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Genetic differentiation in the threatened soft coral *Dendronephthya australis* in temperate eastern Australia

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Abstract The endangered soft coral *Dendronephthya australis* faces substantial population decreases in central eastern Australian waters. Despite uncertainty about the cause of these declines, the population genetics of the species has not been investigated. Genetic analysis suggests that *D. australis* is a single species within the family Nephtheidae, confirming identifications based on morphological characteristics only. Soft coral colonies were distributed from Seahorse Gardens in Port Stephens to Jervis Bay in temperate Australian waters, a distance of some 400 km. Genetic differentiation was observed along this distribution using SNP genotyping. Relatively high levels of genetic differentiation were observed between Jervis Bay and the other sites, indicating limited gene flow between this location and others. Moreover, the genetic distinctiveness, low diversity and heterozygote excess at this southern location suggested that it was subjected to a recent population decline and genetic bottleneck. Colonies at Seahorse Gardens and Ettalong, approximately 150 km south of Seahorse Gardens, displayed greater genetic diversity, making these sites more likely to host ancestral populations and to have acted as refugia. Recent substantial decreases in population sizes at these locations are particularly concerning, and these locations require immediate conservation attention.

Key words: cauliflower soft coral, endangered species, PCR sequencing, population genetics, SNP genotyping.

INTRODUCTION

Soft corals (Cnidaria: Alcyonacea) are a diverse taxon typically associated with tropical coral reef communities. In tropical waters of the Indo-Pacific, soft corals are the second most common group of benthic organisms on reefs (Benayahu & Loya 1981) and play important functional roles in the community as ecosystem engineers (Dai 1990, Maida *et al.* 1995). Some soft corals inhabit temperate to cool waters (e.g. Slattery & McClintock 1995), but relatively little is known of the population structure or functional roles of these corals in temperate habitats.

Species within the soft coral genus *Dendronephthya* are globally distributed and moderately common in habitats with fast currents at depths below 20 m (Fabricius & Alderslade 2001). While they routinely form branched colonies on tropical hard bottom substrata, they can also inhabit a diverse array of habitat, from muddy estuaries to deep oceanic waters (Fabricius & Alderslade 2001).

Commonly called 'cauliflower soft coral', *Dendronephthya australis* Kükenthal, 1905, forms colonies composed of sterile stalks that pass into stems, which distally divide into branches containing groups of densely placed polyps (Verseveldt & Alderslade 1982). This species is unusual within the family because it inhabits soft sediment communities in temperate Australian waters (Poulos *et al.* 2015). *Dendronephthya australis* is unique in that it is geographically limited to waters in eastern Australia. Other species from the family Nephtheidae that resemble *D. australis* morphologically also occur in a similar geographical distribution, but these generally inhabit rocky reef habitats and not sandy bottoms (FSC 2021). Despite their abundance in tropical and temperate habitats, less is known about the reproductive strategies of species within the family Nephtheidae than in other octocoral families (Kahng *et al.* 2011).

Little is known of the taxonomy of *D. australis*. It was first described in 1905 from Port Jackson and Port Hacking in Sydney and then redescribed in more detail from material collected in 1975 from Fly Point in Port Stephens, approximately 200 km north (Verseveldt & Alderslade 1982). In 2011, a whole specimen from Port Stephens was verified to species level by experts at the National Natural History Museum in the Netherlands. All descriptions were

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based solely on morphological characteristics and predominantly focussed on the armature of the polyps and shape of the sclerites in the surface layer of the stem and stalk (Verseveldt & Alderslade 1982). To date, no genetic verification of *D. australis* has been done.

The distribution and occurrence of *D. australis* appears somewhat sporadic, with its presence and abundance fluctuating substantially. More recently, it has significantly declined in area (Larkin *et al.* 2021a). Currently, *D. australis* colonies consistently only occur in abundance at three locations in NSW: Port Stephens and at Brisbane Water (~150 km south of Port Stephens), and newly discovered colonies have been found occurring on the HMAS Adelaide shipwreck located off Terrigal, close to Brisbane Water (Harasti 2021, personal observation). Small numbers of colonies have also recently been observed in two additional sites: a very small (~25 m^2) area near Bowen Island at the mouth of Jervis Bay (~200 km south of Sydney) and in Botany Bay, south Sydney. Persistence of *D. australis* at these latter two sites is unknown, and colonies in the Botany Bay site and HMAS Adelaide were not present at the time of sampling for this research. The abundance of *D. australis* has varied significantly over time, with large distributions and abundances recorded over the past decade (Larkin *et al.* 2021a, b). The size of *D. australis* colonies also vary in size depending on tidal currents (Davis *et al.* 2015) and have been recorded up to 70 cm in height at Port Stephens (Harasti *et al.* 2014).

Targeted mapping surveys in other areas with similar habitat within the region, including the Clyde River, Port Hacking, Port Jackson, Pittwater, the Hawkesbury River and Wallis Lake, did not detect further colonies of the species (Creese *et al.* 2009, Harasti pers. comm 2013). Despite its tenuous occurrence, *D. australis* holds a distinct and important functional role in the communities it inhabits (Corry *et al.* 2018, Finlay-Jones *et al.* 2021). In Port Stephens, it has been linked to increased fish and invertebrate diversity (Poulos *et al.* 2013) and offers crucial habitat for syngnathids (seahorses and pipefish), particularly the endangered White's seahorse *Hippocampus whitei* (Harasti 2016), which are a protected species in Australia (Harasti *et al.* 2014).

Dendronephthya australis was listed in 2021 as a threatened species, with its conservation status listed as endangered in Australia, based on IUCN categories A2ac, B1ab(i,ii,iii,iv) + B2ab(i,ii,iii,iv) (FSC 2021). It is the first soft coral species to be added to threatened species legislation in Australia. Habitat requirements for the species are very specific and are thought to limit suitable locations that colonies could occupy (Poulos *et al.* 2015; Larkin *et al.* 2021a). For example, colonies require a specific set of current velocities (Davis *et al.* 2015) and bathymetry (Poulos *et al.* 2015) for long-term persistence.

Existing colonies are at risk from sand inundation, poor water quality and damage from boat anchoring and moorings. Colonies that occurred on sediments at other sites in the Port Stephens region (Fly Point, Seahorse Gardens and Little Beach) from 2006 to 2010 (Harasti *et al.* 2014) have now disappeared completely. This disappearance coincides with extensive sand movement from beach nourishment initiatives in the estuary (Wainwright 2011). Damage from boat moorings and anchoring has been observed and reported for several sites in Port Stephens (Glasby & West 2018), which also coincided with large declines in *D. australis* abundance (Harasti 2016). In addition, *D. australis* may seasonally suffer predation from *Dermatobranchus* sp., a heterobranch sea slug that aggregates in the Austral spring in the same habitat as *D. australis* (Davis *et al.* 2018) and small colonies are susceptible to other predators such as ovulids (Larkin *et al.* 2021b). More recently, flooding events that lowered salinity for over 1 week in Port Stephens and Brisbane Water have led to further declines.

In light of the uncertainty of the long-term persistence of colonies of *D. australis* in the small number of locations they are found, a study was done to answer the following questions: (i) do genetic analyses confirm previous morphological methodologies used to identify *D. australis* as a distinct species, (ii) are the current populations genetically diverse and (iii) is it possible to identify an ancestral site or a site of genetic refugia for existing populations?

MATERIALS AND METHODS

Mapping *Dendronephthya australis* at known sites in temperate eastern Australia

Dendronephthya australis occurs sporadically in sites spanning approximately 400 km of the temperate east Australian coast. All known sites at the time of sampling were included. To ensure that samples were taken from a representative range of *D. australis* colonies in each site, the extent of the soft coral at the sites were mapped in February 2019 prior to sampling. Mapping of the two sites in Port Stephens estuary (Pipeline and Seahorse Gardens) was done using a diver-towed GPS system that has previously been employed for mapping this type of habitat (Harasti 2016, Poulos *et al.* 2015). Briefly, a video camera (Sony Handycam, HDR-XR550VE Full HD, 12 mega pixels) in a Light & Motion STINGRAY G2+ underwater housing with GPS was held by a diver on SCUBA. The diver then swam forward ~1 m above the substrate in a zigzag manner with the recording camera angled slightly toward the substrate. Underwater video footage was analysed as the presence or absence of data using GPS coordinates that had been downloaded using MapSource software (V.6.16.3, 2010). Seagrass associated with *D. australis* was also

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recorded. The site in Jervis Bay was mapped in the same manner.

Mapping in Brisbane Water (Ettalong and Foreshore Ettalong) was done over three consecutive days in May 2018. Transects were conducted parallel to the water flow in sites where the soft corals had been observed. Other areas based on the habitat suitability model developed by Poulos *et al.* (2015) were also searched. These mainly excluded areas north of the Rip Bridge that are prone to increased turbidity, lower water flow and sediment content with higher organic loads (Gaston, pers. comm). Transects were run at depths of 1–10 m from the Rip Bridge (33°30′25.2″S, 151°20′45.6″E) to Little Box Head (33°32'9.6″S, 151°20′13.2″E) at speeds of 1 to 2 kn. A SpotX dropcam with a GoPro™ Hero 5 Black (Full HD, 12 mega pixels, wide-angle setting) was towed ~1 m above the substrate. This provided a live full HD video feed to an iPad mini. Underwater video footage was analysed as the presence or absence of data using GPS waypoints recorded on the GPS unit of the vessel. Resulting GPS locations of the colonies were copied from the vessel GPS into ESRI ArcGIS (V.10.6.1) to construct a map of the habitat extent.

Tissue collection for genetic analyses

Ten colonies at each site were sampled for genetic analysis, apart from the Foreshore site in Ettalong where only one colony could be identified at the time of collection. Chosen colonies were distributed throughout each site based on the mapping to ensure adequate colony representation. Three (0.5 cm^3) samples containing branches and polyps from separate sections of each colony were taken using scissors sterilised with 75% EtOH between each sample. Tissue samples were placed in individual plastic vials and preserved in 75% EtOH. The EtOH was drained and replaced after 24 h, and the samples were then stored in 100% EtOH until analysis.

Species verification using PCR sequence analysis

Tissue (<2 mm^3) was excised from each coral sample (one per colony) and DNA extracted using a Proteinase-K protocol (Geraghty *et al.* 2013). To ensure that samples were unlikely to be different species, two genetic markers were PCR-amplified: one mitochondrial marker (ribosomal *16S* gene) and one nuclear marker (ribosomal *ITS-1* region). The 16S marker was chosen based on an assessment of the complete mitochondrial genomes of the several species of *Dendronephthya* that were available at the time. We found that among this genus specifically, the 16S gene had the greatest number of SNPs and we therefore opted for 16S as the best chance of species discrimination despite the slow rates of mitochondrial gene evolution in this region.

The *16S* mitochondrial gene was amplified using the novel primers DN1-F: 5'-AGGCTACTTAAGTATA GGGG-3′ and DN1-R: 5'-AACTCTCCGACAATA TTACGC-3′. To generate these primers, several complete mitochondrial genomes of other *Dendronephthya* species were downloaded from GenBank [*D. putteri* (JQ886185); *D. suensoni* (NC_022809); *D. mollis* (NC_020456); *D. gigantea* (FJ372991); *D. castanea* (NC_023343)] and aligned using the MUSCLE alignment algorithm (Edgar 2004) in Geneious (V.10.2.4) software (Kearse *et al.* 2012). The consensus sequence of this alignment was then used to design primers targeting the *16S* region with the built-in Primer3 plug-in (Untergasser *et al.* 2012).

PCR conditions were as follows; an initial denaturation at 98°C for 3 min followed by 35 cycles of 98°C for 0.5 min, 53°C for 0.5 min and 72°C for 1.5 min, and a final extension step at 72°C for 10 min. The nuclear ribosomal *ITS-1* region was amplified using the primers 1w (McFadden & Hutchinson 2004) and 2ss (Chen *et al.* 1996), with PCR conditions described in McFadden *et al.* (2001). All PCRs were amplified using GoTaq® Colourless mastermix (Promega, Madison, WI, USA) and GeneReleaser™ (Bioventures, Murfreesboro, TN, USA) as per the manufacturer's protocols. PCR efficiency was assessed using 1.5% agarose gel electrophoresis.

PCR products were Sanger sequenced in both directions using the amplification primers at the Macrogen Sequencing Facility (Seoul, Korea). Forward and reverse sequences for each PCR sample were edited and aligned using Geneious (V.10.2.4). Sequences were interrogated against the GenBank nucleotide database using BLAST. Representative sequences of the *16S* mitochondrial gene and the nuclear ribosomal *ITS-1* region generated in this study are available in GenBank under accession numbers MN366374 to MN366377, and MN366380 to MN36683 respectively.

Analysis of genetic structure

Single nucleotide polymorphism (SNP) genotyping and filtering

Subsamples of coral tissue (~20 mg) were sent to Diversity Arrays Technology Pty Ltd. (DArT, Canberra, Australia) for DNA extraction and genotyping of single nucleotide polymorphisms (SNPs). As multiple tissue samples were available for most *D. australis* colonies, each colony was represented by two or three samples (hereafter referred to as replicates) for sequencing with the DArTSeq™ protocol (a combination of genome complexity reduction and next generation sequencing methods; Sansaloni *et al.* 2011; Kilian *et al.* 2012; Lambert *et al.* 2013; Rojas *et al.* 2020). DNA samples were digested using a combination of *PstI* and *SphI* restriction enzymes. Adapters specific to these restriction enzymes and a unique barcode were then ligated to each sample for subsequent amplification *via* PCR. Primers specific to the adapter and barcode sequences were used for PCR, with conditions consisting of an initial denaturation at 94°C for 1 min, followed by 30 cycles of 94°C for 20 s, 58°C for 30 s, 72°C for 45 s, and a final extension step at 72°C for 7 min. Equimolar amounts of the amplification products from each sample were pooled and applied to c-Bot (Illumina) bridge PCR followed by sequencing on the Illumina HiSeq 2500 platform (a single sequencing read run for 77 cycles). The final report file generated by DArT consisted of 69 bp fragments

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containing one or more SNPs called by DArT's proprietary pipelines.

The final report file was initially screened to identify replicates with large amounts of missing data. For each replicate, the total amount of missing data was calculated and then compared between replicates for each *D. australis* colony. The replicate with the least amount of missing data was then selected as the representative for the colony. This was done to avoid pseudoreplication in downstream population genetics analyses.

Filtering the SNPs in the reduced dataset was performed using the dartR V.1.1.11 package (Gruber *et al.* 2018) in R V.3.6.0 (R Core Team 2019) with the RStudio (RStudio Team 2018) interface. The 3581 SNPs called by DArT were filtered as follows: (i) an average repeatability of 100%; (ii) removal of additional monomorphic loci (detected following the removal of the replicates); (iii) loci with a call rate of ≥95%; (iv) removal of multiple SNP loci within the same sequence fragment (secondaries) so that the proportion of physically linked loci was minimised (using the 'best' method in the gl.filter.secondaries function); (v) individuals with a call rate of ≥90%; (vi) minor allele frequency of ≤0.05; and (vii) loci that showed departures from Hardy–Weinberg equilibrium (α = 0.05 with Bonferroni correction). As our analyses assumed a dataset of neutral loci, we filtered out loci potentially associated with selection by performing outlier tests using BayeScan version 2.1 (Foll & Gaggiotti 2008) with default settings and OutFLANK V.0.2 (Whitlock & Lotterhos 2015) (performed within the dartR package). For both analyses, a false discovery rate of 0.05 was applied. This dataset of putatively neutral loci was used in subsequent analyses. Where necessary, PGDSpider V.2.1.1.5 (Lischer & Excoffier 2012) and GenAlEx V.6.503 (Peakall & Smouse 2006, 2012) were used to transform the filtered dataset into file formats compatible with the software or R packages used for analysis.

Based on preliminary results, it was decided that two datasets would be analysed separately: (i) all locations (43 colonies) and (ii) Port Stephens and Brisbane Water (PSBW) locations (30 colonies). Both datasets were filtered as described previously.

Basic population parameters, including allelic richness (A_r), observed heterozygosity (H_o) and expected heterozygosity (H_e) averaged over all loci and the inbreeding coefficient (F_{IS}), were calculated using R for each of the sampled locations with the divBasic function in the diveRsity V.1.9.90 package (Keenan *et al.* 2013). For F_{IS}, 1000 bootstrap replicates were used to calculate the 95% confidence intervals (CIs). Deviations from Hardy–Weinberg equilibrium (HWE) were also tested with divBasic, implementing the Fisher's exact test and 2000 Monte Carlo permutations.

Genetic differentiation between sampled *D. australis* locations was tested using a combination of analysis of molecular variance (AMOVA; Excoffier *et al.* 1992), pairwise F_{ST} tests and principal coordinates analysis (PCoA) (Gower 1966). AMOVA was performed in the poppr V.2.8.3 package (Kamvar *et al.* 2014; Kamvar *et al.* 2015). The ade4 implementation of AMOVA was selected and significance was assessed using the randtest function and 999 permutations. Pairwise F_{ST} for the sampled locations was calculated with the StAMPP version 1.5.1 package (Pembleton *et al.* 2013), with 1000 bootstrap replicates to calculate the 95% CIs. CIs that did not contain zero were considered to be statistically significant. Genetic distance between individuals and locations was visualised through the generation of PCoA plots with functions contained in the dartR package. The number of informative axes to retain for the plot was determined by examining a scree plot of eignenvalues (Cattell 1966).

Gene flow between locations was further assessed by admixture analysis, calculating the number of migrants (N_m) and generating relative migration networks. Levels of admixture among *D. australis* colonies were examined using the LEA V.2.6 package ADMIXTURE (Frichot & François 2015). The number of ancestral populations that best explained the genotypic data was identified based on a calculation of the cross-entropy criterion with the snmf function (Frichot *et al.* 2014) in the LEA package (K range 1–10, with 10 repetitions for each value of K). Bar plots of ancestry proportions for each *D. australis* colony were then generated to visually assess admixture. The number of migrants between locations was estimated using the formula $N_m = [(1F_{ST})1]4$ (Slatkin 1993), where F_{ST} is the pairwise F_{ST} value between locations as calculated with StAMPP. Finally, relative migration between locations was examined using the divMigrate function (Sundqvist *et al.* 2016) in the diveRsity package. All three statistics offered in the function [Nei's G_{ST}, Jost's D and Alcala's N_m (Alcala *et al.* 2014)] were calculated with 1000 bootstrap replicates to detect significant asymmetrical gene flow between the sampled locations. Networks were then generated with the qgraph V.1.6.3 package (Epskamp *et al.* 2012).

RESULTS

Mapping *Dendronephthya australis* at known sites in temperate eastern Australia

Two sites in Port Stephens contained colonies of *D. australis*, and samples were collected from both locations. Approximately 30 colonies occurred at the Seahorse Gardens site (32°42′54.35″S 152°9′0.56″E) covering an area of approximately 75 m^2 and approximately 60 colonies were found at the Pipeline site (32°43′2.97″ S 152°8′30.76″E) occurring over an area of 400 m^2. Colonies at Seahorse Gardens occurred at an average of 3-m water depth and were interspersed among *Zostera muelleri* and *Halophila ovalis* seagrass. Colonies at the Pipeline occurred on sandy substrata among sponge and algal habitat in depths of 9–10 m (Figs 1, 2). Recent surveys of these locations in 2021 found that the soft coral has now disappeared completely from both sites (Harasti 2021, unpublished data).

Two soft coral sites were identified in Brisbane Water: one on the Foreshore Ettalong adjacent to the Ettalong beach barbeque area (33°30′59.33″S 151°20′7.67″E) and one on the south-facing shore of Ettalong west of the ferry jetty (33°31′06.36″S

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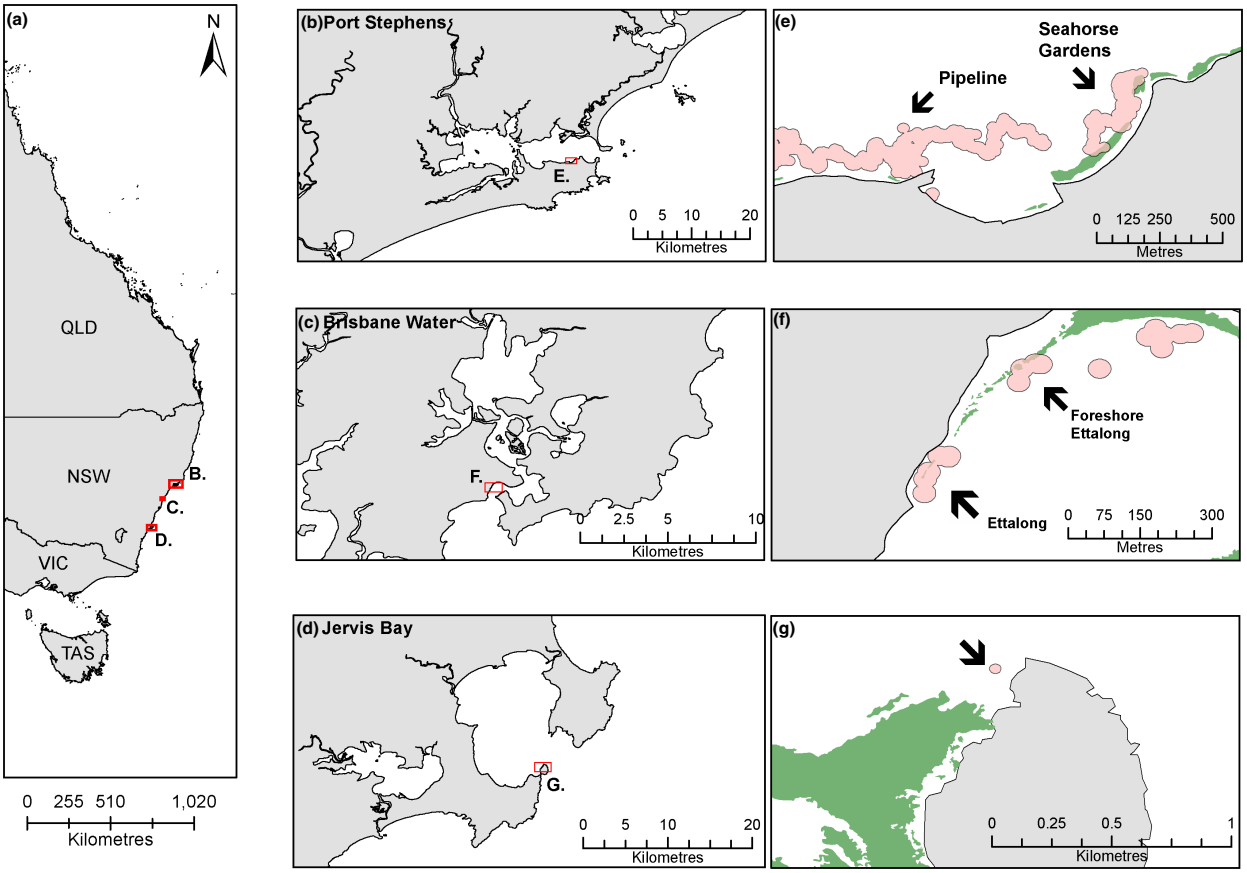

Fig. 1. Map of sites and distributions from which genetic samples of cauliflower soft coral (*Dendronephthya australis*) were obtained. Soft coral habitat is displayed in pink. Seagrass habitat (mixed species including *Zostera muelleri, Halophila ovalis* and *Posidonia australis*) is in green. Locations from which tissue samples were obtained are highlighted with arrows.

151°20′1.04″E). These sites were at depths of 1–4 m, ~1.0 ha in size and were interspersed with low densities of the seagrasses *H. ovalis* and *Z. muelleri*, the macrophytic alga *Sargassum longifolium* and coarse sand substrate. A dense *Z. muelleri* meadow was present immediately north west and at shallower depths from the soft coral locations (Figs 1, 2).

Only one site in Jervis Bay was identified, with a total of 18 colonies (35° 6′45.50″S 150°46′1.49″E). These colonies covered an area approximately 4 m^2 at a depth of 9 m (Newson 2019, pers. comm; Figs 1, 2).

Species verification using PCR sequence analysis

No intersample nucleotide variation was observed for the *16S* and *ITS-1* DNA markers, which spanned 490 and 400 bp respectively. While it is difficult to definitively show that the colonies were the same species using these markers, the DNA markers do not provide any evidence that the specimens are from different species, and the closest matches in the database are *D. australis*. Mitochondrial *16S* sequences shared a >99.8% nucleotide identity to several species of the *Dendronephthya* genus (Accessions: NC_036022, JQ290079, MG018854). Similarly, the *ITS-1* region aligned with an uncharacterised *Dendronephthya* species, sharing ~82% sequence similarity. The relatively low percentage similarity is not surprising given that at the time of this study, there were no DNA sequences belonging to *D. australis* lodged in GenBank. The most parsimonious explanation is that our colonies were *D. australis*, especially considering the morphological and ecological alignment with this species in the colonies assessed.

Analysis of genetic structure

Following filtering, the number of SNPs analysed in all locations and in the PSBW location datasets were 709 and 403 respectively. Outlier analyses did not identify any outlier loci in the 'all locations' dataset, while one outlier locus was identified in the PSBW

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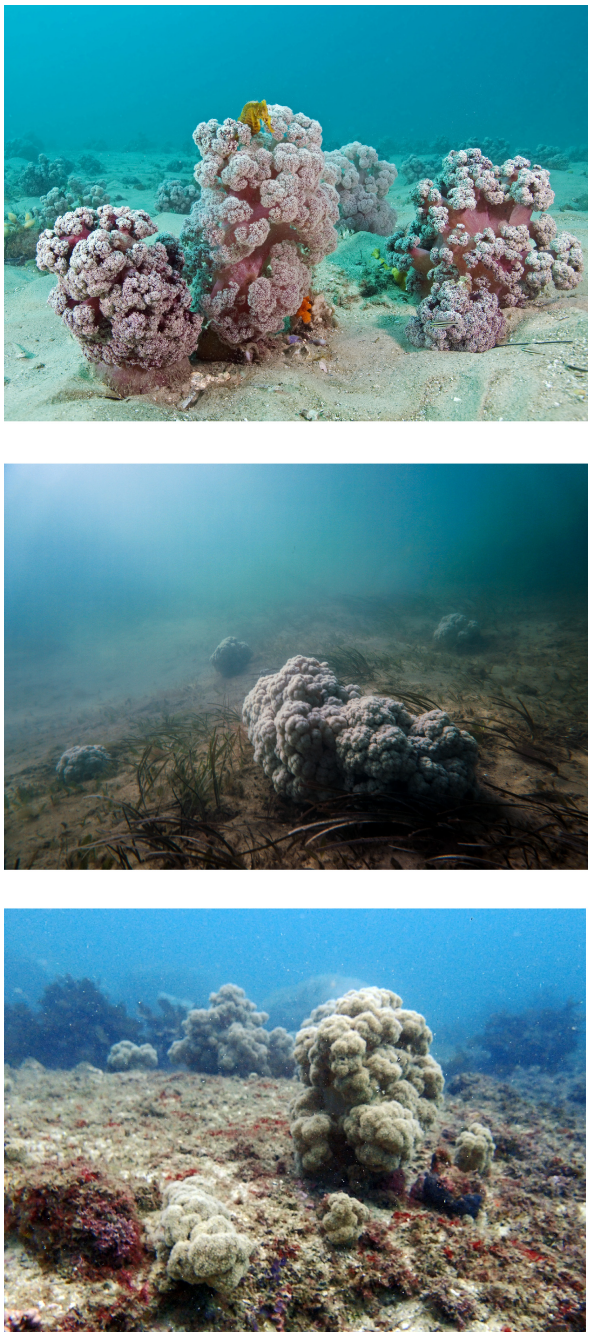

Fig. 2. Underwater photographs of cauliflower soft coral (*Dendronephthya australis*) habitats within each estuary: Port Stephens (top), Brisbane Water (middle) and Jervis Bay (bottom). Note the differences in the substrate habitats (sand, seagrass and rocky reef) that the colonies occur in each estuary.

dataset using OutFLANK and subsequently removed.

All locations were initially analysed collectively. Given the strong influence of the Jervis Bay population on analyses of genetic structure, however, *D. australis* colonies from Port Stephens and Brisbane Water were also analysed separately to allow for finer-scale assessment of genetic structure at these sites.

All locations

Summary statistics calculated for each of the sampled locations indicated differences between the northern (Port Stephens and Brisbane Water) and southern (Jervis Bay) occurrences. Foreshore Ettalong was excluded from these analyses because it comprised a single colony. The three northern locations all displayed similar values for allelic richness (A_r) and observed (H_o) and expected (H_e) heterozygosity (Table 1). These locations did not deviate from Hardy–Weinberg equilibrium (HWE) and had low inbreeding coefficients (F_{IS}), with 95% CIs containing zero. Jervis Bay, on the other hand, showed a lower A_r and higher H_o compared with the northern locations (Table 1). This location also deviated significantly from HWE and displayed a strong heterozygote excess ($F_{IS} = -0.930$).

Analysis of molecular variance revealed significant structuring of *D. australis* populations in eastern Australia ($\Phi_{ST} = 0.745$, $P = 0.001$), with 74.5% of the total variation derived between locations. Calculation of pairwise F_{ST} values and their 95% CIs indicated significant population differentiation between locations (Table 2). In particular, the Jervis Bay and Seahorse Gardens populations were significantly different from all other sampled locations, as well as from each other. However, colonies from Foreshore Ettalong, Ettalong and Pipeline were not genetically discrete. Results of the pairwise F_{ST} tests were illustrated by the PCoA plot, with colonies from Jervis Bay clustering on one side of the plot and colonies from Port Stephens and Brisbane Water clustering on the other (Fig. 3a).

Admixture analysis revealed two ancestral populations that corresponded to the northern and southern occurrences, with estimates of migration rates inferring limited gene flow between them (Figs 4, 5). One population comprised all colonies from Jervis Bay, while the other consisted of the colonies from Port Stephens (Seahorse Gardens and Pipeline) together with colonies from Brisbane Water (Foreshore Ettalong and Ettalong). The estimated number of migrants between Jervis Bay and the other locations was very low, ranging from 0.09 to 0.13 (Table 2; Fig. 5). The number of migrants per generation between Seahorse Gardens and other *D. australis* locations was also low (2.83 to 10.62 migrants).

All three relative migration statistics produced similar results. Hence, the results presented here are only for Alcala's N_m. No significant asymmetry in gene flow was observed between pairs of locations (i.e. no significant difference in rates of immigration

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Table 1. Summary statistics calculated for *Dendronephthya australis* from each of the sampled locations, including allelic richness (A_r), average observed (H_o) and expected (H_e) heterozygosity, the inbreeding coefficient (F_{IS}) and the associated 95% CIs

Dataset	Location	*N*	A_r	H_o	H_e	F_{IS}	F_{IS} 95% CI	HWE
All sampled locations (709 SNPs)	Ettalong	7	1.53	0.21	0.20	−0.067	−0.212, 0.028	1
	Pipeline	11	1.55	0.19	0.20	0.033	−0.027, 0.076	1
	Seahorse Gardens	10	1.52	0.21	0.20	−0.080	−0.218, 0.012	1
	Jervis Bay	14	1.32	0.30	0.16	−0.930	−0.997, −0.825	<0.001
PSBW locations (403 SNPs)	Ettalong	8	1.82	0.31	0.30	−0.023	−0.184, 0.103	1
	Pipeline	11	1.84	0.29	0.30	0.018	−0.046, 0.055	1
	Seahorse Gardens	10	1.79	0.32	0.29	−0.077	−0.214, 0.009	0.943

P-values testing the null hypothesis of no deviation from Hardy–Weinberg equilibrium (HWE) for each location are also presented. Summary statistics for Foreshore Ettalong were not calculated because this location comprised a single colony.

N, number of colonies; PSBW, Port Stephens and Brisbane Water.

Table 2. Pairwise F_{ST} values (95% CIs) (lower matrix) and estimated number of migrants (N_m) (upper matrix) for all *Dendronephthya australis* colonies sampled. ET, Ettalong; FE, Foreshore Ettalong; JB, Jervis Bay; PIP, pipeline; SHG, Seahorse Gardens.

	PIP	ET	JB	SHG	FE
PIP	-	138.64	0.13	10.62	89.04
ET	0.0018 (−0.007, 0.011)	-	0.12	8.00	18.00
JB	**0.6637** (0.636, 0.688)	**0.6735** (0.647, 0.698)	-	0.12	0.09
SHG	**0.0230** (0.012, 0.034)	**0.0303** (0.019, 0.043)	**0.6709** (0.644, 0.695)	-	2.83
FE	0.0028 (−0.03, 0.042)	0.0137 (−0.030, 0.056)	**0.7346** (0.706, 0.762)	**0.0812** (0.037, 0.129)	-

Significant pairwise F_{ST} values are indicated in bold (i.e. 95% CI did not contain zero).

compared to emigration). However, the relative migration rates between Jervis Bay and the other locations were very low (emigration = 0.03; immigration = 0.02; Fig. 5a). The positioning of Jervis Bay far away from the other locations in the network also indicated very limited gene flow. Migration rates between the other locations were similar, with the highest rates between Ettalong and Pipeline. The Foreshore Ettalong location was not included in this analysis because calculations of relative migration require more than one colony per location.

Port Stephens and Brisbane Water locations

All sampled locations in this dataset showed similar summary statistics, with no location deviating from the assumptions of HWE (Table 1). Weak but significant structuring of *D. australis* was detected with AMOVA (Φ_{ST} = 0.039, P = 0.02). The pairwise F_{ST} tests showed significant genetic differentiation between most of the sampled sites (Table 3), with Foreshore Ettalong the only location to display non-significant comparisons. Given the limited sample size from this location, this result was not unexpected. Calculation of the cross-entropy criterion for this dataset indicated that the number of ancestral populations was either four or six. The plot showed a plateau at K = 4 but had a minimum at K = 6 (Fig. 6). Regardless of which K was selected, the admixture plots show that each of the colonies sampled from Pipeline and Foreshore Ettalong were admixed, while some colonies from Ettalong and Seahorse Gardens displayed no evidence of admixture (Fig. 7). These colonies corresponded to those separated from the main cluster in the PCoA plot (Fig. 3b).

The estimated number of migrants between locations ranged from 5.44 to 17.36 (Table 3). No significant asymmetrical gene flow between location pairs was detected by any of the three statistics. The relative migration network (using Alcala's N_m) indicated relatively high levels of gene flow between most pairs of locations (>0.70), although a lower level of emigration from Ettalong into Seahorse Gardens (0.44) was observed (Fig. 5b). As noted previously, Foreshore Ettalong was excluded from this analysis.

DISCUSSION

Molecular work from this study suggests that the populations of soft corals assessed in this research are *Dendronephthya australis*. It should be noted, however, that while all our samples shared identical sequences at 16S, this is not compelling evidence that they represent a single or unique species as

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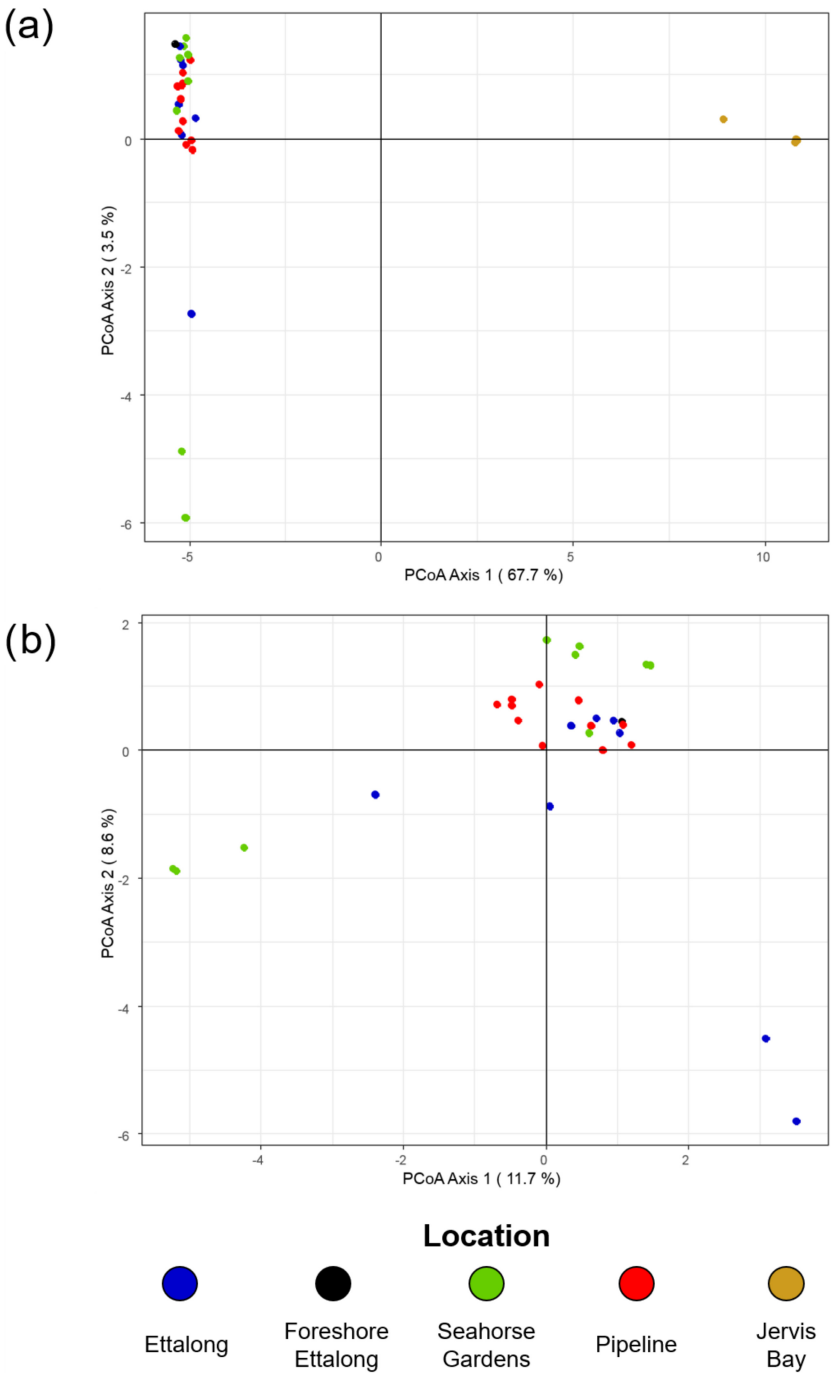

Fig. 3. Genetic similarity of *Dendronephthya australis* colonies from (a) all locations and (b) Port Stephens and Brisbane Water locations as indicated by principal coordinate analysis.

closely related species often share sequence variants (Lee & Song 2000; McFadden & Hutchinson 2004). Issues arise when using single-locus molecular data to validate a species and caution should be taken for the colonies from Jervis Bay in particular, which could represent a separate species. The lack of admixture, very high F_{ST} values and slight differences in ecology (occurring on hard substrata rather than soft sediments) suggest that the Jervis Bay colonies may be reproductively isolated from their more northern counterparts.

This is the first molecular study on *D. australis*, which had previously been identified *via* morphological characteristics (Verseveldt & Alderslade 1982). Accurate family-level taxonomy for soft corals is challenging without a molecular approach because of their relatively simple morphological features and lack of defining characteristics (McFadden *et al.* 2009). Such molecular identification is essential because analyses are increasingly showing that traditional morphological characteristics that have previously been considered pivotal in defining coral

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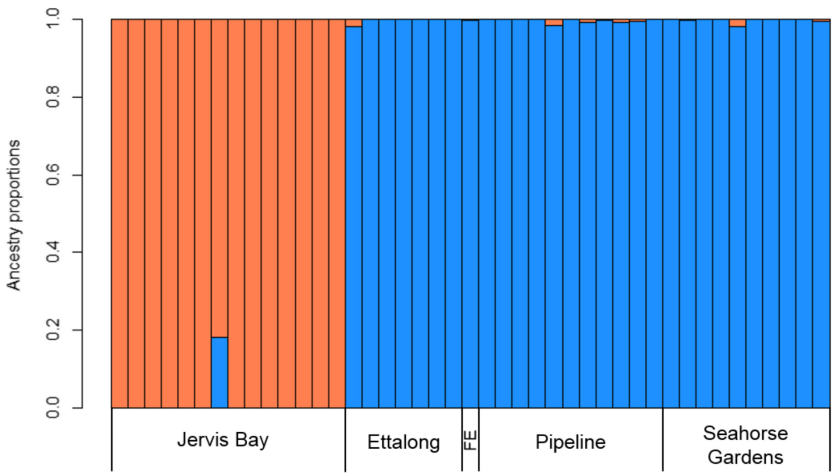

Fig. 4. Admixture plot of calculated ancestry proportions for cauliflower soft coral (*Dendronephthya australis*) colonies from each of the sampled sites based on two ancestral populations ($K = 2$). Each bar represents a single *D. australis* colony. FE, Foreshore Ettalong.

genera may be less important than other traits, thus refining the soft coral phylogeny and taxonomy (McFadden *et al.* 2006). Alignment between morphological and molecular analyses for *D. australis* found in this study simplifies identification of the species and gives confidence in the use of morphological characteristics in future identification for this species in field studies.

Genetic differentiation among sites was evident, particularly between the northern (Seahorse Gardens) and southern (Jervis Bay) sites. Both Seahorse Gardens and Jervis Bay displayed greater genetic divergence with each other and to the remaining sites. Relatively low levels of gene flow were inferred between Jervis Bay colonies and the more northern counterparts. The genetic distinctiveness and low diversity of the Jervis Bay location suggests that these colonies may have either been founded by few individuals or have been subjected to a genetic bottleneck. The heterozygote excess observed at the Jervis Bay site can be indicative of a recent genetic bottleneck because allelic richness is expected to decrease more rapidly than expected heterozygosity under mutation-drift equilibrium (Cornuet & Luikart 1996). The proportion of admixed individual colonies was highest at Pipeline, followed by Ettalong, with Seahorse Gardens having the lowest proportion of admixed individuals. This pattern accords with the pattern of pairwise F_{ST}, with Seahorse Gardens being the most genetically diverged site, implying greater isolation (see N_m; Fig. 5b). Not much can be gleaned from the Foreshore Ettalong site due to the low sample size, but it appears to display a similar admixture pattern to colonies at Ettalong.

The distinctness of the proportions of admixed individuals at sites in this study is surprising when the distance between sites is considered. The maximum distance between the five sites is only ~300 km (Pipeline and Jervis Bay), and the two sites in Port Stephens and Brisbane Water are very close (800 and 100 m apart respectively). While soft corals encompass a variety of sexual reproductive strategies (Kahng *et al.* 2011), the differences observed in our study suggests a potential lack of dispersal of progeny akin to brooders in *D. australis*. Brooding corals release negatively buoyant planulae that typically disperse close to the parent colony (Harrison & Wallace 1990). The planulae are usually more mature than those of broadcast spawners at the time of release (Kahng *et al.* 2011) and archetypally have short competency periods (Farrant 1986), enabling faster settlement. Such parameters usually lead to limited dispersal over time (McEdward 1995) and finer-scale population structure.

No published data exist on the reproduction or maturity status of *D. australis*. The few studies that have assessed reproduction and larval development of species from this family show that most species are gonochoric brooders, with some species brooding internally (e.g. *Litophyton arboretum*, Benayahu *et al.* 1992) and others displaying external brooding (e.g. *Capnella gaboensis*, Farrant 1986). Moreover, prolonged gametogenesis and brooding episodes are typical of temperate soft corals from other families (Benayahu 1991, Ben-David-Zaslow & Benayahu 1998). It is thus highly probable that *D. australis* does not exhibit a discrete and synchronous spawning period, the lack of which as a general reproductive strategy is considered to decrease fertilisation success (Harrison *et al.* 1984, McEdward 1995).

Fertilisation success may also be limited by the numbers of females at each site if *D. australis* is gonochoristic. Many brooding soft coral species that are gonochoristic, including others in the family Nephtheidae, exhibit uneven male to female ratios with a bias toward males (Zeevi Ben-Yosef & Benayahu 1999, Schleyer *et al.* 2004, Hwang & Song 2007). If *D. australis* populations at our sampled sites contain substantially less females than

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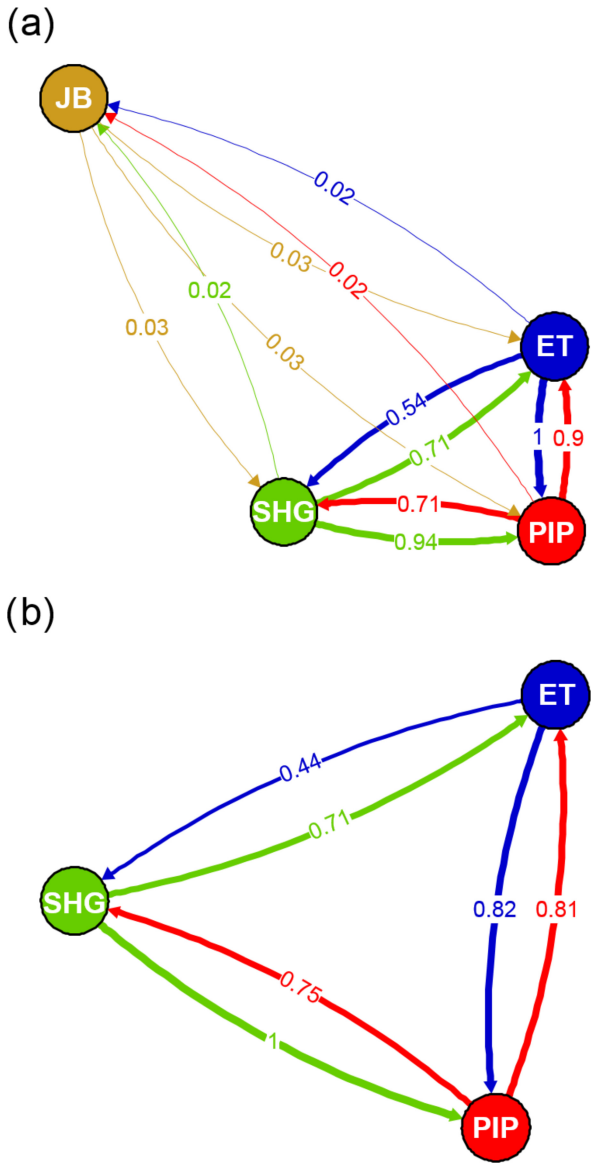

Fig. 5. Relative migration networks of the sampled *Dendronephthya australis* locations using (a) all populations and (b) Port Stephens and Brisbane Water datasets. Migration rates were calculated using Alcala's N_m. The thickness of the arrows is proportional to the rate of migration between each pair of locations. ET, Ettalong (blue); JB, Jervis Bay (gold); PIP, Pipeline (red); SHG, Seahorse Gardens (green).

males, these corals may be more threatened than indicated by mere numbers of colonies. More research is urgently needed to assess the reproductive strategy and biology of *D. australis* because this knowledge is essential to manage and conserve existing populations.

Dendronephthya australis predominantly occurs in areas of high current (Davis *et al.* 2016). Increased water flow associated with the current provides food in the form of phytoplankton, zooplankton and particulate organic matter, which are known food sources for this soft coral (Corry *et al.* 2018). These currents also have the potential to assist in reproduction through increasing dispersal distance of planulae and/or propagules (Werner *et al.* 2007; Álvarez-Noriega *et al.* 2020). Such dispersal does not appear to be the main driver of population structure, however, as modelling suggests the species could have much wider occurrence than its current locations given available suitable habitat (Poulos *et al.* 2015; Larkin *et al.* 2021a). However, recorded evidence of increased colonisation or spread to new areas has to date been limited. The influence of habitat substrate, depth, food availability and water quality on dispersal and subsequent recruitment success is unknown.

Being reliant on specific abiotic and biotic factors makes *D. australis* very susceptible to environmental changes. The species is currently experiencing significant declines across its range, hence its recent listing status of endangered (FSC, 2021), with declines of approximately 70% in the Port Stephens population over the past decade, largely linked to increased sand movement within the estuary (Larkin *et al.* 2021a). Decreased boating and dredging activity near established colonies could be considered to further protect this unique species, especially in locations like Brisbane Water where colonies occur in narrow and shallow boating lanes traversed by large (>10 m) vessels.

If the threats to *D. australis* are not resolved, then there is a very high likelihood that this species will experience localised extinctions. As such, intervention may be required to ensure the ongoing survival of this species, such as relocation of colonies that are at risk, and development of artificial grow-out methods for transplanting individuals back into the wild to

Table 3. Pairwise F_{ST} values (95% CIs) (lower matrix) and estimated number of migrants (N_m) (upper matrix) for *Dendronephthya australis* colonies sampled at Port Stephens and Brisbane Water

	PIP	ET	SHG	FE
PIP	-	17.36	15.78	N/A
ET	**0.0142** (0.005, 0.024)	-	6.23	N/A
SHG	**0.0156** (0.006, 0.025)	**0.0386** (0.026, 0.052)	-	5.44
FE	−0.0191 (−0.058, 0.017)	−0.0134 (−0.059, 0.030)	**0.0439** (0.000, 0.088)	-

Significant pairwise F_{ST} values are indicated in bold (i.e. 95% CI did not contain zero). Negative F_{ST} values were not used in the calculation of N_m as these are equivalent to an F_{ST} of zero.

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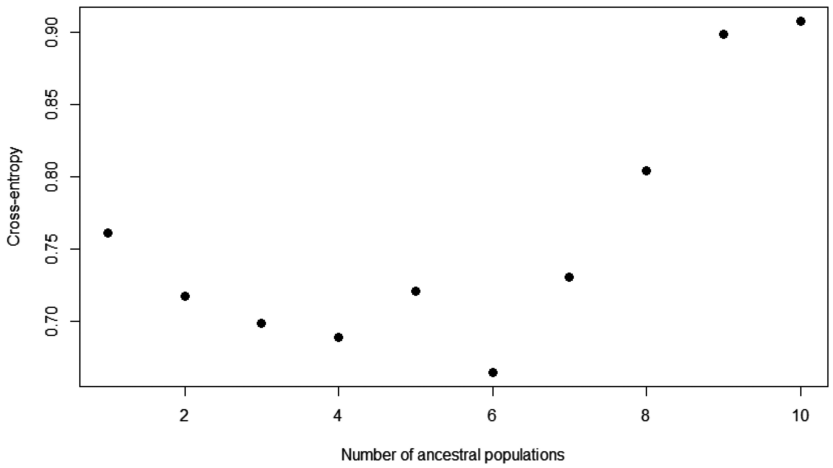

Fig. 6. Plot of the cross-entropy criteria for the number of ancestral populations (*K*) in the Port Stephens and Brisbane Water dataset. Cross-entropy criteria were calculated with the snmf function in the LEA R package.

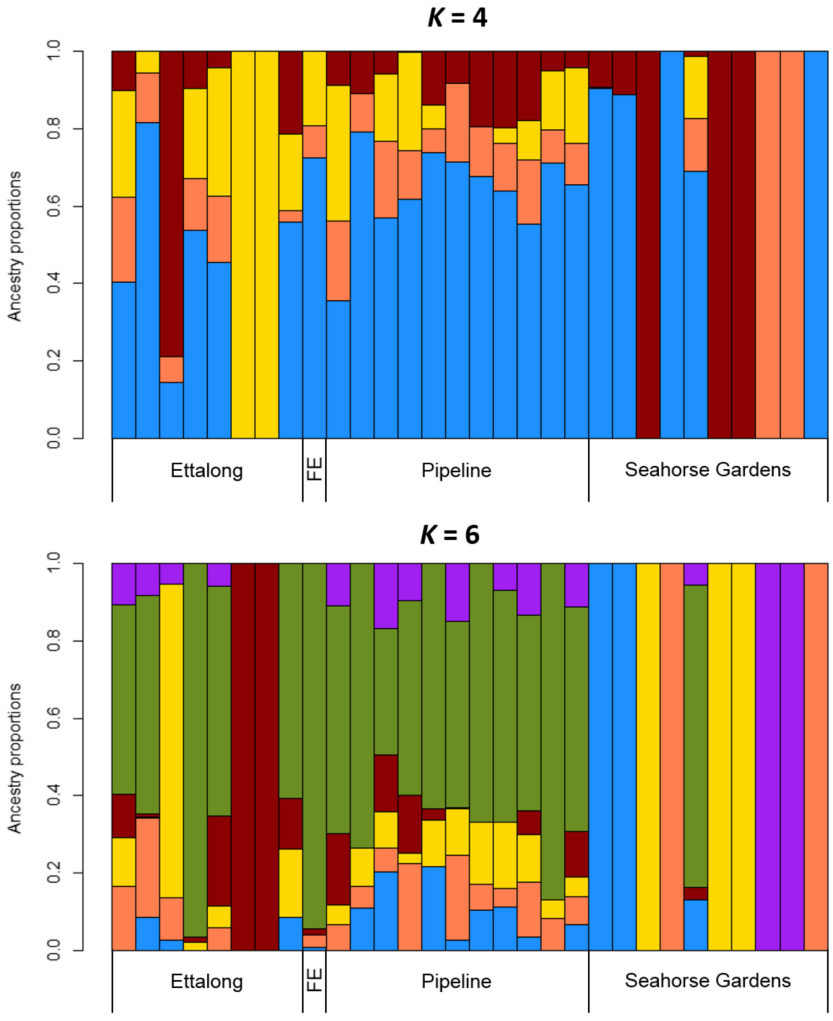

Fig. 7. Admixture plots of the calculated ancestry proportions for the colonies sampled at Port Stephens [Pipeline and Seahorse Gardens] and Brisbane Water [Ettalong and Foreshore Ettalong (FE)] based on different numbers of ancestral populations ($K = 4$ and $K = 6$). Each bar represents a single cauliflower soft coral (*Dendronephthya australis*) colony.

help local populations recover. For this activity, our data on genetic diversity will be useful to ensure appropriate and representative transplantations. Due to the genetic and ecological uniqueness of the Jervis Bay colonies, however, it would be prudent to conduct further research into the population before approving any management strategies such as translocations that may risk this uniqueness.

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AUTHOR CONTRIBUTIONS

Jane Eden Williamson: Conceptualization (equal); data curation (equal); funding acquisition (equal); investigation (equal); project administration (equal); resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Michael R Gillings:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); methodology (equal); resources (equal); supervision (equal); writing – review and editing (equal). **Ryan J Nevatte:** Formal analysis (equal); investigation (equal); methodology (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **David Harasti:** Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Vincent Raoult:** Data curation (equal); investigation (equal); writing – review and editing (equal). **Timothy M Ghaly:** Formal analysis (equal); investigation (equal); methodology (equal); visualization (equal); writing – review and editing (equal). **Adam J Stow:** Methodology (equal); software (equal); supervision (equal); visualization (equal); writing – review and editing (equal). **Timothy M Smith:** Data curation (equal); writing – review and editing (equal). **Troy F Gaston:** Conceptualization (equal); data curation (equal); funding acquisition (equal); resources (equal); visualization (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

There is no conflict of interest.

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