

The Asian Green Mussel *Perna viridis* (Linnaeus 1758): 20 Years after Its Introduction in Trinidad and Tobago

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ABSTRACT

Invasive species are often characterised by the introduction of low numbers of individuals and a small genetic pool which could give rise to a population with a lower genetic diversity than the original population. *Perna viridis* was first reported on the west coast of Trinidad in 1990 in the Point Lisas Industrial estate. They may have been first introduced either as juvenile free floating mussels (spat) or as adults attached to the hulls of ships. This gregarious species spread rapidly throughout the Caribbean to Venezuela, Kingston Harbor (Jamaica) and by 1999 was reported in Tampa Bay (Florida). Genetic analysis was done on tissue samples from Trinidad to determine the genetic similarity of newly collected specimens (2009) with archived specimens (collected 1994). Results suggest that there may not be any genetic variation in the population of *Perna viridis*, even approximately 20 years after initial colonization. These results suggest that there was no evidence of genetic bottlenecks in the population of *P. viridis* from Trinidad.

Keywords: *Perna viridis*; Green Mussels; Trinidad and Tobago; Invasive Species

1. Introduction

The green mussel, *Perna viridis* (Linnaeus, 1758) is native to the tropical waters of the Indo-Pacific region of Asia [1]. It is a dioecious species that reaches sexual maturity within 2 - 3 months and can live for about 3 years. *Perna viridis* thrives in coastal waters (<10 m), in salinities between 16 and 33 ppt, at temperatures between 10°C and 35°C (optimal: 27 to 33 ppt, 26°C to 32°C), and exhibit a wide tolerance range for turbidity and pollution [2,3]. They are often found attached to hard structures in the midlittoral and sublittoral areas in densities of up to 35,200 individuals/m² [4]. *Perna viridis* is characterised by its fast growth and relatively high tolerance to environmental variables such as temperature, salinity and pollution [5-7]. Their well-developed byssal apparatus allows them to form dense clusters which effectively modify the natural ecosystem and out-compete other organisms (Figure 1).

P. viridis was first reported at Point Lisas on the west coast of Trinidad in 1990 [8]. This was also the first record of the species in the Atlantic region. It was thought to have arrived in Trinidad from the Indo-Pacific region either as “spat” in ballast water or as adults attached to

the hulls of ships [8]. Throughout the 1990s, *P. viridis* continued to colonize west coast locations, in particular, the major ports and areas of high shipping activity (Figure 2). This gregarious species spread rapidly throughout



Figure 1. Colonization of mangrove (*Rhizophora mangle*) roots by *Perna viridis* in Caroni swamp, Trinidad (1992) (Photo by John Agard).

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the Caribbean and populations were subsequently reported in Venezuela in 1993 [9], in Kingston Harbor, Jamaica in 1998 [10] and in Tampa Bay, Florida in 1999 [4,11-13] (**Figure 3**). Initial colonization by *P. viridis* was thought to have occurred with the introduction of a small number of individuals. The initial introduction of low numbers and small pool of genetic material could have given rise to a population bottleneck which may have resulted in a new population with a lower genetic diversity, when compared to the original population. A number of conflicting views about the role of genetic bottlenecks in invasion biology have been presented. Traditionally, genetic bottlenecks were thought to decrease the potential for adaptive evolution because of a reduce-

tion in the quantitative variation within a population. However, recent theories suggested that bottlenecks may lead to increase genetic variance in founding populations because of the conversion of epistatic to additive variance, and hence could promote rapid adaptive evolution [14,15].

Invasive species also frequently experience founding effects that reduce their genetic variability (and may account for the loss of genetic variation), as the new population becomes reproductively separated from the main population. This reduced genetic variability may be as a result of significant inbreeding which lowers their ability to evolve, and would inevitably reduce their persistence in the new environment [16]. Additionally, as a result of the loss of genetic variation, the new population may be distinctively different, both genetically and phenotypically, from the parent population. In extreme cases, the founder effect may explain speciation and subsequent evolution of a new species. Kolbe *et al.* (2004) [17] showed that the blending of genetic material from different geographical sources across an invasive species native range resulted in an invasive population of *Anolis sagrei* that was genetically different from the native population.

Twenty years after its initial introduction into Trinidad, the distribution of *P. viridis* can now be described as patchy, with isolated populations along the west coast. Baker *et al.* (2007) [18] similarly described green mussel populations as patchy, in the coastal waters of northeast Florida. In this study, we confirm the identity of *P. viridis* in Trinidad and examine the genetic similarity of newly collected specimens (2009) with archived specimens collected in 1994 to determine whether there have been any genetic variations post-colonization.

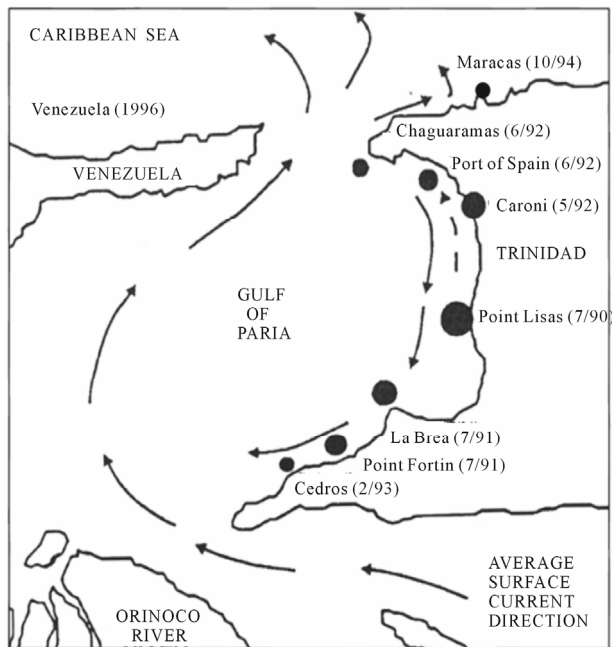


Figure 2. Chronology of colonization of *Perna viridis* in the Trinidad (Adapted from Agard *et al.*, 1990 [8]).



Figure 3. Chronology of colonization of *Perna viridis* in the Caribbean.

2. Method

Samples of *P. viridis* were collected in February 2009, at four sites (Cedros, Point Lisas, San Fernando and Chaguaramas) where its presence was previously [9]. Genetic analysis of tissue samples were done at the Laboratories of Analytical Biology, Smithsonian Institution. Tissue samples were digested with 150 μ L of Autogen M2 buffer and 150 μ L of Autogen M1 buffer with Proteinase K at 56°C in a shaker incubator. DNA extraction was performed using the Autogen Prep 956 Extractor. The DNA was eluted in 50 μ L of Autogen R9 buffer. CO1 amplification was carried out with primers: M13(F)-29-dgLCO1490 and M13(R)-dgACO2198, each at 10 mM concentrate, with 1 μ L DNA extracts in 10 μ L reactions. The annealing temperature in the amplification was 48°C with 36 cycles. PCR runs were done on 1.5% SeaKem LE agarose gels and 1.2% - 1.3% NuSieve GTG agarose gels. The amplified samples were cleaned up

using gel purification and run on a thermal cycler: 10 minutes at 72°C and 2 - 3 minutes at 45°C before adding 1 µL of gelase to each amplified sample and incubated for a few hours at 45°C prior to sequencing. The resulting sequences were mapped onto a NJ tree for analysis using the software Paup. A distance analysis with a Neighbor-joining search was performed.

Genetic results from these samples are compared with reference GenBank samples for various species of *Perna* (Table 1) as well as the archived samples from Trinidad which had been collected in 1994.

3. Results and Discussion

Phylogenetic comparison between species collected from Trinidad in 2009 confirmed their identity as *Perna viridis*, based on comparison with GenBank references for *Perna* species. Comparison of the intraspecific distance with *Perna perna* (0.208), *Perna indica* (0.22), and *Perna canaliculus* (0.252) showed that these were distinctly phylogenetically different from *Perna viridis*. *Perna viridis* (Trinidad) had an average intraspecific distance of 0.002 (0.000 - 0.009). Trinidad samples gave an intraspecific distance ranging between 0.005 and 0.008, when compared to the GenBank *Perna viridis* sample. Similarly the archived Trinidad samples from 1994 gave an intraspecific distance of 0.005, which suggests that there may not be any genetic variation in the population of *Perna viridis*, even 20 years after initial colonization.

Benson *et al.* (2001) [11] also confirmed that the green mussels in Florida were *P. viridis*, and were closely related to *P. viridis* from Trinidad. These results suggest that there was no evidence of genetic bottlenecks in the population of *P. viridis* from Trinidad. Kolbe *et al.* (2004) [17] suggested that the lack of genetic variation in an invasive species decreases its chances of establishment during introduction, which could account for the lower than predicted success of *P. viridis* colonization in Trinidad. This can also be explained by the “Wahlund effect” which occurs when genetically similar family clusters are created, as frequently happens with invasive

species, leading to a reduction of heterozygosity. The underlying cause of this could be the geographic barrier to gene flow from the original population. However, secondary introductions from other colonized areas in the Caribbean may greatly favor the persistence of the species in the region resulting in little loss of genetic diversity.

These results support electrophoretic analysis of allozymes done on specimens in 1992, by the Plymouth Marine Laboratory. Coding for enzymes confirmed the identification of the new arrivals as *Perna viridis* [8]. Interestingly the electrophoretic results also showed that the LAP enzyme which was previously reported in green-lipped mussels (*P. viridis*) from the West Coast of Peninsular Malaysia [19] was not present in any of the specimens analysed from Trinidad (*B. bayne pers. comm.*). This result is unlike any other reported for *P. viridis* and warrants further investigation.

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Table 1. GenBank samples used for NJ tree.

Species	Accession number
<i>Perna canaliculus</i>	DQ343603
<i>Perna indica</i>	FJ428754
<i>Perna perna</i>	DQ351451
<i>Perna perna</i>	DQ351432
<i>Perna viridis</i>	FJ428757
<i>Perna viridis</i>	DQ343582
<i>Perna viridis</i>	DQ343573

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