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Contrasting population structures of three *Pristis* sawfishes with different patterns of habitat use

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Abstract. This research demonstrates how population structure differs in elasmobranchs with different patterns of habitat use. Population structure was assessed using data at microsatellite loci in three species of *Pristis* sawfishes in northern Australian waters. Statistically significant population structure was found in each of *P. clavata* ($F_{ST} = 0.021$, $F'_{ST} = 0.151$, $P < 0.001$) and *P. zijsron* ($F_{ST} = 0.026$, $F'_{ST} = 0.130$, $P < 0.001$), which spend their entire life in marine waters. In contrast, there was no evidence of significant population structure in *P. pristis*, which uses freshwater rivers as juveniles and marine waters as adults ($F_{ST} = 0.004$, $F'_{ST} = 0.029$, $P = 0.210$). When combined with the results of mtDNA analyses from a previous study, the results suggested that dispersal in *P. pristis* is male-biased, whereas both male and female gene flow are restricted at large spatial scales in each of *P. clavata* and *P. zijsron* in Australian waters. The present study has provided the first evidence of sex-biased dispersal in a sawfish.

Additional keywords: conservation genetics, elasmobranch, sex-biased dispersal.

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Introduction

Recent studies into the population structures of elasmobranchs have revealed that many species have matrilineal population structure, although the spatial scales over which structuring occurs differ among species and niches, e.g. at larger spatial scales for pelagic species compared to coastal and or benthic species (e.g. Pardini *et al.* 2001; Keeney *et al.* 2005; Dudgeon *et al.* 2009; Karl *et al.* 2012). The proposed explanation for matrilineal population structure in elasmobranchs is philopatric behaviour of females to parturition sites (e.g. Feldheim *et al.* 2014; Chapman *et al.* 2015). Some species have been shown to exhibit significant structuring in both mtDNA and nuclear (nDNA) markers over broad spatial scales, suggesting that both males and females exhibit regional philopatry (e.g. Lewallen *et al.* 2007; Blower *et al.* 2012; Chapman *et al.* 2015). In contrast, studies of other species have revealed significant structuring in mtDNA, but not nDNA, markers, suggesting that females are philopatric whereas males have wider dispersal (Portnoy *et al.* 2010; Karl *et al.* 2011; Daly-Engel *et al.* 2012). In the context of the present study, dispersal refers to gene flow; the movements of individuals may cover a much larger area than their effective dispersal range. The primary explanation for male-biased dispersal in elasmobranchs is that females do not disperse because of their need to find a suitable parturition site, which may be challenging in an unfamiliar environment (Chapman *et al.* 2015), creating a bias in dispersal. No clear pattern(s) has emerged in terms of which species or niches are more likely to be associated with male-biased dispersal *v.* regional philopatry in both sexes, partially because not all genetic studies separately account for male and female gene flow. This is complicated by other factors such as physical barriers to dispersal and biogeography when comparisons of population structures are made between elasmobranchs from different geographic regions. Few studies have investigated the population structures of closely related elasmobranchs that differ in only one or two critical aspects of their life history, but are otherwise similar; however, such studies could potentially reveal important patterns in population structure.

The present study compares the population structures of three sawfishes in northern Australian waters with different patterns of habitat use, the dwarf sawfish (*Pristis clavata*), the green sawfish (*P. zijsron*), and the largetooth sawfish (*P. pristis*) (formerly the freshwater sawfish, *P. microdon*, in the Indo-West Pacific). *Pristis pristis* has a unique life history when compared

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with *P. clavata* and *P. zijsron*; juveniles utilise freshwater rivers, whereas adults use marine waters (Thorburn *et al.* 2007; Whitty *et al.* 2009). By contrast, *P. clavata* and *P. zijsron* are entirely marine, with juveniles utilising inshore waters and mangrove areas (Peverell 2005; Morgan *et al.* 2015). This critical difference in the habitat use of juveniles (i.e. freshwater rivers *v.* inshore marine waters and mangroves) provides a unique opportunity to investigate whether population structures differ among closely related, sympatric rays with different habitat requirements as juveniles. In fact, a previous study by Phillips *et al.* (2011) found that, although all three species exhibited matrilineal structuring in northern Australian waters, the amount of structure was much higher in *P. pristis* (Phillips *et al.* 2011). The study by Phillips *et al.* (2011) employed a single mtDNA marker and, therefore, was limited by a lack of information on gene flow in the male component of the population. The current study uses microsatellite loci to assess population structures in these three *Pristis* species, providing an account of both male and female gene flow. The results of the current study will be combined with those of the mtDNA data of Phillips *et al.* (2011), to investigate whether *Pristis* sawfishes have male-biased dispersal at large spatial scales in northern Australian waters and whether population structure varies in species with different patterns of habitat use.

Materials and methods

Sampling and sample pooling

Genetic data were generated from tissue biopsies (preserved in 100% ethanol or 20% dimethyl sulfoxide saturated with NaCl) or skin taken from dry rostra from 69 individuals of *P. clavata*, 48 individuals of *P. zijsron* and 112 individuals of *P. pristis* from northern Australian waters, as described in Phillips *et al.* (2011). The majority of samples for each species were taken from juveniles and, in the case of *P. pristis*, samples were collected from known nursery areas. Few nursery sites have been confirmed for *P. clavata* and *P. zijsron* and pupping may be widespread along the Australian coast (Morgan *et al.* 2011, 2015). Microsatellite data were generated for samples of *P. clavata* collected from sites on the western coast ($n = 34$), northern coast ($n = 10$) and the Gulf of Carpentaria ($n = 25$), and for samples of *P. zijsron* from sites on the western coast ($n = 24$), the Gulf of Carpentaria ($n = 18$) and the eastern coast ($n = 6$). For *P. pristis*, data were generated for samples from the western coast ($n = 36$), the northern coast ($n = 8$) and the Gulf of Carpentaria ($n = 68$). Samples from different sites within a single geographic region were pooled for analysis for each species because sample sizes at each site (outside of the western coast) were generally fewer than 10 (Phillips *et al.* 2011; Table S1, Fig. S1, available as Supplementary material).

Genetic methods

Total genomic DNA was extracted from ~5 mg of tissue by using a Masterpure DNA extraction kit (Epicentre Technologies, Sydney, NSW, Australia), according to the manufacturer's protocol in the case of the preserved tissue and following the protocol of Phillips *et al.* (2009) in the case of the rostral tissue. Polymerase chain reaction (PCR) was used to amplify alleles at seven microsatellite loci in *P. pristis* and eight loci in each of

Table 1. Conditions of polymerase chain reaction (PCR) and summary statistics for each locus in *Pristis clavata, P. zijsron* and *P. pristis*

T_a, annealing temperature in the PCR cycling conditions; a range indicates that touch-down PCR was employed; *k*, total number of alleles; *A*, average number of alleles across all assemblages; H_E, average expected heterozygosity; H_O, average observed heterozygosity

Species and locus	Primer (µM)	T_a (number of cycles)	*k*	*A*	H_E	H_O
P. clavata						
Ppe4[A]	0.04	60–40°C (50)	16	11.67	0.912	0.867
Ppe5	0.04	60–40°C (50)	23	15.00	0.927	0.956
Ppe69[A]	0.04	55°C (50)	12	6.67	0.601	0.650
Ppe122	0.04	60–40°C (50)	9	8.00	0.837	0.848
Ppe152[A]	0.04	60–40°C (50)	17	10.33	0.890	0.929
Ppe165	0.20	55°C (50)	16	10.00	0.940	0.889
Ppe179[A]	0.04	60–40°C (50)	15	10.00	0.855	0.851
Ppe186	0.04	60–40°C (50)	16	10.33	0.896	0.787
P. zijsron						
Ppe4[A]	0.20	55°C (35)	14	9.00	0.850	0.904
Ppe88	0.08	55°C (35)	29	15.33	0.930	0.978
Ppe152[A]	0.04	60–40°C (40)	6	3.33	0.348	0.412
Ppe165	0.20	55°C (50)	19	12.33	0.916	0.900
Ppe172[A]	0.20	55°C (40)	16	9.33	0.861	0.842
Ppe179[A]	0.04	64–44°C (50)	13	9.00	0.881	0.930
Ppe180[A]	0.20	55°C (40)	18	9.33	0.841	0.843
Ppe186	0.04	60–40°C (50)	10	6.00	0.720	0.915
P. pristis						
Ppe4[A]	0.04	60–40°C (50)	14	11.00	0.837	0.833
Ppe5	0.04	60–40°C (50)	33	21.67	0.953	0.949
Ppe122	0.04	60–40°C (50)	7	6.33	0.736	0.726
Ppe167	0.20	55°C (50)	27	16.33	0.923	0.892
Ppe172[A]	0.20	55°C (40)	21	15.33	0.915	0.879
Ppe180[A]	0.04	55°C (50)	20	11.67	0.825	0.705
Ppe186	0.04	63–43°C (50)	24	15.00	0.930	0.889

[A]New microsatellite locus.

P. clavata and *P. zijsron* (Table 1). These loci were obtained from a partial genomic microsatellite library developed by Feldheim *et al.* (2010) for the smalltooth sawfish (*P. pectinata*). Five of the loci were reported by Feldheim *et al.* (2010), one by Fields *et al.* (2015) and the following six loci were developed from the library as a part of the current study: *Ppe4*, *Ppe69*, *Ppe152*, *Ppe172*, *Ppe179* and *Ppe180* (Table 2).

The forward primer for each locus was fluorescently labelled with 6-FAM (Geneworks, Adelaide, SA, Australia), VIC, PET or NED (Applied Biosystems, Melbourne, Vic., Australia). PCR amplification was performed in a reaction mixture containing ~10 ng of DNA template, 1 mM of TAQ buffer with 1.5 mM $MgCl_2$ (Roche, Dee Why, NSW, Australia), 0.1 mM of dNTPs (Promega, Alexandria, Vic.), 0.25 U of *Taq* polymerase (Roche), 0.04, 0.08, or 0.2 µM of each primer and adjusted to a final volume of 15 µL with PCR-grade water (Table 1). The alleles were amplified using either a 50-cycle touchdown approach or a single annealing temperature (Table 1). The protocol for the 50-cycle touchdown PCR consisted of an initial denaturation phase at 94°C for 5 min, followed by 50 cycles, with each cycle consisting of 30 s of denaturation at 94°C, 1 min of annealing at the optimised starting temperature (Table 1) with a 0.4°C decrease in each cycle and 30 s of extension at 72°C, followed by a final

Table 2. Characteristics of the newly developed microsatellite loci in *Pristis clavata*, *P. zijsron* and *P. pristis*

Locus	Primer sequence	Repeat motif	Allele size (bp)	GenBank accession number
Ppe4	F: 5′-CCATGAACCCATGAACATTACA-3′ R: 5′-AAGGCATGAAATTACTGCAAA-3′	$(TATC)_{33}TAATC(TATC)_{21}$	118–191	KU562844
Ppe69	F: 5′-GAGAGAACGCGAGCCATAGT-3′ R: 5′-CCCTATTTATCTATCTGTCTTTC-3′	$(TGGA)_{17}$	196–232	KU562845
Ppe152	F: 5′-TGCATCATTTCCAGAAGTACG-3′ R: 5′-TGACCTCGCCTGGAGTAGA-3′	$(TAGA)_{39}$	177–249	KU562846
Ppe172	F: 5′-AGCATCAGTCAGCAGGACATT-3′ R: 5′-CGTTTATGTTTCCAATATGCAC-3′	$(TC)_{12}(AC)_{11}$	150–254	KU562847
Ppe179	F: 5′-CAGCAACATCCAAATCCTGA-3′ R: 5′-TCCATGTACCTGTCCAAATG-3′	$(TAGA)_{23}$	186–250	KU562848
Ppe180	F: 5′-TAATCGGGCGAATAGATTGA-3′ R: 5′-TTTGGGCTTTCAACTGCTG-3′	$(TAGA)_{25}(CAGA)_{16}$	247–403	KU562849

extension at 72°C for 20 min. The PCR protocol for the single annealing temperature consisted of an initial denaturation phase at 94°C for 5 min, followed by 35–50 cycles, with each cycle consisting of 30 s of denaturation at 94°C, 1 min of annealing at the optimised temperature (Table 1) and 30 s of extension at 72°C, followed by a final extension at 72°C for 20 min.

Each PCR product (1 or 2 µL) was added to 15 µL of (Hi-Di) formamide (Applied Biosystems) and 0.1 µL of LIZ-600 size standard (GeneScan, Applied Biosystems) and run on an Applied Biosystems 3730 DNA Analyzer. The sizes of the alleles at each locus were automatically scored using the software GENEMARKER v.1.8 (SoftGenetics Inc., Melbourne) and manually checked for error. Two positive controls were included in all plates to ensure internal consistency in the scoring of alleles and the alleles in ~40% of the samples were blindly scored by two independent researchers.

Data analyses

Linkage disequilibrium and deviation from Hardy–Weinberg equilibrium (HWE) were tested for each of *P. clavata*, *P. zijsron* and *P. pristis* in GENEPOP version 1.2, with a dememorisation number of 10 000, 1000 batches and 10 000 iterations per batch (see Raymond and Rousset 1995) and a Bonferroni correction for multiple tests (Rice 1989). Micro-Checker v.2.2.3 (Van Oosterhout *et al.* 2004) was also used to check for null alleles and genotyping errors for all loci in each species.

Population structure

Population structure was assessed using F'_{ST}, D_{EST} and G'_{ST} overall and between reasonably well sampled regions (i.e. the western coast and the Gulf of Carpentaria). Weir and Cockerham's (1984) F_{ST} was used to assess the extent of genetic differentiation overall and between regional samples for each species in GENEPOP version 4.2 (Raymond and Rousset 1995). Exact tests with 10 000 steps in the Markov chain were used to test the statistical significance of population differentiation in GENEPOP version 4.2 (Raymond and Rousset 1995). The estimates of F_{ST} for each species were standardised (F'_{ST}) according to Meirmans (2006), where the raw estimate was divided by the maximum possible value of F_{ST}. RecodeData version 0.1 (Meirmans 2006) was used to recode the raw F_{ST} values, so as to calculate the maximum values of F_{ST} in FSTAT version 2.9.3.2 (J. Goudet, see http://www2.unil.ch/popgen/softwares/fstat.htm, accessed 8 May 2014). The harmonic mean of Jost's (2008) D_{EST} across all loci was calculated overall and between pairwise samples for each species in SMOGD version 1.2.5 (Crawford 2010). G'_{ST} was determined overall and between pairwise samples for each species in GENODIVE (Meirmans and Van Tienderen 2004) using the method of Meirmans and Hedrick (2011).

Bayesian multi-locus clustering was used to estimate the number of populations of each of *P. clavata*, *P. zijsron* and *P. pristis* in northern Australian waters, as implemented in STRUCTURE ver. 2.3.3 (Pritchard *et al.* 2000). The Bayesian approach distributes the individuals into a given number of populations (*K*) based on the allele frequencies and estimates the posterior probability of the data for each value of *K*. However, because the probability of the data does not always provide an accurate estimate of the number of populations, the second-order rate of change of the probability of the data between successive *K* values (ΔK), was also used when more than one population was likely to be present (see Evanno *et al.* 2005). Simulations were run with the no admixture and admixture models (see François and Durand 2010) and correlated allele frequencies (see Hubisz *et al.* 2009). Simulations were run with 200 000 steps burn-in and 500 000 steps in the Markov chain. The number of populations (*K*) was set from 1 to 10 and the posterior probability of *K* populations was averaged over 10 iterations.

Because it was proposed by Phillips *et al.* (2011) that *Pristis* sawfishes have undergone recent range expansions in northern Australian waters, an analysis of migration-scaled divergence time was conducted to explore the hypothesis that assemblages have diverged; however, not enough time has passed for structure to become apparent in the nDNA because of the characteristics of the markers (see Ballard and Whitlock 2004). Migration-scaled divergence time (*t*) was estimated for the western coast and Gulf of Carpentaria assemblages for each species in IMa2 (Hey and Nielsen 2007). Posterior probability distributions of *t* were generated using the SMM model and the final runs had a minimum of 2 000 000 steps in the Markov Chain Monte Carlo (MCMC) with

the first 1 000 000 steps discarded as burn-in, using only individuals with data for all loci. These settings generated consistent results among three final runs with different seed numbers.

Results

Microsatellite summary statistics

The numbers of homozygotes and heterozygotes at each locus in samples of each of *P. clavata*, *P. zijsron* and *P. pristis* were generally in accordance with those expected under HWE after a Bonferroni correction for multiple tests (see Tables S2, S3 and S4 in the Supplementary material). The exception was *Ppe167* in *P. pristis* for the Gulf of Carpentaria sample (see Table S4 in the Supplementary material). Because the departure from HWE was significant after a Bonferroni correction, all data analyses for *P. pristis* were conducted without *Ppe167*. Micro-Checker did not identify any evidence of errors in genotyping or the presence of null alleles for any loci and there was no evidence of linkage disequilibrium between loci. The sample size from the northern coast for each of *P. clavata* and *P. pristis* was small and comprised primarily of dried rostra and formalin-fixed samples, making amplification of alleles at larger loci difficult.

Levels of polymorphism at microsatellite loci in each of *P. clavata*, *P. zijsron* and *P. pristis* were generally moderate to high. The total number of alleles per locus ranged from 9 to 23 in *P. clavata* (mean = 15.5, s.e. = 1.427), 6 to 29 in *P. zijsron* (mean = 15.6, s.e. = 2.43) and 7 to 33 in *P. pristis* (mean = 19.8, s.e. = 3.61) (Table 1). The expected heterozygosity at each locus was generally moderate to high, ranging from 0.601 to 0.940 in *P. clavata* (average = 0.857, s.e. = 0.039), from 0.348 to 0.930 in *P. zijsron* (average = 0.793, s.e. = 0.068) and from 0.736 to 0.953 (average = 0.866, s.e. = 0.033) in *P. pristis* (Table 1).

Population structure

Statistically significant population structure was found in each of *P. clavata* and *P. zijsron* in Australian waters. In contrast, there was no evidence of significant population structure in *P. pristis* in these waters. The overall values of F'_{ST}, D_{EST} and G'_{ST} for *P. clavata* were 0.151 ($F_{ST} = 0.021$), 0.012 and 0.102 ($P = 0.012$) respectively, with a highly significant ($P < 0.001$) exact test. The overall values of F'_{ST}, D_{EST} and G'_{ST} for *P. zijsron* were 0.130 ($F_{ST} = 0.026$), 0.114 and 0.189 ($P = 0.001$) respectively, also with a highly significant ($P < 0.001$) exact test. In contrast, the values of overall F'_{ST}, D_{EST} and G'_{ST} in *P. pristis* were 0.029 ($F_{ST} = 0.004$), 0.003 and 0.060 ($P = 0.072$) respectively, and the result of the overall exact test was not statistically significant ($P = 0.210$).

The Bayesian clustering method found that there was more than one population of *P. clavata* across northern Australia, although there was some discrepancy in the number of populations between the two different models. Using the admixture model, K and $\Delta K = 2$ had the highest probability, but when using the no admixture model, K and $\Delta K = 3$ had the highest probability (Fig. 1*a*). The pairwise comparisons of F'_{ST} and D_{EST} suggested that the assemblages of *P. clavata* from the western coast and the Gulf of Carpentaria are genetically distinct (Table 3). The Bayesian clustering method found that there were three assemblages of *P. zijsron* across northern Australia, for both K and ΔK using the admixture and no admixture models (Fig. 1*b*). Pairwise comparisons suggested that the assemblages of *P. zijsron* from the western coast and the Gulf of Carpentaria are genetically distinct (Table 3).

In contrast to the results for *P. clavata* and *P. zijsron*, the Bayesian clustering method found that there was most likely a 'single population' of *P. pristis* because $K = 1$ had the highest probability with a negligible standard error (Fig. 1*c*). The pairwise values of F'_{ST}, D_{EST}, G'_{ST} and exact tests for the comparison between the western coast and the Gulf of Carpentaria further supported this finding, providing no evidence of population structure in Australian waters (Table 3). Furthermore, the results of the analysis of migration-scaled divergence, on the basis of microsatellite data, indicated no population divergence in *P. pristis* between the western coast and the Gulf of Carpentaria (Fig. 2*c*). In contrast, population divergence was evident in each of *P. clavata* and *P. zijsron* (Fig. 2*a*, *b*).

Discussion

The present study has demonstrated that population structure differs in Indo-West Pacific *Pristis* sawfishes with contrasting patterns of habitat use. Each of *P. clavata* and *P. zijsron*, which are marine for their entire life cycle, has significant genetic structure in northern Australian waters. In contrast, there was no evidence of significant genetic structure in *P. pristis*, which has strong habitat partitioning with freshwater juveniles and marine adults. These overall results are considered fairly robust, given that the sampling design (e.g. the majority of samples came from the western coast and the Gulf of Carpentaria) and number of microsatellite loci for each species was fairly similar, yet very different overall results were obtained for *P. pristis*. However, it should be noted that the sample size for *P. zijsron* in the Gulf of Carpentaria was small.

Although it cannot be ruled out that the lack of population structure in *P. pristis* was due to low statistical power because only six microsatellite loci were ultimately used, the sample sizes from the western coast and the Gulf of Carpentaria should have been sufficient (e.g. $n > 30$) to detect modest amounts of genetic structure, if present. In addition, all of the loci used in *P. pristis* were also used in at least one of the other study species, but a very different pattern emerged in the results. An analysis of migration-scaled divergence suggested that assemblages of *P. pristis* from the western coast and the Gulf of Carpentaria have not diverged, whereas those of *P. clavata* and *P. zijsron* have; i.e. it does not appear to be a case of recent divergence in *P. pristis*.

Although the microsatellite markers indicated the presence of regional divergence in both *P. clavata* and *P. zijsron* in northern Australian waters, it is not possible to elucidate the number and boundaries of the population for either species. This is because the sample sizes for some regions (e.g. northern coast) and for sites within regions were small. When evaluating the adequacy of the sampling of these two species, and also of *P. pristis*, it is important to remember that *Pristis* sawfishes are endangered or critically endangered (depending on the species) and inhabit remote locations in Australia, which makes it difficult to obtain samples. This difficulty is demonstrated by the fact that the samples used in the present study represent ~10 years of sampling effort (see Peverell 2005; Phillips *et al.* 2011).

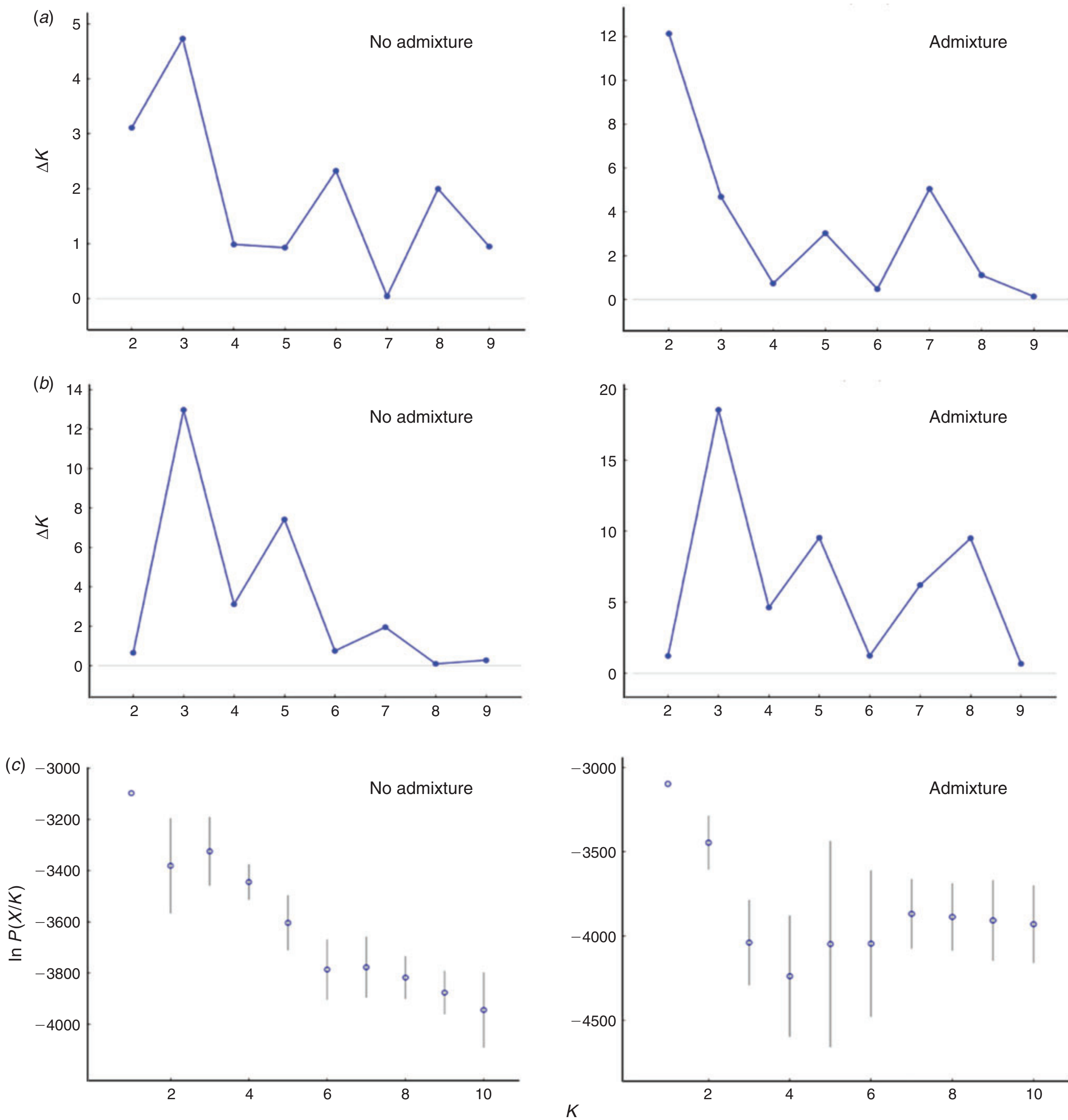

Fig. 1. Estimates of the number of populations (*K*) of (*a*) *Pristis clavata*, (*b*) *P. zijsron* and (*c*) *P. pristis* across northern Australia on the basis of Bayesian clustering of multi-locus genotypes with the no admixture and admixture models. ln P(X/K) is the natural log of the probability of *K* populations given the data (*X*), averaged over 10 iterations for each value of *K*. Error bars indicate the standard deviations from the average value of *K*. Δ*K* is the second-order rate of change between successive values of *K*. The model used in the analysis is indicated in the upper right corner of each graph.

When the results of the present study are combined with those from Phillips *et al.* (2011; i.e. matrilineal structuring in each *Pristis* species that was greatest in *P. pristis*), this suggests that dispersal is male-biased in *P. pristis*, whereas both male and female dispersal in each of *P. clavata* and *P. zijsron* is restricted, at least among regions in northern Australian waters. The present study has provided the first evidence of male-biased dispersal in sawfishes, although this type of dispersal has been found in several sharks (Schrey and Heist 2003; Portnoy *et al.* 2010; Daly-Engel *et al.* 2012). Male *P. pristis* could be migrating directly between geographic regions (e.g. the western coast and the Gulf of Carpentaria) to mate, or there could be a single

breeding ground for the (sampled) Australian assemblages. The movements of males and females could be similar, but the philopatric behaviour of the females to parturition sites would create a bias in effective dispersal and matrilineal structuring across northern Australia (e.g. Encalada *et al.* 1996).

In each of *P. clavata* and *P. zijsron*, it is most likely that it is the reproductive behaviour of males and females that is limiting gene flow over large spatial scales in northern Australian waters rather than other factors that are attributed to creating stock structure in marine species, such as limited dispersal capabilities or physical barriers to dispersal (e.g. Palumbi 1996; Duncan *et al.* 2006; Le Port and Lavery 2012). The different pattern of dispersal in *P. clavata* and *P. zijsron* compared with *P. pristis* is unlikely to be due to species differences in size or vagility. Both *P. pristis* and *P. zijsron* grow up to 7 m in length and, although not as large, *P. clavata* still grows up to 5 m in length (Peverell 2005). The difference is also unlikely to be due to physical barriers to dispersal because male *P. pristis* appear to be capable of moving over large spatial scales in northern Australian waters, as do some other elasmobranchs in northern Australian waters (see Ovenden *et al.* 2009). Regional philopatric tendencies in both sexes have been observed in some other elasmobranchs, such as the Nurse Shark, *Ginglymostoma cirratum* (e.g. Karl *et al.* 2012).

The finding of contrasting population structures in *Pristis* sawfishes with different patterns of habitat use as juveniles provides new insight into the evolution of population structure in elasmobranchs. It would suggest that the reliance of *P. pristis* on freshwater rivers as nursery areas might have played some role in the evolution of male-biased dispersal in this species. Hypotheses for the evolution of sex-biased dispersal in terrestrial species include inbreeding avoidance (see Pusey 1987; Perrin and Mazalov 2000), local resource competition (see Clark 1978; Greenwood 1980), local mate competition (see Hamilton 1967; Dobson 1982), and cooperative behaviour among kin and local resource-enhancement hypotheses (see Perrin and Lehmann 2001; Lawson Handley and Perrin 2007). It has been suggested that male-biased dispersal in elasmobranchs is the consequence of female philopatry to parturition sites, which is an adaptation to ensure that offspring are born at an appropriate time and location to increase the chances of the survival of the pups (Schrey and Heist 2003; Portnoy and Heist 2012). Although this explanation might apply over small spatial scales, it does not fully explain why *P. pristis* has male-biased dispersal over larger spatial scales whereas *P. clavata* and *P. zijsron* exhibit regional philopatry in both sexes.

The precise force(s) driving the occurrence of male-biased dispersal in *P. pristis* is unknown, but may be associated with biogeography or a selective response to the highly localised female philopatry in this species (see Phillips *et al.* 2011). The coastline of north-eastern Australia shifted dramatically during the glacial cycling of the Pleistocene, with the repeated emergence of the Torres Strait land bridge at low sea levels (in the area that is now the Gulf of Carpentaria; see Voris 2000). This cycling from the aquatic Gulf of Carpentaria environment to the terrestrial land bridge meant that habitat availability for each of *P. clavata* and *P. zijsron*, especially for juveniles utilising inshore waters and mangrove areas, fluctuated substantially. In contrast, habitat availability for juvenile *P. pristis* during the Pleistocene may have been more stable (than that for *P. clavata* and *P. zijsron*) because extensive freshwater habitats remained throughout this region, even when the Torres Strait land bridge was present at low sea levels (see Voris 2000). The greater availability and stability of nursery habitat for *P. pristis* in the Gulf of Carpentaria region over evolutionary time may have allowed more time for male-biased dispersal to evolve. The risk to straying males in each of *P. clavata* and *P. zijsron* may have been high in light of the instability of juvenile habitat over time.

The occurrence of male-biased dispersal in *P. pristis*, but not *P. clavata* or *P. zijsron*, could also be a selective response to

Table 3. Pairwise F_{ST}/F'_{ST}, exact test *P*-values, Jost's D_{EST} and G'_{ST} (*P*-value) for microsatellite data between the western coast and Gulf of Carpentaria assemblages of *Pristis clavata*, *P. zijsron* and *P. pristis* in Australian waters

*, F_{ST}/F'_{ST}, G'_{ST} and exact test values that are statistically significant at $P = 0.05$

Species	F_{ST}	F'_{ST}	P	D_{EST}	G'_{ST}
Pristis clavata	0.023*	0.171*	<0.001*	0.106	0.283 (0.006)*
Pristis zijsron	0.012*	0.060*	0.002*	0.018	0.172 (0.001)*
Pristis pristis	0.002	0.011	0.198	0.000	0.010 (0.285)

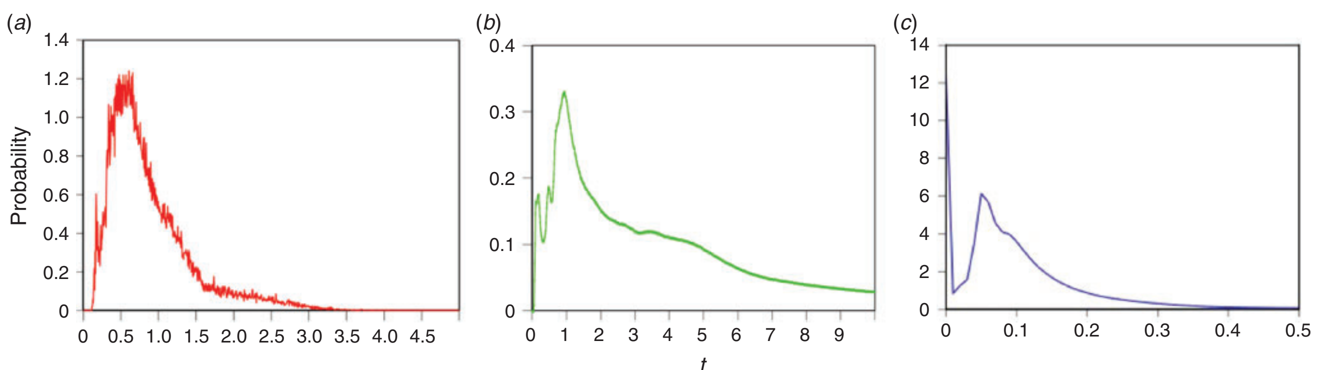

Fig. 2. Posterior probability distributions of mutation-scaled time since divergence (*t*) of the assemblages from the western coast and Gulf of Carpentaria, on the basis of data from microsatellite loci in (*a*) *Pristis clavata*, (*b*) *P. zijsron* and (*c*) *P. pristis*.

stronger and very localised philopatry of female *P. pristis* as a means to avoid inbreeding (see Pusey 1987; Perrin and Mazalov 2000; Phillips *et al.* 2011), especially given the large-scale dispersal of male *P. pristis*. The risk of inbreeding may be high in *P. pristis* if females exhibit very localised philopatry, such as natal philopatry (i.e. Feldheim *et al.* 2014), to freshwater rivers and maternal populations are small in size and fragmented (i.e. a single river or group of nearby rivers; see Cockburn *et al.* 1985; Perrin and Mazalov 2000; Hoarau *et al.* 2005; Feutry *et al.* 2015). The risk of inbreeding in *P. clavata* and *P. zijsron* may be lower as a result of the greater continuity of nursery habitat, which could, theoretically, encourage larger and more continuous assemblages (e.g. isolation by distance) along the coastline. The sandbar shark (*Carcharhinus plumbeus*) is the only other elasmobranch known to have male-biased dispersal at spatial scales similar to *P. pristis* in northern Australian waters (Portnoy *et al.* 2010). However, unlike *P. pristis*, *C. plumbeus* does not utilise freshwater habitats at any stage in its life history. The occurrence of male-biased dispersal in *P. pristis* and *C. plumbeus* could be related to inconspicuous similarities of these two species not shared by *P. clavata* or *P. zijsron*, such as the number or location of breeding sites (i.e. a single site for Australian assemblages) or historic migratory routes related to biogeography.

Additional research into the population structures of closely related species that differ in only one or two critical aspects of their life history are needed to develop a better understanding of the factors that drive the evolution of population structure in elasmobranchs. Studies of the population structures of other elasmobranchs that utilise freshwater environments in northern Australian waters may provide important context for interpreting the basis of male-biased dispersal in *P. pristis*. Future research should attempt to capture the direct movements of adult sawfish through tagging data to determine their residency status in each region (see Chapman *et al.* 2015). In addition, an alternative approach to investigate gene flow with substantially more power should be employed, such as single-nucleotide polymorphisms (SNPs). However, such research would require a large number of SNPs and samples from throughout as much of the geographic range of each species as possible, so as to attempt to overcome the limitations of the current study.

Conservation implications

The *Pristis* sawfishes in Australian waters have been recognised as key assemblages in global conservation efforts for each species (IUCN 2013). Conservation plans for each of *P. clavata* and *P. zijsron* in Australian waters need to consider that philopatric tendencies of males and females increase the risk of localised extirpation (see Leonard 2008). It is also important to note that even if dispersal is effectively philopatric, individuals could be moving large distances from their natal region outside of critical breeding and pupping periods (e.g. Encalada *et al.* 1996; Pratt and Carrier 2001; Bowen *et al.* 2005; Mull *et al.* 2008). Species with wide-ranging male dispersal coupled with female philopatry, such as *P. pristis*, pose a conservation challenge because they are at high risk of extirpation, but what constitutes a 'population' is unclear (e.g. Bowen *et al.* 2005; Sheridan *et al.* 2010). Ideally, the best practice to conserve such a species is to offer continuous protection across all of the habitats they utilise, these being freshwater rivers and marine environments in the case of *P. pristis*. However, in practice, this is often a difficult goal to achieve, particularly in the marine environment because of the large number of agencies, states and countries needed to collectively agree on management practices and enforcement (Musick *et al.* 2000; Crowder *et al.* 2006). For *P. pristis*, the health of freshwater rivers utilised by juveniles should be paramount in conservation efforts (see Phillips *et al.* 2011). Conservation plans for *P. pristis* also require cooperative management (at least) across Australia, taking into account that a decline in the abundance of this species in one region could have an effect on its abundance in other locations via male dispersal (e.g. Sheridan *et al.* 2010).

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