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Mitochondrial control region sequence analyses indicate dispersal from the US East Coast as the source of the invasive Indo-Pacific lionfish *Pterois volitans* in the Bahamas

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Abstract Lionfish are popular aquarium fish from the Indo-Pacific that have invaded the western Atlantic. Two species, *Pterois volitans* and *P. miles*, were well established along the United States east coast before the first lionfish were reported from the Bahamas in 2004, where they quickly dispersed throughout the archipelago by 2007. The source of the Bahamian lionfish invasion has been in question because of the hypothesized low connectivity between Florida and Bahamas reef species as well as the temporal lag in their arrival in the Bahamas. Mitochondrial control region haplotypes (680 bp) were determined and analyzed for lionfish specimens from the Bahamas, North Carolina, and two sites within their native range (Indonesia and the Philippines). Exact tests, pairwise F_{st} and AMOVA analyses all showed no significant differentiation between the Bahamas and North Carolina specimens. The similarity between the Bahamas and North Carolina lionfish was also reflected in a minimum spanning network and neighbor-joining distance tree generated from the data. Sequence analyses also revealed the presence of only *Pterois volitans*, as no *P. miles* were detected in the Bahamian sample. These results indicate that the source of the Bahamian lionfish is egg and larval dispersal from the United States east coast population, and support previous models of reef fish dispersal that suggest a low level of connectivity between the Bahamas and east coast of Florida.

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Introduction

Lionfish (family Scorpaenidae) are striking and attractive reef fishes. They are very popular among aquarists and highly recognizable to the general public because of their ornate striped coloration and flamboyant dorsal and pectoral fins. The two best known species, *Pterois volitans* and *Pterois miles* (hereafter referred to collectively as lionfish) are venomous, predatory reef fishes native to Indo-Pacific waters (Schultz 1986), but because of their popularity as aquarium fish they have been widely distributed and now occur in aquaria on six continents. This worldwide aquarium trade is likely responsible for introducing lionfish to the wild outside their native range, and has been identified as the source of the invasion of lionfish in the western Atlantic (Whitfield et al. 2002; Ruiz-Carus et al. 2006).

Lionfish reproduce by releasing free-floating egg masses that subsequently develop into planktonic larvae (Fishelson

1975; Imamura and Yabe 1996; Morris et al. 2009). It is during these early life history stages that dispersal by ocean currents occurs. The floating egg mass may enhance survival by reducing predation from other planktonic organisms, and it also facilitates broad and rapid dispersal by keeping the eggs concentrated at the surface where wind driven currents are stronger than they are at depth. These characteristics have likely promoted the fast and wide spread dispersal of lionfish in the western Atlantic.

Lionfish were first reported along the east coast of Florida, USA near Fort Worth Inlet in the early to mid-1990s (Courtenay 1995; Ruiz-Carus et al. 2006). By 2000, they had dispersed northward from Florida to Cape Hatteras, North Carolina and have now become established along the United States east coast (Whitfield et al. 2002, 2007; Meister et al. 2005). Winter bottom water temperatures are thought to be the only factor controlling the distribution of lionfish along the east coast since lionfish perish in water below 10°C (Kimball et al. 2004).

Lionfish were first sighted in the Bahamas near Nassau in 2004 (www.reef.org) with additional sightings that year coming from the Abacos, Andros and northern Exuma Islands. Initial reports were that their distribution was restricted to a few isolated sites with population numbers remaining low until late 2006. Surveys coordinated by the Reef Environmental Education Foundation (REEF), reports from fishermen and dive operators, and information provided to NOAA (Whitfield unpublished data) all suggest that lionfish numbers in the Bahamas increased dramatically thereafter, and they spread southward throughout the entire Bahamian archipelago by 2007 (www.reef.org, Schofield and Fuller 2009) (Fig. 1).

Although the timeline of observations points to the east coast of Florida as the initial source of the western Atlantic invasion, the relationship of the US east coast and Bahamian lionfish is uncertain. Simulations of larval transport predicted little connection between Cuba and the Florida Keys (Paris et al. 2005), and a similar situation probably also occurs between southeast Florida and the Bahamas, where the strong northward flowing Gulfstream current is believed to act as a barrier between these separate biogeographic provinces (Briggs 1995). Passive transport models also show a weak connection between the Florida Keys and the northwest Bahamas (Roberts 1997; Cowen et al. 2003). Genetic evidence for a separation between Florida and the Bahamas has been observed in the rock hind, where Florida fish were genetically distinct from those in the Bahamas and other areas in the Atlantic (Carlin et al. 2003). Likewise, recent models of connectivity between damselfish populations suggest little exchange between southeast Florida and the Bahamas (Cowen et al. 2006), and drifters released from the lower Florida Keys over approximately a 2-year period remained in the vicinity of the reef track or

moved along the Florida shelf without crossing to the Bahamas (Hare and Walsh 2007). In addition, lionfish have not dispersed into the Florida Keys, which excludes them as the source of the Bahamas invasion. Therefore, despite the relatively small geographic separation between Florida and the Bahamas, little exchange between their marine benthic and reef faunas is thought to occur.

The apparent absence of strong connectivity and the temporal lag between lionfish sightings in the two areas (2000 for the East Coast and 2004 for the Bahamas) raises questions regarding the source of the Bahamas invasion. The population of Bahamian lionfish is either the result of a completely separate (likely aquarium) introduction within the Bahamas itself or they are the result of dispersal from the United States east coast population. Presently it is also unknown if one, or both, of the nearly morphologically identical species, *P. miles* and *P. volitans*, are present in the Bahamas.

Understanding the pattern of marine connectivity within ocean systems and the implications for marine organisms is a fundamental need for understanding population dynamics of all marine species. Although unfortunate, the lionfish invasion has provided an opportunity to examine the pattern of marine connectivity by simply observing how they are dispersing throughout their new ecosystem. These observations combined with genetics may provide the means to understand, as never before, marine connectivity within the tropical and subtropical western North Atlantic and at the same time, validate model predictions. The implications for control and management of future invasive species, for the placement of marine reserves, and for development of regional initiatives to manage fisheries are enormous.

In previous studies, genetic analyses of the United States east coast lionfish invasion have revealed that both *P. miles* and *P. volitans* are present in the Atlantic, and that the invasive populations of both species are characterized by low genetic diversity as a result of strong founder effects (Hamner et al. 2007; Freshwater et al. 2009). These initial studies were based on sequence analyses of the mitochondrial cytochrome *b* gene, a relatively conservative genetic marker. A more variable mitochondrial DNA marker (the control region or d-loop) has been used for this analysis of the potential connection among invasive aggregations, in order to enhance the detection of differences between populations of interest, and strengthen conclusions regarding connectivity.

Materials and methods

Western Atlantic lionfish specimens were collected off the North Carolina coast and from various locations within the

Fig. 1 Lionfish distribution within the Caribbean, Gulf of Mexico and Atlantic. *Dots* show locations where lionfish have been reported and *arrows* depict the direction of lionfish dispersal or expected dispersal, based on modeling efforts by Cowen et al. (2006) and Paris (unpublished data). The displayed distribution data is derived from REEF, NOAA and USGS (Schofield and Fuller 2009), and represents the geographic extent of lionfish as of January 2009, but not every report in these databases
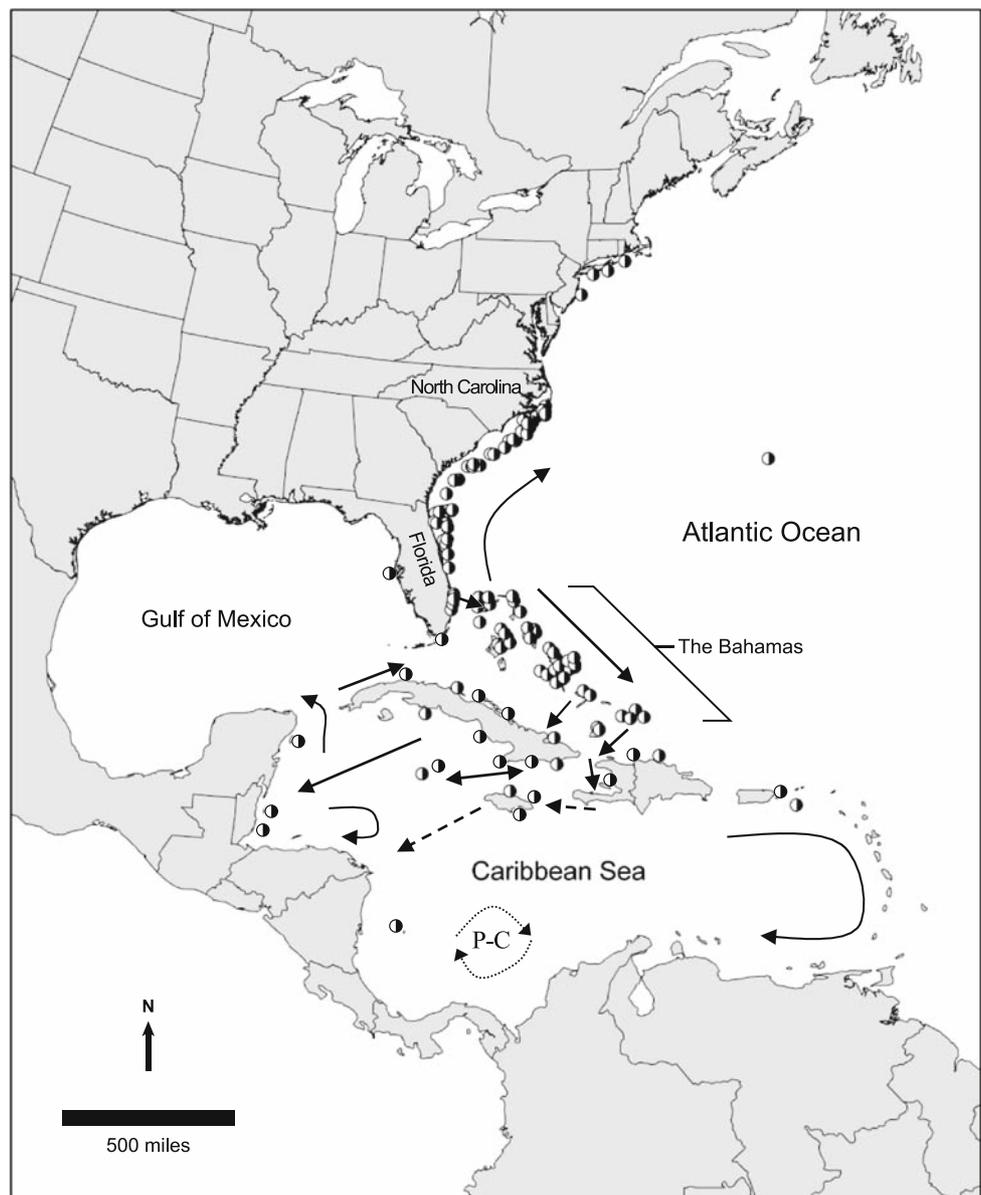
Bahamas. Native range specimens (Western Indonesia, Philippines) were purchased from aquarium fish dealers. Muscle tissue was dissected from the North Carolina and native range fish and preserved in 95 or 70% ethanol, and gill tissue was taken from the Bahamian fish and stored in DMSO buffer (20% DMSO, 250 mM EDTA, saturated with NaCl). Total genomic DNA was extracted from both tissue samples following a modification of the method described by Sambrook and Russell (2000) with a final cleaning step using the QIAquick PCR Purification Kit and protocol (Qiagen Inc., Valencia, CA, USA), or by using a PureGene extraction kit (Qiagen Inc.). A ca. 750 bp portion of the mitochondrial control region (d-loop) was amplified following the basic PCR recipe and thermocycling protocol outlined in Freshwater et al. (2000) but using GOTaq DNA

polymerase and buffer (Promega, Madison, WI, USA) and a 50°C annealing temperature. Amplification products were cleaned with Stratapreps (Statagene, La Jolla, CA, USA) and sequenced in both directions using the Big Dye v.3 kit and manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). The sequence reactions were resolved on an Applied Biosystems 3130*xl* Genetic Analyzer (DNA Analysis Core Facility, CMS, UNCW). A small number of lionfish specimens were amplified and sequenced using primers L-PROF (Meyer et al. 1994) and 12SAR-H (Palumbi 1996). Two lionfish specific primers, LionA-H (5′-CCA TCT TAA CAT CTT CAG TG-3′) and LionB-L (5′-CAT ATC AAT ATG ATC TCA GTAC-3′), were designed based on these initial sequences, and the remaining specimens amplified and sequenced using these lionfish

specific primers. Contigs were made from forward and reverse sequences using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA), and an alignment generated in MacClade (Maddison and Maddison 2000). All new sequences were deposited in GenBank (accession numbers FJ516407–FJ516454).

Distinct d-loop haplotypes were identified and haplotype and nucleotide diversity (Nei 1987) and average number of pairwise differences between samples (Nei and Li 1979) were estimated using Arlequin (v. 3, Excoffier et al. 2005). Haplotype frequency distributions for each population were compared using an exact test of population differentiation (Raymond and Rousset 1995). The haplotype correlation measure, Φ_{st}, was calculated for each pair of populations, and the significance of the observed Φ_{st} estimates were evaluated by permuting haplotypes between populations 1,000 times and recalculating Φ_{st}. The observed Φ_{st} was deemed significant if less than 5% of the permuted Φ_{st} estimates were equal to or greater than the observed value. Population differentiation was also examined using a hierarchical analysis of molecular variance (AMOVA, Excoffier et al. 1992) as implemented in Arlequin.

A minimum spanning network for the observed haplotypes was generated by Arlequin. A neighbor-joining tree based on Tamura-Nei corrected distances (Tamura and Nei 1993) was also generated using PAUP* (Swofford 2002), and subjected to bootstrap analysis (5,000 replications).

Results

A 680 bp section of the mitochondrial d-loop was analyzed from 127 invasive Bahamian lionfish. Incorporation of these sequences in an existing data set derived from other western Atlantic and Indo-Pacific lionfish (334 *Pterois volitans* and 42 *P. miles* d-loop sequences, including the partial sequences of Kochzius and Blohm 2005) revealed that all the Bahamas specimens were *P. volitans* (data not shown). D-loop haplotypes for the Bahamas fish were compared with invasive North Carolina *P. volitans* specimens, and native range specimens from Western Indonesia and the Philippines (Table 1). Thirty-six haplotypes were identified in the 70 Indo-Pacific specimens (two haplotypes were present in both the Western Indonesia and Philippines

samples), while only nine total were observed in the 391 invasive *P. volitans* specimens. The 127 Bahamas and 264 North Carolina specimens shared seven haplotypes, and both populations had one unique haplotype, so that eight were found in each. The majority of invasive specimens (78%) exhibited one of the two dominant haplotypes (Figs. 2, 3). No haplotypes were shared between the native Indo-Pacific and invasive western Atlantic populations. All haplotype diversity and nucleotide diversity values, as well as the mean pair-wise difference between haplotypes, were smaller in the Bahamas and North Carolina populations than they were in the Western Indonesia and Philippines populations (Table 1).

An exact test of haplotype frequencies resulted in significant differences ($P < 0.00001$) among all populations except for the comparison of the Bahamas and North Carolina ($P < 0.21645$). Similarly, significant Φ_{st} estimates ($P < 0.00001$) were observed in all pair-wise population comparisons except that for the Bahamas and North Carolina ($P < 0.27832$). A hierarchical AMOVA was performed with the specimens divided into three groups—Bahamas/North Carolina, Western Indonesia, and Philippines as suggested by the results of the exact test. The largest proportion of variation (59.73%) occurred between groups using this hierarchical arrangement (Table 2). The among-populations within-group component, which is composed of variation between the Bahamas and North Carolina, was the only component found not to be significantly different.

A geographic partitioning of haplotype variation is also noticeable in the minimum spanning network and neighbor-joining distance tree generated from these data. Two main clusters are evident in the minimum spanning network (Fig. 2). One includes haplotypes found in Bahamas, North Carolina and Western Indonesian fish, and most connections between haplotypes require the inclusion of only one to a few non-sampled intermediate haplotypes. The other cluster includes haplotypes found in mostly Philippines, but also some Western Indonesian specimens, and many of the inter-haplotype connections require a large number of hypothetical intermediates. The presence of these two clusters is likewise reflected in the two major clades resolved in the haplotype distance tree (Fig. 3). The Bahamas/North Carolina/Western Indonesia clade is well supported (bootstrap proportion value [BP] = 84) by the bootstrap analysis.

Table 1 Measures of genetic variation for native range and invasive populations of *Pterois volitans*

	n	Number of haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Mean pair-wise differences
Bahamas	127	8	0.648 ± 0.028	0.0033 ± 0.0020	2.23 ± 1.24
North Carolina	264	8	0.704 ± 0.018	0.0038 ± 0.0022	2.56 ± 1.38
Western Indonesia	42	26	0.962 ± 0.017	0.0132 ± 0.0069	8.92 ± 4.20
Philippines	28	12	0.886 ± 0.041	0.0153 ± 0.0080	10.38 ± 4.88

Fig. 2 *Pterois volitans* d-loop
haplotype network. Individual
haplotypes are labeled numeri-
cally following "H", and the
number of specimens sharing
that haplotype in a geographical
region is shown. *Lines* connect-
ing haplotypes represent one
nucleotide difference and *small
open circles* represent intermedi-
ate haplotypes not found in the
sampled specimens
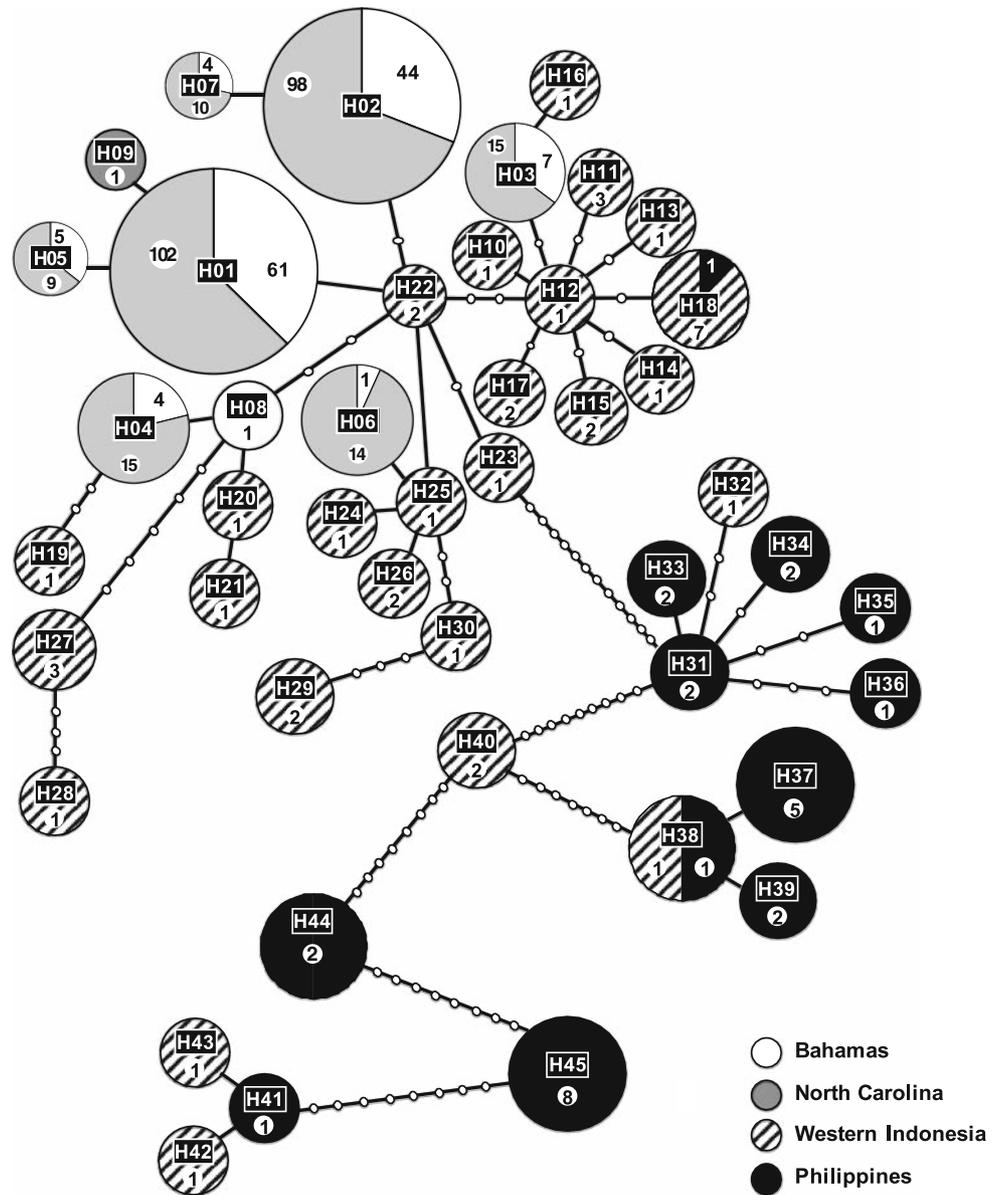
The Philippines–Western Indonesia clade is less well sup-
ported (BP = 73), but it is split into two subclades that have
good support (BP = 80 and 85).

Discussion

A previous study using cytochrome *b* haplotypes showed
low levels of genetic diversity (founder effect) associated
with the invasive population along the United States east
coast (Hamner et al. 2007). A portion of the east coast sam-
ples from North Carolina, and native range specimens from
Western Indonesia and the Philippines, were reanalyzed
utilizing the more sensitive d-loop marker to confirm these
earlier findings, and to allow comparisons between the
North Carolina and Bahamas populations.

As expected, d-loop sequences generated from *P. voli-
tans* specimens included in the Hamner et al. (2007) study
were more variable than those for cytochrome *b*. The mean
pair-wise difference between d-loop haplotypes of US east
coast *P. volitans* specimens were nearly an order of magni-
tude greater than that for cytochrome *b* haplotypes (Hamner
et al. 2007, Table 1). Likewise, only three cytochrome *b*
haplotypes were detected in the 158 US east coast speci-
mens sequenced by Hamner et al. (2007) while eight d-loop
haplotypes were found in these same specimens.

The genetic composition of the Bahamas and North Car-
olina lionfish populations was very similar based on d-loop
sequences (Table 1). Seven of nine haplotypes were shared
between the two areas and the majority of fish in both sam-
ples exhibited one of the two dominant haplotypes. All
analyses revealed non-significant levels of differentiation,

Fig. 3 *Pterois volitans* d-loop haplotype neighbor-joining tree. Bootstrap proportion values are shown for branches when >50. *Circles* with included numbers represent the frequency and geographical distribution of each haplotype
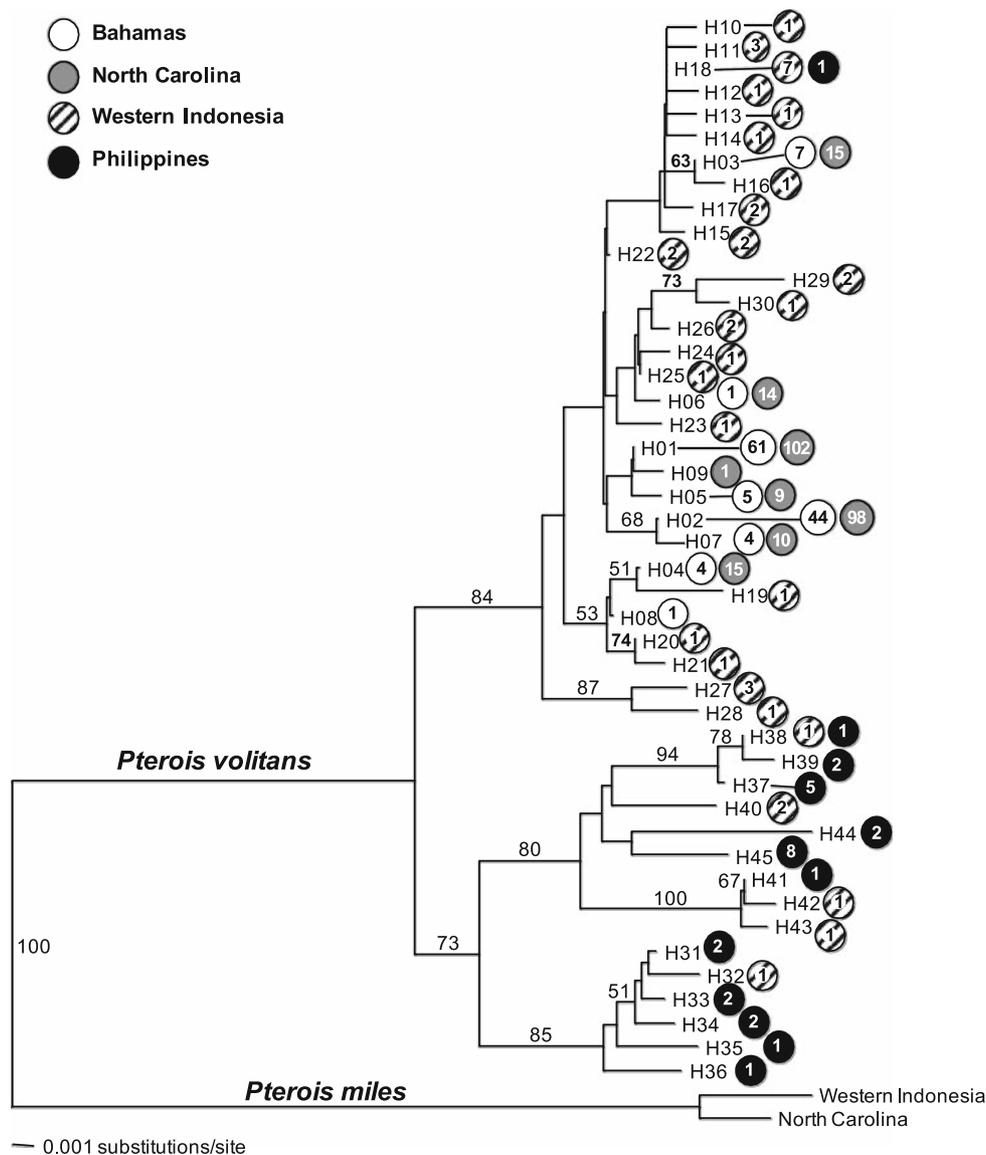
Table 2 Results of analysis of molecular variance (AMOVA) for four sampled populations divided into three "groups" (North Carolina/Bahamas, Western Indonesia and Philippines)

Source of variation	df	Sum of squares	Variance components	Percentage of variation	P value
Among groups	2	326.493	2.60930	59.73	0.00001
Among populations					
within groups	1	1.454	−0.00179	−0.04	0.29521
Within populations	457	804.760	1.76096	40.31	0.00001
Total	460	1132.707	4.36848		

suggesting that the two populations are genetically homogeneous. Measures of genetic variation among native range lionfish populations were substantially greater than those for the invasive North Carolina and Bahamas populations (Table 1). The reduced levels of within population d-loop diversity in the invasive populations relative to the native

range, reinforces the conclusion drawn from previous cytochrome *b* analyses (Hamner et al. 2007), and support the presence of a strong founder effect in the western Atlantic invasive populations.

Intentional and/or accidental aquarium releases are the most likely vector for the introduction of lionfish into the

western Atlantic (Whitfield et al. 2002; Semmens et al. 2004; Ruiz-Carus et al. 2006), and the genetic homogeneity of the Bahamian and North Carolina populations suggests that they ultimately trace back to the same introduction. It is possible that the absence of a genetic difference between the Bahamas and North Carolina populations is the result of different aquarium releases of fish that had been collected in both cases from the same locations within the native range. However, this is highly unlikely given the variability of the native range populations revealed by this analysis, and considering the geographically distant and genetically distinct regions of the Indo-Pacific where lionfish are collected for the aquarium trade (Africa, Indonesia, Philippines, etc.). In addition, the earliest reports of US lionfish are more than a decade prior to the first documented lionfish in Bahamian waters. Therefore, the best explanation for the lack of genetic differentiation between the North Carolina and Bahamian lionfish populations is that the initial source of lionfish in the Bahamas is dispersal from the US east coast population across the Florida Straits.

Preliminary results of Lagrangian biophysical modeling coupled with high resolution data from the Regional Ocean Modeling System indicate that dispersal of lionfish could have occurred from the Florida east coast to the Bahamas between about 26–27°N latitude (CB Paris and L Cherubin, unpublished data). These models as well as data from the Intra-Americas Seas drifter tracks (http://www.iaslinks.org/ias-rto.html) show rare crossing events at this location that would require a species to have a greater dispersal potential than typical coral reef fishes (Fig. 1). The free-floating egg masses and larvae of lionfish are believed to enhance their dispersal capabilities, but the absolute duration of this dispersal phase is unknown because the duration of development from an egg to embryo to larvae has not been determined (Morris et al. 2009).

Cowen et al. (2006) used biophysical models based on the life history traits of damselfish to develop maps of "demographic connectivity" within the Caribbean. Confirmed reports (NOAA, REEF, USGS) demonstrate that lionfish dispersal throughout the Bahamas, and now into the Caribbean (Chevalier et al. 2008; Guerrero and Franco 2008), has occurred following the modeled population linkages described in Cowen et al. (2006), but at a faster rate (Fig. 1). If this trend continues, lionfish are expected to disperse throughout the Caribbean, Gulf of Mexico, and the Florida Keys, with the last location likely to be the Panama-Colombia gyre, the most isolated sub-region in the Caribbean (Fig. 1) (Richardson 2005; Cowen et al. 2006). Although more research is clearly necessary to build and test an accurate model of predicting lionfish dispersal (i.e., basic information on early life history stages, such as hatching time, pelagic larval duration, larval vertical behavior, survivorship, etc.), it is likely that even very low levels of connectivity would allow the effective dispersal of lionfish as is evidenced by this study.

Despite the Bahamas lionfish resulting from an expansion of the US east coast population, the two may not be currently functioning as a single large population. The low connectivity predicted by model simulations (Cowen et al. 2006), and time lag between the establishment of the US east coast population and Bahamas invasion indicate only sporadic, rather than consistent exchange between the Bahamas and southeast Florida populations.

If the east coast of Florida is assumed to be the source of both the Bahamas and North Carolina lionfish populations, the lack of genetic differentiation between them would mean that the genetic composition of the southeast Florida source population was temporally stable between the invasions of the two new locations. Although the development of an age-size relationship in lionfish has yet to be completed (J. Potts personal communication, J. Morris personal communication), the cytochrome *b* analyses of Hamner et al. (2007) and Morris and Freshwater (2008) suggest temporal genetic stability in the source of the North Carolina population during 2001–2005. Three cytochrome *b* haplotypes with percentages of haplotype A: 89.9%; haplotype C: 7.6%; haplotype E: 2.5%, were found in the 158 2004–2005 collected *P. volitans* specimens analyzed by Hamner et al. (2007). The same three haplotypes were found in the 81 *P. volitans* specimens analyzed by Morris and Freshwater (2008), and in nearly the same proportions (A: 87.7%, C: 3.7%, E: 8.6%). The majority of 2004–2005 collected specimens are estimated to represent a year class range of 2001–2003, while the majority of the 2006 collected specimens represent the 2003–2005 year classes. The timing of the initial Bahamas introduction suggests that it may have originated from 2003 to 2004 year class fish. A more comprehensive analysis of temporal genetic stability in the US east coast lionfish population using the more variable d-loop sequences is in progress and will provide additional insight into this question.

Interestingly, this study of Bahamas lionfish did not detect any specimens of *P. miles*. Hamner et al. (2007) found that 7% of the US east coast specimens collected during 2004 and 2005 were *P. miles*. Further analyses of North Carolina specimens collected during 2006 found nearly the same proportion of *P. miles* (10%, Morris and Freshwater 2008). The absence of *P. miles* in the Bahamas may indicate that the southern distribution limit of this species along the US east coast is too far north for even sporadic dispersal into the Bahamas. Alternatively, it may be that their population numbers are too few for them to have thus far overcome the obstacles to dispersal across the Florida Strait.

The northward distribution of lionfish along the US east coast is facilitated by the north flowing Gulf Stream current. It is unlikely that lionfish populations north of the

Bahamas can disperse southwestward against the fast moving northerly Gulf Stream current. However, the Gulf Stream may promote dispersal in the opposite direction from the Bahamas northward. As the lionfish population has spread throughout the Bahamas, and especially in the western islands, it is probably now functioning as a source of recruits to the Bermuda population (McClean et al. 2002). Although an unfortunate introduction, the dispersal of lionfish into new locations provides an important glimpse of marine connectivity within the entire western North Atlantic. This information will be valuable for understanding the management of threatened populations and control of other invasive species.

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