Mitochondrial DNA (mtDNA) Haplotype Diversity of the Invasive Lionfish (*Pterois volitans*) in Barbados

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L'ADN Mitochondrial (ADNmt) Haplotype Diversité des Invasives Apparaissant (*Pterois volitans*) à la Barbade

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EXTENDED ABSTRACT

The introduction and spread of Indo-Pacific lionfishes (*Pterois volitans* and *P. miles*) in the Western Atlantic (WA) since the 1990s has stimulated research examining their dispersal from the US east coast to Bermuda and across marine connectivity barriers into the Bahamas and subsequently throughout the Caribbean (Freshwater et al. 2009, Schofield 2009, 2010, Betancur-R et al. 2011). A strong founder effect has been noted in the WA populations of both species which show low genetic diversity compared with the native source populations in Western Indonesia (Freshwater et al. 2009). A secondary founder effect has been suggested to explain the observed population break and further reduction in genetic diversity of *P. volitans* as the invasion of this species has progressed with a fairly significant time lag between the invasion of northern WA locations and those of the Caribbean (Betancur-R et al. 2011). Only three or four d-loop haplotypes have been found in the northwestern and central Caribbean populations (Grand Cayman, San Andrés, Santa Marta) compared with a total of nine in the northern WA group (North Carolina, Bahamas and Bermuda) (Betancur-R et al. 2011) (Table 1). To date there have been no genetic analyses of lionfish published from the eastern Caribbean, a region further isolated from the rest of the Caribbean by a significant connectivity barrier (see Cowen et al. 2006). This study therefore represents the first report of the application of DNA sequencing to determine the species and haplotypic composition of the invasive lionfish in this region. We hypothesised that only *P. volitans* will have reached Barbados, and that there will be a second population break and further reduction in genetic diversity with the continued spread of this species across the eastern Caribbean connectivity barrier.

Lionfish were collected in Barbados from the first reported arrival (24 Nov, 2011) until September 2013 and muscle tissue was preserved frozen or in DMSO for subsequent DNA extraction. A 680 bp fragment of the mitochondrial control region (d-loop) was amplified using 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)] following Freshwater et al. (2009). Sequencing of all amplicons was performed by Molecular Cloning Laboratories (California, USA) and sequences were matched against published mtDNA control region sequences using BioEdit. Measures of genetic diversity were calculated and analyses of molecular variance (AMOVA) were undertaken using Arlequin v. 3.5.

All 178 amplicon sequences (679 bp) aligned with those of red lionfish (*Pterois volitans*) confirming our expectation of a single species lionfish invasion in Barbados to date. However, contrary to our expectations, a total of six haplotypes were detected, showing neither any indication of the secondary founder effect apparent in other Caribbean lionfish populations studied to date, nor a further reduction in haplotype diversity as expected (Table 1). All haplotypes detected in Barbados matched published WA lionfish haplotypes H01, H02, H03, H04, H05 and H07 (GenBank accession numbers FJ516409–FJ516413 and FJ516415 registered by Freshwater et al. 2009). Consistent with other studied WA populations, two haplotypes were dominant; H01 found in 30.3% of our samples and H02 found in 65.7%, whilst the other haplotypes were relatively rare, each being found in less than 2% of samples. Calculated measures of genetic variation for the population in Barbados were low (e.g. haplotype diversity (HD) = 0.478) compared with those of native *P. volitans* populations (HD = 0.886 to 0.962, Betancur-R *et al*. 2011) but similar to other WA invasive populations (Table 1).

Despite differences in the presence of the rare haplotypes, there was no significant population structuring detected between the Year 1 pioneer (comprising H01, H02, H04) and Year 2 established (comprising H01, H02, H03, H04, H05 and H07) populations in Barbados (AMOVA: F_{ST} -0.0164, $p = 0.947$), nor among the three different coastal samples around the island (AMOVA: F_{ST} = -0.0118, $p = 0.692$, Figure 1). However, the presence of haplotypes H03, H05 and H07, which appeared in the second year of the invasion and have not previously been reported from the southern Caribbean (San Andrés, Santa Marta) populations raises the interesting question of whether Barbados received lionfish from one or more

sources. The bimodal size structure of the Barbados population in Year 2 indicates non-continuous recruitment which would support the idea of two separate invasion pulses to this up-current, upwind location. These pulses could conceivably have been from different sources. An analysis of molecular variance between the pioneer population in Barbados (Year 1) and the nearest populations within the Caribbean and Northern groups indicated no significant difference between Barbados and the southern Caribbean population, whilst there was a significant difference between Barbados and the Northern population (AMOVA: Barbados vs Santa Marta F_{ST} = 0.00092, p = 0.324; Barbados vs Bahamas F_{ST} = 0.0734, p < 0.001), suggesting that the first pulse could have come from the southwest. An examination of the chronology of reported sightings (USGS-NAS database at http://nas.er.usgs.gov) indicates that lionfish were approaching Barbados in 2010 simultaneously from the southwest along the South American coastline and from the northwest down the Lesser Antilles island chain. However, from these data it would appear that the group spreading down the island chain from the north arrived first. This is also supported by the fact that the fish carrying H03 and H05 haplotypes, although not found until Year 2, were in fact mature fish (274-289 mmTL) and therefore likely arrived in the first pulse the year before. Since the other northern haplotype (H07) was found in the second year in a small fish (160 mmTL), this suggests that the second pulse also came from the northern populations. However, an analysis of molecular variance across all combinations of groups (Caribbean vs Northern, Barbados vs Caribbean, Barbados vs Northern) fails to detect any significant differences among groups (AMOVA F_{CT}: p > 0.05 in all cases) although populations within groups showed significant variation (AMOVA F_{CS}: p < 0.01 in all cases). A more extensive genetic analysis using samples from more locations will be required to better resolve the route of invasion and relatedness of island populations in the Eastern Caribbean.

KEY WORDS: Lionfish, P*terois volitans*, invasive species, MtDNA d-loop, Barbados

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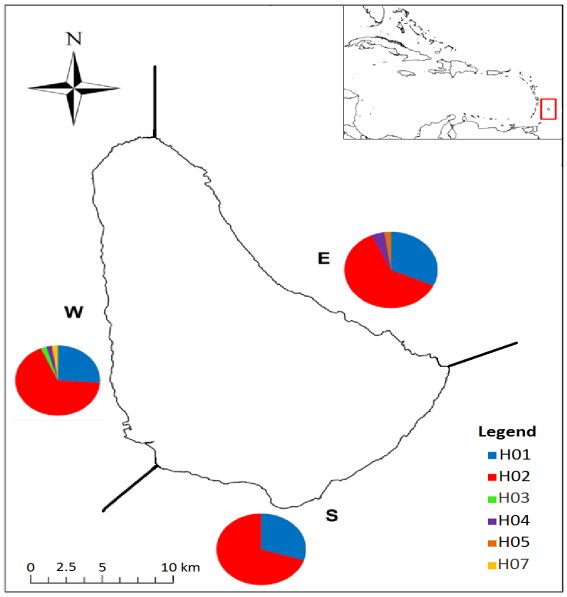

Figure 1. Red lionfish (*P. volitans*) haplotypic composition by coast in Barbados based on the 680 bp d-loop fragment sequences. Inset map shows the location of Barbados in the Eastern Caribbean.

Table 1. Summary of invasive Indo-Pacific red lionfish, *Pterois volitans*, d-loop haplotype presence/absence and haplotype diversity (HD) across the Western Atlantic populations

Group	Location	n	Haplotypes										HD
			H01	H02	H03	H04	H05	H06	H07	H08	H09	Total	
Northern	North Carolina[1]	264	✓	✓	✓	✓	✓	✓	✓		✓	8	0.704
	Bermuda[2]	45	✓	✓	✓			✓	✓			5	0.627
	Bahamas[1]	127	✓	✓	✓	✓	✓	✓	✓	✓		8	0.648
Central Caribbean	Grand Cayman[2]	79	✓	✓	✓	✓						4	0.432
	San Andrés[2]	50	✓	✓		✓						3	0.541
	Santa Marta[2]	169	✓	✓		✓						3	0.524
Eastern Caribbean	Puerto Rico[3]	118	✓	✓	✓	✓						4	0.449
	Barbados[4]	178	✓	✓	✓	✓	✓		✓			6	0.478

Data from [1] Freshwater et al. (2009), [2] Betancur-R et al. (2011), [3] Velez-Zuazo et al. (unpubl.), [4] this study.