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The genetic structure of *Nautilus pompilius* populations surrounding Australia and the Philippines

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Abstract

Understanding the distribution of genetic diversity in exploited species is fundamental to successful conservation. Genetic structure and the degree of gene flow among populations must be assessed to design appropriate strategies to prevent the loss of distinct populations. The cephalopod *Nautilus pompilius* is fished unsustainably in the Philippines for the ornamental shell trade and has limited legislative protection, despite the species' recent dramatic decline in the region. Here, we use 14 microsatellite markers to evaluate the population structure of *N. pompilius* around Australia and the Philippines. Despite their relative geographical proximity, Great Barrier Reef individuals are genetically isolated from Osprey Reef and Shark Reef in the Coral Sea ($F_{ST}=0.312$, 0.229, respectively). Conversely, despite the larger geographical distances between the Philippines and west Australian reefs, samples display a small degree of genetic structure ($F_{ST}=0.015$). Demographic scenarios modelled using approximate Bayesian computation analysis indicate that this limited divergence is not due to contemporary gene flow between the Philippines and west Australia. Instead, present-day genetic similarity can be explained by very limited genetic drift that has occurred due to large average effective population sizes that persisted at both locations following their separation. The lack of connectivity among populations suggests that immigrants from west Australia would not facilitate natural recolonization if Philippine populations were fished to extinction. These data help to rectify the paucity of information on the species' biology currently inhibiting their conservation classification. Understanding population structure can allow us to facilitate sustainable harvesting, thereby preserving the diversity of genetically distinct stocks.

Introduction

Throughout the world, many commercial marine species are experiencing significant population declines (Hutchings 2000; Worm *et al.* 2006; Neubauer *et al.* 2013; Watson *et al.* 2013). The harvesting of marine animals began 42,000 years ago (O'Connor *et al.* 2011), up to 50,000 years after the anthropogenically triggered extinctions of large mammals seen on land (Koch & Barnosky 2006). Human access to the marine environment is no longer technologically inhibited, and defaunation of

the oceans is now occurring (McCauley *et al.* 2015). Present extinction rates are probably a thousand times higher than extinction rates in the absence of human actions (Pimm *et al.* 2014). Identifying species with a high risk of extinction (Davidson *et al.* 2012) can facilitate policy changes and prevent declines. An international agreement between governments that was developed to regulate declines is the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). CITES aims to ensure that the survival of species is not threatened by international trade, and the convention is reliant on biological data to construct a specific framework for participating countries to implement (CITES 2015). Unfortunately, the absence of biological information and global industrial data can prevent qualification for CITES protection (De Angelis 2012).

Population connectivity data can be used by CITES to design effective conservation strategies for marine resources. Contemporary movement and migration among marine populations have been monitored using tracking devices, but success has been variable (see Semmens *et al.* 2007). Movement among populations can be estimated using population genetic analyses (Pearse & Crandall 2004). Although specific individuals cannot always be tracked, molecular techniques do allow the detection of gene flow among populations through patterns of shared genetic variation (Levin 2006; Cowen & Sponaugle 2009). For vulnerable species, knowledge of the genetic structure of populations can be used to inform sustainable harvesting practices, prevent local extinctions and preserve the diversity of genetically distinct stocks (Carvalho & Hauser 1994). Oceans are considered to present few physical barriers to gene flow so that widely separated areas can remain connected, making absolute vicariance rare in a marine environment (Palumbi 1994; Mirams *et al.* 2011). Population structure and speciation have, however, been attributed to oceanic features, such as salinity (Rocha 2003; Lessios *et al.* 2003), depth and temperature (Zardi *et al.* 2007). Ocean fronts (Galarza *et al.* 2009) and currents (White *et al.* 2010) have also been shown to represent major barriers to gene flow, suggesting that marine environments may contain more barriers to dispersal than is generally appreciated.

Oceanic features with the potential to act as a barrier to movement can result in genetic structure that ranges from panmixia (Lessios *et al.* 2003) to complete separation (Baums *et al.* 2012). The inference of demographic history over long time scales can answer questions regarding long-term gene flow between, and distribution of genetic diversity among, populations (Semmens *et al.* 2007). This ability to infer a species' movements can be used in various areas of conservation, for example, to evaluate whether marine protected areas are appropriate (i.e. to assess that species movement does not extend beyond protected boundaries; Grüss *et al.* 2011), to trace the origins of a catch (Hobson 1999), or to ensure that illegal catches are not being missold to the consumer (Griffiths *et al.* 2013).

Despite the technology and conservation strategies available, population declines continue in most species (Neubauer *et al.* 2013). The poor success of attempts to aid the recovery of commercial marine species (Hutchings 2000) suggests that anthropogenic pressure is too high and that there are gaps in scientific knowledge that must be resolved to enable effective conservation (Sale *et al.* 2005). A key example of a species currently lacking biological and population connectivity data is the shelled cephalopod, *Nautilus pompilius*. Nautiloids (*Nautilus* spp. Linnaeus and *Allonautilus* spp. Ward and Saunders) are heavily overfished for the ornamental shell trade. The long-term effects of fishing on populations, or their ability to recover, are unknown (De Angelis 2012). An 80% decline in catch per unit effort for *N. pompilius* during 1980–2010 has been reported from Philippine fisheries (Dunstan *et al.* 2010). The current deficiency of data on the species' biology has inhibited the development of appropriate legislative mechanisms to prevent the species' overexploitation and decline. Understanding migration and its effect on population dynamics is important when deciding on appropriate protection measures for populations through *in-situ* means, such as marine protected areas (Grüss *et al.* 2011).

Cephalopod migration, however, is poorly understood, and this is exacerbated by the difficulties associated with tracking them (Stark *et al.* 2005; Semmens *et al.* 2007). In coleoids (octopus, cuttlefish, squid), juveniles are often too small to be tagged, tag placement in adults can be difficult,

and capture rates fluctuate and are often reliant on fishermen reporting catches (Sauer *et al.* 2000). Improvements in technology, with concomitant decreases in device size and cost, will aid progress in this area.

Historical movement of *N. pompilius* populations has been investigated using molecular approaches; hypothesised differentiation of *N. pompilius* populations was confirmed by comparing variation at *cytochrome c oxidase subunit I (COI)*. Populations separated into three geographically distinct monophyletic clades, which comprise a west Australian/Indonesian clade, an east Australian/Papua New Guinean clade and a west Pacific clade (Wray *et al.* 1995; Sinclair *et al.* 2007, 2011; Bonacum *et al.* 2011; Williams *et al.* 2012). The observed population structure was proposed to be the result of dispersal from an ancestral population in the Philippines (Wray *et al.* 1995). Historical expansions of nautiloid distribution will have been restricted by at least three biogeographical barriers (Crick 1993): water depth, distance between adjacent shelf seas and sea temperature. These constraints are also relevant to modern *Nautilus* and *Allonautilus*, whose movements are limited to some extent by their morphology. Nautiloids are typically found between depths of 130 and 700 m (Dunstan *et al.* 2011), remaining close to the reef for protection from predators. The internal arrangement of the *Nautilus* shell means that their deepest position in the water column is limited by their risk of shell implosion. As a consequence, the maximum depth at which an individual would be encountered is considered to be approximately 800 m (Saunders & Wehman 1977; Kanie *et al.* 1980). These limitations create a dispersive barrier and, despite small geographical distances, genetic differentiation has occurred (Sinclair *et al.* 2007), furthered by the absence of a juvenile larval stage that might aid dispersal (Saunders & Landman 2010). Understanding gene flow between current populations can help to inform sustainable fishing and aid the design of specific genetic management, and yet nothing has been known about the current connectivity of *N. pompilius* populations. Understanding population connectivity will help to establish the impact that fishing is having across their distribution following *N. pompilius* population declines in the Philippines (Barord *et al.* 2014).

Here, we use molecular techniques and statistical analyses to assess the connectivity of *N. pompilius* populations surrounding Australia and the Philippines. These data will contribute towards rectifying the information deficiency that currently inhibits the legal classification of *N. pompilius* as an endangered species, and our findings can be used to assess the species' qualification for CITES listing. Approximate Bayesian computation (ABC) analysis has enabled a powerful assessment of the species' genetic diversity and made it apparent that, despite the persistence of high levels of variability that are usually associated with large populations, there is limited gene flow into shrinking contemporary populations. This implies that overfishing currently threatens some populations with extinction.

Materials and Methods

Sample collection

Australian samples were collected from seven reefs in the Indo-Pacific Ocean (Fig. 1) under an Australian Fisheries Management Authority Scientific Permit (number 1002548). West Australian (WA) samples were taken from four reefs: Clerke Reef, Imperieuse Reef, Ashmore Reef and Scott Reef. East Australian (EA) samples consisted of Osprey Reef and Shark Reef in the Coral Sea, and the Far North Great Barrier Reef. Samples from the Philippines (PH) were collected from three locations: Tinitian, Roxas and Palawan. Collections were made under a Gratuitous Permit from the Department of Agriculture in the Republic of the Philippines.

N. pompilius was caught using traps positioned on the reefs at a depth of ~200 m (Sinclair *et al.* 2011) baited with ~1 kg of uncooked chicken (*Gallus gallus*), set at dusk and collected at dawn. Tissue collection was non-lethal: a 1–2 cm-long labial tentacle sample was collected before each individual was released. Tentacles were immediately placed into a 20% DMSO (dimethylsulfoxide), 100 mM EDTA pH 8, saturated NaCl₂ solution and stored at 4°C in the field. Samples were later washed in TE buffer (1 M Tris-HCl pH 7.5; 0.5 M EDTA pH 8.0; Sambrook *et al.* 1989) and placed into 1 ml absolute ethanol in the laboratory for storage at room temperature (Sinclair *et al.* 2011).

Microsatellite Genotyping and Validity

Genomic DNA was extracted using Qiagen DNeasy tissue kits (QIAGEN Ltd, Manchester, UK). DNA concentration was quantified using a fluorometer (Fluostar Optima) and its quality assessed with electrophoresis on a 1% agarose gel. Fourteen polymorphic *N. pompilius* microsatellite loci (Williams *et al.* 2015) were selected based on satisfactory results from quality checks, and used to genotype all 215 individuals sampled. PCR amplification was performed in 2- μ l PCR reactions, including 10 ng air-dried DNA, 0.2 μ M reverse primer, 0.2 μ M forward fluorescent primer (6FAM, HEX, VIC or PET labelled) and 1 μ l Qiagen Multiplex Master mix. Multiplexes were amplified under the following profile: 95°C for 15 min, followed by 44 cycles of 94°C for 30 s, 56°C for 90 s, 72°C for 90 s and finally 72°C for 10 min. PCR products were analysed on an ABI 3730 48-well capillary DNA analyser (Applied Biosystems Inc.) using LIZ GS500 size standard (Applied Biosystems Inc.). Allele sizes were assigned using the GENEMAPPER v3.7 software (Applied Biosystems Inc.).

Relatedness between individuals was estimated with SPAGEDI (Hardy & Vekemans 2002) using Queller and Goodnight's (1989) measure of relatedness. Relatives were removed and departure from Hardy–Weinberg equilibrium (HWE; $P < 0.05$) and linkage disequilibrium (LD) were calculated using GENEPOP (Raymond & Rousset 1995; Rousset 2008). LD was assessed using 1000 iterations per population and P -values corrected using the False Discovery Rate adjustment (FDR; Verhoeven *et al.* 2005). Corrections were made on a population-by-population basis to avoid overinflating the number of tests in which the correction was required. Each microsatellite locus was assessed to estimate the frequency of null alleles and identify scoring errors due to stutter using MICRO-CHECKER (Van Oosterhout *et al.* 2004). Null allele frequency per locus was estimated using CERVUS v3.0 (Kalinowski 2005). To assess genotyping error rate, 60% of samples were re-extracted and re-genotyped across all loci. Error rates per reaction were calculated according to Hoffman & Amos (2005).

Population Structure

Three Bayesian clustering methods were used to determine the most likely number of genetic clusters within the data set: STRUCTURE (Pritchard *et al.* 2000), TESS (Durand *et al.* 2009) and GENELAND (Guillot *et al.* 2005). The software STRUCTURE was run with an admixture model and no prior information on the sampling locations (Supplementary Material Table 1). To avoid the influence of kinship on inferred structure, all individuals within a population with a relatedness of 0.5 were removed from the dataset before analysis (Queller & Goodnight 1989). Plotting the natural logarithm of the posterior probability (P_p) of K given the data over multiple runs determined the predicted number of clusters (Supplementary Material Fig. 2), and this was compared with ΔK (Evanno *et al.* 2005) as determined in STRUCTURE HARVESTER v.0.6.93 (Earl & VonHoldt 2011). Independent runs for all datasets were averaged in CLUMPP v.1.1.2 (Jakobsson & Rosenberg 2007) using the *Greedy* algorithm with 10,000 repeats to develop a consensus value for K . Graphical representation was produced in DISTRUCT v.1.1 (Rosenberg 2003). Bayesian clustering of TESS was run without admixture and K was inferred from the modal value of the replicate with the highest likelihood. A correlated allele frequency model was used in GENELAND and the burn-in length was based on the appearance of the posterior density log, as suggested by the software manual. The number of proposed clusters was selected from the highest mean $\log P_p$ (Guillot *et al.* 2009).

MICROSATELLITE ANALYSER (Dieringer & Schlötterer 2003) was used to calculate pairwise F_{ST} values (Weir & Cockerham 1984) between sampling locations, with Bonferroni corrections applied. To test for an association between F_{ST} and geographical distance, Mantel's test for isolation by distance (IBD) was performed in SPAGED1 with 10,000 randomisations. A regression of the spatial distance against $F_{ST}/(1-F_{ST})$ was performed (Rousset 1997). Jost's differentiation index (D_{est}) values (Jost 2008) across loci were calculated using DEMETICS (Gerlach *et al.* 2010).

Approximate Bayesian Computation Methods

To test alternative hypotheses that could explain the genetic similarity between PH and WA samples (see Results), we conducted an ABC analysis (Beaumont *et al.* 2002). ABC aims to obtain the joint posterior distribution of complex models for which the likelihood function can be difficult or impossible to solve analytically, allowing a great flexibility in the scenarios being investigated (Marjoram & Tavaré 2006). Its rationale is to bypass the need of an exact likelihood function by comparing summary statistics from observed data to the summary statistics obtained by simulating the models of interest (Beaumont 2010; Csilléry *et al.* 2010).

We compared three evolutionary scenarios (Fig. 3). Model IWOM assumes that an ancestral population of size N_A split t generations ago into two daughter populations, PH and WA, of effective sizes N_1 and N_2 , respectively (Fig. 3b). Model IM (Nielsen & Wakeley 2001) is equivalent to Model IWOM with the adjustment that populations PH and WA have constantly exchanged migrants since their split at rates m_{12} and m_{21} , respectively (Fig. 3c). A null model was also tested as Model PAN; this assumes that PH and WA are part of the same panmictic population of effective size N_p (Fig. 3a). Note that to compute summary statistics comparable to the observed data (i.e. from two different populations), we set Model_{PAN} using Model_{IWOM} parameters but fixed the divergence time to 1 generation – effectively modelling the samples as a panmictic population throughout their whole history.

The prior distributions were uniform for all demographic parameters and the same range was used for common parameters between models (Table 2). For all demographic models, we assumed that microsatellites evolved under a stepwise-mutation model. Mean mutation rates across loci were extracted from a normal prior distribution, and single-locus mutation rates were drawn from a Gamma distribution as parameterized in ABC TOOLBOX (Wegmann *et al.* 2010), using uniform priors for the two parameters of the distribution. To avoid effects of substructure within the WA clade, and due to similar sample size, Ashmore Reef was chosen to represent the WA clade.

Summary statistics and simulations

ABC analyses were conducted using the package ABC TOOLBOX (Wegmann *et al.* 2010). One limitation of ABC is that models can appear more or less likely dependent on the range of the parameter values and the weight assigned to them by the priors (Sousa *et al.* 2012). Exploratory simulations were therefore performed with varied sets of priors to allow an assessment of their effect on the posterior distribution and ensure that the whole posterior was contained within the final prior range.

We used FASTSIMCOAL (Excoffier & Foll 2011; Excoffier *et al.* 2013) to run one million coalescent simulations of our dataset of 14 microsatellites under each model. ARLSUMSTAT (Excoffier & Lischer 2010) was used to calculate a set of 30 summary statistics (within and between populations; Supplementary Material Table 2), chosen based on those shown to be informative in previous studies (Palero *et al.* 2009; Sousa *et al.* 2012; Butlin *et al.* 2013). To reduce the high dimensionality of the summary statistics, we used a partial least-squares (PLS) transformation (Wegmann *et al.* 2009) to extract their orthogonal components. PLS identifies components to explain variability of response variables (model parameters) by maximising the covariance matrix of predictor (raw summary statistics) and response variables (Wegmann *et al.* 2009).

Model choice and parameter estimation

For model comparison, marginal densities comparable between models were produced using the PLS-transformed summary statistics for the rejection step, while all raw summary statistics were used to perform the post-sampling adjustment step using the ABC-GLM (General Linear Model) in ABC TOOLBOX (Wegmann *et al.* 2009). We retained the 5% of simulations closest to the observed data. We checked that our observed summary statistics (for both PLS components and raw summary statistics) fell within the distribution of summary statistics from the simulations retained. Bayes Factors and P_p were derived from the model choice procedure (Supplementary Material Table 3). P -values were taken as an indication of each model's ability to explain the data.

To validate our model choice procedure, we simulated 1000 pseudo-observed data sets for each model using the original priors. The original results files (of one million simulated data sets for each model) were used to perform our model choice procedure using each of the 1000 pseudo-observed data sets in turn. To test the robustness of discrimination between models using our model choice procedure, each pairwise comparison of simulated and observed data for the two models was performed. A model's original data were compared with the pseudo-data of the same model and those of the model being compared. Posterior probabilities were compared with a logistic regression. Confidence in model choice was calculated by estimating the FDR (Verhoeven *et al.* 2005): the frequency of the P_p being equal to or larger than the real P_p of the best model.

For parameter estimation, the distance step and post-sampling adjustment were both carried out using PLS components. This was performed independently for each model because different PLS components are extracted for each model. The GLM method implemented in ABC TOOLBOX (Leuenberger & Wegmann 2010) was used for post-sampling adjustment. Parameter estimation was verified by ensuring P -values were reasonably large (>0.05 as suggested in the ABC TOOLBOX manual) and checking that posterior distributions were within the prior ranges (Supplementary Material Fig. 3). The pseudo-observed data were also used to check for uniformity of the posterior quantiles; a departure from uniformity suggests a parameter is over- or underestimated.

Results

Genotyping Validation

A genotyping error rate of zero was determined between replicates. No evidence was found for frequent allelic drop-out across any loci in any sampling locations. A shortage of heterozygous genotypes was found for locus *Npom08* in the Osprey Reef and Scott Reef populations, possibly resulting from scoring errors due to stutter. This locus was retained in analyses due to its quality in other reef populations. The estimated frequency of null alleles was low for all loci (≤ 0.05). Departure

from HWE was not detected consistently across all sampling locations at any loci. No pairs of loci consistently showed LD in all populations, suggesting that no loci were physically linked. F_{ST} values ranged from -0.04 to 0.35, D_{est} ranged from -0.04 to 0.75 (Table 1). IBD analysis revealed no overall association between F_{ST} and geographical distance and was not significant ($r^2 = 0.139$; $P = 0.095$).

Population Structure

Shark Reef was grouped with Osprey Reef for analyses based on their close geographical location and high degree of relatedness (Table 1); they will hereafter be referred to as Osprey Reef. Results incorporating spatial data in TESS (Fig. 2a) and GENELAND (Supplementary Material Fig. 1) returned three and five genetic clusters, respectively. TESS returned the first cluster, including PH and all WA reefs, the second cluster of the Great Barrier Reef, and third cluster of Osprey Reef. GENELAND divided samples into five genetic clusters of: (i) PH, (ii) Ashmore, Imperieuse and Clerke Reefs, (iii) Scott Reef, (iv) Great Barrier Reef and (v) Osprey Reef.

Plots of ΔK and $\text{LnP}(K)$ generated from STRUCTURE results indicated four as the most likely number of genetic clusters present in the full dataset (Fig. 2). The first two genetic clusters consisted of populations PH and WA, the third cluster consisted of EA Great Barrier Reef, and cluster four consisted of Coral Sea's Osprey Reef (Fig. 2b). Sub-setting the data to look for further division within clusters returned validating results (Fig. 2c and 2d).

Approximate Bayesian computation analysis

The model comparison gave strong support to model_{IWOM} ($P_p = 1.0$, Supplementary Material Table 3) and the FDR was low (0.2%), indicating with high confidence that the data were not the result of sustained migration between the Philippines and WA (see also Supplementary Material Fig. 4). Moreover, this model fitted the data well (the observed summary statistics lay within the range of both the untransformed and PLS-transformed post-rejection simulated summary statistics), indicating that its best score was not a result of a bad fit by all models to the data. Parameter estimation under

model_{IWOM} enabled estimation of the ancestral effective population size (median: 2,035,120; highest posterior density (HPD95 low, high): 62,816.9, 4,508,320), which was smaller than the estimated current population sizes of PH (median: 3,080,000) and WA (median: 2,610,000). The distribution of posterior quantiles did not show strong departures from uniformity, which is indicative of a lack of bias in parameter estimation (Wegmann *et al.* 2009).

Discussion

We detected population structure among east Australian sampling sites, indicating genetic isolation of Osprey Reef and Shark Reef from the Great Barrier Reef. West Australian samples revealed limited population structure but with significant pairwise F_{ST} and D_{est} between Scott Reef and the other west Australian reefs. The genetic similarity found between the Philippines and west Australia was unexpected. Further investigation modelling different demographic scenarios revealed that this similarity was not the result of migration, but may be attributable to ancestral population sizes that were until recently large (population declines have been shown in areas under fishing pressure; Barord *et al.* 2014), and consequently exhibiting limited genetic drift.

Mechanisms for population structure

Results from software STRUCTURE, TESS and GENELAND showed Osprey Reef and Shark Reef populations in the Coral Sea to be genetically distinct from the Great Barrier Reef, west Australia, and Philippine populations. Ocean physiography (the physical geography of the ocean floor) appears to have been influential in this differentiation. Ocean depths in the Coral Sea between Osprey Reef and the Great Barrier Reef exceed 1700 m (Dunstan *et al.* 2011), and while movement through open water is feasible, it leaves individuals vulnerable to predation (Yomogida & Wani 2013). The response of *N. pompilius* individuals to attacks by teleosts showed that that they retreated into their shells and demonstrated no defence or escape response (Saunders & Landman 2010). The Great Barrier Reef was shown to be distinct, not only from Osprey Reef but also from the western populations, which supports

the conclusions of previous evolutionary studies using partial *COI* sequences (Sinclair *et al.* 2007, 2011; Bonacum *et al.* 2011; Williams *et al.* 2012).

West Australian results were not consistent across software; STRUCTURE and TESS assigns the Philippines and west Australia to the same genetic population, whereas GENELAND designates the Philippines and Scott Reef as separate genetic clusters. Geographically, Scott Reef is located between Ashmore and Clerke Reef, and the differentiation is seen between Scott Reef and surrounding west Australian reefs, despite shallower surrounding sea depths. F_{ST} measures deviation from panmixia, D_{est} measures deviations from total differentiation (Whitlock 2011); both F_{ST} and D_{est} values distinguish Scott Reef as a separate genetic cluster.

Due to their residing depth, data on currents at the surface cannot explain *N. pompilius* dispersal patterns (Biuw *et al.* 2007). Currents have been shown to impact individual positions on a reef (O’Dor *et al.* 1993), with recorded movements of up to 6 km that may have been facilitated by currents (Dunstan *et al.* 2011). However, *N. pompilius* has also demonstrated strong resistance to currents and an ability to utilise them to obtain food (O’Dor *et al.* 1990). The overall impact of currents on the species’ population distribution is poorly documented.

No significant correlation was found between F_{ST} and linear geographic distance (Rousset 1997). IBD has been shown in other cephalopods (Pérez-Losada *et al.* 2002; Kassahn *et al.* 2003; Cabranes *et al.* 2008) but, like *Nautilus*, the octopus *Octopus vulgaris* (Moreira *et al.* 2011), the cuttlefish *Sepia esculenta* (Zheng *et al.* 2009) and the squid *Loligo pealeii* (Buresch *et al.* 2006) have all demonstrated genetic distances disproportionate to geographical distances. It has been hypothesised that this was in each case due to natal philopatry (Kassahn *et al.* 2003; Buresch *et al.* 2006). It has been speculated that this behaviour does not occur in nautiloids (Crook & Basil 2013), but gaps remain in our knowledge of *N. pompilius* ecology. Despite lacking the lensed eye and vertebrate-like brain of other cephalopods (including dedicated lobes for learning and memory), *N. pompilius* has been shown to be

capable of both spatial learning and navigational strategy (Crook *et al.* 2009; Crook & Basil 2013). Migration on a small scale is not completely unfeasible, but seems unlikely at the scale investigated in our study.

Divergence without migration

Our ABC model does not support the possibility of sustained migration between the Philippines and west Australia as an explanation for the genetic admixture shown in the structural analyses. Movement of individuals between the two sites was unknown, but depth limitations of *N. pompilius* are indicative of isolation over such a geographical distance; it is possible that the genetic similarity observed has resulted from incomplete lineage sorting. Marker data can be misleading about relationships among populations due to the retention and stochastic sorting of ancestral polymorphisms. This is especially likely if the effective population size is large relative to lineage length (the time since the populations split; Maddison & Knowles 2006). Alleles can then persist in both populations due to limited genetic drift. Model_{IWOM} indicated extremely large ancestral and current effective population sizes, resulting in a lower probability of alleles becoming fixed before divergence (Pamilo & Nei 1988). Current population size estimates for the Philippines (median = 3,190,920) and Ashmore Reef (median = 2,562,800) suggest that genetic drift has yet to have a significant impact. Such large current population estimates are potentially due, in part, to substructure within the sampled areas. Sampling from the Philippines was conducted in several locations, the connectivity between which was assumed but not confirmed. West Australian samples were from the most northern of these reefs, Ashmore Reef; gene flow was previously established between the sampled west Australian reefs (Williams *et al.* 2012). This local gene flow may have inflated the population estimate for Ashmore Reef, reflecting the population size of the NW shelf populations.

Population density estimates made using baited remote underwater video systems were calculated as 13.6 and 0.03 individuals per km² for Osprey Reef and Bohol Sea (Philippines), respectively (Barord *et al.* 2014). Using different methods to generate these results produced predictably dissimilar

abundance data. Additionally, Barord *et al.* (2014) documents evidence of a sudden population size reduction, but our data showed that allelic richness in the Philippines was no lower than at other locations sampled (Fig. 4). It is possible that the genetic consequences of population reduction have yet to take effect; low fecundity and long developmental time (Carlson *et al.* 1984; Landman *et al.* 2010) of nautiloids results in a long generation time compared to other cephalopods. Fishing for shells is relatively new, with no cultural or historical significance in studied areas such as Palawan (Dunstan *et al.* 2010), and so it is possible that we will see a genetic response to exploitation that is not yet detectable with our current set of markers.

The allelic richness in Philippine samples also supports the proposed direction of colonisation from the progenitor population located in the Philippines (Wray *et al.* 1995). There is lower allelic richness in the Great Barrier Reef, with a further decline in the more genetically distinct Osprey and Shark Reefs of the Coral Sea (Fig. 4). The time split estimation (median = 296,495 generations/1,660,366 years based on a generation time of 5.6 years (Saunders & Landman 2010)) by model_{IWOM} indicates that current populations have been evolving independently for a similar time as the accepted species of modern *N. pompilius* (Kröger *et al.* 2011).

Management implications

The absence of migration between the Philippines and west Australia highlights the need for mechanisms to protect these populations as discrete conservation units (Moritz 1994). Environmental differences between sites have not been measured and so it is unknown if resulting divergent selection has occurred, but as a unique population with the possibility of local adaptation, adequate protection for the Philippines is imperative to the long-term survival of this genetic cluster. The absence of contemporary migration indicates that it is unlikely that the Philippines would be repopulated, should fishing in this area continue to extinction. Informative results must now reach policy makers to enable legislative protection.

The variation seen in IBD within cephalopods demonstrates the need for species-specific range studies, especially when results are extrapolated for fisheries management. As fin fish stocks decline and the fishing industry targets novel resources, it is likely that cephalopod stocks will experience increased fishing pressure (Dillane *et al.* 2005). The data supporting the need for *Nautilus* and *Allonautilus* protection is ever increasing (Dunstan *et al.* 2010; Bonacum *et al.* 2011; Williams *et al.* 2012; De Angelis 2012; Barord *et al.* 2014). Overexploitation is threatening marine species worldwide (Hutchings 2000; Worm *et al.* 2006; Doukakis *et al.* 2009; Neubauer *et al.* 2013; Watson *et al.* 2013) and our study highlights the need for multiple or finer-scale markers to determine connectivity patterns and establish adequate protection. For example, mitochondrial DNA data on the west Australian reefs (Williams *et al.* 2012) suggested that the population was panmictic, but the higher-resolution data presented here reveals substructure. Our results show how management plans should incorporate discrete management units and should account for more than separation by geographic distance.

Conclusions

A range of molecular studies has been conducted on coleoids (Allcock *et al.* 2015), including population structure analysis using minisatellites, microsatellites and mitochondrial DNA (Dillane *et al.* 2005; Zheng *et al.* 2009; Moreira *et al.* 2011), but this is the first study to use microsatellite markers in a nautiloid. We had hypothesised genetic division between east and west Australia based on previous evolutionary studies on these populations (Sinclair *et al.* 2007, 2011; Bonacum *et al.* 2011; Williams *et al.* 2012), but we found a greater degree of genetic similarity between samples from the Philippines and west Australia than had been previously considered. Our conclusion that this similarity was not the result of migration emphasises the need to reduce over-exploitation and prevent the local extinction of *N. pompilius* in the Philippines. Protection for *Nautilus* and *Allonautilus* under CITES would decrease the incentive for continued exploitation. In relatively inaccessible species, genetic data can provide an insight into migration and population dynamics. Such genetic studies should be utilised to develop efficient species-specific management plans for declining populations.

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Enforcing these, in collaboration with legislative protection, is imperative for the conservation of marine populations (Neubauer *et al.* 2013).

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Author Contributions

RCW and WS obtained samples. RCW performed the genotyping. RCW and DAD assessed the marker quality. RCW, BCJ and LD analysed and interpreted the data. WS, DAD, TB oversaw the project. RCW drafted the manuscript and all authors contributed edits and comments.

Data accessibility

Microsatellite primer sequences for *N. pompilius* are available through NCBI: accession numbers HG918068-HG918111. Microsatellite genotyping data and the geographic information for the Mantel's test are available in Dryad, doi:10.5061/dryad.j251f.

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Population	Philippines	Ashmore Reef	Scott Reef	Clerke Reef	Imperieuse Reef	Great Barrier Reef	Osprey Reef	Shark Reef
Philippines ($N = 27$)	-	0.015*	0.044*	0.014*	0.024*	0.130*	0.330*	0.237*
Ashmore Reef ($N = 29$)	0.136*	-	0.018*	-0.004	0.006	0.121*	0.319*	0.228*
Scott Reef ($N = 30$)	0.263*	0.097*	-	0.015*	0.015*	0.173*	0.354*	0.268*
Clerke Reef ($N = 32$)	0.114*	-0.028	0.084*	-	0.004	0.124*	0.322*	0.234*
Imperieuse Reef ($N = 31$)	0.158*	0.044	0.111*	-0.042	-	0.144*	0.343*	0.255*
Great Barrier Reef ($N = 13$)	0.527*	0.485*	0.586*	0.494*	0.528*	-	0.312*	0.229*
Osprey Reef ($N = 45$)	0.693*	0.696*	0.740*	0.703*	0.745*	0.5*	-	0.012
Shark Reef ($N = 8$)	0.651*	0.612*	0.736*	0.678*	0.731*	0.483*	-0.014	-

Parameters	Prior range	Mean	Median	Mode	HPD95 low	HPD95 high
ARG_K	Uniform [0 - 6]	1.78785	1.62815	1.35678	0.105534	3.72541
MUT_U	Uniform [10^{-6} - 5×10^{-4}]	5.00E-05	4.11E-05	2.61E-05	-2.53E-07	0.000122236
N_A	Uniform [0 - 5×10^6]	2.17E+06	2.04E+06	1.31E+06	62816.9	4.51E+06
N_1	Uniform [0 - 5×10^6]	3.08E+06	3.19E+06	3.79E+06	1.05E+06	4.99E+06
N_2	Uniform [0 - 5×10^6]	2.61E+06	2.56E+06	2.09E+06	665830	4.77E+06
t	Uniform [0 - 10^6]	353845	296495	105529	-2511.56	839452



