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Research Article

Investigating the cryptogenic status of the sea squirt *Didemnum perlucidum* (Tunicata, Ascidiacea) in Australia based on a molecular study of its global distribution

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Abstract

Didemnid species are assessed as species with a high invasive potential for Australia and as such are listed as target species for both state and national monitoring programs. The presence of the sea squirt *Didemnum perlucidum* (Monniot, 1983) was first documented in Australia in 2010 and has since then been detected extensively throughout the state of Western Australia and in the Northern Territory. These detections have raised important questions as to the origin and potential impact of this species in Australia. The current study was initiated to review the current known global geographic range of *D. perlucidum* and to obtain specimens that could support molecular studies aimed at evaluating the potential origin of this species in Australia. Characterization of 5’ *COI* mitochondrial sequences from 286 specimens revealed a remarkably low level of genetic diversity across the current known range of *D. perlucidum* and the existence of one main widespread genetic haplotype. Such findings suggest that all locations sampled in this study may in fact represent introductions of *D. perlucidum* and that the natural native range of the species remains unknown. Our demonstration that specimens (*n*=187) originating from across a broad expanse of the Australian West Coast were comprised of a single haplotype also lends support to the hypothesis that *D. perlucidum* is a species that has been introduced recently into Australia. This hypothesis is supported by the fact that *D. perlucium* distribution in Australia is mostly confined to artificial structures, it has displayed invasive characteristics, and its presence is now being detected across an increasingly wide geographical area. Given the demonstrated low level of genetic *COI* variation across its known global distribution, lack of clarity around its native range, and limited availability of data on this species globally, we recognize the requirement for further work to more fully elucidate the exact origins and patterns of distribution of *D. perlucidum* in Australia. This study represents the most comprehensive mapping of the current global distribution of *D. perlucidum* conducted to date and will hopefully motivate further studies aimed at elucidating this species biology, origin, high-risk routes and impacts.

Key words: Didemnidae, cytochrome c oxidase I, colonial sea squirt, introduced

Introduction

The human-mediated transport of species outside of their native ranges is recognised to have dramatically increased during the last century due to industrialization, human population growth, and globalization (Elton 1958; Carlton 2011). Global anthropogenic influence and the lack of baseline surveys undermine certainty in assigning new records to a native or introduced status. Species that cannot be reliably assigned to either category are referred to as “cryptogenic” (Carlton 1996, 2011). One example of a cryptogenic species is *Didemnum perlucidum* (Monniot, 1983), a colonical ascidian that was first documented in Australia from the Swan River, Perth, in 2010 (Smale and Childs 2012).

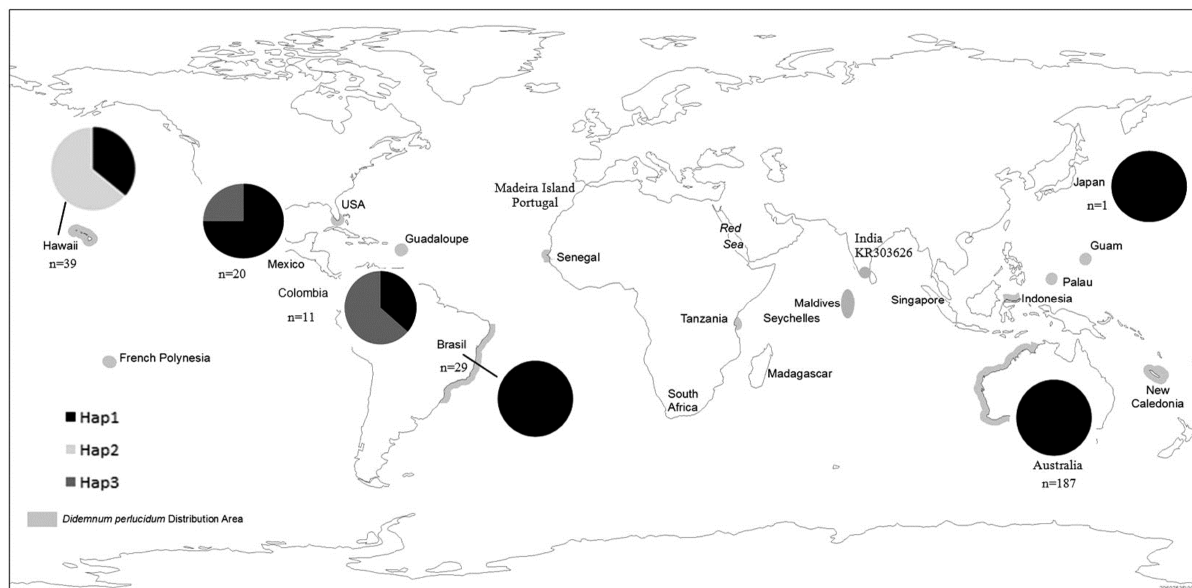

Figure 1. Map showing worldwide distribution of *D. perlucidum*, number (n) of colonies and haplotypes observed per country sampled in this study. Pie charts indicate the proportion of the three identified haplotypes (Hap1, Hap2 and Hap3). A fourth haplotype (Hap4) reported from India by Jaffar Ali et al. (2015) is indicated by GenBank Accession number KR303626. For detail on sampling locations within countries please refer to Table 1. Locations from where *D. perlucidum* had been previously identified include Guadeloupe (Monniot 1983), Tahiti in French Polynesia (Monniot et al. 1985), Brazil (Rocha and Monniot 1995), Belize (Goodbody 2000), Venezuela (Rocha et al. 2010), Senegal (Monniot and Monniot 1994), Tanzania (Monniot and Monniot 1997), the Maldives, Indonesia and Phillipines (Monniot and Monniot 2001), New Caledonia (Monniot and Monniot 1996), Palau (Lambert 2002), Guam (Lambert 2003), Hawaii (Godwin and Lambert 2000), Panama (Carman et al. 2011), USA (Lambert 2002), Galapagos Islands in Ecuador (Witman and Smith 2003), Madeira Island in Portugal (Canning-Clode et al. 2013). Countries with recent ascidians or invasive species surveys and where *D. perlucidum* has not been recorded to date include the Red Sea (Shenkar 2012), South Africa (Rius et al. 2014), Madagascar (Monniot 2012) and the Seychelles (Floerl et al. 2006).

Globally, ascidians are among the taxa with the highest reported record of introduced species (Lambert 2002; López-Legentil et al. 2015; Pagad et al. 2015) and according to a risk-based approach, didemnid species are listed as target species for both state and national introduced marine pest monitoring programs in Australia. Following its first detection in Australia, monitoring programs supported by molecular identification techniques have documented the distribution of *D. perlucidum* throughout the state of Western Australia (WA) and in the Northern Territory (NT). Given the absence of previous records of *D. perlucidum* in Australia, a country from which ascidian diversity has been extensively described (Kott 2005; Shenkar and Swalla 2011), and its demonstrated invasive characteristics (Smale and Childs 2012; Bridgwood et al. 2014; Muñoz et al. 2015), *D. perlucidum* is being managed in Australia as an introduced marine pest.

A primary aim of the current study was to review the current known global distribution of *D. perlucidum* and collect biological specimens from across its global range. Whilst the species has been reported from numerous locations across the Atlantic, Pacific, and Indian Oceans (Figure 1), its native range remains undetermined (Lambert 2002; Muñoz et al. 2015). We further sought to use this specimen collection as a basis to investigate the molecular diversity across the range of *D. perlucidum* in an attempt to identify the likely native range and potential origin of the species in Australia. The use of genetic information has previously proven of great value in resolving the status of “cryptogenic” species (Holland 2000; Cristescu 2015). Resolution of this question is generally based on the principle that genetic diversity of a species is often relatively high in its native range due to an accumulation of mutations within natural populations over a long timeframe. When species are first introduced into a new location, only a small subset of that genetic diversity is initially transported and becomes established, which creates a founder effect (Dlugosch and Parker 2008) that is reflected in a reduced genetic diversity within the new expanding population. As

Table 1. Location, type of substrate and GPS coordinates of sampling sites, number of *D. perlucidum* colonies sampled (*n*) and haplotypes.

	Sampling location	Substrate	GPS	*n*	Haplotype
Australia	1. Esperance port, Western Australia	settlement array	33°52′18″S 121°54′07″E	2	1
	2. Waterfront Marina, Albany, Western Australia	Pontoon	35°01′52″S 117°53′16″E	2	1
	3. Port Geographe Marina, Busselton, Western Australia	Pontoon	33°37′52″S 115°23′36″E	1	1
	4. Fremantle port, Western Australia	settlement array	32°02′58″S 115°44′23″E	14	1
	5. Geordie Bay, Rottnest Island, Western Australia	Pontoon	31°59′29″S 115°31′23″E	1	1
	6. Point Walter, Swan River, Western Australia	Seagrass	32°00′40″S 115°47′12″E	1	1
	7. Hillarys Marina, Western Australia	Pontoons	31°49′32″S 115°44′17″E	71	1
	8. Mindarie Marina, Western Australia	Pontoons	31°41′25″S 115°42′00″E	2	1
	9. Batavia Coast Marina, Geraldton, Western Australia	Pontoon	28°46′03″S 114°36′39″E	1	1
	10. Houtman Abrolhos Islands, Western Australia	Pontoons	28°42′93″S 113°49′17″E	12	1
	11. Exmouth, Western Australia	Pontoons	21°57′22″S 114°08′35″E	22	1
	12. Cygnet Bay, Western Australia	oyster farm	16°23′27″S 122°55′38″E	8	1
	13. Dampier, Western Australia	settlement array	20°39′46″S 116°42′04″E	45	1
	14. Darwin, Northern Territory	oyster farm	11°92′52″S 136°80′17″E	5	1
Japan	15. Kochi new port, Kochi Prefecture, Shikoku	dock	33°26′10″N 133°26′36″E	1	1
Hawaii	16. Keehi Lagoon, Oahu	floating dock	21°18′59″N 157°53′09″W	39	1 and 2
Mexico	17. Veracruz, Gulf of Mexico	buoys	19°11′17″N 96°07′19″W	12	1 and 3
	18. Club Nautico Mazatlan, Sinaloa, Gulf of California	ropes and docks	23°10′55″N 106°25′28″W	8	1
Colombia	19. International Marina of Santa Marta	ropes tied to floating docks	11°14′31″N 74°13′05″W	11	1 and 3
Brazil	20. Florianópolis, Santa Catarina	oyster farm	27°44′08″S 48°33′52″W	28	1

the genetic geographical variation within species is often stronger than any morphological variation, it can provide a useful basis for the assignment of potential origin (Estoup and Guillemaud 2010; Geller et al. 2010). This is particularly true for recently introduced populations, as subsequent long-term multiple introduction events from different sources is known to increase genetic diversity and “blur the genetic signal” that would allow to track the origin of introduced populations (Sakai et al. 2001; Dlugosch and Parker 2008). This study reports on the evaluation of *COI* diversity across 286 specimens of *D. perlucidum* assembled from across the known global distribution of this species in an attempt to better understand the cryptogenic status and likely origins of this potential invasive marine pest in Australia. Understanding the origins of potential invasive marine pests remains fundamental to their risk-based management.

Materials and methods

Sampling

Tissue samples were collected by hand (snorkelling, scuba diving or from artificial structures pulled from the water) from individual (discontinuous) colonies of *D. perlucidum* at locations in Australia and internationally between 2012 and 2015 (Table 1). Samples were preserved in 70–100 % ethanol and transported to the Marine Research Laboratories of the WA Government Department of Fisheries (DoF) at Hillarys for processing. The *COI* sequence from one colony previously collected in Japan, was also made available to this study.

COI amplification and sequence analysis

Approximately 5 mg of ascidian tissue (with tunic) from each sample was transferred to an micro-centrifuge tube, homogenised using a micropestle and 200µl of lysis buffer (Fisher Biotec), and incubated overnight with 20µl of proteinase K (Fisher Biotec) at 60ºC. DNA was extracted using a Fisher Biotec Favorgen FavorPrep Tissue Genomic DNA Extraction Mini Kit, following the manufacturer's instructions (Fisher Biotec, Australia, Wembley, West Australia). PCR amplification of mtDNA *COI* haplotypes was performed using the Tun_forward and Tun_reverse2 primers developed by Stefaniak et al. (2009). PCR reactions were conducted in 25 µl containing 2 µl DNA, 1.25 mM of each dNTP, 62.5 mM MgCl2, 2.5 µl of 10x reaction Buffer, 2.5 µM of each primer, one unit of Taq DNA polymerase (Fisher Biotec) and PCR-grade water (Fisher Biotec). PCR conditions consisted of an initial incubation at 94 ºC for 1 min, followed by 5 cycles of 94 ºC for 40 s, 45 ºC for 40 s and 72 ºC for 60 s; 35 cycles of 94 ºC for 40 s, 51 ºC for 40 s, 72 ºC for 60 s; and a final extension step of 72 ºC for 5 min. PCR reactions were conducted in an Applied Biosystems (ABI) 2720 thermal cycler. A negative control, with no template DNA added, was included in all PCR assays. PCR products were separated by electrophoresis using 1.5 % agarose (Fisher Biotec) gels stained with ethidium bromide (Fisher Biotec) alongside a 100 base pairs (bp) molecular weight marker (Axygen Biosciences, Union City, California, USA) and visualised under UV light.

Sequencing of unpurified PCR products was performed using the service provided by the Australian Genome Research Facility (AGRF) in Perth. All samples were sequenced in both directions and consensus sequences generated using the Sequencher® 5.0 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI USA). Sequences were aligned, analysed and trimmed in BioEdit 7.1.3.0 (Hall 1999). To our sequence set, we added three *D. perlucidum* haplotypes of the *COI* gene available from the NCBI GenBank database (JQ731735, JQ731740 and KR303626).

Results

A total of 187 samples were collected from within Australia and 99 samples were collected from locations elsewhere (Table 1), spanning the known geographical range (Figure 1). All specimens were confirmed as *D. perlucidum* based on sequence comparison of the CO1 "barcode" region. Despite previous records of *D. perlucidum* in Palau (Monniot and Monniot 1996) and Kaneohe Bay in Hawaii (Coles et al. 2002), and the suspected presence of *D. perlucidum* fouling mussel farms in Singapore, no *D. perlucidum* samples were available when researchers revisited these locations. One sample collected in another previously-reported *D. perlucidum* location, Zanzibar in Tanzania (Monniot and Monniot 1997), was identified as *Lissoclinium fragile* (Van Name, 1902), a white colonial Didemnidae frequently mistaken with *D. perlucidum* during sampling.

Analysis of 511 bp of the *COI* region of 286 *D. perlucidum* samples revealed three distinct haplotypes. Sequences obtained from 182 samples collected at 13 sites in WA and from five samples collected at one site in the NT were represented exclusively by a previously reported haplotype for WA (JQ731735) which we refer to for convenience as Hap1. The 29 sequences obtained from one location in Brazil, the one sequence obtained from Japan and the eight samples collected in the Mexican Pacific coast also belonged to Hap1. This haplotype was the most frequent overall (247/286) and was present at all locations sampled, in Australia and abroad (Table 1, Figure 1).

In contrast, samples collected from Hawaii were dominated (25/39) by a second haplotype (Hap2), which was also represented by a single colony collected from a vessel incursion in New South Wales (NSW), Australia (JQ731740). Five of the 12 samples collected from the Mexican Caribbean coast, and seven of the eleven samples collected from Colombia, revealed a previously unreported haplotype (Hap3, GenBank Accession number KU883151) (Figure 1, Table 1). We did not identify any sequences matching the KR303626 haplotype (Hap4) reported for *D. perlucidum* in India (Jaffar Ali et al. 2015). Hap2 and Hap3 were found to differ from Hap1 by only 5 base pairs and 1 base pair, respectively, while the Indian Hap4 differs from Hap1 by 3 base pairs.

Discussion

In the present work, we report on the current presence of established populations of *D. perlucidum* in Australia and internationally (Table 1). Our study greatly added to the known range of *D. perlucidum* in Australia through identification of six new location records in addition to the ones reported by Bridgwood et al. (2014). New locations in WA included Esperance, Albany, Rottnest Island, Mindarie, the Houtman Abrolhos Islands, and Exmouth. Detection from artificial structures at locations within the marine reserve areas of the Houtman Abrolhos Islands and Rottnest Island are of particular relevance, as such areas are classed as High Value Asset areas within the state. In addition, this study

represents the first documented characterisation of populations previously known to be established in the NT. Overall these findings highlight the ability of *D. perlucidum* to colonise across the wide range of environmental conditions encountered across this wide geographical range. Indeed, the identification of *D. perlucidum* from Esperance represents the southern-most detection of this organism in the world to date.

From a global perspective, this study further adds to the known distribution of *D. perlucidum*, by documenting the first reports of *D. perlucidum* from Japan and Mexico. It is also the first documented characterisation of colonies previously suspected (F. Brown, University of Sao Paulo, Brazil, personal communication) to be present at Santa Marta, Colombia. Detection at Kochi in southern Japan represents the most northern latitude from which this organism has been detected to date. Whether *D. perlucidum* is able to persist at the climatic extremes of Esperance and Kochi throughout the year remains unclear, as the species has been shown to be strongly influenced by seasonal changes in temperature (Munoz et al. 2015). The detection in Japan also represents the first documented report of the tropical invasive species *D. perlucidum* co-occurring with the temperate invasive species *Didemnum vexillum* (Kott, 2002), within the native range of *D. vexillum*.

Detection from the east coast of Mexico adds to previous reports of *D. perlucidum* from US waters in the Gulf of Mexico area (Lambert 2002). The detection of *D. perlucidum* from the west coast of Mexico is, as far as the authors are aware, the first reported for the Gulf of California. Attempts to collect further samples in this area was complicated by weather conditions and the fact that the disappearance of *D. perlucidum* mats, as well as other four introduced ascidians namely *Botryllus schlosseri* (Pallas, 1766), *Botrylloides nigrum* (Herdman, 1886), *B. violaceus* (Oka, 1927) and *Polyclinum constellatum* (Savigny, 1816), is common in this area after weather events such as tropical depressions, storms and hurricanes, and consequent floods (MA Tovar-Hernandez, personal observation). This kind of tropical disturbance may explain the apparent disappearance of *D. perlucidum,* or any other look-alike ascidians, from Singapore, Palau and Kaneohe Bay in Hawaii.

The great majority of colonies of *D. perlucidum* from locations reported in this study were obtained from artificial structures from within ports, harbours, and marinas. In WA, the exception to this was the invasive *D. perlucidum* population found established and heavy fouling seagrass meadows in the Swan River. Despite the awareness and obligation to report *D. perlucidum* populations in Australia, this species has not been noticed from natural habitats such as marine reefs. This is consistent with the fact that the species has been historically reported in association with sites under anthropogenic influence (Monniot 1983; Lambert 2002; Kremer et al. 2010; Bridgwood et al. 2014). Indeed, the presence of *D. perlucidum* has not been noticed to date from natural substrates in the countries sampled outside Australia such as Hawaii, where researchers are routinely involved in the survey of marine protected areas. Despite the numerous surveys of ascidian species from natural and artificial substrates such as Monniot (1983) in Guadeloupe, Monniot and Monniot (1997) in Tanzania, Lambert (2003) in Guam, and Lambert (2002) in Palau, this species was found mainly restricted to artificial structures like pylons and buoys, and locations with high abundance of dead coral. The fact that there are few records for the occurrence of *D. perlucidum* in natural substrates supports the previous notion that the native range of the species remains unknown (Lambert 2002; Monniot and Monniot 1997).

In order to attempt to resolve the cryptogenic status of *D. perlucidum* in Australia we examined the molecular diversity of specimens obtained at the *COI* locus. *COI* was selected as an appropriate marker due to both it being used routinely in our laboratory as the basis for *D. perlucidum* identification and the fact that it has been demonstrated to be suited to the global-scale study of diversity amongst other closely related ascidians (Stefaniak et al. 2012). Overall the results of this study indicated a striking lack of *COI* genetic diversity across the known range of *D. perlucidum*. Limited genetic variation within colonial organisms with a largely asexual life cycle such as ascidians has been noticed at introduced locations and is suggested to represent an ecological advantage as it reduces inter-colony conflict, enhancing colonizing potential (Smith et al. 2012). The haplotype detected in Australia was also shown to be the predominant haplotype worldwide. The level of genetic variation well below that reported in natural populations of other colonial ascidians supports the notion that the entire range of *D. perlucidum* reported in this study may reflect introductions from an as yet unidentified historical or contemporary natural population. This is supported by the fact that similar low *COI* haplotype diversity coupled to a predominant haplotype is also a pattern consistently observed for other introduced ascidians species such as *Clavelina lepadiformis* Mueller, 1776 (Turon et al. 2003), *Clavelina oblonga* Herdman, 1880 (Rocha et al. 2012), *Botryllus schlosseri* (Bock et al. 2012) and the closely related *D. vexillum* (Stefaniak et al. 2012; Ordóñez et al.

2015). However, we recognise that only through more comprehensive sampling, in tropical areas and within the *D. perlucidum* native range, could the association between low *COI* diversity and introduced populations of this species be confirmed. Teske et al. (2014) points out that in ascidians lacking a long-lived dispersal phase, and where the number of individuals establishing new populations might be small, it can be difficult to distinguish between recently established, natural, and introduced populations. The lack of understanding of the native range of introduced marine pests is also not unique to *D. perlucidum*, with similar situations existing for other introduced ascidians in Australia such as *Botryllus schlosseri* (Kott, 2005), *Styela plicata* Lesueur, 1823 (Torkkola et al. 2013) and *Ciona intestinalis* Linnaeus, 1767 (Zhan et al. 2010).

Coupled with the lack of previous records of *D. perlucidum* despite numerous surveys of ascidians in Australia (Kott 2001, 2005; McDonald et al. 2005), evidence of invasive properties, and a widening detected distribution restricted mainly to artificial infrastructure, the detection of only a single haplotype of *D. perlucidum* in established populations of this species in Australia adds further support to the current hypothesis that *D. perlucidum* has been introduced relatively recently and likely has spread within this region. This study represents the most comprehensive mapping of the current global distribution of *D. perlucidum* and has resulted in the collection of a comprehensive biological specimen collection. Evaluation of *COI* molecular diversity across its global range lends support to the hypothesis that, like a number of other cryptogenic ascidian marine pest species, *D. perlucidum* appears to have been introduced into man-made environments across its global range. Future work is planned using finer scale population genetic markers in order to further resolve the pattern of likely spread of this species within Australia. Such information remains fundamental to understanding the distribution pathways of marine pests so that their introduction, dissemination and potential impacts can be effectively managed.

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References

Bock DG, MacIsaac HJ, Cristescu ME (2012) Multilocus genetic analyses differentiate between widespread and spatially restricted cryptic species in a model ascidian. *Proceedings of the Royal Society B: Biological Sciences* 279: 2377–2385, http://dx.doi.org/10.1098/rspb.2011.2610

Bridgwood SD, Muñoz J, McDonald JI (2014) Catch me if you can! The story of a colonial ascidian's takeover bid in Western Australia. *BioInvasions Records* 3: 217–223, http://dx.doi.org/10.3391/bir.2014.3.4.02

Canning-Clode J, Fofonoff P, McCann L, Carlton JT, Ruiz G (2013) Marine invasions on a subtropical island: fouling studies and new records in a recent marina on Madeira Island (Eastern Atlantic Ocean). *Aquatic Invasions* 8: 261–270, http://dx.doi.org/10.3391/ai.2013.8.3.02

Carlton JT (1996) Biological invasions and cryptogenic species. *Ecology* 77: 1653–1655, http://dx.doi.org/10.2307/2265767

Carlton JT (2011) The inviolate sea? Charles Elton and biological invasions in the world's oceans. In: Richardson DM (ed), Fifty Years of Invasion Ecology: The Legacy of Charles Elton. First edition. Blackwell Publishing, UK, pp 25–34

Carman MR, Bullard SG, Rocha RM, Lambert G, Dijkstra JA, Roper JJ, Goodwin A, Carman MM, Vail EM (2011) Ascidians at the Pacific and Atlantic entrances to the Panama Canal. *Aquatic Invasions* 6: 371–380, http://dx.doi.org/10.3391/ai.2011.6.4.02

Coles SL, DeFelice RC, Eldredge LG (2002) Nonindigenous marine species in Kane'ohe Bay, Oahu, Hawaii. Bishop Museum Technical Report No. 24, 364 pp

Cristescu ME (2015) Genetic reconstructions of invasion history. *Molecular Ecology* 24: 2212–2225, http://dx.doi.org/10.1111/mec.13117

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431–49, http://dx.doi.org/10.1111/j.1365-294X.2007.03538.x

Elton CS (1958) The Ecology of Invasions by Animals and Plants. Methuen, London, http://dx.doi.org/10.1007/978-1-4899-7214-9

Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology* 19: 4113–4130, http://dx.doi.org/10.1111/j.1365-294X.2010.04773.x

Floerl O, Johnston O, Richmond MD (2006) Baseline survey of Port Victoria and surroundings: Introduced species of the Seychelles: Final Report. NIWA Client report CHC2006-050 Project IUC05502 Prepared for The World Conservation Union (IUCN), New Zealand, 66 pp

Geller JB, Darling JA, Carlton JT (2010) Genetic Perspectives on Marine Biological Invasions. *Annual Review of Marine Science* 2: 367–393, http://dx.doi.org/10.1146/annurev.marine.010908.163745

Goodbody I (2000) Diversity and distribution of ascidians (Tunicata) in the Pelican Cays, Belize. *Atoll Research Bulletin* 480: 1–33, http://dx.doi.org/10.5479/si.00775630.480

Godwin LS, Lambert G (2000) New records of Ascidiacea (Urochordata) in the marine invertebrate fouling community of O'ahu, Hawaii. *Bishop Museum Occasional Papers* 64: 59–61

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98

Holland BS (2000) Genetics of marine bioinvasions. *Hydrobiologia* 420: 63–71, http://dx.doi.org/10.1023/A:1003929519809

Jaffar Ali HA, Gopi V, Akram AS, Arshan MLMK, Rani TVJ (2015) Molecular Based Identification of Colonial Ascidians of Mandapam Coast, India through Sequencing mt-DNA. *International Journal of Science and Humanities* 1(2): 895–908

Kott P (2001) The Australian Ascidiacea Part 4, Aplousobranchia (3), Didemnidae. *Memoirs of the Queensland Museum* 47: 1–407

Kott P (2005) Catalogue of Tunicata in Australian waters. Australian Biological Resources Study, Canberra, 331 pp

Kremer LP, Rocha RM, Roper JJ (2010) An experimental test of colonization ability in the potentially invasive *Didemnum perlucidum* (Tunicata, Ascidiacea). *Biological Invasions* 12: 1581–1590, http://dx.doi.org/10.1007/s10530-009-9571-8

Lambert G (2002) Nonindigenous Ascidians in Tropical Waters. *Pacific Science* 56: 291–298, http://dx.doi.org/10.1353/psc.2002.0026

Lambert G (2003) Marine biodiversity of Guam: the Ascidiacea. *Micronesica* 35–36: 584–593

López-Legentil S, Legentil ML, Erwin PM, Turon X (2015) Harbor networks as introduction gateways: contrasting distribution patterns of native and introduced ascidians. *Biological Invasions* 17: 1623–1638, http://dx.doi.org/10.1007/s10530-014-0821-z

McDonald JI, Fromont J, Kendrick G (2005) Sponge and ascidian communities of the Recherche Archipelago. Final Report to CSIRO Strategic Research Fund for the Marine Environment. Unpublished Report

Monniot F (1983) Ascidies littorales de Guadeloupe I. Didemnidae. *Bulletin du Musee d'Histoire Naturelle de Paris, 4th series*, 5: 5–49

Monniot F (2012) Some ascidians from the southern coast of Madagascar collected during the "AtimoVatae" survey. *Zootaxa* 3197: 1–42

Monniot C, Monniot F (1994) Additions to the inventory of Eastern tropical Atlantic Ascidans: arrival of cosmopolitan species. *Bulletin of Marine Science* 54: 71–93

Monniot F, Monniot C (1996) New Collections of Ascidians from the Western Pacific and Southeastern Asia. *Micronesica* 29(2): 133–279

Monniot F, Monniot C (1997) Ascidians collected in Tanzania. *Journal of East African Natural History* 86: 1–35, http://dx.doi.org/10.2982/0012-8317(1997)86[1:ACIT]2.0.CO;2

Monniot F, Monniot C (2001) Ascidians from the tropical western Pacific. *Zoosystema* 23: 201–383

Monniot C, Monniot F, Laboutte P (1985) Ascidians of the port of Papeete (French Polynesia); Relation to the environment and to intercontinental transport by navigation (French). *Bulletin du Museum National d'Histoire Naturelle, 4th series, Section A: Zoologie, Biologie et Ecologie Animales* 7: 481–495

Munoz J, Page M, McDonald JI, Bridgwood SD (2015) Aspects of the growth and reproductive ecology of the introduced ascidian *Didemnum perlucidum* (Monniot, 1983) in Western Australia. *Aquatic Invasions* 10: 265–274, http://dx.doi.org/10.3391/ai.2015.10.3.02

Ordóñez V, Pascual M, Fernández-Tejedor M, Pineda MC, Tagliapietra D, Turon X (2015) Ongoing expansion of the worldwide invader *Didemnum vexillum* (Ascidiacea) in the Mediterranean Sea: high plasticity of its biological cycle promotes establishment in warm waters. *Biological Invasions* 17: 2075–2085, http://dx.doi.org/10.1007/s10530-015-0861-z

Pagad S, Hayes K, Katsanevakis S, Costello MJ (2015) World Register of Introduced Marine Species (WRIMS). Assessed at http://www.marinespecies.org/introduced on 2015-11-15

Rius M,Clusella-Trullas S, McQuaid CD, Navarro RA, Griffiths CL, Matthee CA, von der Heyden S, Turon X (2014) Range expansions across regions: interactions of climate change, physiology and genetic diversity. *Global Ecology and Biogeography* 23: 76–88

Rocha RM, Monniot F (1995) Taxonomic and ecological notes on some Didemnum species (Ascidiacea, Didemnidae) from São Sebastião Channel, south-eastern Brazil. *Revista Brasileira de Biologia* 55(4): 639–649

Rocha RM, Guerra-Castro E, Lira C, Pauls SM, Hernández I, Perez A, Sardi A, Pérez J, Herrera C, Carbonini AK, Caraballo V, Salazar D, Diaz MC, Cruz-Motta JJ (2010) Inventory of ascidians (Tunicata, Ascidiacea) from the National Park La Restinga, Isla Margarita, Venezuela. *Biota Neotropica* 10: 209–218, http://dx.doi.org/10.1590/S1676-06032010000100021

Rocha RM, Kremer LP, Fehlauer-Ale KH (2012) Lack of COI variation for *Clavelina oblonga* (Tunicata, Ascidiacea) in Brazil: Evidence for its human-mediated transportation? *Aquatic Invasions* 7: 419–424, http://dx.doi.org/10.3391/ai.2012.7.3.012

Shenkar N (2012) Ascidian (Chordata, Ascidiacea) diversity in the Red Sea. *Marine Biodiversity* 42: 459–469, http://dx.doi.org/10.1007/s12526-012-0124-5

Shenkar N, Swalla BJ (2011) Global diversity of Ascidiacea. *PLoS ONE* 6: e20657, http://dx.doi.org/10.1371/journal.pone.0020657

Sakai A, Allendorf F, Holt J (2001) The population biology of invasive species. *Annual Reviews of Ecology, Evolution and Systematics* 32: 305–332, http://dx.doi.org/10.1146/annurev.ecolsys.32.081501.114037

Smale DA, Childs S (2012) The occurrence of a widespread marine invader, *Didemnum perlucidum* (Tunicata, Ascidiacea) in Western Australia. *Biological Invasions* 14: 1325–1330, http://dx.doi.org/10.1007/s10530-011-0167-8

Smith KF, Stefaniak L, Saito Y, Gemmil CEC, Cary SC, Fidler AE (2012) Increased Inter-Colony Fusion Rates Are Associated with Reduced COI Haplotype Diversity in an Invasive Colonial Ascidian *Didemnum vexillum*. *PLoS ONE* 7: e30473, http://dx.doi.org/10.1371/journal.pone.0030473

Stefaniak L, Lambert G, Gittenberger A, Zhang H, Lin S, Whitlatch RB (2009) Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. *Aquatic Invasions* 4: 29–44, http://dx.doi.org/10.3391/ai.2009.4.1.3

Stefaniak L, Zhang H, Gittenberger A, Smith KF, Holsinger K, Lin S, Whitlatch RB (2012) Determining the native region of the putatively invasive ascidian *Didemnum vexillum* Kott, 2002. *Journal of Experimental Marine Biology and Ecology* 422–423: 64–71, http://dx.doi.org/10.1016/j.jembe.2012.04.012

Teske PR, Sandoval-Castillo J, Waters JM, Beheregaray LB (2014) Can novel genetic analyses help to identify low-dispersal marine invasive species? *Ecology and Evolution* 4: 2848–2866, http://dx.doi.org/10.1002/ece3.1129

Torkkola J, Riginos C, Liggins L (2013) Regional patterns of mtDNA diversity in *Styela plicata*, an invasive ascidian, from Australian and New Zealand marinas. *Marine and Freshwater Research* 64: 139–145, http://dx.doi.org/10.1071/MF12289

Turon X, Tarjuelo I, Duran S, Pascual M (2003) Characterising invasion processes with genetic data: an Atlantic clade of *Clavelina lepadiformis* (Ascidiacea) introduced into Mediterranean harbours. *Hydrobiologia* 503: 29–35, http://dx.doi.org/10.1023/B:HYDR.0000008481.10705.c2

Witman JD, Smith F (2003) Rapid community change at a tropical upwelling site in the Galapagos Marine Reserve. *Biodiversity and Conservation* 12: 25–45, http://dx.doi.org/10.1023/A:1021200831770

Zhan A, MacIsaac HJ, Cristescu ME (2010) Invasion genetics of the *Ciona intestinalis* species complex: from regional endemism to global homogeneity. *Molecular Ecology* 19: 4678–4694, http://dx.doi.org/10.1111/j.1365-294X.2010.04837.x