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Evidence of a human-mediated invasion of the tropical western Atlantic by the 'world's most common brittlestar'

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Approximately three million years ago the Isthmus of Panama formed an impenetrable land barrier between the tropical eastern Pacific Ocean and the tropical western Atlantic Ocean. Since this time, isolated geminate species have evolved from once contiguous populations, either side of the barrier. One such organism whose distribution is divided by the Isthmus is the tropical brittlestar *Ophiactis savignyi*, once suggested to be the most common brittlestar in the world. Rather than showing a genetic pattern consistent with a history of isolation, we show that this species has recently dispersed between the Pacific Ocean and the western Atlantic Ocean. This conclusion is based upon a phylogenetic analysis using sequences of the COI mitochondrial DNA gene from these populations. Identical haplotypes between oceans, and a genetic signature of population expansion, provide compelling evidence that the western Atlantic contains at least one cluster of haplotypes recently derived from the Indo-Pacific. Inadvertent human-aided translocation of individuals, presumably in ballast water or fouling communities, is strongly implicated as a mechanism for dispersal between oceans. We believe that cryptic marine invasions are likely to be common and our awareness of them will rapidly increase as systematic and phylogeographic knowledge of marine taxa grow.

Keywords: Isthmus of Panama; mitochondrial DNA; marine conservation; *Ophiactis savignyi*

1. INTRODUCTION

Studies of vicariant speciation in the marine environment are often confounded since barriers to gene flow are usually cryptic and highly ephemeral (Benzie 1999; Palumbi 1994). An exception to this is the relatively well studied Isthmus of Panama, a land barrier that separates the tropical Atlantic Ocean from the tropical Pacific Ocean. The evolutionary effects on the once contiguous marine populations that existed across this region, prior to its formation three million years ago (Keigwin 1982), have been profound, with many geminate species evolving in allopatry either side of the barrier (review in Knowlton & Weigt 1998). As a direct result of the closing of the Isthmus, marine biodiversity increased by *ca*. 45–60% in the surrounding Atlantic and Pacific regions (McKinney 1998). The genetic legacy of the recent connection of these oceans is still evident amongst many taxa, including eastern Pacific coral genera that are more closely related to those of the western Atlantic than to those of the western Pacific (Grigg & Hey 1992). Several studies have used the known geological age of the closing of the Isthmus to calibrate molecular clocks (Lessios 1979) and investigate the biogeography of tropical marine taxa (Knowlton & Weigt 1998; Lessios & Weinberg 1993). The integrity of the Isthmus as a barrier to gene flow is of paramount importance for maintaining the independent evolution of the populations and incipient species either side of it. It has been predicted that if the isolatory effects of the Isthmus of Panama disappear, a loss of up to 38% of marine biodiversity may result through ecological and genetic processes (McKinney 1998).

One such organism, whose populations were apparently divided by the Isthmus, is the small tropical ophiactid brittlestar *Ophiactis savignyi*. This species was first described from the Red Sea (Müller & Troschel 1842) and then subsequently from other tropical marine regions of the world. It is a common species, and noted as being the 'most common brittlestar in the world' by Clark (1946, pp. 210), being associated with coral reefs and sea grass ecosystems (Hendler *et al.* 1995). Several well-known studies of the species have taken place from western Atlantic locations (Mladenov & Emson 1990) and these have been used to investigate its intriguing life history, which includes both asexual and sexual life cycles. Dispersal primarily occurs via planktotrophic larvae that live for approximately one month before developing into benthic adults (P. V. Mladenov, personal communication). Dispersal can also occur via juveniles, which can drift or raft (Hendler *et al.* 1999). However, this mode of dispersal is unlikely to facilitate long- or even medium-range movement and it is more likely an adaptation to finding local ecological optima within reef systems (Hendler *et al.* 1999). Most importantly, this species is known to inhabit fouling communities of boats (Clark 1919) and other man-made oceanic structures (De Felice 1999). Due to the scale and speed of shipping, this species is potentially able to disperse globally within a matter of a single or few generations, easily crossing natural marine barriers that once isolated populations and catalysed their independent evolutionary trajectories. In fact, Clark (1919) suggested that the distribution of this species could indeed be artificial.

We compare mitochondrial DNA (mtDNA) markers

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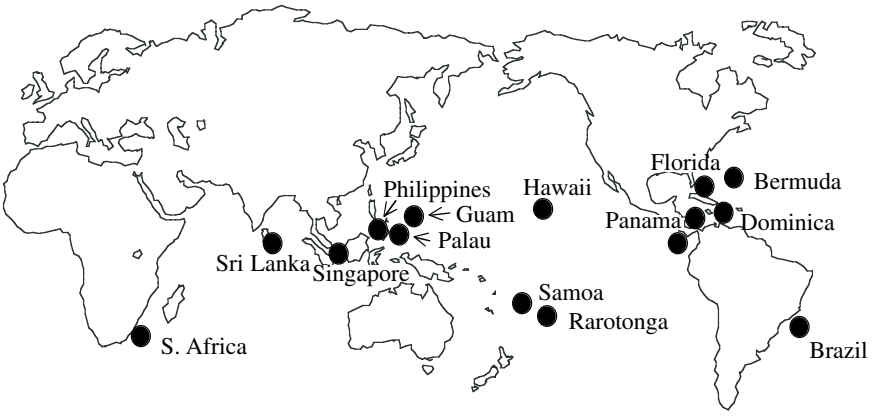

Figure 1. Location of samples for this study.

from western Atlantic and Indo-Pacific populations of *O. savignyi*. We tested whether populations separated by the Isthmus exhibit genetic divergence on a scale that is similar to other echinoderm species that span the Isthmus (Lessios 1979), and reflect three million years of isolation, as predicted by historical processes. Alternatively, we investigated whether recent dispersal between these regions is evident from our data, as predicted by their fouling behaviour. We discuss our results in the light of possible natural and anthropogenically mediated scenarios.

2. MATERIAL AND METHODS

(a) *Sample collection*

Samples of *O. savignyi* were collected by wading, snorkelling and scuba-diving and preserved in absolute ethanol. As sponge inhabitants are clonal only one specimen per sponge was used in the analysis. For the same reason, one sample from each cluster found on algae was used. Using this approach we sampled specimens from five localities in the Caribbean and western Atlantic (Bermuda $n = 9$, Florida $n = 6$, Dominica $n = 9$, Panama $n = 7$, Brazil $n = 5$) and eight localities in the tropical Pacific (Panama $n = 6$, Hawaii $n = 8$, Palau $n = 3$, Guam $n = 1$, Philippines $n = 2$, Singapore $n = 3$, Rarotonga $n = 7$ and Samoa $n = 7$) and two localities from the tropical Indian Ocean (South Africa $n = 2$, Sri Lanka $n = 4$) (figure 1). Other Indian Ocean populations were previously found to be of a separate evolutionary lineage and may constitute a cryptic species (Roy & Sponer 2001); these populations were not included in this analysis. We used *Ophiactis algicola, O. lymani* and *O. quinqueradia* as outgroup comparisons.

(b) *DNA extraction and sequencing*

DNA was extracted from a piece of arm, 2 mm long, using 5% Chelex 100 (Walsh *et al.* 1991) and incubated at 65 °C for 2–4 h. After brief vortexing and spinning the solution was boiled for 8 min and spun down at 13 000 *g*. The supernatant was transferred to a new tube and used for PCR.

All amplifications were carried out using internal mtDNA COI gene primers COIa and COIf (Palumbi *et al.* 1991). The PCR products were separated from excess primers and oligonucleotides in Qiaquick spin columns (Qiagen) and subsequently used in cycle sequencing.

Cycle sequencing was carried out as 20 µl reactions with the double-stranded product from above and the Big Dye (Applied Biosystems) labelling mix, using a temperature profile of 15 s at 96 °C, 30 s at 55 °C and 4 min at 60 °C for 30 cycles, with an initial denaturing step at 94 °C for 1 min. Sequences were run overnight on a 377 ABI automated sequencer (Perkin Elmer).

Sequences have been submitted to GenBank with accession numbers AF 331527–30, 331533, 331534, 331536, 331537, 331539–73, 331591, 331592, 331594, 331595, 331600, 331601, 331603–16, 331629, 331632, 331634–36, 480892–480905.

(c) *Sequence alignment and phylogenetic data analysis*

Chromatographs were verified by eye and aligned visually using the program SeqApp (Gilbert 1992). Unweighted maximum-parsimony trees were constructed by comparing 500 base pairs (bp) of the COI gene, in Paup* (Swofford 2000). Bootstrap analyses were undertaken using 1000 heuristic search replicates. In addition to a phylogenetic tree a parsimony network was constructed using the programme TCS (Clement *et al.* 2000). TCS calculates the most parsimonious network at the 95% confidence level. The programme collapses sequences into haplotypes and calculates the frequencies of haplotypes. These frequencies are used to estimate the haplotype outgroup probabilities which correlate with haplotype age.

Frequency distributions of Kimura 2 parameter distances (Kimura 1980) between pairs of haplotypes (mismatch distribution) were estimated from the sequence data using Arlequin (v. 2.000; Schneider *et al.* 2000). This distribution is usually multimodal in populations at demographic equilibrium, but is unimodal in populations that have recently experienced a sudden expansion (Rogers & Harpending 1992). A parametric bootstrap approach was used to estimate *p*-values, under the assumption that the distribution of mismatches are not different from the expansion model (Schneider *et al.* 2000) and provide a guide to how well the data fit the expansion model. Nucleotide and gene diversities were calculated in Arlequin (v. 2.000; Schneider *et al.* 2000).

3. RESULTS

Thirty-seven trees of equal length were recovered from the analysis (figure 2) which differed only in the ordering of tips. Bootstrap analysis gave high overall support to clades. Ingroup taxa were well supported as a monophyletic clade when compared with congeneric taxa. We tested for evolutionary rate heterogeneity amongst ingroup and outgroup taxa by comparing trees, using maximum-

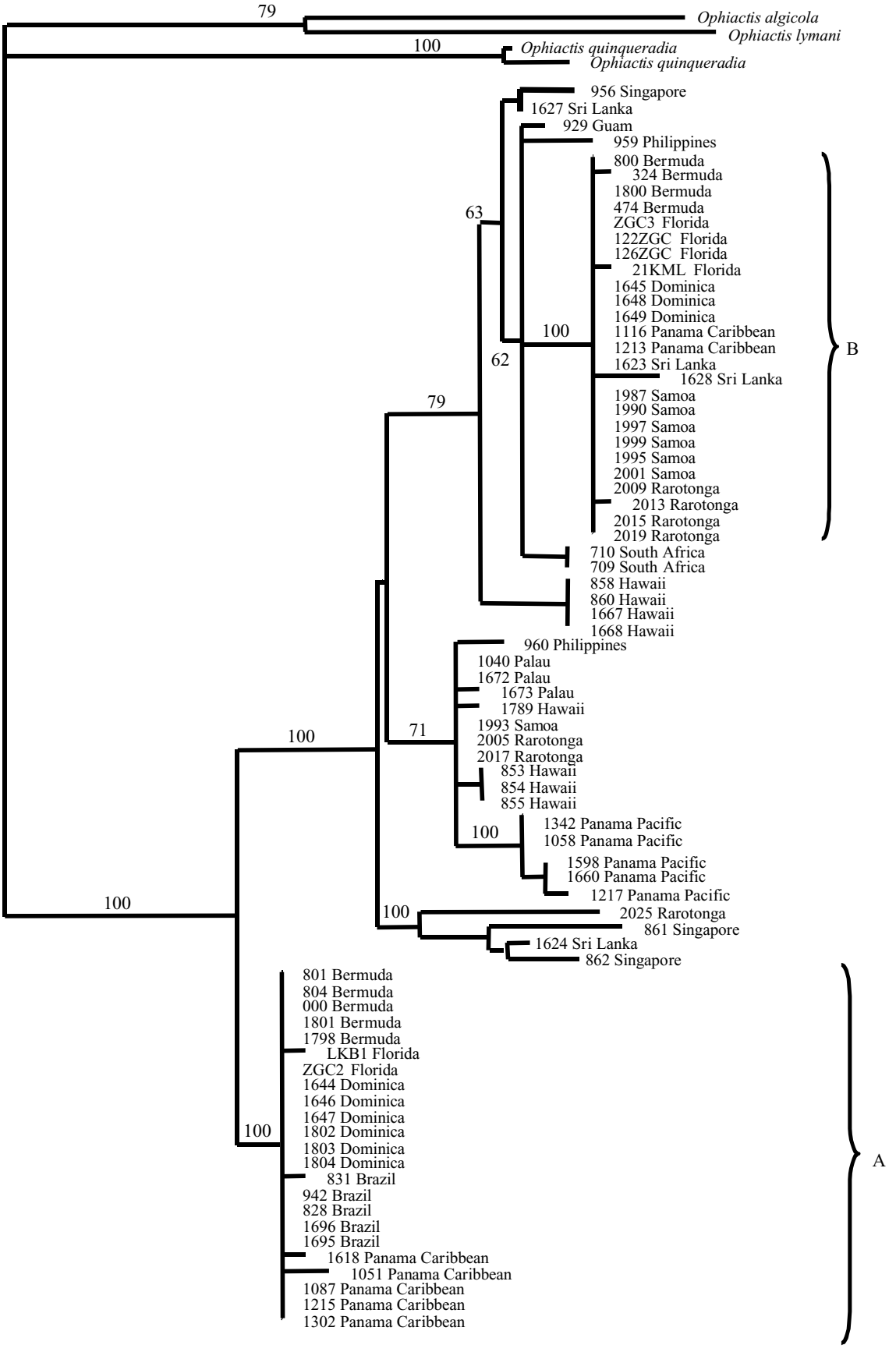

Figure 2. One of 37 maximum parsimony trees based upon 500 bp of the COI mtDNA gene. Trees had an equal length of 308 steps, consistency index of 0.785, retention index of 0.91 and homoplasy index of 0.21. Bootstrap support is indicated above branches. The two lineages A and B are indicated.

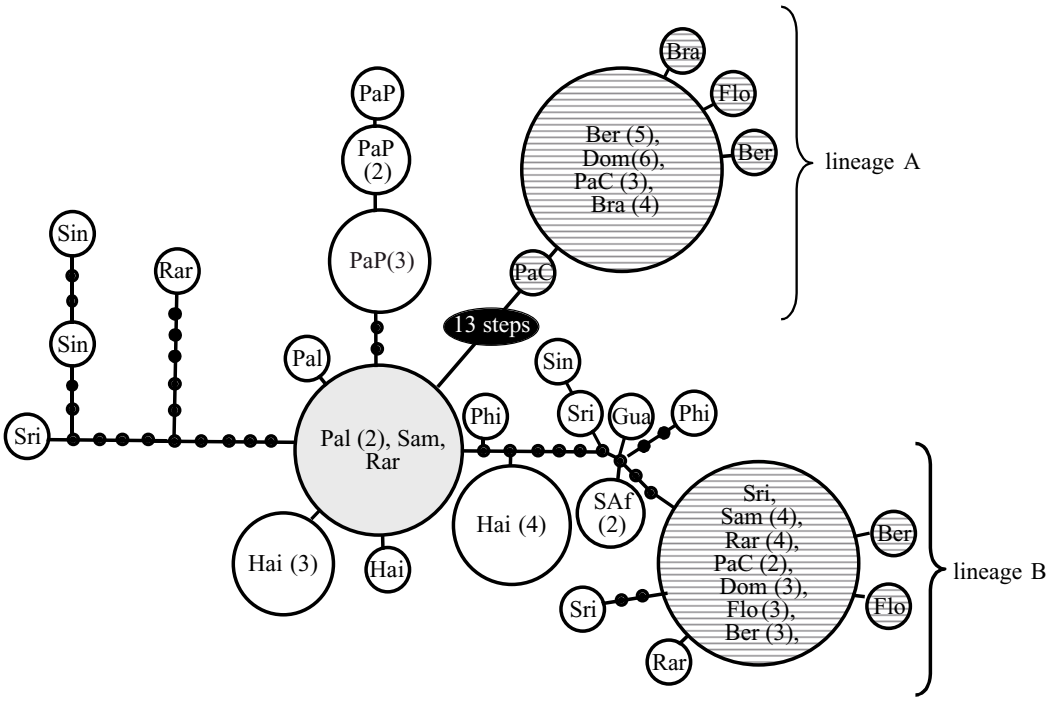

Figure 3. TCS parsimony network of haplotypes, with Atlantic lineages indicated. Maximum connection length with 95% probability was limited to nine steps, hence the connection of lineage A cannot be made with certainty. The putative ancestral haplotype is shaded in grey. Hatched circles represent those from the Atlantic and open circles are those from the Indo-Pacific. Small black filled circles indicate missing haplotypes. Abbreviations: Ber, Bermuda; Flo, Florida; Dom, Dominica; PaC, Panama Caribbean; Bra, Brazil; SAf, South Africa; Sri, Sri Lanka; Pal, Palau; Phi, Philippines; Gua, Guam; Sam, Samoa; Rar, Rarotonga; Hai, Hawaii; PaP, Panama Pacific.

likelihood scores with and without the enforcement of a molecular clock. No evidence for an unequal rate of evolution for the COI gene was revealed from this analysis ($\chi^2 = 58$, d.f. = 81, $p > 0.05$).

Atlantic individuals grouped into two lineages, A and B (figures 2 and 3), that differed by 1.9% (±1.0%) sequence divergence (Nei & Li 1979). Lineage A was apparently monophyletic and differed from Pacific haplotypes by 2.6% (±1.3%). This level of divergence is comparable with other echinoderm species whose populations have been isolated by the formation of the Isthmus. This implies that lineage A was derived *in situ* within the nascent western Atlantic, congruent with geological processes. Lineage B, however, shared its most frequent haplotype with Sri Lanka, Rarotonga and Samoa of the Indo-Pacific (figure 2). This haplotype does not represent an ancient ancestral haplotype since it is highly improbable that no change (nucleotide substitutions) would have occurred over 500 bp over three million years ($p \ll 0.01$) given an evolutionary rate of 1.5% per million years (congruent with other studies of invertebrates that span the Isthmus; see Knowlton & Weigt (1998) for a review). Rather, this result strongly suggests that the distribution of this haplotype is caused by recent dispersal between the Pacific and Caribbean, that postdates the closing of the Isthmus.

(a) *Mismatch distributions*

Gene and nucleotide diversity of Atlantic lineages (figure 2) were extremely low ($h^2 = 0.32 \pm 0.12$, $\pi = 0.0007 \pm 0.0007$ and $h^2 = 0.29 \pm 0.16$, $\pi = 0.0006 \pm 0.0008$ for A and B, respectively) compared with Indo-Pacific haplotypes ($h^2 = 0.93 \pm 0.03$, $\pi = 0.018 \pm 0.01$). The mismatch distribution of Atlantic samples as a whole was significantly different to that expected under an expansion model ($p = 0.02$). However, this was due to the inclusion of the two divergent lineages of haplotypes, essentially producing a bimodal distribution of sequence substitutions. When each lineage was analysed independently, neither were significantly different to that expected under an expansion model ($p = 0.29$ and 0.37, for A and B, respectively; figure 4). Furthermore, relative frequencies of haplotype differences appeared similar (figure 4) suggesting that the onset of expansion of both lineages was synchronous and is ongoing. Pacific haplotypes did not fit the expansion model ($p < 0.05$; figure 4) suggesting that the demographic expansion of the Atlantic was not linked to a worldwide phenomenon of population expansion because Pacific populations appear to have enjoyed a history of long-term demographic equilibrium.

4. DISCUSSION

Ophiactis savignyi exhibited a strong phylogenetic structure amongst haplotypes over its range. Within the Indo-Pacific, however, little phylogeographic structure was evident, with local haplotypes often more similar to haplotypes of distant regions than to others within the same locality. An identical haplotype was found amongst Palau and the South Pacific, and between the South Pacific and Sri Lanka. A phylogenetically similar haplotype was found between South Africa and Guam. These data suggest a history of massive long-distance dispersal and recent mixing amongst the western Pacific and the Indian Ocean populations. Conversely, eastern Pacific haplotypes sug-

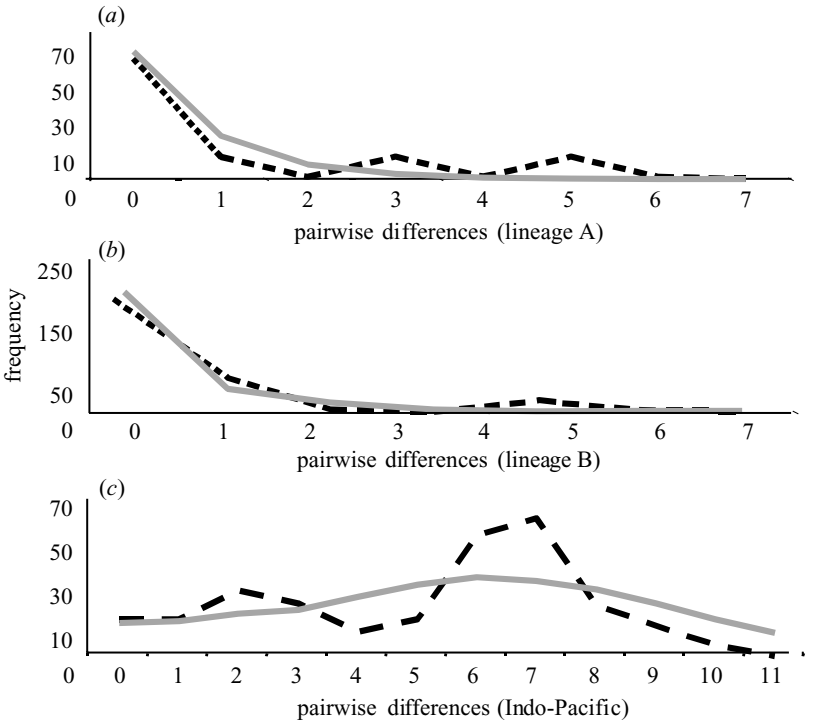

Figure 4. Mismatch distribution of haplotype distances. Expected distributions are indicated by the solid grey line and observed distributions are given as the hatched broken line.

gest a history of long-term isolation showing monophyly and strong phylogenetic structure (figures 2 and 3). This region is often cited as being isolated from the rest of the Pacific due to the large area of open and deep ocean, often known as the eastern Pacific barrier (EPB; Ekman 1953).

Atlantic mtDNA haplotypes grouped into two phylogenetic lineages, A and B, which were more similar to Pacific haplotypes than to each other. The distribution of an identical haplotype in the Indian, Pacific and Atlantic oceans provided strong evidence of recent dispersal of haplotypes between these regions (figures 2 and 3). To our knowledge, the occurrence of a single haplotype in every ocean for a gene of equivalent mutation rate to COI, has not been documented previously.

Natural long-distance movement of this species is likely to be dictated by ocean currents moving larvae away from natal sites (Hendler *et al.* 1999). We explored the possibility that recent dispersal by currents between the Atlantic and Indo-Pacific could explain our findings. Although this tropical species is highly unlikely to be able to disperse around Cape Horn (South America) due to the cold waters of the circum-Antarctic current, dispersal around the Cape of Good Hope (South Africa) may be more probable, as warm waters of the Agulhas current travelling south along the eastern side of South Africa are able to flow into the southeastern Atlantic (Briggs 1974; Gordon 1985; de Ruijter *et al.* 1999). However, the Benguela cold current, and upwelling off southwestern Africa, present a major barrier to the dispersal of even highly vagile tropical Indian Ocean taxa into the Atlantic (Bowen *et al.* 2001). The Benguela current originated in the Miocene period but has experienced fluctuations in its intensity ever since the beginning of the Pleistocene period (Shannon 1985) and may have allowed dispersal of tropical taxa into the Atlantic during times of minimal strength. Although we cannot discount this possibility we do not favour this explanation of our result. First, we would expect that several opportunities for dispersal would have existed in the past and therefore ancient western Atlantic haplotypes of Indo-Pacific affinity should be apparent throughout the phylogeny. Second, if gene flow had occurred via this route we would expect many diverse Indo-Pacific haplotypes to be represented in the western Atlantic since dispersal opportunities for several dispersing larvae would presumably have existed at any one time. Third, there is no evidence of very recent gene flow in other tropical taxa via this route. For example, although Lessios *et al.* (2001) found evidence for ancient gene flow via this route for tropical *Diadema* spp. (Echinoidea) there is no evidence that gene flow has occurred for the past one to two million years. Furthermore, extant species of this group exhibit a wide range of thermal tolerance, lending themselves well to dispersal within the cold waters of the southeastern Atlantic. *Amphipholis squamata*, a small ophiuroid often sympatric with *O. savignyi*, shows a deep biogeographic break between tropical eastern and south–southwestern South African populations (R. Sponer and M. S. Roy, unpublished data) suggesting that gene flow between tropical Indian Ocean and Atlantic Ocean has not occurred for several millions of years. Unlike either of these species the distribution of *O. savignyi* is highly restricted to warm waters, suggesting that it is unlikely to survive the low temperatures of the Benguela cold current. Lastly, and most importantly, the occurrence of a single haplotype over such a wide area, spanning several biogeographic regions and barriers to dispersal (South Pacific, Indian Ocean and western Atlantic), would require an unprecedented natural dispersal potential as yet unknown in the marine realm.

We conclude that lineage B is derived from a recently introduced haplotype from the Indo-Pacific via anthro-

pogenic means. In support of this assertion is its low gene and nucleotide diversity that are congruent with a recent founding event and suggest only a single introduction of a related cluster of haplotypes. This intriguing pattern may be explained by the behaviour of *O. savignyi*, which is known to live within sponges to densities of up to 3000 per litre (Hendler *et al.* 1995). Importantly, these aggregations are highly clonal, since reproduction within them is by fission. We therefore suggest that rather than by a single larva, lineage B is more likely to have colonized the western Atlantic by a single sponge inhabiting colony of several thousand clonemates, a few (e.g. 324 Bermuda and 21KML Florida) subsequently developing unique single point mutations in the COI gene that were picked up in our analysis. The hypothesis that mutational events in this gene are recent, is supported by a lack of genetic variation amongst Caribbean individuals of lineage B for the 16S mtDNA gene (data not shown). As combined asexual and sexual cycles are a dominant feature in the reproduction of this organism, the presence of uniquely derived haplotypes within the Caribbean within only a few hundred years of introduction may be explained by high somatic mutation rates, and fixation to homoplasmy in this species (Howell *et al.* 1996; Gill *et al.* 1995). Furthermore, this lineage shows a large and ongoing radiation as evidenced by its low genetic diversity and huge range (Bermuda and throughout the Caribbean) as expected for an invading population in the process of establishing itself. Intriguingly, lineage A also displays comparably low diversity indices with an enormous range (Brazil, throughout the Caribbean and Bermuda) suggesting that perhaps it too is radiating from a recent founding event from a single colony. Although our data do not support this conclusion, it remains to be seen whether haplotypes identical to those in lineage A might be found in the Pacific after exhaustive sampling. If both lineages were introduced from the Pacific then perhaps the entire species range in the Atlantic could be artificial, as referred to by Clark (1919), and it is experiencing ecological release in this region. This hypothesis suggests that introduction of the species into the western Atlantic occurred prior to their description in the 19th century from the western Atlantic, or that those original descriptions were taxonomically incorrect.

We predict that cryptic marine invasions are extremely widespread and will become more common, especially amongst species that are able to foul vessels, or those with long-lived planktonic larvae that may be transported within the ballast water of large ships. Approximately ten billion tonnes of shipping ballast water are carried around the world annually (Rigby 1999) providing an efficient mechanism for the transfer and dispersal of most marine taxonomic groups (Carlton & Geller 1993). This process serves to homogenize once isolated marine regions by erasing past evolutionary patterns, and ongoing evolutionary processes, that have served to produce global marine biodiversity (McKinney 1998). Our poor systematic knowledge of marine organisms has confounded our appreciation of the scale of the problem, which has grave ecological and economic consequences (Carlton 1989).

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