Multiple dispersal vectors drive range expansion in an invasive marine species

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Multiple dispersal vectors drive range expansion in an invasive marine species

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Abstract

The establishment and subsequent spread of invasive species is widely recognised as one of the most threatening processes contributing to global biodiversity loss. This is especially true for marine and estuarine ecosystems, which have experienced significant increases in the number of invasive species with the increase in global maritime trade. Understanding the rate and mechanisms of range expansion is therefore of significant interest to ecologists and conservation managers alike. Using a combination of population genetic surveys, eDNA plankton sampling and hydrodynamic modelling we examined the patterns of introduction of the predatory Northern Pacific seastar (*Asterias amurensis*) and pathways of secondary spread within southeast Australia. Genetic surveys across the invasive range reveal some genetic divergence between the two main invasive regions and no evidence of ongoing gene flow; a pattern that is consistent with the establishment of the second invasive region via a human-mediated translocation event. In contrast hydrodynamic modelling combined with eDNA plankton sampling demonstrated that the establishment of range expansion populations within a region is consistent with natural larval dispersal and recruitment. Our results suggest that both anthropogenic and natural dispersal vectors have played

an important role in the range expansion of this species in Australia. The multiple modes of spread combined with high levels of fecundity and a long larval duration in *A. amurensis* suggests it is likely to continue its range expansion and significantly impact Australian marine ecosystems.

Introduction

Many invasive species often go undetected and can establish extensive ranges before being detected (Pyšek & Richardson 2010). Thus the history of range expansion is often inferred from current distributions (Carlton & Cohen 2003; Thresher *et al*. 2003; Estoup & Guillemaud 2010) or reconstructed from historical records (Lyons & Scheibling 2009). Studies have revealed that after colonisation, rates of range expansion can vary markedly between species. For example, invasions of zebra mussels (*Dreissena polymorpha*) in North America (Johnson & Padilla 1996) and cane toads (*Rhinella marina*) in Australia (Phillips *et al.* 2006; Rollins *et al.* 2015) showed rapid population growth and range expansion soon after introduction, whereas a review of 105 introduced weeds in New Zealand revealed considerable lag phases between initial introduction and range expansion (Aikio *et al.* 2010). Yet, it can be difficult to identify the vectors by which invasive species expand their range, or understand the relative importance of these mechanisms, when patterns of range expansion are reconstructed using historical records alone. The use of population genetic surveys (utilising highly variable molecular markers) combined with an understanding of dispersal mechanisms (both natural and anthropogenic) offers a powerful means to better resolve introduction events, patterns of range expansion and population connectivity.

Colonisation of new habitat beyond a species current range requires an effective dispersal strategy. Range expansions may occur through contiguous dispersal where the population expands into adjacent habitats over short distances, or by non-contiguous dispersal, where the population expands into disjunct habitats far from the source over long distances (Shigesada *et al.* 1995; Berthouly-Salazar *et al.* 2013). The latter is usually facilitated by rare long-distance natural dispersal events or mediated by anthropogenic vectors. Several recent studies have indicated that invasive range expansions often occur by a combination of both these strategies, collectively termed stratified dispersal (Darling & Folino-Rorem 2009; Bronnenhuber *et al.* 2011). These dispersal mechanisms are known to affect genetic structure between established populations, while also producing distinctive patterns of genetic differentiation during range expansion (Nei 1975; Shigesada *et al.* 1995; Ramakrishnan *et al.* 2010). Whilst the dispersal strategy is important to successful range expansion, so is the species ability to respond to new environmental conditions experienced at the range edge.

Expansion into new environments exposes introduced species to a wide host of selective pressures, encompassing both abiotic and biotic factors (Sakai *et al.* 2001). Populations containing high levels of standing genetic variation are suggested to be better able to resist detrimental founder effects and adapt to these new environments (Lee 2002; Bock *et al.* 2015; Sherman *et al.* 2016). However, sequential bottlenecks and the effects of genetic drift on small founding populations will act to deplete a population's level of genetic diversity and adaptive potential (Klopfstein *et al.* 2006; Peacock *et al.* 2009). Despite theoretical predictions for a loss of genetic diversity, high propagule pressure, multiple introductions from different sources and stratified dispersal are potential mechanisms that can maintain genetic diversity during range

expansion (Dlugosch & Parker 2008; Tobin & Blackburn 2008; Bronnenhuber *et al.* 2011; Berthouly-Salazar *et al.* 2013). These theoretical expectations, together with the genetic patterns arising from different dispersal mechanisms (see Ramakrishnan *et al.* 2010), underscore the important role population genetic approaches have in reconstructing expansion pathways and patterns of connectivity within invasive ranges.

Marine coastal environments are among the most heavily invaded ecosystems globally (Ruiz *et al.* 1997; Roman & Palumbi 2004; Molnar *et al.* 2008; Reusch *et al.* 2010). Many of these introductions occurred through commercial shipping, or via introductions arising from aquaculture, fisheries and the aquarium trade (Grosholz 2002). Numerous benthic marine species reproduce through broadcast spawning and undergo a pelagic larval stage. The associated high levels of fecundity, large number of propagules and long larval duration (from a few days to several months) can facilitate the rapid establishment and range expansion of these species when introduced into new areas (Johnson & Carlton 1996; Johnson & Padilla 1996). As such, the dispersive life history stages of many marine invaders can result in yearly range expansions over hundreds of kilometers (Lyons & Scheibling 2009). Propagule dispersal of most marine species is largely determined by prevailing coastal currents and local hydrodynamic regimes, with planktonic larvae primarily limited to managing their vertical position within the water column (Paris & Cowen 2004). Fluctuations in local ocean currents and abiotic conditions will therefore be important in determining the extent of invasive marine range expansions. Consequently, there is a need to integrate information about introduction history, life history, hydrodynamic regimes, dispersal vectors and empirical genetic data in order to better understand the factors influencing dispersal

(see Werner *et al.* 2007; Pfeiffer-Herbert *et al.* 2007; Galindo *et al.* 2010) and predict potential future range expansions of invasive marine species (Connolly & Baird 2010).

The Northern Pacific seastar, *Asterias amurensis* is a benthic marine predator native to the coastal regions of the northwest Pacific (Fisher 1930) that also has a substantial introduced range in Southeast Australia (Byrne *et al.* 2013). In its native range, *A. amurensis* undergoes episodic 'boom-bust' population cycles, similar to fluctuations in population size and density observed for several other Asteroidea (e.g. Uthicke *et al.* 2009; Kayal *et al.* 2012). These population booms' can cause significant damage to commercial fisheries and aquaculture in the native range (Kim 1969; Nojima *et al.* 1986), leading to this species now being recognised as one of the most potentially damaging invasive species in Australian waters (Byrne *et al.* 2013; Parry & Hirst 2016). Believed to have first been introduced to Australia in southeast Tasmania (Derwent River estuary) from central Japan via ballast water discharge in the 1980s (Buttermore *et al.* 1994; Ward & Andrew 1995), it was then introduced to mainland Australia (Victoria) from Tasmania in the mid-1990s (Murphy & Evans 1998). Since its introduction into Victoria it has, until recently, remained largely confined to a single large embayment, Port Phillip Bay. However, a number of recent incursions have been recorded at several locations along the Victorian coastline east of Port Phillip Bay, suggesting this species is currently undergoing a range expansion (Holliday 2005; Hirst *et al.* 2013; Richardson & Sherman 2015). However, species distribution models based on current and future climate scenarios using habitat temperature data and larval thermal tolerances suggest that the northern extremes of the potential invasive range would be limited under a warming scenario (Byrne *et al.* 2016). Yet, we lack empirical

data describing dispersal modes, pathways and population connectivity in the invasive range.

The initial introduction of *A. amurensis* was linked to the capacity of its planktonic larvae to remain viable in ballast water for long periods (Ward & Andrew 1995). Adult *A. amurensis* spawn during the winter months and fertilization of eggs and sperm occurs externally in seawater (Byrne *et al.* 1997). The long-lived planktotrophic (feeding) larvae are capable of remaining in the water column for up to 120 days prior to settlement (Bruce *et al.* 1995). Previous hydrodynamic modelling suggests that *A. amurensis* larvae have the potential to disperse over relatively large distances in southeastern Australia assisted by oceanographic processes (Dunstan & Bax 2007). Yet, it is unclear whether the recent expansion within Victoria represents a natural range expansion facilitated by planktonic larval dispersal, or is the product of human-mediated physical translocation events of larval, juvenile or adult seastars.

In the present study we integrate information from a range of sources to examine the processes that have contributed to the range expansion of *A. amurensis* since its initial introduction to Australia. We assess patterns of genetic structure across the current invasive range to: *i*) examine genetic connectivity between the two invasive regions and among invasive sites within regions, and *ii*) identify the origin of range expansion sites. We also investigate the potential contribution of larval dispersal to the formation of new populations using a combination of hydrodynamic (dispersal) modeling and eDNA plankton sampling to determine the vector/s responsible for range expansion along the Victorian coastline.

Materials and Methods

Population genetic sampling and microsatellite genotyping

318 adult *A. amurensis* were collected from 10 sites across its invasive range within Australia (Fig. 1; Table 1) between April 2011 and May 2012 for population genetic analysis. This encompassed four sites in south east Tasmania, four from Port Phillip Bay, Victoria and two range expansion populations in Victoria: San Remo (SR) and Tidal River (TR). At each location between 17-39 (mean 31.8 ± 2.13 SE, Table 1) adult seastars were hand caught; a sample of tube feet was removed (~100) and immediately stored in 95% ethanol.

Total genomic DNA was extracted from tube feet samples using DNeasy kits (Qiagen), according to the manufacturer's protocol. We amplified 14 polymorphic microsatellite loci (Aamr02, Aamr04, Aamr05, Aamr06, Aamr08, Aamr10, Aamr11, Aamr17, Aamr18, Aamr21, Aamr30, Aamr33, Aamr34, Aamr37) following the PCR protocol described in Richardson *et al.* (2012). Fragment analysis was performed using an ABI 3130xl Genetic Analyser, incorporating LIZ 500 (-250) size standard (Applied Biosystems). To minimise differences due to capillary electrophoreses and ensure reproducibility ~10% of samples were re-genotyped and rescored. Allele calling was automated using GeneMapper, v3.7 (Applied Biosystems) then manually checked. MICROCHECKER v2.2.3 (Van Oosterhout *et al.* 2004) was used to test for the presence of null alleles across all loci and found evidence of null alleles at Aamr04, Aamr17 and Aamr37 in two populations. We used FreeNA (Chapuis & Estoup 2007) to correct potential null alleles using the ENA method and compute pairwise F_{ST} for both the original and corrected data sets. We found no difference in pairwise estimates of F_{ST}

between the data sets (Table S1, S2) and therefore used the original data set for all further analyses.

Population genetic analysis

We tested for departures from Hardy-Weinberg equilibrium (HWE) for each locus within each site, and linkage disequilibrium (LD) (Weir 1979) using GENEPOP v4.2 (Raymond & Rousset 1995). Exact tests were calculated using the Markov chain method (dememorization = 1000, batches = 100, iterations = 1000) to estimate the exact *P*-value at each locus. Probabilities across multiple loci were combined using Fisher's method and significance levels were adjusted based on the sequential bonferroni correction (Rice 1989). Significant departures from HWE were detected in 11 out of 131 single locus tests across 14 loci after correction for multiple comparisons. These departures were evident at six loci (Aamr04, Aamr10, Aamr17, Aamr18, Aamr34, Aamr37) in five populations (MI, MT, PA, TR, CY) and all are characterised by heterozygosity deficits and may represent the presence of null alleles at several of these loci. Of 803 pairwise tests for LD only three were significant after correction for multiple tests (Aamr04–Aamr05 in TR; Aamr10–Aamr37 in PA and MT). As associations among loci were not consistent across sites, these are unlikely to represent true linkage.

We used the diveRsity package (Keenan *et al.* 2013) in R (R Development Core Team 2011) to calculate: observed heterozygosity (H_O), expected heterozygosity (H_E), Allelic richness (A_R; corrected for the smallest sample size, SR, n = 17, Table 1), and the inbreeding coefficient (F_{IS}). Confidence intervals for F_{IS} estimates (95% CI) were obtained from 1000 bootstrap replicates.

There have been criticisms of G_{ST} (and its relative F_{ST}) as measures of differentiation when a locus's mutational effect is greater than that of migration; giving rise to underestimates of both parameters (Hedrick 2005; Jost 2008; Wang 2015) We used CoDiDi (Wang 2015) to examine the correlation coefficient (r_{GH}) between locus H_S (its diversity) and G_{ST} to evaluate whether our loci are likely to give unbiased estimates of genetic differentiation. When there is no correlation between H_S and G_{ST} (i.e. the same G_{ST} over a range of H_S), loci will provide useful information about the demographic history of a population (Wang 2015). The 14 microsatellite loci used here showed no correlation between H_S and G_{ST} (r_{GH} = -0.156, P = 0.594; Fig. S1) so levels of genetic differentiation were determined using global and pairwise F_{ST} (Weir & Cockerham 1984). We adopted a hierarchical approach where F_{ST} was calculated: 1) encompassing all sites across the range and 2) for sites within each region separately. F_{ST} estimates were calculated in the diveRsity package, with 95% CIs calculated from 2000 bootstrap replicates. A hierarchical Analysis of Molecular Variance (AMOVA) was also carried out to partition the genetic variance within and between regions using the program ARLEQUIN v3.5.1.5 (Excoffier & Lischer 2010).

We used STRUCTURE v2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2003) to investigate the spatial genetic structure within the invasive range and determine the number of distinct genetic clusters represented by the samples. STRUCTURE implements a Bayesian model-based, Markov chain Monte Carlo (MCMC) approach that assigns individuals to clusters (K) based on their genotypes. Individuals can be assigned admixture proportions (q) encompassing multiple genetic groups when their genotypes exhibit admixture from more than one genetic group. We ran 10 independent simulations for each value of K between 1 and 10, each with a burn-in length of 100,000

steps followed by 500,000 MCMC iterations. For all simulations we adopted the admixture ancestry model (i.e. the genetic composition of individuals can be a mixture from different populations) with correlated allele frequencies and no location prior. We implemented the Evanno (ΔK) method (Evanno *et al.* 2005) for inferring the most likely number of genetic groups using STRUCTURE HARVESTER v0.6.94 (Earl & vonHoldt 2011). We used CLUMPAK (Kopelman *et al.* 2015) to merge runs for each *K* and build the final plot.

To test whether range expansion along the Victorian coast showed a pattern of Isolation by distance we used the adegent (Jombart 2008) and ade4 (Dray *et al.* 2007) packages in R to perform a Mantel test of pairwise genetic differentiation (F_{ST}) against pairwise Euclidean distance. Significance was evaluated using 10,000 permutations. We then used GENECLASS2 (Piry *et al.* 2004) to assign individuals from range expansions sites (SR and TR) back to putative source regions (Port Phillip Bay or Tasmania). Individuals from all sites in within Port Phillip Bay and Tasmanian, respectively, were used as the reference for each region. Assignment statistics were computed for each individual from the range expansion sites, following Rannala & Mountain (1997); assignment probabilities were calculated following 10,000 Monte-Carlo resampling iterations with an alpha of 0.01 (Paetkau *et al.* 2004).

Hydrodynamic modelling

The dispersal potential of *A. amurensis* larvae in Victorian coastal waters (Bass Strait) was examined using hydrodynamic and dispersion models with Port Phillip Bay assumed to be the dominant larval source in the region. The dispersal of buoyant particles – simulating the behaviour of larvae exported from Port Phillip Bay – was

modelled using a combination of the SHOC (Sparse Hydrodynamic Ocean Code) model – for hydrodynamics – (Hertzfeld, 2006) and particle tracking model – for dispersal – (Condie *et al*, 2005) accessed within the Connie2 interface (http://www.csiro.au/connie2). Particles were released at the entrance to Port Phillip Bay at a constant rate of 25 particles per day over a 61-day period during August and September (2014) using observed forcing conditions (tidal, wind/current direction and velocity and localized wave behavior) for this period. We ran the model during these months because this simulated the period during which larvae are naturally present in the water column and coincided with the period during which plankton sampling was undertaken (see section below). Each particle had a maximum life span of 100 days, consistent with the estimated larval duration of *A. amurensis* larvae in Bass Strait (Bruce *et al.* 1995). The release rate is indicative only and provides an effective simulation of the dispersal footprint of buoyant particles released within Bass Strait.

Distribution of A. amurensis *larvae in Victorian coastal waters*

The presence of *A. amurensis* larvae in Victorian coastal waters was examined by sampling zooplankton assemblages from 34 locations along the coast (Fig. 1; Table S3). Zooplankton was sampled in surface waters during August - September 2012 and September 2014. This corresponds with the period where *A. amurensis* larvae are most abundant in the water column (Bruce *et al.* 1995, Johnson et al. 2004) following peak spawning in July - August (Byrne *et al.* 1997). We sampled Port Phillip Bay at the start (August) and end (September) of the survey to confirm that *A. amurensis* larvae were present in the water column during our sampling period. Collection dates for each site are given in Table S3. Zooplankton assemblages were sampled using a 90 µm-mesh plankton net (mouth diameter 0.48 m; length 3.25 m, with cod-end containing 90 µm-

mesh windows) towed at 2.5 knots for 5 minutes (~400m) to a depth of 0.5m. This mesh size is sufficient to sample all planktonic life-stages of *A. amurensis*, including eggs (approx 105 µm in diameter), embryos (105-150 µm in diameter) and larvae (150 µm, gastrula stage; 4.5 mm, brachiolaria stage) (Kashenko 2005). All developmental stages are hereafter referred to as larvae for simplicity. Prior to each plankton tow the net was towed without the cod-end for one minute to wash residual contents from the previous sample and reduce potential cross contamination between samples and sites. There was no evidence of cross-contamination between samples (see Hirst *et al.* 2013). Plankton samples were fixed in *RNAlater* (Ambion) immediately and stored at < 4°C prior to DNA extraction.

All zooplankton samples were prepared for molecular analysis in the laboratory following (Giblot-Ducray & Bott 2013). Briefly, environmental DNA (eDNA) was extracted from filtered zooplankton samples using the methods of (Ophel-Keller *et al.* 2008; Bott *et al.* 2009). eDNA was analysed using the *A. amurensis* quantitative polymerase chain reaction (qPCR) assay described in Bott *et al.* (2010) and analysed on an ABI HT 7900 sequence detection system (Applied Biosystems) using QuantiTect™ qPCR mastermix (Qiagen). Each qPCR assay was run with positive (serial dilution DNA standards) and negative plate controls and analysed using ABI SDS 2.3 software (Applied Biosystems). qPCR results are given as cycle threshold (Ct) values. The Ct value represents the qPCR cycle number at which the fluorescence signal passes a fixed threshold. The threshold is manually set and based on the proprietary normalised reporter (ΔRn) in the QuantiTect™ qPCR mastermix. It is set to be the point where DNA amplification rises above the baseline, i.e. number of qPCR cycles vs magnitude of the fluorescence signal intensity (ΔRn). Quantity of *A. amurensis* DNA per sample was

estimated via the absolute quantification method. A standard curve was generated using known *A. amurensis* DNA standards (known DNA quantities in serial dilution). The Ct of eDNA samples are then plotted on the standard curve to provide an estimation of *A. amurensis* DNA in each eDNA sample. Assuming a constant flow rate, the concentration of *A. amurensis* DNA was converted to pg/m^{-3} for each sample (calculation provided in Table S3).

Results

Genetic diversity and population structure

All 14 microsatellite loci used were polymorphic, with the number of alleles per locus ranging from 3 to 11 (mean = 6.5 ± 0.59 SE). Levels of genetic diversity were similar across all sites and regions with H_E ranging from 0.49-0.59 (mean = 0.54 ± 0.010 SE), and A_R ranging from 3.52-4.13 (mean = 3.74 ± 0.055 SE) (Table 1). We find no significant difference in either diversity indices between Tasmanian and mainland Victorian sites, or the recent range expansion sites (SR and TR). Inbreeding coefficients (F_{IS}) were positive for all sites (and significantly different from zero for 6 of 10 sites; Table 1) indicating potential heterozygous deficits. Values were higher on average in Victoria (mean F_{IS}, 0.138 ± 0.027 SE) than in Tasmania (mean F_{IS}, 0.090 ± 0.017 SE). Within Victoria, mean F_{IS} was higher in Port Phillip Bay (0.149 ± 0.042 SE) than the range expansion sites (0.117 ± 0.002 SE), however, F_{IS} is not significantly different between the ranges (t = 1.51, df = 7.66, P = 0.17) or range expansion sites (t = 0.77, df = 3, P = 0.50).

We detected significant levels of genetic differentiation among sites with a global F_{ST} = 0.033 (95% CI, 0.023–0.445). We find significant levels of genetic differentiation within Victoria with F_{ST} = 0.028 (95% CI, 0.014–0.044), however, within Tasmania we found no significant differentiation among sites, F_{ST} = 0.007 (95% CI, -0.004–0.020). Our AMOVA analysis revealed significant differentiation among regions (F_{CT} = 0.03, P < 0.01), among populations within regions (F_{SC} = 0.01, P < 0.01) and within populations (F_{ST} = 0.04, P < 0.01). Most of the genetic variation was attributed to differences among individuals within populations (96%) while only 1% was attributed to differences between populations within regions and 3% due to differences between regions (Table S4). Pairwise F_{ST} estimates between all sites revealed the greatest level of differentiation was between the Victorian and Tasmanian sites, with F_{ST} ranging between 0.010 and 0.084 (95% CI, 2000 bootstraps; Table 2). Levels of genetic differentiation were lower among sites within regions, with pairwise F_{ST} ranging from 0.004–0.076 in Victoria and 0.001–0.011 among sites in Tasmania. The range expansion site, TR, is most similar to MI (F_{ST}, 0.010) and MT (F_{ST}, 0.021), while the range expansion site, SR, is most similar to MI (F_{ST}, 0.007) and LF (F_{ST}, 0.010).

The STRUCTURE analysis revealed three (K = 3) genetically distinct clusters within the Australian invasive range (Fig. 2), based on the distribution of ΔK (largest ΔK; Fig. S2). One genetic cluster encompasses all Tasmanian sites; another represents the majority of Victorian sites, including the two range expansion sites (SR and TR), while the third genetic cluster is predominantly made up of individuals from a single site (PA) in Victoria.

We did not detect a significant pattern of Isolation by distance for any sites along the Victorian coast, including range expansion sites (r = -0.135, P = 0.58). We conducted individual level genotype assignment tests for 52 range expansion samples from SR and

TR to identify their most likely source region. Assignment probabilities between Port Phillip Bay and Tasmanian sites were similar for most individuals, which suggests that the relatively low levels of structuring between sites within and between regions provided limited power to accurately assign individuals back to source populations. (Table S5).

Hydrodynamic modelling

The hydrodynamic and dispersal models indicate buoyant particles released from Port Phillip Bay are predominantly transported east and southeast along the coastline towards Wilsons Promontory (Fig. 3a). There is limited dispersal to the west of Port Phillip Bay. The greatest concentration of particles occurs directly offshore from the entrance to Port Phillip Bay in the Bass Strait. There is a clear dilution effect with increasing distance from Port Phillip Bay, with only a small proportion of particles (<2%) released reaching the coastline of Wilsons Promontory, where the furthest range edge populations is located, by the end of the simulation. The model simulation predicts some exchange between Port Phillip Bay and Western Port, predominantly via the western entrance to Western Port. Very few particles dispersed eastwards around Wilsons Promontory peninsula within the 100-day simulation (Fig. 3a).

Distribution of A. amurensis *larvae in plankton samples*

We amplified *A. amurensis* DNA in 20 of the 34 eDNA plankton samples collected (Fig. 3b; Table S3), indicating the presence of *A. amurensis* larvae in these samples. *Asterias amurensis* DNA was recorded in 17 of 21 plankton samples collected between Port Phillip Bay and Wilsons Promontory, including within Western Port , Andersons Inlet

and the TR estuary (Fig 3b). No *A. amurensis* larvae were detected in samples east of Wilsons Promontory or in the three samples west of Port Phillip Bay. The quantity of *A. amurensis* DNA amplified varied by several orders of magnitude (Table S3, Fig 3b) indicating large variation in the density and/or size of larvae sampled. *Asterias amurensis* DNA concentrations were highest in Port Phillip Bay, and adjacent to Port Phillip Bay in Bass Strait, but diminished with increasing distance from the bay (Figure 3b; Table S3).

Discussion

This study provides evidence that both anthropogenic and natural larval dispersal have been important vectors of range expansion during the *A. amurensis* invasion in Australia. Our combined data supports the previous hypothesis that the Port Phillip Bay introduction on mainland Australia (Victoria) resulted from a human assisted translocation event from the original introduction in southeast Tasmania. Port Phillip Bay sites exhibit some similarity in genetic diversity/structure with Tasmanian sites (suggesting the Tasmania population was its most likely source); however, they also show some genetic divergence, indicating no ongoing gene flow between these sites since the potential translocation event. The genetic divergence between the Tasmanian and Victorian ranges suggests Port Phillip Bay is the most likely origin of recent range expansion sites along the Victorian coast. By incorporating the population genetic survey with dispersion modeling and information on the distribution of *A. amurensis* larvae in Victorian coastal waters, we are further able to demonstrate that the recent range expansion is consistent with the dispersal of larvae from Port Phillip Bay. As such,

multiple dispersal vectors – both anthropogenic and natural larval dispersal – are likely to have played an important role in the range expansion of this species in Australia.

Population structure between invasive regions

Inferences of colonisation history can be made based on observations of changes in genetic diversity and differentiation between sites, with more recently established populations often showing lower diversity and greater differentiation compared to longer established populations (Ramakrishnan *et al.* 2010). Levels of genetic diversity observed within the introduced range were not significantly different between Tasmania and Victoria (Table 1). Measures of genetic differentiation (F_{ST}), AMOVA and STRUCTURE analysis indicate significant differentiation between the Tasmanian and Victorian ranges. Each range forms a separate distinct genetic cluster based on STRUCTURE analysis (Fig. 2), albeit it, with some individuals showing proportional membership to multiple clusters. Likewise, pairwise F_{ST} between the ranges indicates significant genetic differentiation (F_{ST} = 0.033). Our reported levels are similar to those from other echinoderms that show significant differentiation (*Evechinus chloroticus*, F_{ST} = 0.012, Nagel *et al.* 2015; *Patiria miniata*, F_{ST} = 0.040, Keever *et al.* 2009). Together with the documented invasion history, these analyses suggest that sites from both regions likely share a recent common history. However, based on genetic data alone we are unable to completely rule out two separate introduction events from a common origin into the two Australian regions. The significant divergence observed between these ranges, however, also suggests there is no ongoing gene flow between sites in Tasmania and Victoria, allowing for some divergence to occur between these invasive range sites.

Low levels of genetic differentiation (non-significant) were observed among Tasmanian sites, indicating high levels of population connectivity throughout the Derwent and Huon River estuaries. This is supported by STRUCTURE analysis that indicates high connectivity and dispersal within the Tasmanian range that effectively homogenises sites. This pattern may have arisen through a process of contiguous dispersal – essentially diffusive short-distance dispersal – leading to a gradual increase in the geographic extent of the initial introduced population, which now encompasses all Tasmanian sites surveyed. Interestingly, we find greater, and significant, genetic differentiation among Victorian populations (F_{ST} = 0.0275) compared to Tasmanian (F_{ST} = 0.0067). STRUCTURE assigned individuals from sites within Port Phillip Bay to two genetic clusters, with the smaller of these predominantly made up of individuals sampled at PA. This site also exhibits significant genetic differentiation to all other sites surveyed, apart from MT, which also has individuals assigned to this second genetic cluster. This signature could also be the result of a second introduction into Australia from the native range. Evidence is mounting that multiple introductions from different native range populations is a reasonably common feature of invasive species (Kolbe *et al.* 2004; Dlugosch & Parker 2008; Rosenthal *et al.* 2008). However, additional work characterising the genetic variation across the native range is needed before we can test this hypothesis. Murphy & Evans (1998), based on AFLPSs suggested that the establishment of a Victorian population in Port Phillip Bay originated from Tasmania ~10 years after the original introduction in the Derwent estuary, facilitated by the transfer of larvae in ballast water. Our population genetic results are consistent with this hypothesis; the genetic signature most likely reflects the founding of the Port Phillip Bay population from the original introduction in Tasmania via human-mediated

dispersal, with subsequent divergence between the regions due to an absence of gene flow.

Range expansion by larval dispersal

Recent studies have focused on the genetic signature during invasive range expansions and revealed the importance of natural dispersal mechanisms to this process (Darling & Folino-Rorem 2009; Ramakrishnan *et al.* 2010; Bronnenhuber *et al.* 2011). Along with dispersal modes, the number of migrants (propagules) from source populations is also known to affect genetic structure (Nei 1975; Whitlock & McCauley 1990; Lockwood *et al.* 2005; Simberloff 2009). Population genetic analysis (both STRUCTURE and pairwise F_{ST}) presented here indicates that Victorian range edge samples from SR and TR are most similar to those from Port Phillip Bay (Table 2, Fig. 2), specifically the southern Port Phillip Bay population of MI; unsurprising given MI is the closest site to the entrance of Port Phillip Bay. Additionally, range edge sites exhibit similar levels of genetic diversity (H_E and A_R; Table 1) compared to Port Phillip Bay suggesting they are not genetically depauperate. Together these pieces of evidence suggest Port Phillip Bay as a potential source of range expansion recruits. Interestingly, TR exhibits significant genetic differentiation to all other Port Phillip Bay populations (apart from MI) and the expansion site SR. Noncontiguous dispersal – the process of founding new sites relatively far from a single source – could generate the higher differentiation seen between TR and other Victorian sites. However, it is difficult to assess what constitutes noncontiguous dispersal compared to the maximum extent of contiguous dispersal based on genetic data alone. Separating these two can also be confounded in marine species with long-lived planktonic larval phases, as they have the ability to disperse over relatively large distances (Lyons & Scheibling 2009). Despite finding no signature

of isolation by distance (which may indicate noncontiguous dispersal), we cannot rule out contiguous dispersal from Port Phillip Bay as the source of recruits. Interestingly, individuals from SR also show some similarity to several Tasmanian sites from the Derwent River (LF, SB, RB), but not the Huon River estuary (CY) based on pairwise F_{ST} estimates (Table 2.). This likely reflects similarity due to the Port Phillip Bay population being founded from the initial introduction in Tasmania.

Previous studies have revealed the utility of hydrodynamic and dispersion models for estimating larval dispersal routes in invasive species (Gilg & Hilbish 2003; Connolly & Baird 2010). Here, thorough the application of an integrative approach that combines dispersion modelling with information on the distribution of *A. amurensis* larvae in zooplankton assemblages we are able to examine the contribution natural larval dispersal has to invasive range expansion. Our modelling of buoyant particles released from Port Phillip Bay predicts larvae will be transported predominately eastwards along the Victorian coast by the prevailing winter currents (including into Western Port, Andersons Inlet and the TR estuary) within the 100-day lifespan for particles used in the simulation (Fig. 4a), (Bruce *et al.* 1995). There is likely to be some variation around the extent of the dispersal front predicted by the larval dispersal model as *A. amurensis* larvae are planktotrophic and food availability will affect the ultimate dispersal extent (potentially increasing or decreasing dispersal extent relative to the amount of food present in the water column). Nevertheless, the model serves as a useful predictor of the potential dispersal pathway from Port Phillip Bay. The model also predicts limited dispersal west of Port Phillip Bay and east of Wilsons Promontory. Surface currents in this region are influenced primarily by the prevailing winds and tides, and flow principally eastwards diverging south of Wilsons Promontory towards

the north coast of Tasmania as part of a gyre that dominates central Bass Strait (Greer *et al.* 2008). Consequently, Wilsons Promontory may act as a geographical barrier restricting the natural dispersal of larvae further east along the Victorian coast. Additional work modeling dispersal routes originating from range expansion sites, under the scenarios suggested by Byrne et al. (2016), would help identify natural biogeographic barriers to dispersal, as well as sites that may act as important sources of larvae for northern and southern range expansion.

Previous work has shown that using genetic techniques to identify inconspicuous larvae in plankton samples is particularly useful for understanding larval dispersal in echinoderms (Medeiros-Bergen *et al.* 1995). For example, Uthicke *et al.* (2015) documented the distribution of *Acanthaster planci* larvae up to 100km away from recent adult outbreaks. The pattern of *A. amurensis* incursions at Andersons Inlet, SR and TR is entirely consistent with natural range expansion via larval dispersal with *A. amurensis* DNA detected at each of these locations. Overall the distribution of *A. amurensis* larvae in the water column was consistent with hydrodynamic and dispersion modelling. *Asterias amurensis* larvae were detected in coastal waters between Port Phillip Bay and Wilsons Promontory (Fig. 4b) but not directly west of the entrance to Port Phillip Bay or east of Wilsons Promontory. Although the eDNA detection method does not allow us to directly quantify the number of larvae present, it provides a useful proxy of larvae density. High concentrations of *A. amurensis* DNA adjacent to the entrance to Port Phillip Bay indicate high quantities of larvae are likely exported into Bass Strait through tidal flushing. These levels progressively declined with increasing distance from the bay; consistent with a dilution effect as larvae are dispersed further from their original source; as also predicted by the dispersal

modelling. While this analysis is consistent with Port Phillip Bay being the source of the larval pool, contribution to this pool may come from other sites or previously unidentified populations outside of Port Phillip Bay. However, range expansion sites outside of Port Phillip Bay underwent eradication programs soon after the detection of adult individuals and before zooplankton sampling (no adults recorded at Andersons Inlet and SR since 2005 and 2011, respectively; most TR individuals eradicated by August 2012, Hirst *et al.* 2013). Consequently, Port Phillip Bay remains the only major known source of larvae in Victorian waters. If vectors other than natural larval dispersal were important in spreading *A. amurensis* along the coast of Victoria, we would also expect to find populations at locations not consistent with larval dispersal, such as to the west of Port Phillip Bay or east of Wilsons Promontory.

Conclusions

The integration of three complementary approaches implicates multiple dispersal vectors in the establishment and expansion of the *A. amurensis* invasive range in Australia. The establishment of a mainland population in Victoria likely occurred through human-mediated translocation event (non-contiguous dispersal) from the initial introduction in Tasmania (most likely via ballast water transfer). The two invasive regions of Victoria and Tasmania appear to be isolated from each other and have undergone significant genetic differentiation. The range expansion eastward along the Victorian coast is entirely consistent with natural larval dispersal and cannot simply be attributed to other dispersal vectors. In Australian coastal waters, adult *A. amurensis* appear to have quite restricted habitat preferences (i.e. limited to protected embayments or coastlines) and therefore have not been effective in moving along the open coastline as adults (Dunstan and Bax 2007). We have demonstrated that dispersal

of larvae by oceanographic currents and the translocation of larvae/juveniles/adults are critical to movement of this species beyond its current restricted range. As such, they should be considered as part of any future risk assessment for this species. The *A. amurensis* invasion in Australia provides important insights into the mechanisms of dispersal and drivers of range expansion that are not only essential for understanding its invasion, but are broadly applicable to marine invasive species dispersal. We propose that the combined use of population genetics, hydrodynamic modeling and eDNA plankton surveillance will be an effective approach for the study of dispersal in a wide range of marine invasive species.

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Data Accessibility

Microsatellite genotypes, STRUCTURE input and parameter files, population genetic analysis script and dispersion model outputs are available on Dryad: http://dx.doi.org/10.5061/dryad.7kb45/1

Author contributions

All authors contributed to experimental design. MFR, CDHS and AJH contributed to interpretation of the data. MFR wrote the paper, collected population genetic samples, extracted DNA, conducted microsatellite genotyping and all analysis. CDHS contributed to population genetic analysis and writing of the manuscript. AJH conducted plankton sampling and analysis and contributed to writing of the manuscript. RSL conducted hydrodynamic and dispersion modelling. NJB conducted eDNA extraction and analysis and contributed to writing the manuscript.

Fig. 1. Sampling sites of *A. amurensis* used in this study from the invasive range in Australia. (a) SE Australia, black boxes highlight the areas sampled. (b) The Derwent and Huon River estuaries, SE Tasmania; (c) Port Phillip Bay and the Victorian coast, mainland Australia. Sites where adult individuals were collected for population genetic analysis are donated by triangles. Grey circles denote sites where zooplankton was collected for the identification of *A. amurensis* larvae. The code for each population genetic sampling site is as in Table 1.

Fig. 2. STRUCTURE analysis of *A. amurensis* collected from Victoria and Tasmania. Each individual is represented by a vertical line, which shows the admixture proportion (*q*) to each of the identified genetic clusters (represented by the three different colours).

The number of genetic groups was determined from the maximum value of Δ*K* as per Evanno et al. (2005).

Fig. 3 (a) 100-day simulation of continuous larval release (total of 1,525 particles) from Port Phillip Bay between August and September shown as probability of buoyant particle visits per cell. The dispersion model provides an indication of the likelihood of *A. amurensis* larvae presence and key dispersal pathways. (b) Bubble plot showing the quantity of *A. amurensis* DNA (pg/m^{-3}) recorded at each zooplankton sampling site along the Victorian coast. Orange bubbles indicate that *A. amurensis* larvae were detected in the plankton sample and are scaled based on the amount of DNA detected. Grey circles denote plankton samples where no *A. amurensis* larvae were detected.

Table 1. Comparisons of genetic diversity between Victorian and Tasmanian populations of the invasive seastar *Asterias amurensis*. Site codes in parentheses; *N*, number of individuals sampled; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; A_R, allelic richness.

Sampling site	*N*	Latitude	Longitude	A_R	H_E	H_O	F_{IS}
Mud Island (MI)	39	-38.2667	144.7667	3.75	0.51	0.43	**0.155**
Mornington (MT)	31	-38.2160	145.0346	3.81	0.55	0.46	**0.169**
Portarlington (PA)	24	-38.1167	144.6500	4.13	0.59	0.45	**0.235**
Williamstown (WT)	32	-37.8990	144.9100	3.77	0.51	0.49	0.035
San Remo (SR)	17	-38.5167	145.3667	3.52	0.49	0.43	0.115
Tidal River (TR)	35	-39.0306	146.3214	3.74	0.52	0.46	**0.118**
Victoria (mean)				*3.79*	*0.53*	*0.45**	*0.138*
Cygnet (CY)	37	-43.1700	147.0967	3.67	0.56	0.49	**0.131**
Lindisfarne (LF)	35	-42.8478	147.3531	3.79	0.55	0.51	0.074
Ralphs Bay (RB)	37	-42.9637	147.4438	3.65	0.56	0.53	0.054
Sandy Bay (SB)	31	-42.9010	147.3570	3.52	0.55	0.49	**0.100**
Tasmania (mean)				*3.66*	*0.56*	*0.51**	*0.090*

*Significant at $P < 0.01$; two-sample t-test; Bold font indicates significant difference (95 % CI do not overlap zero)

Table 2. Pairwise estimates of genetic differentiation F_{ST} (95 % CI; 2000 bootstraps) for Victorian and Tasmanian population of the invasive seastar *Asterias amurensis*.

	MI	MT	PA	WT	SR	TR	CY	LF	RB	SB
MI	-									
MT	0.0040	-								
PA	**0.0613**	0.0239	-							
WT	0.0108	0.0124	**0.0725**	-						
SR	0.0069	0.0186	**0.0756**	0.0204	-					
TR	0.0096	**0.0209**	**0.0574**	**0.0289**	**0.0247**	-				
CY	**0.0557**	**0.0413**	**0.0837**	**0.0436**	**0.0295**	**0.0549**	-			
LF	**0.0308**	**0.0224**	**0.0620**	**0.0346**	0.0099	**0.0354**	0.0088	-		
RB	**0.0262**	**0.0266**	**0.0658**	**0.0267**	0.0139	**0.0269**	0.0091	0.0032	-	
SB	**0.0583**	**0.0415**	**0.0835**	**0.0446**	0.0182	**0.0605**	0.0070	0.0009	0.0106	-

Bold font indicates significant difference (95 % CI do not overlap zero)

Supplementary Information

Fig. S1. Graph showing the correlation between locus H_S and G_{ST}. Triangles represent each microsatellite loci used in this study. The correlation coefficient (r_{GH}) and *P*-value are shown in the insert.

Fig. S2. Graph showing ΔK for each K (number of populations) used in the STRUCTURE analysis as per Evanno et al. (2005). We chose the number of genetic groups represented in our data based on the maximum value of ΔK.

Table S1. Pairwise estimates of genetic differentiation F_{ST} (Weir 1996) for Victorian and Tasmanian population of *A. amurensis* calculated using null allele corrected and uncorrected allele frequency datasets using the ENA method implemented in FreeNA

Table S2. Pairwise estimates of genetic differentiation D_{EST} (95 % CI; 2000 bootstraps) for Victorian and Tasmanian population of *A. amurensis*.

Table S3. Plankton sampling locations and detection of *A. amurensis*, as pg/5min tow. Date of plankton tow, locations as Latitude and Longitude, threshold of detection in qPCR (ct) and quantity of DNA amplified.

Table S4. *Asterias amurensis* population differentiation results from the AMOVA analysis

Table S5. Output of range expansion individual assignment tests contucted in GENECLASS2. Assigned population (where sampled from), VIC,PA and TAS column indicates probability of individual being assigned to that region, No. of loci used and thir names respectivly. Program parameters given at the bottom of the table