinata* is distributed throughout the Caribbean inhabiting shallow coral rubble habitats. Reproductively, *O. echinata* is a gonochoric spawner with planktonic larvae and no sexual dimorphism (Hendler et al. 1995). *Amphipholis squamata* (Chiaje, 1829) (Fig. 2) is a small, bioluminescent, polychromatic brittle star in the family Amphiuridae. *A. squamata* is found in all of the world's oceans except the polar areas. It occupies a variety of habitats (algal turf, bryozoans, coral rubble, gravel, under boulders, sand, and mud) from the intertidal zone to at least 1,330 m depth (Hendler et al. 1995). *A. squamata* is a simultaneous hermaphroditic brooder (Hendler et al. 1995) and hence does not have free-living larvae. The cosmopolitan distribution of this species is surprising given that benthic marine invertebrates, such as brittle stars, typically rely on larvae for dispersal. However, detailed genetic studies of previously regarded cosmopolitan species usually discover the presence of cryptic species and/or a species complex (Todaro et al. 1996; Sponer et al. 2001; Le Gac et al. 2004; Glatzel & Königshoff, 2005; Boissin et al. 2008a; b).

Factors controlling, mechanisms of larval dispersal and the identification of dispersal barriers or biogeographic boundaries have important ecological and biogeographical consequences in population dynamics and the continuity of species (Vance 1973; Gilg & Hilbish 2003; Baums et al. 2006; Galarza et al. 2009). The reproductive strategies of organisms also play a major role in the population dynamics and the distribution of the species (Ramirez 2002). All reproductive phases can be influenced both by phylogeny and environmental conditions that exert selective pressure on life-history traits (Vance 1973). By using two species of ophiurids with different

reproductive patterns and modes of development, it is possible to isolate the effect of the shared phylogenetic history of the two species because the mode of development of marine invertebrates is thought to influence levels of population structure and the species distribution via differences in dispersal ability. Observed differences between the population structure of the two species may be solely attributed to their life histories rather than to phylogenetically influenced traits.

Genetic studies of brittle stars have been limited to taxonomic issues and molecular physiology based on aspects related to organ and system functions. For example, studies have focused on the energy allocated for regeneration by comparing the relative feeding levels of *Ophiocoma echinata* (Pomory & Lawrence 1999). Mladenov (1985) studied the development and metamorphosis of *Ophiocoma pumila* and Deheyn (2000) studied the seasonal variation and the effects of environmental factors in bioluminescence of *Amphipholis squamata*. These studies highlighted the important ecological roles but for a better understanding of the function of species within a community or ecosystem, studies incorporating the effects of the reproductive strategies are needed.

Several recent studies have compared the population genetic structure of ophiurids that have different life histories and modes of development. Mladenov & Emson's (1990) study highlighted the importance of life histories in the determination of genetic population structure of two closely related brittle stars, *Ophiocomella ophiactodes* and *Ophiocoma pumila* in Jamaica. Allozyme electrophoretic data indicated a sibling species relationship between these species (Mladenov & Emson 1990). The levels of genotypic diversity of *Ophiocoma pumila* were concordant to expectations for a sexually reproducing species. In contrast, the genetic diversity of *Ophiocomella ophiactodes* was significantly lower than expected for a sexually reproducing species. Sponer & Roy (2002) examined the phylogeography of *Amphipholis squamata*, using restriction fragment length polymorphisms (RFLP), patterns of nuclear and mitochondrial DNA from 16 coastal populations in New Zealand. They found that the dispersal ability of *A. squamata* is restricted regionally but sporadic long-distance dispersal through rafting increases local genetic variation and they discovered the presence of four major mtDNA lineages (A-D). The *A. squamata* complex is distributed worldwide but has limited dispersal potential.

Paradoxically, it undergoes selfing at extreme rates (Boissin et al. 2008b) which causes loss of genetic variability, but presumably is highly adaptable to new habitats.

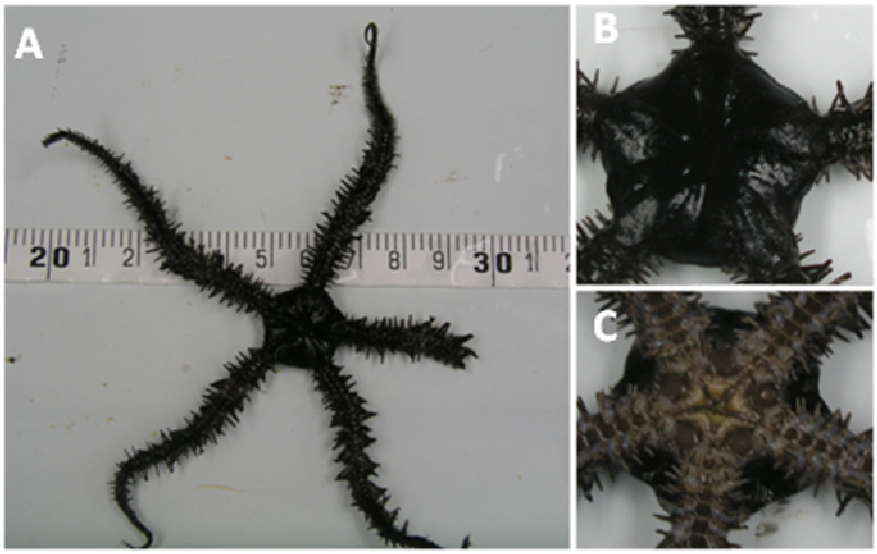

Figure 1. A. Details of the brittle star *Ophiocoma echinata*; B, dorsal; C, ventral. Photos by M. Rojas

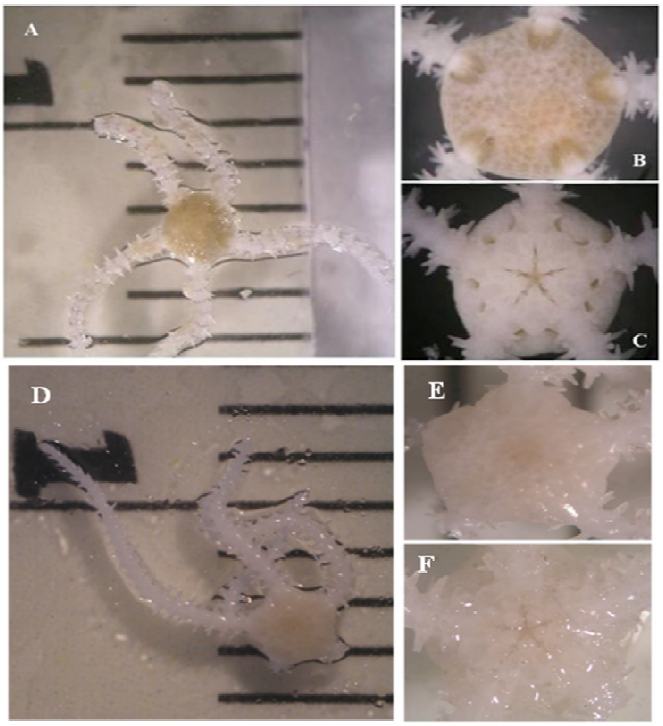

Figure 2. Details of the brittle star *Amphipholis squamata* showing Individual with 2 mm disk diameter. Lineage II: A, General View; B, dorsal; C, ventral. Lineage I: D, General View; E, dorsal; F, ventral. Photos by M. Rojas

2. MATERIALS AND METHODS

2.1. Sampling

To answer the questions or to test the hypothesis that there are significant genetic differences in population structure between two brittle stars with contrasting life histories, populations of the two ophiuroids species were sampled at two different geographic scales (Figs 3, 4): 1) a fine-scale sampling in southwest Puerto Rico to examine reef-to-reef population connectivity and, 2) a wider scale sampling at geographically distant locations throughout the Caribbean to maximize the possibility of detecting significant population structure. Within La Parguera, southwestern Puerto Rico, specimens were collected from the following mangrove cays: Enrique, Collado, Mario, Pelotas, Media Luna, San Cristobal and Turrumote. Additional samples were collected from the Caribbean: Puerto Rico islands of Mona, Culebra, and Vieques. For the large-scale study, ophiurids were collected from Belize, Navassa, Jamaica, Dominican Republic, Guana (BVI), Guadeloupe, Dominica, Barbados, St. Vincent, Key Largo (Florida), Curaçao, and Panama and Western Atlantic: Andros (Bahamas).

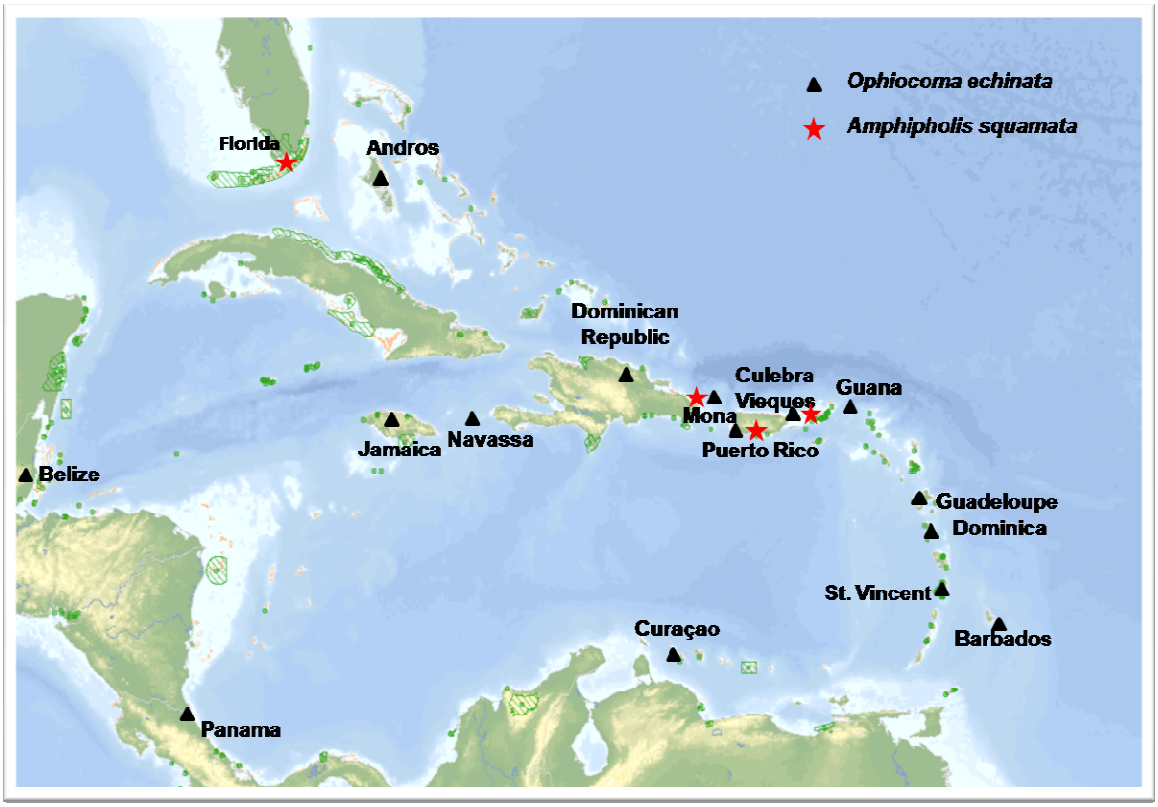

Figure 3. Sample locations in the Caribbean region. *Ophiocoma echinata* are indicated by black triangles and *Amphipholis squamata* are indicated by red stars.

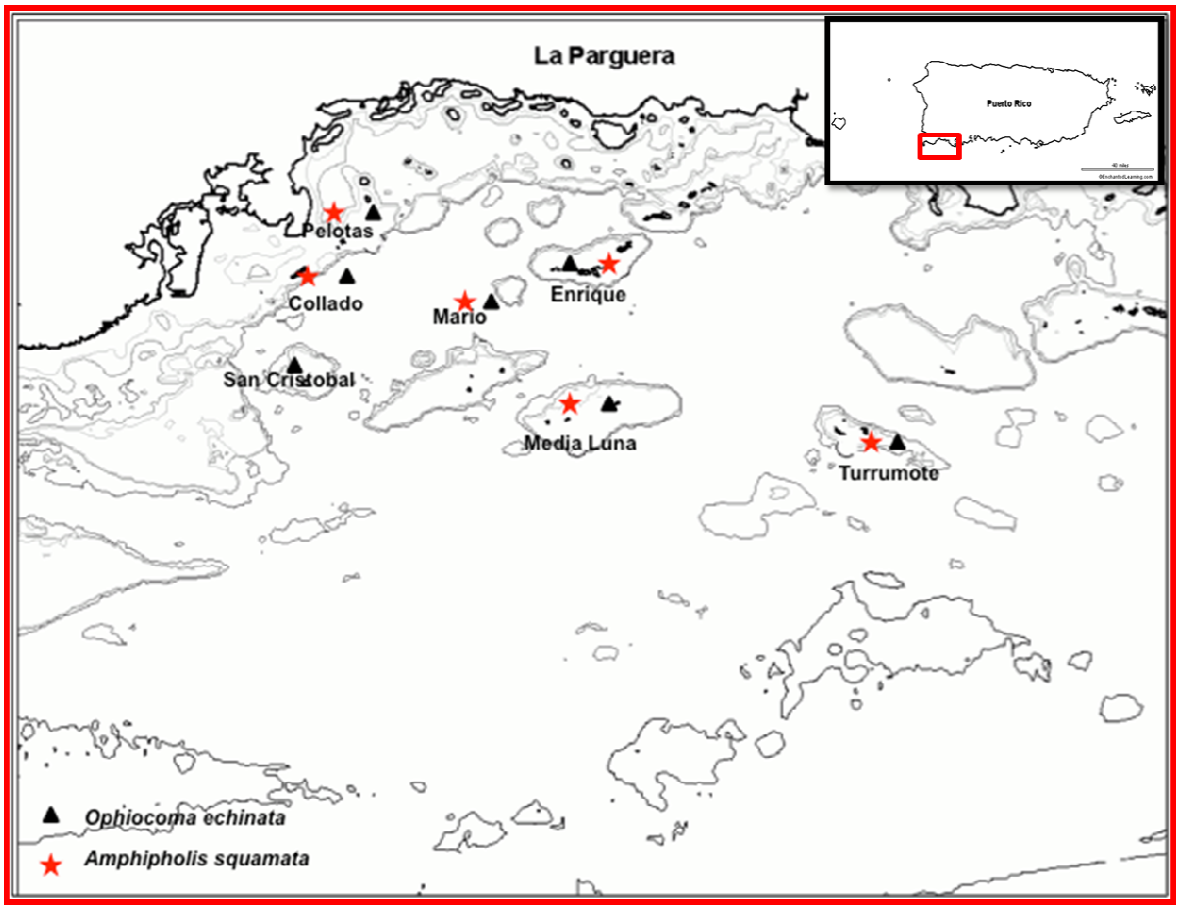

Figure 4. Sample locations in La Parguera, southwestern Puerto Rico. *Ophiocoma echinata* are indicated by black triangles and *Amphipholis squamata* are indicated by red stars.

Table 1. Sample localities of *Amphipholis squamata*

Localities	Reef	Latitude	Longitude	# Samples
La Parguera, PR	Enrique	N17°57.298’	W67°02.618’	12
	Media Luna	N17 °56.484’	W67°02.422’	4
	Turrumote	N17 °50.075’	W67 °01.047’	12
	Collado	N17°57.228’	W67°04.733’	11
	Pelotas	N17°57.542’	W67°04.416’	4
Vieques, PR	La Esperanza	N18°05’43.16”	W65°28’29.54”	8
Culebra, PR	Carlos Barbosa Reef	N18°19’31.52”	W65°19’49.45”	12
Mona Island, PR	Sardinera Beach	N18°05’21.60”	W 67°56’15.08”	11
Florida	Key Largo	N25°07’12.84”	W80°24’11.20”	7

Table 2. Sample localities of *Ophiocoma echinata*

Localities	Reef	Latitude	Longitude	# Samples
Belize		N17°12'25.72"	W88°15'53.57"	5
Navassa Island	Jamaica Channel	N18º 24.82'	W 75º 1.82'	5
Jamaica	Discovery Bay	N18°27'58.66"	W77°23'58.90"	8
Dominican Republic	Las Terrenas	N19°19'36.16"	W69°33'02.75"	12
La Parguera, PR	Media Luna	N17 °56.484'	W67°02.422'	12
	Pelotas	N17°57.542'	W67°04.416'	10
	Mario	N17 °57.176'	W67°03.416'	10
	San Cristobal	N 17°56.645'	W 67°04.63'	10
Vieques, PR	La Esperanza	N18°05'43.16"	W65°28'29.54"	10
Culebra, PR	Carlos Rosario Reserve	N18°19'31.52"	W65°19'49.45"	26
Mona Island, PR	Sardinera Beach	N18°05'21.60"	W 67°56'15.08"	4
	Carmelitas Beach	N18°10'09.40"	W 67°93'64.70"	6
Andros, Bahamas		N24º 54.81'	W77º 53.23'	5
Guana Is., British Virgin Is.	North Bay lagoon	N18º 28.91'	W64º 34.49'	5
Guadeloupe	Saint-François	N16°14'37.31"	W61°16'56.52"	12
Dominica	Scotts Head	N15°12'41.48"	W61°22'32.91"	9
Barbados	Speightstown	N13°15'14.06"	W59°39'34.81"	2
St. Vincent	Wallilabou	N13°15'50.70"	W61°15'34.43"	1
Curaçao	Jan Thiel	N12°03'53.99"	W68°53'05.27"	12
Panama	Isla Grande	N9°36'43.42"	W82°38'50.65"	3

Collection of specimens were made while snorkeling or SCUBA diving. *Amphipholis squamata* was collected by examination of algae, while *O. echinata* was collected by sorting through coral rubble. Specimens were placed in containers of seawater for transport to the laboratory where they were labeled and preserved in 100 % ethanol for DNA extraction, following the recommendations of Hendler et al. (1995). Individual specimens were identified using the taxonomic keys of Fell (1960), and Hendler et al. (1995). DNA was extracted from freshly collected and/or stored specimens using a DNeasy Tissue Kit (Qiagen, Inc.) following the protocol for animal tissue. DNA extractions were stored at –20 °C.

2.2. Primers and PCR amplification

Standard PCR amplifications were performed in an Eppendorf mastercycler. Portions of the mitochondrial gene 16S rRNA (16S) were amplified with the universal primers LR-j-12887 (alias 16Sbr) and LR-N-13398 (alias 16Sar; Simon et al. 1994). The nuclear ribosomal region, internal spacer region (ITS-1) was amplified with universal nuclear ribosomal primers. following Fotlz et al. (2004). The PCR mix for the mtDNA and nuclear DNA was identical for *O. echinata* and *A. squamata*, and contained 1.0 µl of each primer (10 µM/µl each), 25 µl 2X PCR Taq MasterMix and 22 µl of $_{dd}$H2O in each tube. Each reaction contained 1.0 µl of DNA template, for a final volume of 50 µl in each PCR tube. The PCR success was verified by running 5µl of the amplicon on a 1% TBE agarose gel electrophoresis and visualized under UV light and captured digitally. The same PCR conditions were used to amplify the 16S and ITS-1 genes of *O. echinata*: initial denaturation at 94 °C for 2 min, then at 94 °C for 30 sec, annealing at 45 °C for 30 sec, extension at 72 °C for 60 sec, repeated 30 times, and the final extension at 72 °C for 10 min. The same PCR conditions were used to amplify the 16S and ITS-1 genes of *A. squamata*: initial denaturation at 94 °C for 3 min, then at 94 °C for 30 sec, annealing at 49 °C - 60°C for 30 sec, extension at 72 °C for 30 sec for 35 cycles, and the final extension at 72 °C for 5 min. PCR reactions were cleaned from excess dNTPs, primers and other impurities by the enzymatic treatment ExoSap. Three µl of the EXOSAP solution (0.45µl SAP, 1u/µl; 0.30 µl Exonuclease, 20u/µl; 2.25 µl of ddH20) were added for every 12 µl of PCR product and incubated at 37 °C for 30 min and at 80 °C for 15 min. The quality and quantity of the ExoSap method the product was evaluated in a 1% agarose gel. Sequencing reactions with each of the primers were prepared with

the Big Dye sequencing chemistry. Ethanol precipitated sequence reactions were loaded in an ABI 3130xl Genetic Analyzer. DNA sequencing trace files were processed with CodonCode Aligner for base calling, quality assessment, contig assembly, visualization and manual editing.

2.3. Genetic Analysis

Edited DNA sequences were imported in MacClade 4.08 (Maddison & Maddison 2005) to derive a homologous alignment. Aligned sequences were imported to DnaSP (Librado & Rozas 2009) for general statistical analysis, including the estimators of nucleotide diversity π and θ. DNA neutrality tests such as Tajima's D (Tajima 1989) were computed to test for deviation from the neutral model of molecular evolution (Kimura 1968), as implemented in DnaSP. Gene genealogies of the samples will be derived using NJ (Saitou & Nei 1987) as implemented in MEGA4 (Tamura et al. 2007). Haplotype networks based on the parsimony method were constructed with TCS (Clement et al. 2000). For population structure analysis, Φ_{ST} were calculated based on the average number of pairwise nucleotide differences within and between island populations in Arlequin v3.5 (Excoffier et al. 2010). The analyses of molecular variance AMOVA (Excoffier et al. 1992) were performed in Arlequin. The significance of Φ-statistics was assessed by 10,000 permutations of groups and haplotypes. The Bonferroni correction was used for multiple testing errors in population pairwise comparisons. Haplotypes from each species were imported into PAUP* 4.0b10 (Swofford 2002) to construct maximum likelihood (ML) genealogies and to MrBayes v.3.2 (Ronquist & Huelsenbeck 2003) to construct the MCMC-based Bayesian (BI) genealogies. Bayesian analysis was run for 2,000,000 generations, four independent chains, sampling every 1000 generations and discarding 15% of the sampled trees. Clade support for ML was evaluated with 100 bootstrap replicates (Felsenstein 1985) using the fast step-wise search and for BI with posterior probabilities (p*P*). Data specific models of nucleotide evolution were evaluated in ModelTest (Posada & Crandall 1998) by the AIC criterion and were applied to the ML, BI, AMOVA tests and pairwise F_{ST} comparisons.

3. RESULTS

3.1. *Ophiocoma echinata*

A portion of the mitochondrial gene 16S (493 bp) and a portion of the nuclear ribosomal gene ITS-1 (417 bp) was obtained from 163 and 153 specimens, respectively, from 16 locations of the wider Caribbean area (Fig. 3, 4). Overall, 66 haplotypes were identified from 153 sequences of 16S mitochondrial gene and 25 haplotypes from 163 sequences of ITS-1 nuclear gene of *O. echinata*. The nucleotide diversity indices π and θ were similar for all the locations, varying from 0.01 to 0.02 (Table 3). A relatively high haplotype diversity (Hd) and relatively low nucleotide diversity was estimated. Tajima's D neutrality tests showed significantly negative departures from neutrality in La Parguera, Mona, Jamaica, Guadeloupe and Curaçao indicative of past population expansion (Aris-Brosou & Excoffier 1996). *Ophiocoma echinata* ITS-1 sequences exhibited few polymorphic sites so most of the genetic diversity values were close to 0. Overall the genetic diversity for *O. echinata* in the Caribbean sample area was low. For the 16S gene, the highest values of π were found in *O. echinata* at Mona population and θ in La Parguera population (Table 3). The lowest values of π of *O. echinata* were found in the samples from Navassa Island and θ in Andros Island (Table 3).

Table 3. Genetic diversity and summary statistics of *Ophiocoma echinata* based on 16S/ITS-1 sequences. N: number of samples, H: number of haplotypes, S: segregation sites, Hd: haplotype diversity, π: nucleotide diversity, θ: Theta and Tajima's D. Significant values are represented by ***, ** and * for $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

Localities	N	H	S	Hd	π	θ	Tajima's D
Belize	3/5	1/1	0/0	0.0000/0	0.0000/0	0.0000/0	0.0000/0
Jamaica	8/8	6/1	8/0	0.8929/0	0.0066/0	0.0101/0	-1.7012*/0
Navassa Island	5/5	3/1	2/0	0.7000/0	0.0020/0	0.0026/0	-0.9726/0
Dominican Republic	7/11	3/2	4/0	0.7143/0	0.0037/0	0.0044/0	-0.5976/0
Mona Island, PR	9/10	9/1	120/0	1.0000/0	0.0992/0	0.1310/0	-1.5836*/0
La Parguera, PR	42/43	16/1	23/0	0.5923/0	0.0033/0	0.0165/0	-2.5922***/0
Vieques, PR	6/10	2/1	3/0	0.3333/0	0.0029/0	0.0051/0	-1.2331/0
Culebra, PR	26/26	7/1	8/0	0.5200/0	0.0021/0	0.0667/0	-1.9120/0
Andros Island	4/5	2/1	1/0	0.5000/0	0.0016/0	0.0014/0	-0.6124/0
Guana Island, BVI	5/5	2/1	3/0	0.0039/0	0.0033/0	0.0039/0	-1.0485/0
Guadeloupe	11/12	6/1	12/0	0.7273/0	0.0065/0	0.0119/0	-2.0299**/0
Dominica	9/8	5/1	24/0	0.7222/0	0.0205/0	0.0276/0	-1.4668/0
Barbados	2/2	2/1	1/0	1.0000/0	0.0000/0	0.0000/0	0.0000/0
St. Vincent	1/1	1/1	0/0	1.0000/0	0.0000/0	0.0000/0	0.0000/0
Curaçao	12/12	7/2	10/3	0.8333/0.1667	0.0050/0.0014	0.0091/0.0038	-1.8740*/-1.6293
Panama	3/3	1/1	0/0	0.0000/0	0.0000/0	0.0000/0	0.0000/0

3.1.1. Network Analysis

Twenty-five distinct haplotypes were derived from ITS-1. Maximum parsimony networks (Fig. 5) were constructed with the TCS computer program (Clement et al. 2000) for *O. echinata* and *A. squamata* for ITS-1 nuclear gene and 16S mitochondrial gene respectively. Due to the high number of haplotypes of *O. echinata* 16S gene and *A. squamata* ITS-1, the parsimony networks are not presented. The TCS analysis suggested that the most common haplotype of *O. echinata* was also the most ancestral to the other sequences. Two alternative connections or loops to each group were created, suggesting the presence of homoplasy in the data set. Hap 1 and Hap 5 are the most widespread geographically and most numerous of all haplotypes found in *O. echinata.*

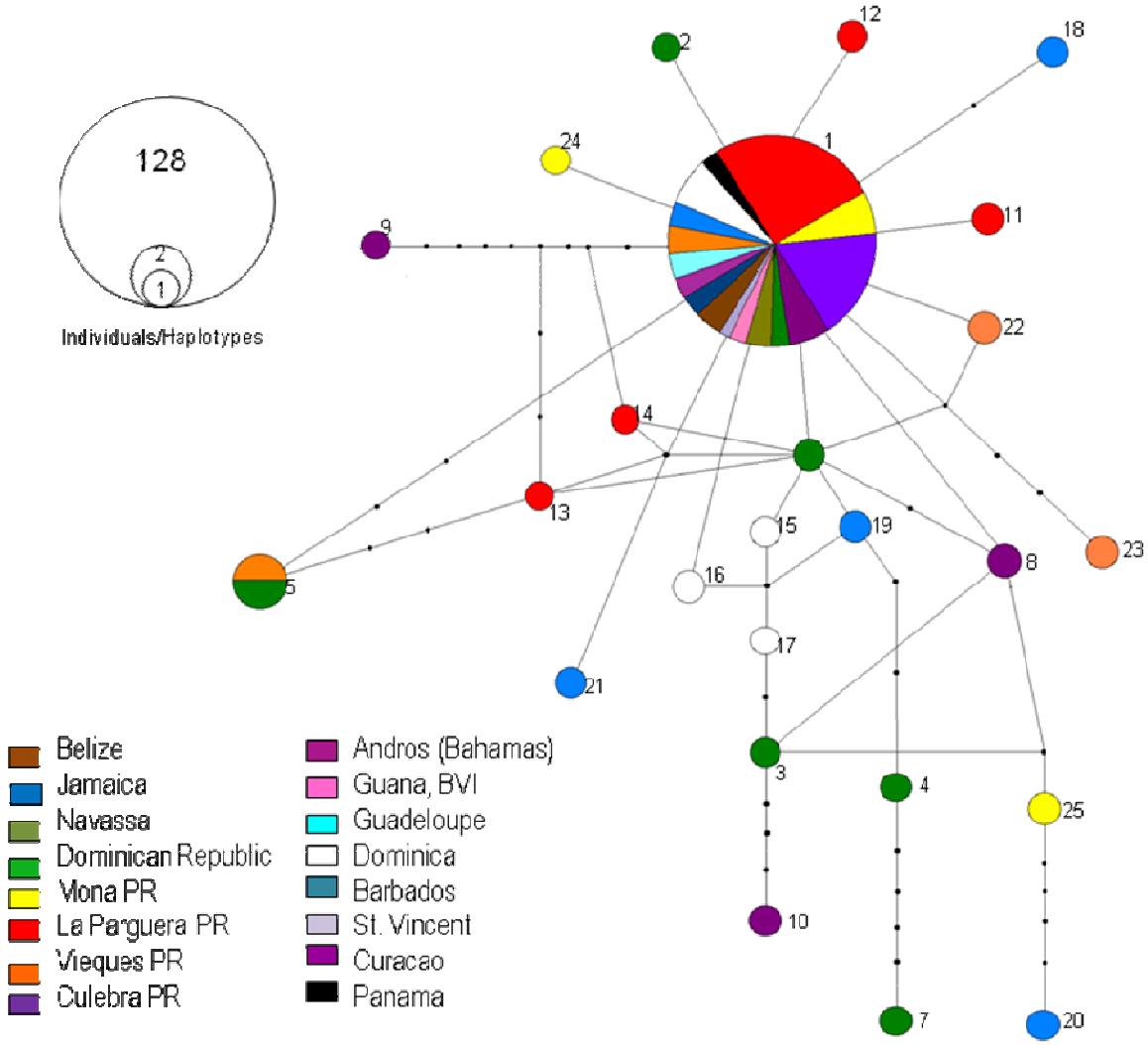

Figure 5. Parsimony network tree of 25 haplotypes of *O. echinata* based on ITS-1. Each circle represents a unique haplotype; the size of the circle is proportional to the observed number of sequences for the corresponding haplotype, the number close to each circle represents the number of the haplotype. The minimum number of mutational steps is represented by the small black circles.

3.1.2. Genealogy

Genealogies based on the 16S gene (Fig. 6) were constructed with ML and BI, corrected with the Tamura & Nei substitution model. The specific attributes of the model are as follows: unequal base frequencies (A = 0.26, C = 0.20, G = 0.22, T = 0.32), probability of invariable sites = 0.76, gamma distribution parameter of $\alpha = 2.7467$ and Ti/Tv ratio = 0.7580. There was very little resolution in both the ML and BI tree. ML and BI genealogies (Fig. 7) for ITS-1 gene constructed with the Tajima & Nei substitution model. The specific model attributes are as follows: unequal base frequencies (A = 0.19, C = 0.25, G = 0.32, T = 0.25), probability of invariable sites = 0, gamma distribution parameter of $\alpha = 0.0395$ and Ti/Tv ratio = 0. Similarly to the 16S trees, there was no resolution in the ITS-1 topology.

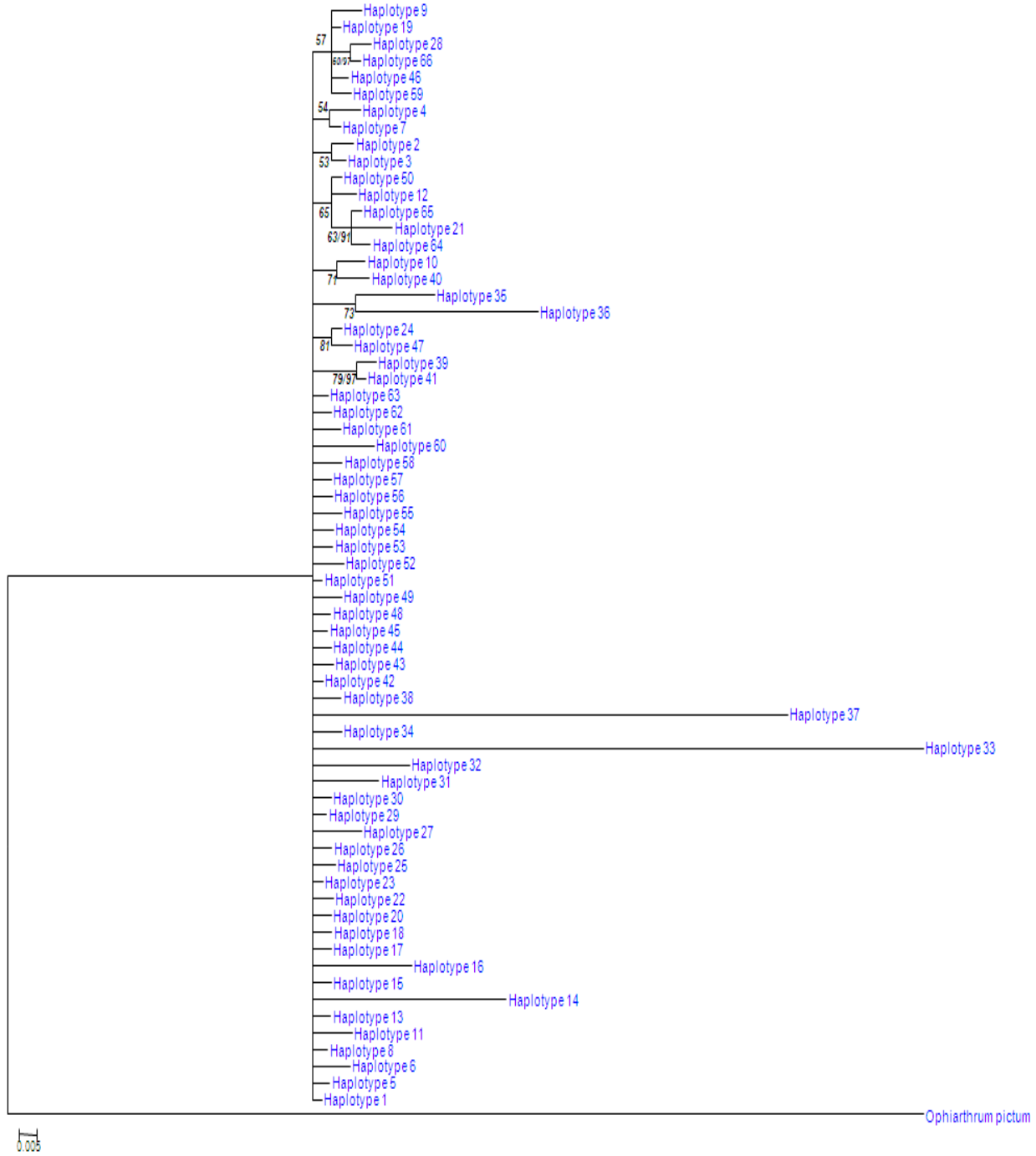

Figure 6. Maximum likelihood genealogy of 66 *Ophiocoma echinata* haplotypes based on 16S.Bootstrap Values and Bayesian posterior probabilities are in ***bold italics*** below. *Ophiarthrum pictum* was used as outgroup.

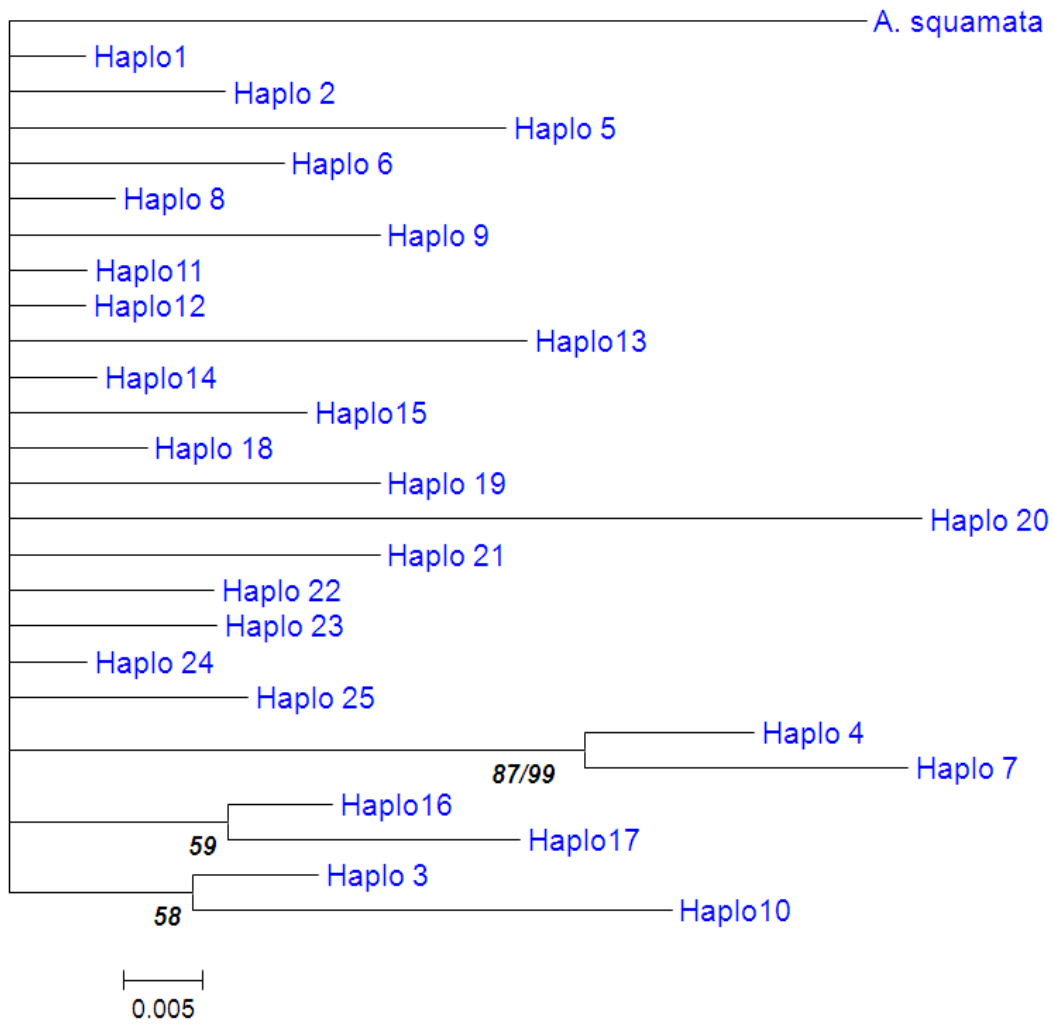

Figure 7. Maximum likelihood genealogy of 25 *Ophiocoma echinata* haplotypes based on ITS-1. Bootstrap values and Bayesian posterior probabilities are in ***bold italics*** below. *Amphipholis squamata* was used as outgroup.

3.1.3. Analysis of Molecular Variances (AMOVA)

An AMOVA test was applied to a total of 16 populations of *O. echinata* across the wider Caribbean: Belize, Navassa, Jamaica, Dominican Republic, Mona PR, La Parguera PR, Vieques PR, Culebra PR, Guana BVI, Andros, Guadeloupe, Dominica, Barbados, St. Vincent, Curaçao and Panama (Tables 4, 6). Variance analysis of *O. echinata* indicated that variation within populations was larger (99.22%) than among populations (0.78%). These results yield an F_{ST} estimate of 0.00781 for 16S gene and 0.0000 for ITS-1 gene indicating no significant population differentiation (F_{ST} = -0.01215; *P* value <0.32649). However, some populations were significantly different across the Caribbean (Table 5). No differentiation was detected among the pairwise comparisons in ITS-1 (Table 7).

Table 4. Analysis of molecular variance (AMOVA) of *O. echinata* based on 16S gene. Comparisons were made within the western Atlantic and Caribbean basin, among populations and within populations. Populations were sampled from Belize, Navassa, Jamaica, Dominican Republic, Mona PR, La Parguera PR, Vieques PR, Culebra PR, Guana BVI, Andros, Guadeloupe, Dominica, Barbados, St. Vincent, Curaçao and Panama.

Source of Variation	**d.f.**	**Sum of Squares**	**Variance components**	**Percentage of variation**	F_{ST}	***P* value**
Western Atlantic and Caribbean						
Among populations	15	28.375	0.00438 Va	0.24		
Within populations	137	253.850	1.85292 Vb	99.76		
Total	152	282.224	1.85730		**0.00236**	**< 0.38319**
La Parguera (Fine scale)						
Among populations	3	1.865	-0.00636 Va	-0.93		
Within populations	38	26.160	0.68842 Vb	100.93		
Total	41	28.026	0.68207		**-0.00932**	**<0.88954**

Table 5. Pairwise F_{ST} comparisons (corrected by the Tamura & Nei model) of *O. echinata* 16S gene populations assigned: 1. Belize, 2. Jamaica, 3. Navassa, 4. Dominica Republic, 5. Mona PR, 6. La Parguera PR, 7. Vieques PR, 8. Culebra PR, 9. Andros, 10. Guana, 11. Guadeloupe, 12. Dominica, 13. Barbados, 14. St Vincent, 15. Curaçao and 16. Panama. Significant values ($p<0.05$) in **bold**, * Significant after Bonferroni correction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0.00000															
2	-0.19975	0.00000														
3	-0.13441	-0.07863	0.00000													
4	0.02263	0.07145	0.11519	0.00000												
5	-0.18478	0.00293	-0.07993	-0.02985	0.00000											
6	-0.19743	0.01445	-0.04710	**0.10795**	**0.17437***	0.00000										
7	-0.15385	-0.03615	-0.00171	0.11740	-0.05284	-0.00561	0.00000									
8	-0.17381	0.02818	-0.01826	**0.17646**	**0.11767***	-0.00882	0.04011	0.00000								
9	-0.09091	-0.08782	-0.01151	0.10101	-0.11634	-0.09736	-0.02778	-0.11137	0.00000							
10	-0.13208	-0.02549	0.00058	0.11220	-0.07448	-0.02033	0.00649	0.00644	-0.15352	0.00000						
11	-0.19106	-0.00837	-0.04904	**0.05925**	**0.01693**	0.01667	-0.05460	0.01816	-0.12043	-0.05176	0.00000					
12	-0.16269	0.00530	-0.06666	0.02755	0.00384	**0.12965**	-0.01748	**0.12163**	-0.08512	-0.04271	0.03408	0.00000				
13	0.25000	-0.20249	0.07893	0.13410	-0.26891	0.00027	0.03583	0.13454	0.14134	0.01403	-0.10073	-0.21207	0.00000			
14	0.00000	-1.05658	-1.00485	-0.60127	-1.01024	-0.99540	-1.00000	-0.93876	-1.00000	-1.00000	-1.00950	-0.95971	-1.00000	0.00000		
15	-0.17712	0.00267	-0.05618	**0.08081***	**0.02677**	0.01251	-0.01007	0.02379	-0.10425	-0.03385	0.00151	0.03768	-0.05695	-0.97124	0.00000	
16	0.00000	-0.19975	-0.13441	0.02263	-0.18478	-0.19743	-0.15385	-0.17381	-0.09091	-0.13208	-0.19106	-0.16269	0.25000	0.00000	-0.17712	0.00000

Table 6. Analysis of molecular variance (AMOVA).of *Ophiocoma echinata,* based on the ITS-1 gene. Comparisons were made within the western Atlantic and Caribbean basin, among populations and within populations. Populations were sampled from Belize, Navassa, Jamaica, Dominican Republic, Mona PR, La Parguera PR, Vieques PR, Culebra PR, Guana, Andros, Guadeloupe, Dominica, Barbados, St. Vincent, Curaçao and Panama.

Source of Variation	d.f.	Sum of Squares	Variance components	% of variation	F_{ST}	*P* value
Western Atlantic and Caribbean						
Among populations	15	0.758	-0.00068 Va	-1.21		
Within populations	147	8.388	0.05706 Vb	101.21		
Total	162	9.147	0.05638		**-0.01215**	**<0.45357**
La Parguera (Fine scale)						
Among populations	3	0.234	0.00071 Va	1.00		
Within populations	39	2.739	0.07024 Vb	99.00		
Total	42	2.973	0.07096		**0.01004**	**<0.70772**

Table 7. Pairwise F_{ST} comparisons (corrected by the Tajima & Nei model) of the *O. echinata* ITS-1 gene. 1. Belize, 2. Jamaica, 3. Navassa, 4. Dominican Republic, 5. Mona PR, 6. La Parguera PR, 7. Vieques PR, 8. Culebra PR, 9. Andros, 10. Guana BVI, 11. Guadeloupe, 12. Dominica, 13. Barbados, 14. St. Vincent, 15. Curaçao and 16. Panama. Significant values ($p<0.05$) in **bold**.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0.00000															
2	0.00000	0.00000														
3	0.00000	0.00000	0.00000													
4	-0.18705	-0.03165	-0.08911	0.00000												
5	0.00000	0.00000	0.00000	-0.00917	0.00000											
6	0.00000	0.00000	0.00000	0.15203	0.00000	0.00000										
7	0.00000	0.00000	0.00000	-0.00917	0.00000	0.00000	0.00000									
8	0.00000	0.00000	0.00000	0.08626	0.00000	0.00000	0.00000	0.00000								
9	0.00000	0.00000	0.00000	-0.08911	0.00000	0.00000	0.00000	0.00000	0.00000							
10	0.00000	0.00000	0.00000	-0.12532	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000						
11	0.00000	0.00000	0.00000	0.00826	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000					
12	0.00000	0.00000	0.00000	-0.03165	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000				
13	0.00000	0.00000	0.00000	-0.32530	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000			
14	0.00000	0.00000	0.00000	-1.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000		
15	-0.18919	-0.03733	-0.09272	0.00966	-0.01617	0.12851	-0.01617	0.07017	-0.09272	-0.12821	0.00000	-0.03733	-0.32663	-1.00000	0.00000	
16	0.00000	0.00000	0.00000	-0.18705	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-0.18919	0.00000

3.2. *Amphipholis squamata*

Individuals of *A. squamata* from two color varieties (beige and black) were collected under boulders from Key Largo, Florida and in the crevices of *Halimeda opuntia* algae in Puerto Rico. A portion of the gene 16S (624 bp) and a portion of the ITS-1 gene (548 bp) was obtained from 63 and 81 specimens, respectively, from five locations (Figs 3, 4). High levels of polymorphism were observed in all five locations of *A. squamata,* 45 haplotypes were identified from 63 16S sequences and 56 haplotypes were identified from 81 ITS-1 sequences. These data showed high haplotype diversity (Hd) and relatively low nucleotide diversity (π) in both genes. Tajima's D was significantly negative for both genes in Culebra and La Parguera (Table 8), indicative of past population expansion (Aris-Brosou & Excoffier 1996).

Table 8. Genetic diversity and summary statistics of *Amphipholis squamata* based on 16S/ITS-1 sequences. N: number of samples, H: number of haplotypes, S: segregation sides, Hd: haplotype diversity, π: nucleotide diversity, θ: Theta and Tajima's D, Significant values are represented by ***, ** and * for P < 0.001, P < 0.01 and P< 0.05, respectively.

Localities	N	H	S	Hd	π	θ	Tajima's D
Key Largo FL	3/7	3/2	21/3	1.0000/0.4762	0.03234/0.0029	0.0000/0.0017	0.0000/-0.2749
Mona, PR	8/11	5/4	16/8	0.7857/0.4909	0.0133/0.0033	0.0150/0.0139	-0.4732/-2.1233**
Parguera, PR	32/43	18/9	193/29	0.8367/0.3776	0.0869/0.0042	0.1521/0.0116	-1.6266*/-2.5160***
Vieques, PR	8/4	1/4	0/34	0.0000/1.0000	0.0016/0.0152	0.0018/0.0158	0.0000/-0.4025
Culebra, PR	12/6	2/1	97/0	0.1667/0	0.0465/0	0.0657/0.0020	-2.3140***/0.3106

3.2.1. Network Analysis

The haplotype network constructed with the 16S gene (Figure 8) revealed clustering of haplotypes of the same geographic origin, and almost every sequence was a unique haplotype. Forty-five haplotypes (37 singletons) divided into three separated haplotype networks and 13 unconnected haplotypes to any network. Network 1 included 27 haplotypes from a total of 38 ophiurids. Network 2 included three haplotypes (1 ophiurid from Turrumote and 3 ophiurids from Collado, both locations in La Parguera, PR) and network 3 included three haplotypes from nine individuals all collected from Culebra PR. Unconnected haplotypes correspond to samples collected from Enrique (n=5), Collado (n=5), Turrumote (n=1), Pelotas (n=1) and Florida (n=1). Haplotype 45 was the representative haplotype (n=5) for *A. squamata*, and was restricted to Culebra.

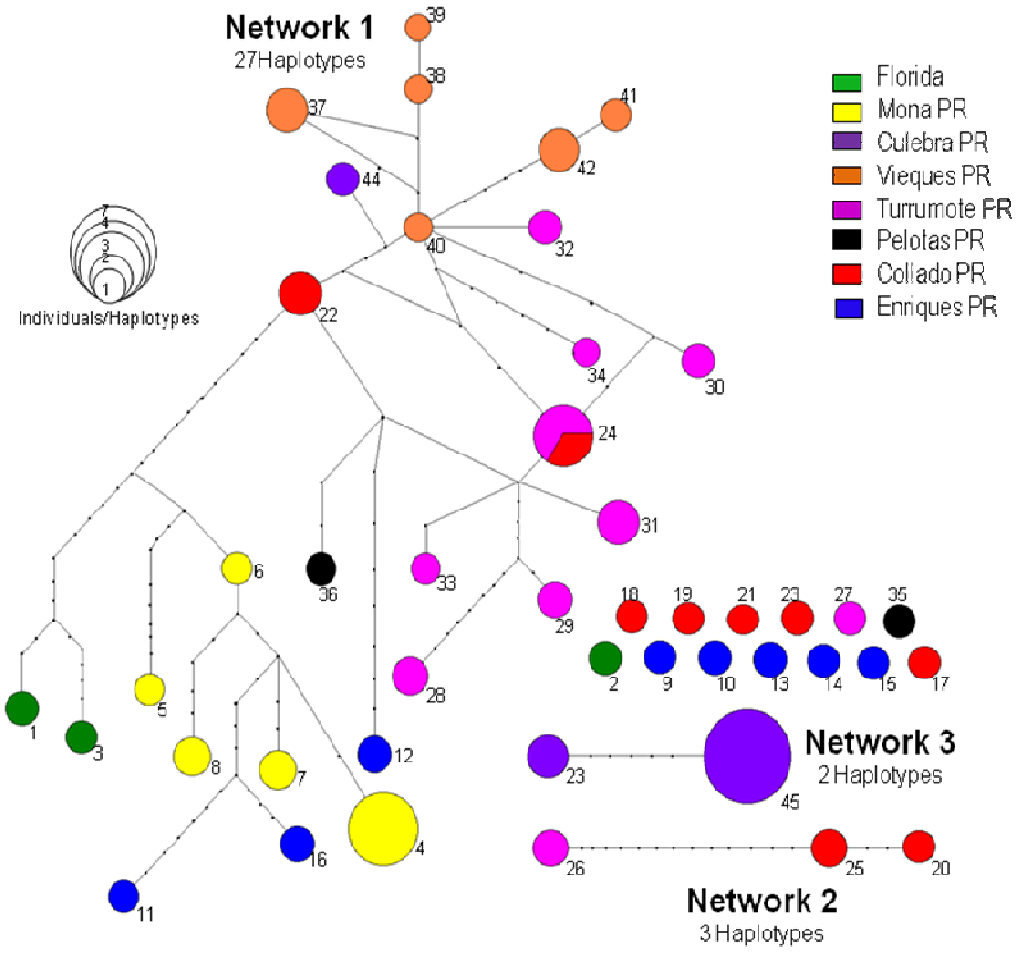

Figure 8. Parsimony network tree of 45 *A. squamata* haplotypes based on the 16S gene. Each circle represents a unique haplotype; the size of the circle is proportional to the observed number of sequences for the corresponding haplotype, the number close to each circle represent the number of the haplotype. The minimum number of steps is represented by the small black circles; all locations are in Puerto Rico except those from Florida.

3.2.2. Genealogy

ML and BI genealogies based on 16S gene (Figs. 9) were constructed with the Tamura & Nei substitution model as suggested by Model Test. The following model attributes were selected: unequal base frequencies (A = 0.32, C = 0.19, G = 0.16, T = 0.33), probability of invariable sites = 0, gamma distribution parameter of α=0.8576 and Ti/Tv ratio = 0. Gene genealogy analysis of *A. squamata* based on ML and BI genealogies of 16S gene revealed the presence of at least two highly differentiated lineages (Fig. 9). The most common lineage (lineage II) corresponds to Lineage 5 (E. Boissin, pers. comm.) and is genetically more diverse than the less common lineage (Table 8). The clade comprised of mostly sequences from Culebra is highly supported (Pp =100, Bp = 100). These 2 clades correspond to the sequence groups recovered in the haplotype analysis (Fig 8). The tree splits in two well supported lineages: Lineage I (bootstrap value 100/100%) and II (bootstrap value 75/100%). II reported as E (tropical lineage of *A. squamata*) by Boissin (unpub. Data). This lineage is subdivided in additional well-defined clades but there is no indication that these subclades are defined geographically. The ITS-1 genealogy (Fig. 10) does not support these lineages.

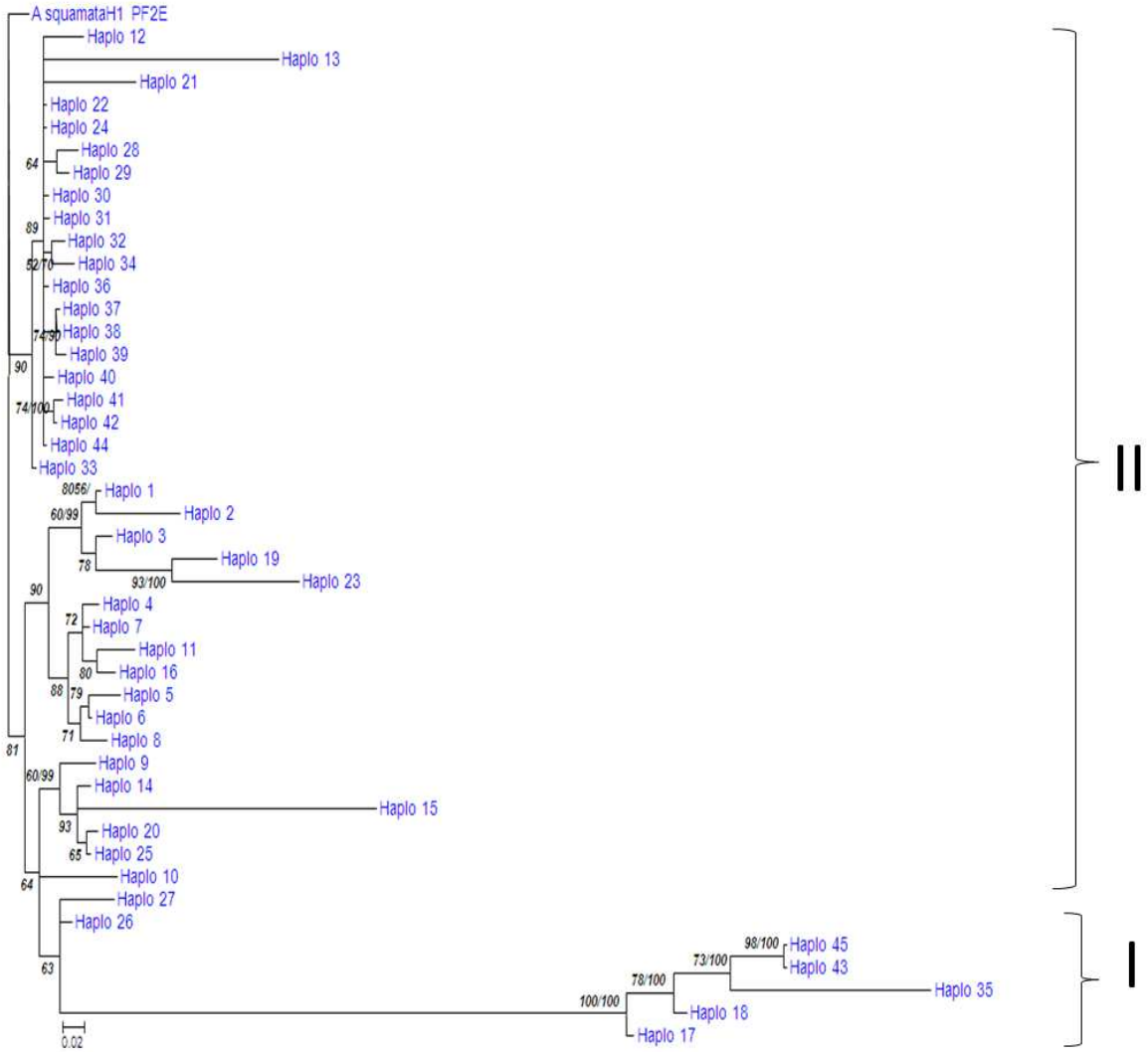

Figure 9. Bayesian genealogy of *Amphipholis squamata* based on 45 haplotypes of the 16S gene. Bootstraps values/*Bayesian* posterior probabilities are in ***bold italics***. When one support value is provided, it represents Bayesian p*P*. *Amphipholis squamata* (*A. squamata*H1 PF2E; Lineage E) from France (Boissin unpub. Data) was used as the outgroup.

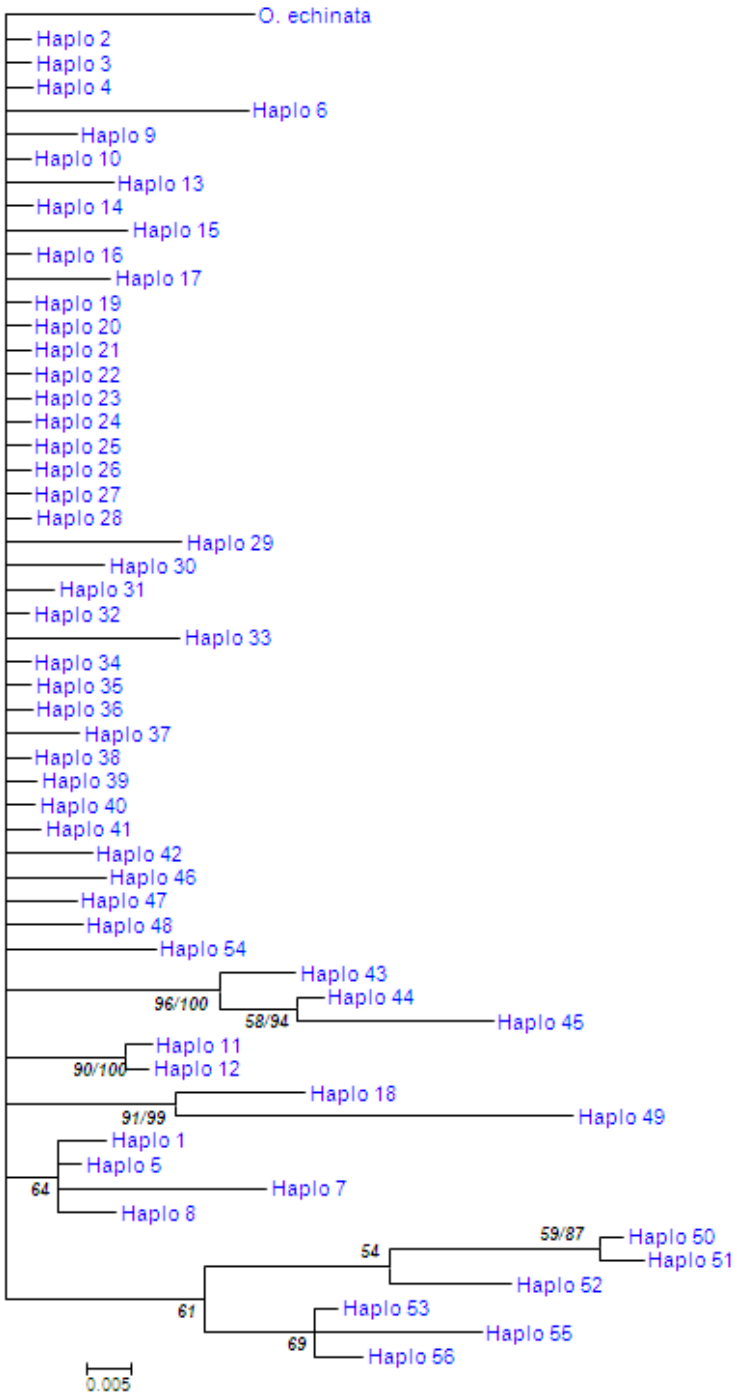

Figure 10. Bayesian genealogy of *Amphipholis squamata* based on 56 haplotypes of the ITS-1 gene. Bootstraps values and Bayesian posterior probabilities are in ***bold italics*** below. When one support value is provided, it represents Bayesian p*P. Ophiocoma echinata* was used as outgroup.

3.2.3. Analysis of Molecular Variances (AMOVA)

Analysis of molecular variance (AMOVA) for *A. squamata* revealed that the among-population variance component (63.87%) was significantly larger than the within-population component (36.13%), indicating population structure (Table 9). The F_{ST} values were significant for the 16S gene (F_{ST} = 0.63867; P = 0.00000) and the ITS-1 gene (F_{ST} = 0.25302; P <0.00000), suggesting the presence of population structure. As inferred from the AMOVAs the haplotype network analysis showed similar patterns (Fig. 8). For *A. squamata*, the overall F_{ST} was significant for the sampled area locations (Mona Island, La Parguera, Vieques, Culebra and Florida) (Table 9, 11). Based on 16S gene almost all pairwise F_{ST} comparisons (Tables 10, 12) indicated significant differentiations but consistently the Mona population was the most differentiated of all.

Table 9. Analysis of molecular variance (AMOVA) of *A. squamata* populations based on the 16S gene. Comparison were made within the Caribbean basin, among populations and within populations. Populations were sampled from: Florida and from four islands of Puerto Rico (Mona, La Parguera, Culebra, and Vieques)

Source of Variation	d.f.	Sum of Squares	Variance components	% of variation	F_{ST}	P value
Western Atlantic and Caribbean						
Among populations	4	1155.140	25.92983 Va	63.87		
Within populations	58	850.850	14.66965 Vb	36.13		
Total	62	2005.980	40.59948		**0.63867**	**<0.000001**
La Parguera (Fine geographic scale)						
Among populations	3	129.590	3.28420 Va	14.85		
Within populations	28	527.489	18.83890 Vb	85.15		
Total	31	657.079	22.12310		**0.14845**	**<0.01369**

Table 10: Pairwise F_{ST} comparisons (corrected by the Tamura & Nei model) of *A. squamata* based on16S. 1. Florida, 2. Mona PR, 3. La Parguera PR, 4. Culebra PR, 5, Vieques PR ($p<0.05$) in **bold, *** significant after Bonferroni correction.

	1	2	3	4	5
1	0.00000				
2	**0.55638***	0.00000			
3	0.13229	**0.13584**	0.00000		
4	**0.85186***	**0.87923***	**0.71703***	0.00000	
5	**0.83376***	**0.79547***	-0.00011	**0.89066***	0.00000

Table 11. Analysis of molecular variances (AMOVA) of *A. squamata* based on ITS-1. Comparisons were made within the Caribbean basin, among populations and within populations. Populations were sampled from: Florida, Mona PR, La Parguera PR, Culebra PR, Vieques PR.

Source of Variation	**d.f.**	**Sum of Squares**	**Variance components**	**% of variation**	F_{ST}	***P* value**
Western Atlantic and Caribbean						
Among populations	4	18.113	0.33768 Va	25.30		
Within populations	66	65.797	0.99692 Vb	74.70		
Total	70	83.910	1.33460		**0.25302**	**<0.000001**
La Parguera (Fine Geographic Scale)						
Among populations	4	6.684	0.07572 Va	6.72		
Within populations	38	39.945	1.05119 Vb	93.28		
Total	42	46.629	1.12691		**0.06719**	**<0.05083**

Table 12. Pairwise F_{ST} comparisons (corrected by the Tajima & Nei model) of *A. squamata* based on the ITS-1 gene. 1. Florida, 2. Mona PR, 3. La Parguera PR, 4. Culebra PR, 5, Vieques PR ($p<0.05$) in **bold**, * significant after Bonferroni correction.

	1	2	3	4	5
1	0.00000				
2	0.09925	0.00000			
3	0.03430	-0.01764	0.00000		
4	0.13758	-0.03635	-0.06363	0.00000	
5	**0.53955***	**0.54118***	**0.61786***	**0.58392**	0.00000

Summarizing *Ophiocoma echinata* and *Amphipholis squamata* exhibited differences in their genetic population structure across the sampling range (Tables 4, 6, 9, 11). The most striking differences were in the levels of gene diversity (Tables 3, 8), regional differentiation, and lineage divergence (Figs. 6, 7, 9, 10). Within localities, gene diversity was higher in *A. squamata* than in *O. echinata* because divergence between haplotypes was greater and the number of haplotypes within regions was higher (Figs. 5, 8). Also, more haplotypes were shared among sampling regions in *O. echinata* than in *A. squamata*. Together, these results suggest that more occasional gene flow, which helps maintain higher levels of genetic diversity, might occur more among populations of *O. echinata* than of *A. squamata.*

4. DISCUSSION

4.1. *Ophiocoma echinata*

As expected for a broadcast spawner, the brittle star *Ophiocoma echinata* does not exhibit significant population structure over the sampled range in the Caribbean and Western Atlantic. Based on the low levels of genetic variability, low population divergence, and number of shared haplotypes, *O. echinata* is a good disperser over the sampled range, we could approach this wire distribution from a phylogenetic age perspective. Geographical barriers in the Caribbean are not prominent, even though dispersal abilities of marine organisms, principally species with planktonic stage larvae, are hard to determine (Lessios et al. 2001). Usually, a species that is characterized by long dispersal potential exhibits high rates of genetic connectivity (Arnt & Smith 1998).

Based on molecular data, differences in population structure and levels of gene flow have been attributed to the length of pelagic larval duration in many diverse marine organisms (Hunt 1993; Hellberg 1996; Arnt & Smith 1998; Richards et al. 2007). Ophioplutei larvae generally have a planktonic existence of several weeks to months (Hendler 1975; 1991). Given a long pelagic larval duration for *O. echinata* it is reasonable to assume dispersal by ocean currents across large geographic distances (Thorson 1950; Shulzitski et al. 2009). Additionally, the ability for post-larval dispersal by rafting on algae has been documented (Hendler et al. 1999).

Currents have been implicated in the high gene flow observed for several planktotrophic coastal species such as sea stars, crabs, brittle stars and amphipods (Hunt 1993; Bunch et al. 1998; Richards et al. 2007) the larval transport has been suggested to be an important mechanism behind the low spatial genetic differentiation in many marine organisms (Stenseth et al. 2006). Because the distribution of planktonic larvae is influenced by the velocity and direction of water currents from spawning sites and the circulation of coastal waters direction (Williams et al. 2009) and the extent of transport varies depending on larval longevity and behavior and ocean circulation (Nunes et al. 2009) larval dispersal has long been recognized as a homogenizing force in marine systems (Shulzitski et al. 2009; Stenseth et al. 2006).

The high rates of connectivity agree with previous studies in echinoderms: the sea urchins, *Eucidaris tribuloides, Tripneustes ventricosus* and *Diadema antillarium*, (Lessios et al. 1999; 2001; 2003) and *Echinometra lucunter* (McCartney et al. 2000) do not exhibit significant differentiation between populations in the Caribbean. Some genetic differences appear when samples are compared between Western and Eastern Caribbean (corals; Vollmer & Palumbi 2006, Baums et al. 2006), but bigger differences can be detected between Western and Eastern Atlantic (Lessios et al. 2001; Nunes et al. 2009) or between Caribbean and south of Amazon (Nunes et al. 2009). Salas et al. (2003) also suggested a restriction in connectivity along the Central America Caribbean coastline because of higher self-recruitment between Costa Rica and Panama, however no genetic difference between Panamá and Belize ophiuroid populations was observed (Tables 5, 6). These results highlight the importance of explicitly considering sampling scale and life history when evaluating phylogeographical patterns.

In conclusion there is a link from larval dispersal to population connectivity in *O. echinata* because the processes that control the dispersal of individuals from one site to another demographically connect many populations (Cowen & Sponaugle 2009).

The neutrality test (Table 3) showed significant negative values of Tajima's D for La Parguera, Mona Island, Jamaica, Guadeloupe and Curaçao, which indicate an excess of rare variation a pattern of genetic expansion indicative of past population expansion or positive selection at these areas (Aris-Brosou & Excoffier 1996).

4.2. *Amphipholis squamata*

4.2.1. Genetic diversity

Amphipholis squamata was characterized by the high number of haplotypes encountered In Puerto Rico and south Florida (Table 8). The high number of haplotypes agrees with previous findings in genetic studies of *A. squamata* (Poulin et al. 1999; Sponer et al. 2001; Boissin et al. 2008a), which established high levels of genetic divergence between specimens located only 100 m to 3 km apart. The Puerto Rico and South Florida populations of *Amphipholis squamata* exhibited two mitochondrial lineages (I and II) with high clade support (Fig. 9), and very high genetic divergence between the two lineages (15.6%, uncorrected distance). Up to date, this is the highest divergence value reported for this species which are comparable to the ones calculated using a more rapidly evolving mitochondrial marker (COI) between species of echinoderms (K 2-P = 5–14%; McCartney et al. 2000). Other high genetic divergences have been reported between lineages A and B (11.5-12%, corrected distance) which occur in the Mediterranean Sea and Western Atlantic (Boissin et al. 2008a). The reciprocal monophyly of the 2 mitochondrial lineages is additionally supported by the ITS-1 data, supporting the idea of different species or subspecies previously posed by Le Gac et al. (2004) and Boissin et al. (2008a). Deeply divergent lineages have been reported from all genetic studies of *A. squamata:* two lineages in the English Channel (Sponer et al. 2001), four lineages in New Zealand among which two were previously found in the English Channel (Sponer & Roy 2002), and two lineages in the Mediterranean Sea, resembling genetically those from the English Channel (Boissin et al. 2008a). Lineage II of the current study was observed previously by Sponer et al. (2001) from Hawaii and Sydney, Australia and by Boissin (unpubls. Data) in French Polynesia, Reunion Is. and Japan, and was catalogued as the "tropical" lineage E supporting the claim for a worldwide distribution of *A. squamata*. Lineage I is a new genetic lineage for *A. squamata* and was found exclusively in samples from Puerto Rico (Culebra Is. and La Parguera).

Even with these high genetic differences between lineages, the specimens of *A. squamata* are morphologically uniform (Fig. 2). A morphometric study of *A. squamata* using scanning electron microscopy did not found any diagnostic differences among biogeographic groupings

(Sponer 2002). There have been reports of numerous color varieties in *A. squamata* (Deheyn et al. 1997; Deheyn et al. 2000) that led some authors (Deheyn & Jangoux 1999) to suggest that these color varieties represent sibling species. (Dupon et al. 2000) suggested that there is a link between color and strength of bioluminescence and that bioluminescence is as good indicator of genetic variability Differences in specimen color were noted in the current study, however, there was no color-specific clade supporting the assertion that there is a relation between color and clades, therefore rejecting the claim that color varieties are sibling species (Sponer et al. 2001; Le Gac et al. 2004).

The presence of multiple divergent genetic lineages in *A. squamata* adhere to the criteria of the phylogenetic species concept (Cracraft 1989), where clusters of organisms can be delimited by fixed DNA differences and within each cluster there is a pattern of ancestry and descent. The suggestion that different clades of *A. squamata* correspond to distinct taxa has been promoted by Sponer et al. (2001) and further supported by Sponer & Roy (2002), Le Gac et al. (2004) and Boissin et al. (2008a). Boissin et al. (2008a) concluded that *A. squamata* consists of at least four species at the northwestern area of the Mediterranean and New Zealand. Almost all clusters have a geographic origin (Fig. 9) however; no isolation by distance pattern was detected (Boissin et al. 2008a).

In conclusion, *A. squamata* is characterized by substantial levels of cryptic diversity (Boissin el at. 2008a). The haplotype analysis (Fig. 8) revealed multiple networks which could represent multiple lineages. According to these criteria, *A. squamata* as currently defined, constitutes at least four species in the Mediterranean Sea (Boissin et al. 2008a). Because of the high divergence (Tables 9, 11) between the two main clades (Figs. 9, 10) we suggest the presence of two morphologically similar species from the Caribbean area, one of which (Culebra and La Parguera PR) is a new species.

4.2.2. Population structure

In contrast to *O. echinata* the brooder *A. squamata* exhibits a higher degree of population structure and genetic differentiation (F_{ST} = 0.25302; P <0.00000) (Tables 9, 11), despite the small number of locations. Almost all pairwise population comparisons indicated significant differentiations (Tables 10, 12). The most prominent distinction among populations was observed between Florida and Puerto Rico and between Mona Island and other Puerto Rico localities (Tables 10, 12). The presence of strong population disjunction could be explained due to the large geographical distance between the localities and limited of dispersal capabilities in this brooding species (Sponer & Roy 2002). *A. squamata* has a high capacity for successful colonization because of the ability to self-reproduce as hermaphrodites and high rates of selfing (Hendler 1995; Dupon et al. 1999; Boissin et al. 2008b). Conversely, Culebra PR haplotypes suggest a history of long term isolation showing a separate lineage of *A. squamata*.

At a finer geographic scale, significant population structure (F_{ST} = 0.14845, P = <0.01369) was found for *A. squamata* (Table 11, 9) suggesting that exchange of genetic material between the individuals has been occurring between some reefs and not in others. Differential genetic exchange may be explained because of variations in current speed, tidal flow, size of reefs, depth, and substrate are some of these variables which affect the distribution and abundance of organisms (Baums et al. 2006). The rationale for examining the reef-to-reef connectivity patterns in the geographically fine-scale portion of the study, is the differences in water motion which have been observed between different reefs separated by a distance as little as 1km in La Parguera (McGehee 1994). Differences in water motion between the back reef (characterized with low water motion) and the fore reef (characterized with high water motion) are significantly different not only within a reef but also between reefs (McGehee 1994; Mercado-Molina, 2007). The local variability in hydrodynamic regimes may have been responsible for the genetic differences found between La Parguera reefs in *A. squamata* (Vollmer & Palumbi 2006 and Garcia & Schizas 2010 In review). If the movement and settlement of coral planulae are influenced by the fine scale hydrodynamics in La Parguera, it is possible that the small young brooders are also susceptible to the local water flow regimes.

There is no correlation between geographical and genetic distances as the pairwise F_{ST} tests (Table 10, 12) showed no evidence for increasing genetic differentiation with geographic distance. Genetic homogeneity in large geographic distances has been reported for brooding marine invertebrates (Sponer & Roy 2002; Richards et al. 2007). Given that *A. squamata* lacks a pelagic larval stage, this species must rely on dispersal of benthic juveniles and adults probably have a greater chance for passive dispersal due to the small size of individuals to maintain population connectivity. Passive transport of rafting adults has been suggested as a means of dispersal for brittle stars (Hendler et al. 1999). Furthermore, *A. squamata* has been reported to occur in dense aggregations on sea grasses and algae, increasing the likelihood that individuals could be dislodged and carried by ocean currents to other geographic localities. If this is the case, long distance transport of this species is likely to be dictated by ocean currents moving larvae away from the origin locations (Hendler et al. 1999). However, the brooder dispersal mode of *A. squamata* is improbable to facilitate long or inclusive medium range movement over short periods of time and it is more likely a cosmopolitan adaptation to finding local ecological optima within reefs (Hendler et al. 1999). Boissin et al. (2008a) concluded that the dispersal ability of *A. squamata* is regionally restricted but occasionally there are some long distances dispersal events. Moreover there are some reports of this species inhabiting fouling and manmade structures at the sea (Roy & Sponer 2002), another potential source of dispersal at the adult stage. Geographically close populations of *A. squamata* showed significant differentiation despite the short distances separating them in agreement with previous studies of brooding echinoderms (Arnt & Smith 1998) and the selfing reproduction of *A. squamata* which can reduce the genetic diversity and affect the population structure (Boissin et al. 2008b). Life history traits could influence the population structure (Mladenov & Emson 1990; Hellberg 1996; Sponer & Roy 2002; Teske et al. 2007; Boissin et al. 2008b).

In summary, *A. squamata* is characterized by unexpected levels of genetic diversity and represents a possible complex of two cryptic species. *A. squamata* which originated from Culebra seem to be genetically isolated and belong to a new distinct genetic lineage. Paradoxically, within each lineage, unexpected levels of genetic continuity maintaining genetic characteristics were estimated for a brooding invertebrate over large geographic distances.

Additional work is needed to further document biodiversity in these isolated biogeographic regions in order to better understand the dynamic physical processes and environmental conditions driving this diversity.

4.3. Conservation genetics

Genetic structure provides an estimate of population connectivity that is relevant over the long term (Shulzitski et al. 2009). The high levels of genetic connectivity between reefs in populations of *O. echinata* and the presence of multiple lineages in *A. squamata* have important implications for the management and conservation of Caribbean habitats (Palumbi, 2003). The concept of spatially complex linkages (or connections) among populations has been recognized in the value and design of Marine protected areas (MPA) networks (Crowder et al. 2000; Gerber et al. 2003; Palumbi, 2003; Fogarty & Botsford 2007). With the ultimate goal of conserving biodiversity or maximizing fishery yields, most theoretical efforts to design maximally effective reserves now consider the role of connectivity via larval exchange (Stenseth et al. 2006) and, in some cases, adult movement. Some efforts have collected what limited empirical data are available on potential larval dispersal distances to make explicit recommendations regarding reserve size and spacing (Kinlan & Gaines 2003; Palumbi 2003; Shanks et al. 2003). Dispersal ability is an important determinant of phylogeographical patterns in marine species. Other factors that may impede the effectiveness of marine reserves is the overreliance of biological data on solely one or few species. The coral reef ecosystem is inhabited by 1000's of species (many of them are not even formally described) but usually data is limited to the species that we intend to protect. In this study, two co-occurring ophiurid species have different life histories and different patterns of genetic population structure. Presence of multiple genetic lineages further complicates the use of additional species in the design of marine reserves. However, this problem cannot be overlooked since many marine species are eventually found to consist of either multiple genetic lineages or several cryptic species (Knowlton 1993; 2000).

5. CONCLUSIONS AND FUTURE WORK

Factors controlling mechanisms of larval dispersal and the identification of dispersal barriers or biogeographic boundaries have important ecological and biogeographical consequences in the population dynamics and the continuity of the species (Vance 1973; Baums et al. 2006).

Based on molecular data, differences in population structure and estimated gene flow have been attributed to the duration length of pelagic larvae in diverse marine organisms. However the degree to which dispersal potential is achieved can vary greatly, particularly at moderate to high levels of potential dispersal (Arndt & Smith 1998). The current study documents one example of how life histories can have profound influences on the geographic allocation of populations as observed in planktotrophic stage that showed wide distribution through the Caribbean.

The study shows that two species of brittle stars with contrasting life histories but similar ecologies differ in their genetic population structure. Thus, neither similar ecologies nor distribution appear to be a good predictor for a common population history, highlighting the importance of life history for population structure studies.

Recent environmental and anthropogenic threats have led to the call for increased spatial management of populations and ecosystems, and much effort with regard to population modeling is currently directed at designing effective marine reserves. Results of this study might assist managers in the designation of marine protected areas (MPAs), since levels and patterns of genetic connectivity between MPAs can be better estimated by using multiple species and contrasting life histories.

Future work will be investigate the genetic lineages of *Amphipholis squamata* to delineate the species present in Caribbean waters, finding out whether the mitochondrial clades are capable of hybridizing, comparing nuclear with mitochondrial genes.

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