Marine Biodiversity Hub

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Examination of connectivity of hammerhead sharks in northern Australia

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Important Disclaimer

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EXECUTIVE SUMMARY

There is increasing concern about the conservation and sustainable use of hammerhead sharks nationally and globally, with documented declines in many parts of their range. Several hammerhead species have been recently added to international conventions such as the Convention on International Trade in Endangered Species (CITES), and the Convention on the Conservation of Migratory Species of Wild Animals (CMS). Within northern Australia, three species of hammerhead regularly occur: scalloped hammerhead (*Sphyrna lewini*), great hammerhead (*Sphyrna mokarran*) and winghead shark (*Eusphyra blochii*). Commonwealth and State Governments have been involved in processes to assess the conservation status and management of hammerhead sharks in Australian waters, with *S. lewini* listed as 'Conservation Dependent' under the Environment Protection Act in 2018.

A key information gap in managing Australia's hammerhead sharks is whether Australian stocks are shared with neighbouring countries, especially Indonesia and Papua New Guinea, where there is heavy fishing pressure. Hammerhead sharks are known to be highly migratory and the extent of connectivity between countries is relevant to management approaches. This project aimed to explore the connectivity of hammerhead sharks across northern Australia, and with Indonesia and PNG using an integrated, multi-method approach including satellite tagging, genetics, and parasite analysis.

Fourteen hammerhead sharks were tagged with satellite tags including six *S. mokarran* and eight *S. lewini*. This sample included ten males and four females. All tagged individuals were adults, but were small with none greater than 2.8 m total length. All individuals except one survived capture and handling, but only three tags remained attached for the intended tracking duration (180 days) with other tags being scraped off, suffering attachment failure, or failing to report. In general, relatively localised movements were observed from all tagged individuals with the furthest latitudinal distance moved being <250 km, and the longest distance between capture and tag-release locations of 169 km.

Genetic samples were collected from 359 *S. lewini*, 233 *S. mokarran* and 142 *E. blochii* and three types of genetic markers were used: mitochondrial DNA, microsatellites, and single nucleotide polymorphisms (SNPs). Results for *S. lewini* revealed that Indo-Pacific populations (eastern and northern Australia, Papua New Guinea - PNG, Philippines, Taiwan, Fiji) are distinct from those from Western Australia (WA) and the Central Pacific. North-eastern Australia, Indonesia and Papua New Guinea samples indicated high gene flow between NT, Indonesia, the Australian east coast and PNG, but no significant gene flow between Indonesia and WA. Collectively, these samples revealed limited gene flow between Australia-Indonesia-PNG and other regions. Genetic samples from *S. mokarran* were only available from northern Australia and showed no evidence of structuring. Genetic samples from *E. blochii* were available from northern Australia and PNG with results unable to distinguish any pattern beyond panmixia.

Parasite fauna were investigated as biological tags to help identify population structure. For this analysis samples were obtained from the Northern Territory, Queensland, northern NSW

and from Lombok in Indonesia. A total of 266 individuals were examined from northern Australia (57 *S. mokarran* and 209 *S. lewini*) and 27 *S. lewini* from Indonesia. Analysis of parasite assemblages found in *S. mokarran* revealed significantly different assemblages between the NT, QLD, and NSW. Similarly, parasite assemblages of *S. lewini* from Australian waters were significantly different between the NT, QLD and NSW. Comparison between Australian and Indonesian parasite faunas was restricted only to internal parasites, but showed some level of differentiation suggesting limited connectivity. However, for all aspects of this component shark size appeared to affect parasite assemblages with a considerable change in parasites found in animals over 2 m total length.

Synthesis of findings from all three methods for scalloped hammerheads showed there was some evidence to suggest stock structuring and limited movement between Australia, Indonesia, and PNG, but this finding is subject to uncertainty. Uncertainty is driven largely by the failure to encounter large (i.e. > 3 m) individuals which are most likely to move the greatest distances and create connectivity. Tracking and parasite fauna data suggest limited movement for individuals up to ~2.8 m, while genetic data indicate connectivity, but limited gene flow between some areas, specifically between Western Australia and other parts of Australia and Indonesia/PNG. Data generated during this project did not identify any use of Australian Marine Parks and did not provide any new information on biologically important areas for these species.

Four stock structure hypotheses were tested for *S. lewini* against these results, but no single hypothesis was supported (Table 1). The most supported hypothesis was connectivity along continental shelves, with limited connection across deep water. The lack of connectivity between WA and other parts of the study area was surprising, and was not considered in the original stock structure hypotheses (Figure 1). This result will add complexity to the approach to management of this species in Australian waters. The stock structure of *S. mokarran* appeared to be similar to that of *S. lewini* within Australian waters, but connections to WA and Indonesia were not tested.

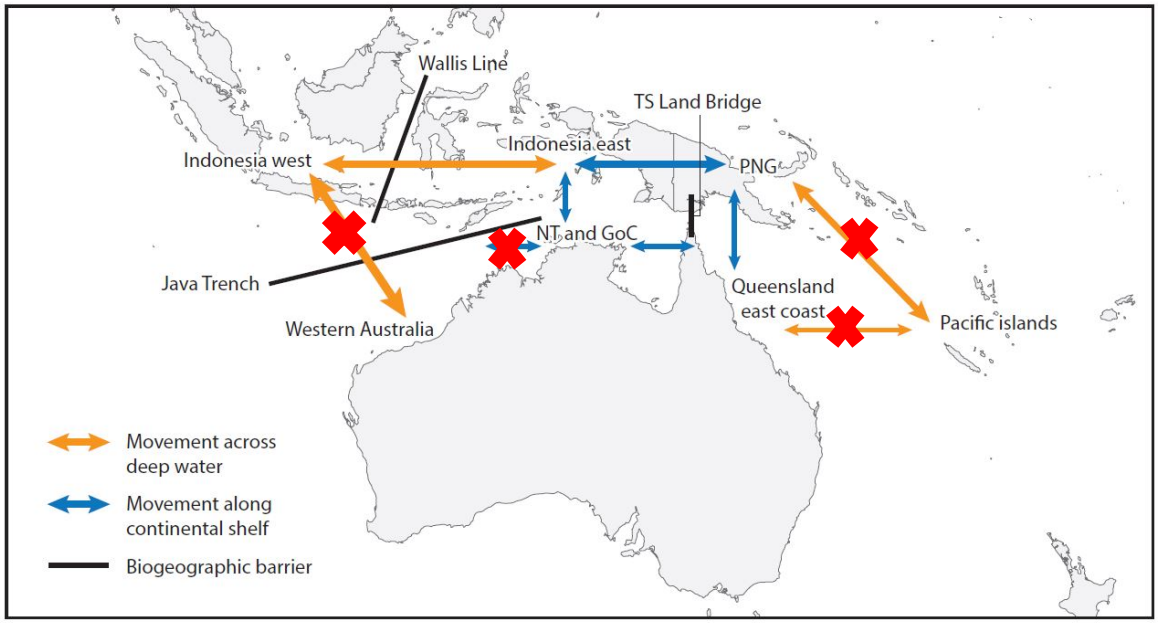
The failure to reliably capture and sample large adults within Australian waters in this study limits the conclusions that can be drawn and indicates that further research is required. The presence of juveniles and sub-adults in coastal areas indicates parturition is occurring locally, but it is unclear where adult and pregnant individuals reside. This information is critical to fully understanding whether the Australian population is sustained by adults resident within or outside Australian jurisdictions. In addition, further sampling in regional neighbouring countries such as Indonesia and Papua New Guinea, which were limited in this project, would help refine our understanding of population connectivity. For example, satellite tagging of large females encountered outside Australia could reveal whether these individuals move to Australia at any stage, especially for the purposes of pupping. Prioritised collection of genetic samples from large hammerheads within the region could help refine current analyses and permit additional genetic studies which may help further define stock structure. Finally, additional research is needed to more fully understand the biology and ecology of great hammerhead and winghead sharks. Future research should use as many samples and methods as possible from as many locations as possible to refine our understanding of

hammerhead populations within and beyond Australia to inform State and Territory, Commonwealth and international cross-jurisdictional management.

Table 1. Support for four stock structure hypotheses for hammerhead sharks based on three different methodological approaches.

Method	Stock structure hypothesis				Comments
	Panmictic across region	Limited movement	Continental shelf movements	East-west Australia stock divide and continental shelf movements	
Scalloped hammerhead					
Satellite tracking	No	Yes	Yes	Yes	No large adults tagged, still a question as to location of adult females in Australian waters
Genetics	No	No	Partial (WA, Pacific differs from rest of region)	Partial (WA differs from rest of Australia)	
Parasites	No	Partial	Yes	Partial	Differences in sizes between areas sampled
Great hammerhead					
Satellite tracking	No	Yes	Yes	Yes	No large adults tagged
Genetics	Partial	No	Yes	No	Best support for Model 3 (continental shelf movements)
Parasites	Not tested	No	Possibly	Not tested	Low sample sizes
Winghead					
Genetics	Partial (Some evidence northern PNG separate)	No	Yes	No	Best support for Model 3 (continental shelf movements)

Figure 1. Revised population connections for scalloped hammerheads based on the results in this report from hypothesised connections Chin et al. (2017).



1. INTRODUCTION

Three species of hammerhead shark occur across northern Australia: great hammerhead (*Sphyrna mokarran*), scalloped hammerhead (*Sphyrna lewini*), and the winghead (*Eusphyra blochii*). In particular, the *S. lewini* is of specific conservation interest to the Commonwealth Government and State and Territory fisheries agencies given its listing as *Conservation Dependent* under the EPBC Act. In addition, *S. lewini* and *S. mokarran* have been added to the Appendices of the Convention on International Trade of Endangered Species (CITES), and the Convention of Conservation of Migratory Species of Wild Animals (CMS). These listings have policy ramifications for how these species are managed in Australian waters.

A key information gap in designing effective management is an adequate understanding of stock structure, movement, and migration of hammerhead sharks across northern Australia, and connectivity to adjacent countries such as Papua New Guinea (PNG) and Indonesia. Preliminary information from genetic studies and fisheries data suggest stocks may be shared across northern Australian jurisdictions and neighbouring countries (Chin et al. 2017), which would require management across jurisdictions. However, more data are required to clarify stock structure and movement patterns to confirm whether hammerhead sharks are moving between Australian states, Indonesia, and PNG, and the frequency of these movements.

This project addressed this knowledge gap by providing information to inform the stock structure and connectivity of hammerhead sharks across northern Australia and with adjacent countries. Three approaches were used: (1) satellite tracking, (2) genetic structure, and (3) parasite fauna studies. This integrated, multiple method approach provides a more robust account of population structure than studies that rely on a single method (Catalano *et al.* 2014; Welch *et al.* 2015). To assist in the design and implementation of this research, a desktop study (i.e. Chin et al. (2017)) collated existing information on hammerheads in northern Australia, and developed a number of hypotheses on stock structure (Figure 2, Table 2) to be tested in this project.

This report provides a summary of the implementation and outcomes of the three approaches and discusses the implications of the results for stock structure and connectivity of the hammerhead sharks in northern Australia. The results of the project should provide managers with greater understanding of how hammerhead sharks are connected, enabling them to develop assessment and management systems related to both domestic and international policy requirements.

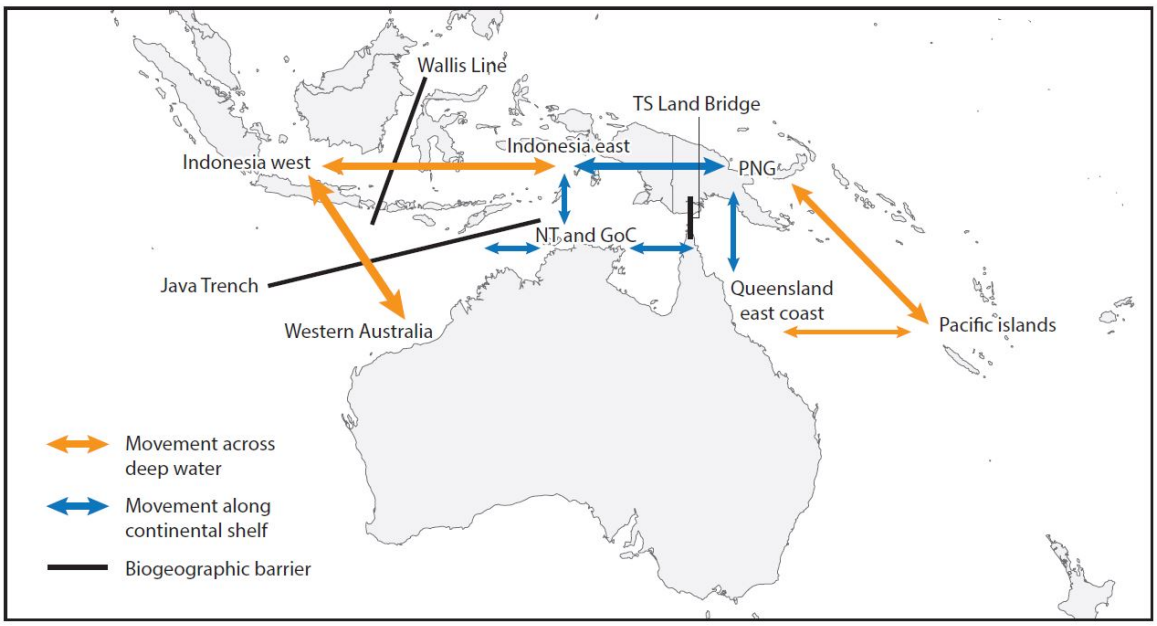


Figure 2: Potential movement and connectivity pathways of hammerhead sharks in northern Australia relative to potential stock structure hypotheses (From Chin et al. (2017)).

Table 2. Description and assessment of conecptual models developed to explain distribution and population structure of *S. lewini* in the Assessment Region from previous research. From Chin *et al.* (2017).

Hypothesis	Description	Current support	Future research results that would support hypothesis
Panmictic population throughout region	Adults move freely through the region; adult females likely to return to natal nursery areas in northern Australia, PNG and Indonesia to give birth	Genetic connection between Australia and Indonesia(Ovenden *et al.* 2009; Ovenden *et al.* 2011); size and sex structure data (Fig. 2); ability to travel across deep water (from other regions)(Kohler *et al.* 1998; Ketchum *et al.* 2014b). **Moderate support**	*Genetic analysis* Tests comparing Australian, Indonesian, PNG and Pacific island samples show no differences with any type of marker (mtDNA, microsatellites, SNPs). *Telemetry and tagging* Tracking results of adults show movements from Australian waters into Indonesian and PNG waters
Limited movement	Adults remain in restricted geographic areas (e.g. adults from Queensland coast move offshore to edge of shelf or Coral Sea Reefs) but rarely move to other areas.	Limited current support, contradicts genetic data	*Genetic analysis* Tests comparing Australian, Indonesian, PNG and Pacific island samples show significant differences between regions (possibly including within Australia) with any type of marker (mtDNA, microsatellites, SNPs). *Telemetry and tagging* Tracking results shows movement of adults to offshore areas but no long distance movements between countries. *Fishing or diver surveys* Sampling Australian shelf edge habitats and offshore seamounts identifies significant populations of adults (especially pregnant females).
Continental shelf movement	Adults move along the margins of continental shelves, including northwards from Australia into eastern Indonesia (eastern Banda Sea) and PNG	Genetic connection to Indonesia(Ovenden *et al.* 2009); size and sex structure data; evidence of residency to continental shelves in other regions; ability to move large distances **Moderate support**	*Genetic analysis* Tests show connectivity between Australian samples and eastern Indonesia (eastern Banda Sea and West Papua) and PNG, but not western Indonesia and Pacific Islands. *Telemetry and tagging* Tracking results shows movements along continental shelves, but not across deep water.
East-West Australian stock divide and continental shelf movements	Similar to the previous hypothesis but Torres Strait land bridge divides stocks to the east and west, with adults moving northwards into Indonesia (from WA, NT) or PNG (from Qld)	Similar to previous hypothesis; Torres Straight Land Bridge has caused population structuring in other sharks and teleost species(Flood *et al.* 2014) **Moderate support**	*Genetic analysis* Tests show (1) connectivity between eastern Indonesia (eastern Banda Sea and West Papua) from NT and WA only, and PNG from eastern Queensland only; (2) no genetic connectivity with western Indonesia and Pacific Islands; 3) no genetic connectivity between eastern Queensland and the rest of northern Australia. *Telemetry and tagging* Tracking results shows movements along continental shelves but not through Torres Strait Land Bridge or across deep water

2. SATELLITE TAGGING AND TRACKING

Satellite tracking is a widely used method for investigating the broad-scale movements of marine species including large sharks (Hammerschlag *et al.* 2011). This technology is used to understand the broad-scale movement patterns of individuals.

Generally, hammerhead sharks are thought to be highly migratory, and have been reported as making long distance movements of several hundred to thousands of kilometres in the Caribbean and eastern United States (Guttridge et al. 2017), eastern Pacific (Ketchum *et al.* 2014b) and southern Africa (Diemer *et al.* 2011). Nevertheless, more localized movements of hammerheads sharks have also been recorded. SPOT tag data from the Gulf of Mexico indicated linear movement of 387 km over 81 days (Drymon and Wells 2017). There is also growing evidence of hammerhead shark site attachment, particularly in juvenile individuals and philopatry by pregnant females in specific locations (Guttridge et al. 2017), including suspected use of coastal habitats as nursery areas (Duncan and Holland 2006; Diemer *et al.* 2011; Brown *et al.* 2016). Here we used satellite tracking to investigate whether individuals captured in northern Australia would display cross-jurisdictional movements to inform national management.

2.1 Satellite tracking methods

There are two commonly used satellite tags to study shark movements: pop-off satellite archival tags and position only tags. Both of these tag types were used in this study. Archival tags record environmental variables such as depth, water temperature, and light levels, and are programmed to release from the individual at a specific time. When the tag detaches from the animal it floats to the surface and transmits data to the ARGOS satellite network. Algorithms are used to reconstruct movements using these data, but location estimates can have errors of up to 100 kms and thus are most useful for indicating large scale movements. A drawback of this approach is that these tags can be prematurely shed by tagged animals, and is a common issue in satellite tagging studies. Position only tags are usually positioned on a body part that will break the water surface, such as attached to a shark's dorsal fin and when the tag breaks the surface the tag transmits a signal to the ARGOS satellite network. Position only tags can provide more accurate position estimates than archival tags, but the tag must be at the surface for sufficient time to transmit a signal and an ARGOS satellite must be in 'view' of the tag. Thus, while position estimates may be more accurate, they may be infrequent and may diminish over time if the tag is damaged or bio fouled. Additionally, if the animal does not surface for sufficient periods of time, no data may be received at all (Drymon and Wells 2017).

2.1.1 Capture and tagging

Sphyrna lewini and *S. mokarran* were captured through fishery independent sampling in Western Australia (WA) and Queensland (QLD) (Figure 3) between July 2016 and January 2018. Fisheries staff, commercial fishers, charter fishers and Indigenous Rangers were engaged to collect anecdotal information to target sampling efforts. Fishing gear used included heavy tackle rod and reel, longlines and drumlines. Longlines were 200 m long,

bottom set longlines set with 2.5 m long gangions that included a 1 m leader that was either 600 lbs monofilament or stainless steel wire, terminating in a 16/0 or 18/0 Mustad tuna circle hook. Gangions were set at 10 m intervals which equated to 16-18 gangions per line. Drum lines consisted of a 15 kg weight connected to a surface marker float, with either a bottom set gangion (identical to longline gangions), or a surface set gangion connected to a second float tied to the drumline mark float by a 2.5 m length of rope. Longlines and drumlines were typically set at depths >20 m and soaked for one hour. Surface set drumlines were closely monitored for indications of captures. Captured individuals were secured with a tail rope, sexed and measured and individuals in good condition were tagged with either a Wildlife Computers Inc. miniPAT archival tag, or a Wildlife Computers SPOT6 position only tag. Archival tags were set to monitor movements over a 180 day period. The release condition and location of each tagged shark was recorded.

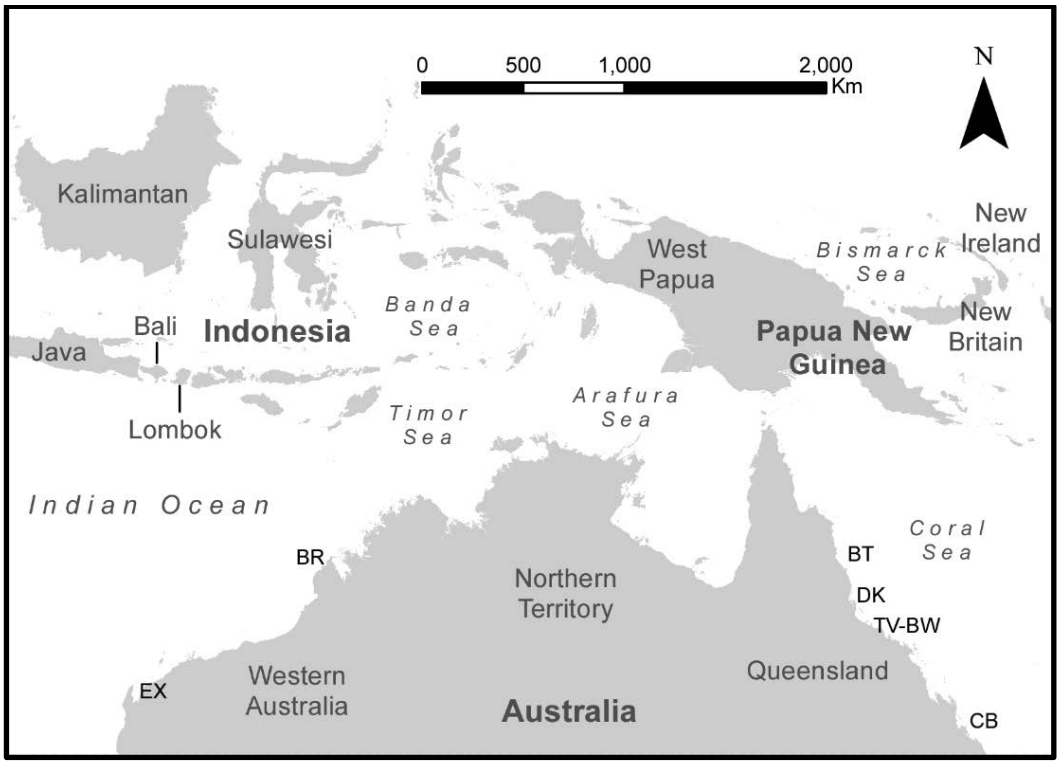


Figure 3. Satellite tagging expeditions occurred in Western Australia, the Northern territory and Queensland, with hammerheads successfully tagged in Exmouth (EX), Broome (BR), Batt Reef (BT), Dunk Island (DK), Townsville (TV), Bowen (BW) and Capricorn Bunker Group (CB).

2.1.2 Data analysis

Data for all tags were retrieved from the ARGOS platform which applied a Kalman filter algorithm to generate location estimates. Accuracy of location estimates for position only (SPOT6) tags was categorised into estimated error location classes with the following error margins: Class 3, < 100 m; Class 2, < 250 m; Class 1, 500 m to 1500 m; Class 0, >1500 m, and additional classes A and B which indicate location estimates derived from 3 or fewer tag messages which prevent estimation of accuracy. As a conservative approach, the maximum

error magnitude of location data for each tag was used in determining animal movement extents (see below).

In addition, for archival tags the maximum depth recorded by the tag was also noted. To process archived temperature, depth and light level data, the Wildlife Computers GPE3 State-Space model was used to generate estimates of animal locations using an animals' swim speed of 0.7m/s based on data from Lowe (1996) and Payne *et al.* (2016). For premature releases of archival tags, data were analysed to identify whether release was triggered due to tag failure, mortality, or for unknown reasons. Data from mortality events (n = 1) were excluded from further analysis.

Location estimates from SPOT6 tags and archival tags were visualised on Google Earth™ and erroneous location estimates (e.g. a single position 100s of km from other positions with a time stamp that was biologically unrealistic) were removed. The maximum error estimate from the remaining location estimates was identified from the ARGOS data and noted for use in further analysis. Location estimates for both tag types were analysed to identify coarse movement extents of tagged animals.

To represent the activity space of tagged hammerhead sharks throughout the tagging period, a Maximum Extent of Location Estimates (MELE) was derived. A KMZ file of locations was uploaded to Google Earth™ and a polygon constructed to encompass the maximum 99% likelihood position for every location estimate. Given the magnitude of error in location estimates, MELE areas were recorded only to the nearest 100 km^2.

2.2 Satellite tracking results

In total 14 hammerhead sharks were tagged between June 2016 and October 2018 (6 *S. mokarran* and 8 *S. lewini*), with four individuals tagged with position only SPOT6 tags, and ten with miniPAT archival tags (Table 3). Tagging with archival miniPAT tags was spread evenly between species, with five of each tagged. However, SPOT6 tags were predominantly deployed on *S. lewini* (n = 3).

All sharks tagged were at a size where they should be sexually mature. Ten of the tagged sharks were male. The four female sharks were all tagged with archival miniPAT tags and included three *S. mokarran* and one *S. lewini* (Table 3). All individuals were tagged in either Queensland (n=10) or the Western Australia (n=4). One individual was identified to have died after release and was excluded from analyses. This animal was opportunistically tagged on board a fisheries research vessel using different long line gear and extended soak times that differed from the fishing techniques applied in this study.

2.2.1 Tag performance

Tag retention and data transmission were limited with only four archival miniPAT tags achieving the full 180 day deployment. Tag shedding is a common issue in satellite tagging studies, and may be exacerbated by the behavior of hammerhead sharks. The mean number of days tracked (monitoring period) for animals tagged with position only SPOT6 tags was

110 days, but monitoring periods varied widely (292 days for one *S. mokarran*; while only 15 and 24 days for *S. lewini*). One SPOT6 tag deployed on a *S. lewini* failed to report any data.

The mean number of days tracked (monitoring period) for animals tagged with archival miniPAT tags was 87 days, but monitoring period varied from 13 to 180 days. Overall, miniPAT tag deployments were more successful on *S. lewini* with a mean monitoring period of 120 days compared with 47 days for *S. mokarran*. However this outcome was reversed for position only SPOT6 tags, with a 292 day deployment for a *S. mokarran* compared with a mean of 20 days for the two SPOT6 tagged *S. lewini*.

It should also be noted that some location data from tags deployed within the Exmouth Gulf (Figure 4) were compromised. Locations estimates could not always be generated as the proximity of land from both sides, turbidity, shallow depth (with limited bathymetric data) and enclosed water body with different sea temperature characteristics from the adjacent Indian Ocean, inhibited the model. Nevertheless, the depth profiles (hammerheads staying shallower than 16 m depth) and proximity of tag release locations to tag deployment locations suggest that their movements were localized to the shallow waters of the Gulf.

Table 3. Biological, capture, and movement details of tagged hammerhead sharks. GHH, great hammerhead (*S. mokarran*); SHH, scalloped hammerhead (*S. lewini*).

Species	Tag type	Tag ID	Size	Sex	Location	Site	Deployment date (AEST)	Linear distance between deployment and transmission	Monitoring duration (days)	Maximum extent of location estimates (nearest 100 km^2 for archival tags)	Max depth (if available)
SHH	SPOT6	163630	2	M	QLD	Cleveland Bay	16/08/2017	NA	15	254	NA
GHH	SPOT6	163631	2.4	M	QLD	Dunk Island	13/09/2017	NA	292	432	NA
SHH	SPOT6	163632	1.8	M	QLD	Dunk Island	12/09/2017	NA	24	677	NA
SHH	SPOT6	163633	1.8	M	QLD	Hull River	7/11/2017	NA	NA	NA	NA
SHH	miniPAT	163619	2.1	M	WA	Exmouth Gulf	13/05/2017	6	180	NA	16
GHH	miniPAT	163620	2.8	F	QLD	Batt Reef	28/07/2017	31	17	5000	56
GHH	miniPAT	163625	2.5	F	QLD	Holbourne Island	14/04/2017	121	90	23600	120
SHH	miniPAT	163626	1.4	M	QLD	Hull River	12/09/2017	38	45	10100	40
SHH	miniPAT	163627	2.1	M	QLD	Exmouth Gulf	13/05/2017	16	15	2800	16
GHH	miniPAT	163628	3	M	QLD	Batt Reef	7/12/2016	3	13	NA	50
SHH	miniPAT	131652	1.85 FL	F	WA	Exmouth Gulf	16/01/2018	48.6	181	6800	40
	miniPAT	131658	2.25	M	WA	Exmouth Gulf	18/01/2018	23.6	181	9200	30
GHH	miniPAT	139075	2.1 FL	M	QLD	Broome	25/07/2016		3	NA	72
GHH	miniPAT	139077	2.2	F	WA	Exmouth Gulf	18/01/2018	169	66	18700	16

2.2.2 Movements and activity space

Data from both tag types indicated relatively localised movements. The furthest distance between tag deployment and release locations was 169 km over 66 days for a 2.2 m long female *S. mokarran* tagged in Exmouth Gulf, Western Australia (Table 3). In Queensland, the longest distance between tag deployment and release location was 121 km for a 2.5 m long *S. mokarran* (tag #163625) at liberty for 90 days (Table 3; Figure 5). The remaining tagged individuals showed more restricted movements. In particular, position only SPOT6 tag tracks ranged from 254 to 677 km^2 (Table 3; Figure 4). No movements across national or international jurisdictional boundaries were recorded.

Activity spaces of hammerheads tagged with archival miniPAT tags were larger than from position only SPOT6 tagged individuals, but this is largely due to inherent error in location estimates generated by archival tags being greater. Consequently, actual activity spaces are likely to be much smaller. The activity spaces of these animals fitted with miniPAT tags ranged from 2,800 km^2 to 23,600 km^2 (Figure 5B). This individual moved into the Coral Sea and dived to a depth of 120 m, the deepest depth recorded by any of the tagged animals (Table 3). The distance recorded between tag deployment and release was <50 km for all three of the tags that were retained for 180 days (Table 3). In particular, sharks tagged in Exmouth Gulf showed movements restricted to the Gulf and adjacent island (e.g. Figure 6). Furthermore, even if MELE values are taken as accurate, the latitudinal north-south distance encompassed by the animal with the largest activity space was still less than 250 km (for *S. mokarran* #139077 tagged in WA). There was also no discernable relationship between how long an individual was at liberty and the size of its activity space. A linear regression of monitoring duration compared to MELE area had an extremely low R Square value of <0.001, and a *P* value of 0.944.

The observed localised movements differ from those documented in many previous studies. For example, Ketchum *et al.* (2014a) revealed that *S. lewini* moved large distances across open ocean regions. It is therefore possible that individuals found in Australia could be connected to Indonesia, Papua New Guinea and more broadly into the Pacific and Indian Oceans.

These studies indicate that hammerhead movement patterns may vary according to location and animal size/age. For example, hammerhead sharks tracked in the Caribbean and eastern United States were primarily > 3 m TL (Guttridge et al. 2017). In contrast, a hammerhead shark showing more localized movements in the Gulf of Mexico was a smaller animal (1.87 m TL) (Drymon and Wells 2017). These differences could be indicative of ontogenetic differences in movement and space use. In coastal waters of northern Australia, hammerhead sharks are predominantly smaller individuals <2.5 m TL, with large female hammerheads appearing to only visit coastal regions during parturition (Chin et al. 2017). This pattern is consistent with information provided by fishers consulted this study who indicated that encountered hammerheads were mainly small, and that larger animals tended to occur seasonally and far offshore.

The location of this study and the size of tagged animals may account for the observed movements. Tagging was focused on coastal areas of northern Australia (Western Australia to Queensland), which include expansive, contiguous areas of relatively shallow, continental shelf habitat. These habitats have relatively stable temperatures and productivity driven by coastal rivers. This is very different from Caribbean island archipelagos and the Galapagos Islands where different temperature regimes, oceanic current systems, bathymetry and ecological processes such as ocean upwelling regulate marine ecosystems, and thus may affect shark movement and migrations (Guttridge et al. 2017). Furthermore, the relatively small size of animals tagged in this study may explain the observed limited movements. Many sharks show ontogenetic changes in movement patterns and habitat use, with adults having different patterns to juveniles and sub adults (Grubbs 2010). The hammerhead sharks tagged in this study were mainly between 2.0 and 2.4 m TL, and while these animals were larger than reported estimated size at maturity, they were considerably smaller than the maximum sizes these species attain (3.5 m for scalloped hammerhead sharks; 4.5 m TL for great hammerhead sharks). It is possible that the individuals tagged in this study had yet to adopt the patterns of larger individuals which may exhibit wider ranging movements.

To gain a more comprehensive account of hammerhead movements and migrations, tagging of large (>3 m TL) individuals is needed. The challenge is to be able to locate and successfully capture and tag these individuals. Information from fishers indicated these animals are mainly large females which seasonally visit coastal areas for parturition. Unfortunately, while sampling efforts occurred in these areas during these periods, no >3 m TL animals were acquired. This may indicate that the number of large females in the region is limited, or that effort wasn't extensive enough to encounter these individuals.

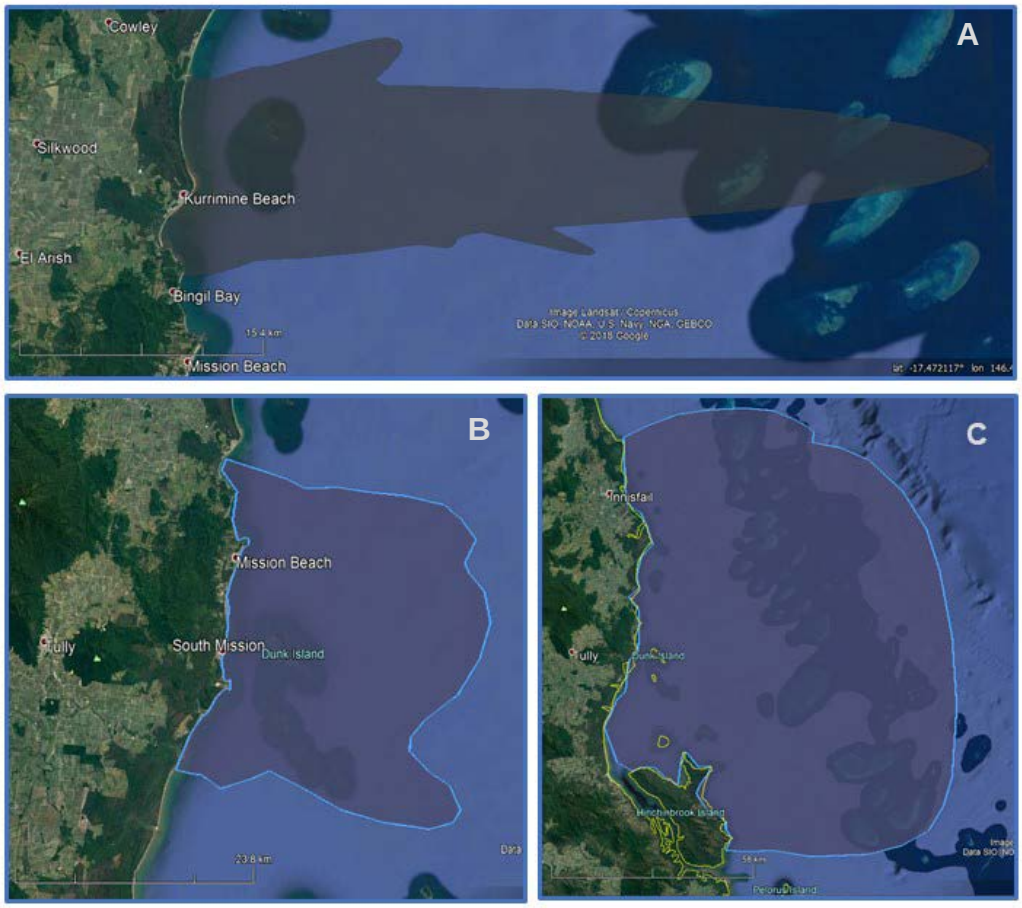


Figure 4. Representative movements of *S. lewini* and *S. mokarran* from Queensland. A. 2.4 m long male *S. mokarran* tagged with position only SPOT6 Tag 136361 showing limited latitudinal movements that are concentrated around the Kurrimine Beach area over 292 days. B. 1.8 m long male *S. lewini* tagged with SPOT6 Tag 136362 showing localised movements around the Mission Beach area over 25 days. C. 1.4 m long male *S. lewini* tagged with archival miniPAT Tag 163626 showing localised movements around the Mission Beach area over 45 days. Note that larger extent of position estimates reflects the reduced accuracy of position estimates generated by archival tags compared with position only SPOT6 tags.

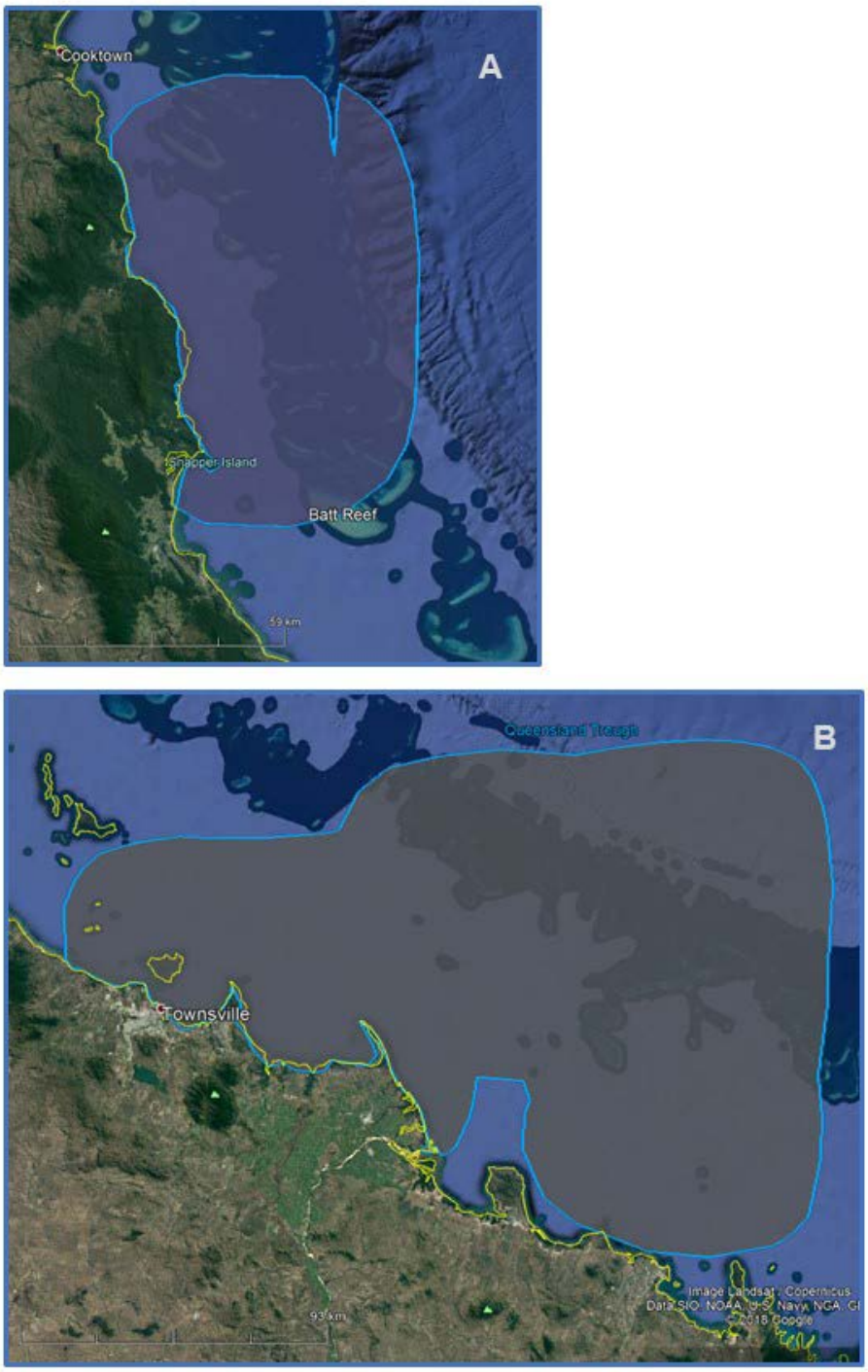


Figure 5. Representative movements of *S. mokarran* from Queensland. A. 2.8 m long female *S. mokarran* tagged with an archival miniPAT Tag 136620 showing relatively localised movements around Batt Reef in north Queensland. B. 2.5 m long female *S. mokarran* tagged with a miniPAT Tag 163265 showing movements from Holbourne Island to Townsville over 90 days.

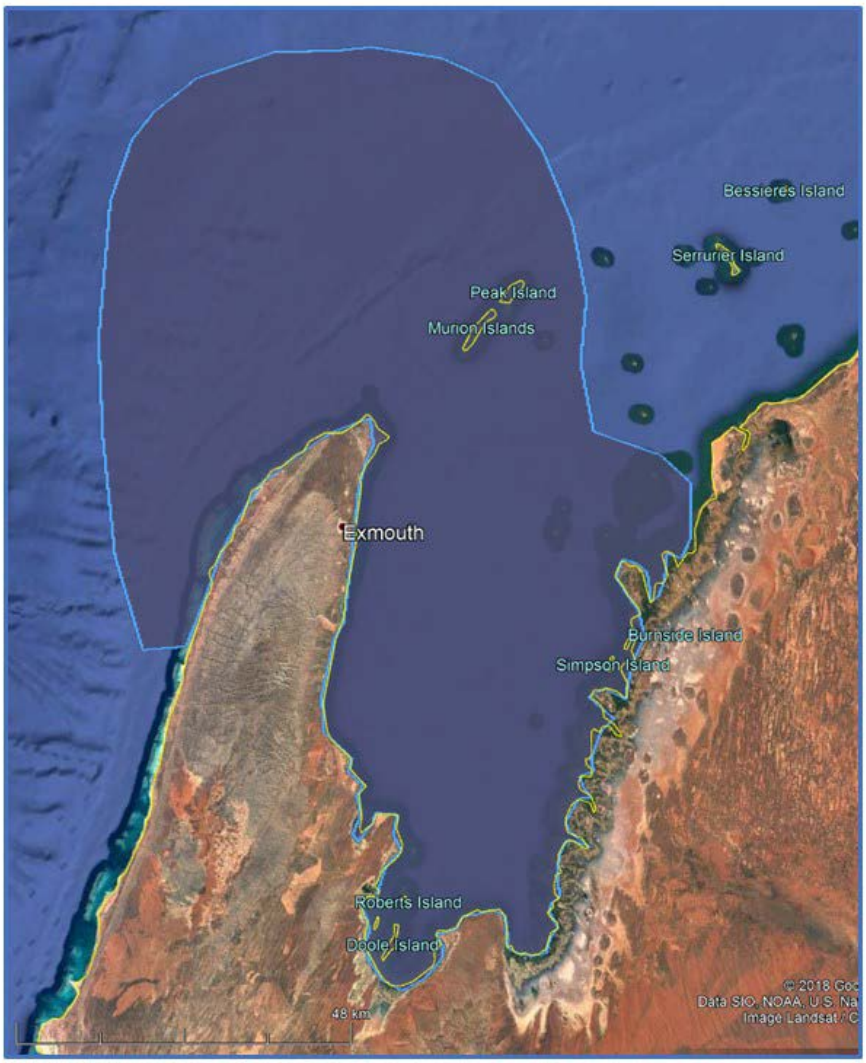


Figure 6. Representative movements of a *S. lewini* tagged in Western Australia. A 1.85m long female tagged with an archival miniPAT tag 131652 and monitored for 181 days. The location estimates indicate relatively localised movements, however location estimates form this area were compromised by model inaccuracy. Depth data indicate a maximum depth of 40 m, suggesting the animal did not venture further seawards than the Murion Islands.

3. GENETIC INVESTIGATION OF POPULATION STRUCTURE

Genetic analyses are widely used to differentiate between and identify discrete populations, and are often used in multi-method approaches for examining the population structure of fishes (Welch *et al.* 2015). In wild populations, genetic drift and variation can lead to divergence of genetic structures over time. This process of differentiation takes longer in long-lived species, and can also depend on the level of gene flow between populations (Jones and Wang 2012). Species with high connectivity, and hence gene flow, will tend to have homogenised genetic structures within their populations. In contrast, individuals in populations with low gene flow will tend to have more distinct genetic structures. These differences can help managers identify the size and boundaries of populations, and the level of connectivity between them.

Comparing genetic profiles from samples collected across a wide area can help define stock discrimination at different scales ranging from ocean basins to regional seas and coastlines. Different types of genetic analyses provide different levels of resolution in identifying discrete stocks. Coarse techniques that use mitochondrial DNA can identify highly diverged populations, however for longer lived species with 'conservative' DNA and/or lower gene flow, higher resolution genetic techniques such as microsatellites or single nucleotide polymorphisms (SNPs) are needed to identify discrete stocks.

The genetic structure of hammerhead populations across northern Australia has been previously investigated, suggesting a large homogenous population (Ovenden *et al.* 2011; Chin *et al.* 2017).

3.1 Scalloped hammerhead

3.1.1 Methods

A total of 436 *S. lewini* DNA samples were obtained from nine locations across the central Indo-Pacific (see Figure 7). Samples were obtained through several collaborative projects (Australian Centre for International Agricultural Research, ACIAR; and Green (2019)) and from the previously published studies of Daly-Engel *et al.* (2012) and Ovenden *et al.* (2009). Therefore, collection occurred over a significant timespan; between 1999 and 2016; however, there was no repeat sampling of any spatial location.

Sampling of individuals from Indonesia (IN), New South Wales, Australia (NSW), Western Australia (WA) and Princess Charlotte Bay, Australia (PCB) is outlined in (Ovenden *et al.* 2009), while samples collected from Philippines and Taiwan (PHTW) are described in Daly-Engel *et al.* (2012). Samples from Papua New Guinea (PNG) were collected on-board fishing vessels by fisheries observers and from coastal fisheries during dedicated surveys as part of an ACIAR project (#FIS/2012/102). For sharks landed by commercial and coastal fishers, a piece of vertebrae or muscle was collected. Samples from Northern Territory, Australia (NT) were collected and supplied by Grant Johnson (Department of Primary Industry and Resources). Samples from Fiji (FJ) were part of Amandine Marie's University of South Pacific 2017

study (A. Marie, pers. comm.) while the samples from Townsville, Australia (TSV) were collected via similar methods as described in the tagging section of this report.

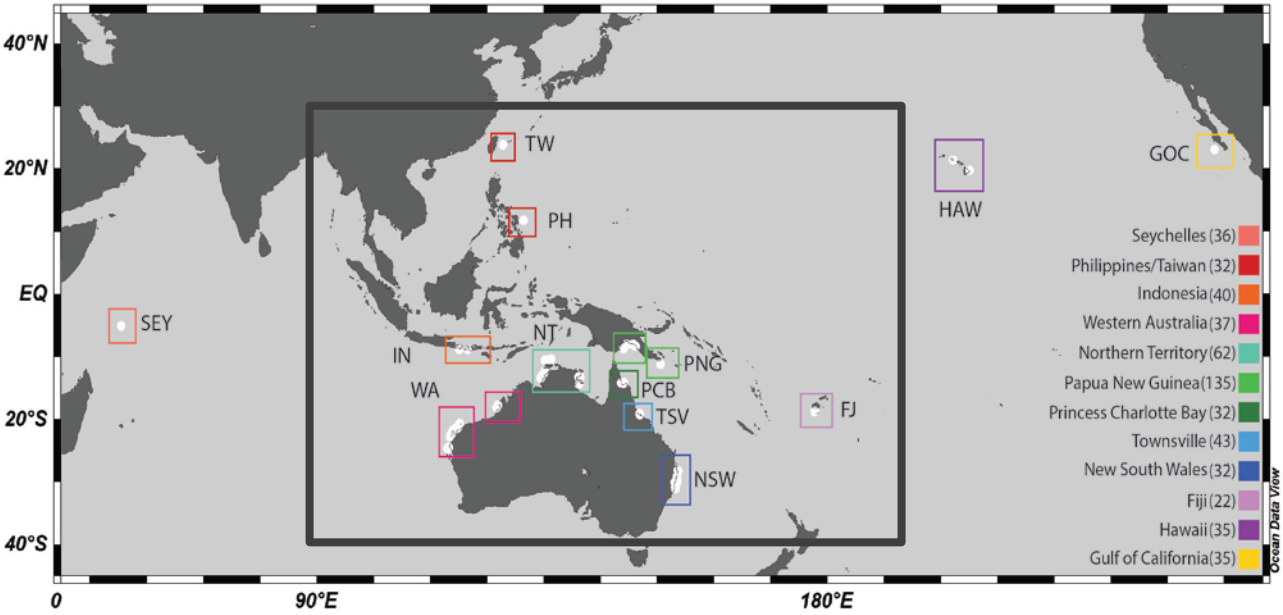


Figure 7. Sample collections for *S. lewini* within the Indian and Pacific Oceans. Colour squares represent location of sample collection, white dots represent sample collection sites, numbers in brackets indicate total sample size (for sample size per marker type). Samples included in the black box are considered here for the central Indo-Pacific analyses (map as per Green 2019).

DNA was extracted from all samples using the Wizard® SV Genomic DNA Purification system (Promega, Australia); tissue extractions were undertaken using SV mini-columns following modifications to the manufacturer's instructions, including an overnight digestion with Proteinase K, at 55°C. Total genomic DNA (gDNA) was eluted in DNAse free water. Archival DNA aliquots are stored at 80°C at the CSIRO marine laboratories.

Mitochondrial DNA (mtDNA)

To estimate the genetic similarity between samples from various locations, (polymerase chain reaction) two portions of mtDNA (i.e. a maternally inherited genetic marker) were PCR amplified and the gene regions sequenced. A 964 bp of the control region (CR) and 853 bp of the NADH dehydrogenase subunit 4 (ND4) were sequenced resulting in a total concatenated sequence of 1 817 base pairs.

Microsatellites (Msats)

Microsatellite loci were one of two types of nuclear markers used to test for population homogeneity across sample locations. Samples were genotyped using nine polymorphic Msat loci initially described in (Nance *et al.* 2009).

Single Nucleotide Polymorphisms (SNPs)

We used a reduced-representation next generation sequencing (NGS) approach to obtain SNPs from across the *S. lewini* genome. This enabled us to simultaneously target and capture a subset of similar regions across the genome for many samples. We sent genomic DNA to the Diversities Arrays Technology Pty. Ltd (Canberra, Australia) for library preparation and sequencing using the standard DArTSeq Protocol. DArTSeq is a genotype-by-sequencing approach that uses Diversity Arrays (DArT) restriction enzymes (Jaccoud *et al.* 2001) and next-generation sequencing on an Illumina platform (Sansaloni *et al.* 2011).

Statistical analyses for genetic markers

All statistical analyses for the three marker types (including haplotype and allele frequency calculations, estimates of observed heterozygosity, tests for genetic homogeneity, FST analyses and Discriminant analysis of principal components (DAPC)) were described as part of Green (2019). Extensive filtering of raw SNP loci (that resulted from the proprietary DArTSeq pipeline) was also undertaken in Green (2019). Mitochondrial DNA haplotype frequencies, microsatellite allele frequencies, SNP input files and sample metadata files will be deposited on the CSIRO Data Access Portal (https://data.csiro.au/dap/home?execution=e1s1) in December 2019.

3.1.2 Results and Discussion

The number of samples successfully collected and analysed for each marker type is described in Table 4. Differences in sample size per location and per marker are likely due to several factors that affected sequencing and genotyping success, primarily sample age and storage condition which resulted in poor quality gDNA from some sampling locations. Due to reduced sample sizes within Philippines and Taiwan, individuals from these sampling locations were grouped to create a more robust sample size. Separate pairwise analysis indicated no significant difference in allele frequencies between locations (hereafter abbreviated PHTW); hence samples were combined (data not shown).

A total of 359 individuals from 12 populations were successfully amplified at both mtDNA regions, resulting in 43 haplotypes (Green 2019). A large break between haplotypes with 19 mutations separated all individuals from SEY and some individuals from IN, PHTW and PNG (Figure 8). Upon further investigation these haplotypes were identified as very similar to those of the previously described CR 'Atlantic Ocean' haplotype of *S. lewini* (Quattro *et al.* 2006; Quattro *et al.* 2013). Overall, most other haplotypes were found to be shared amongst individuals from different sampling locations. In central Indo-Pacific samples, numbers of haplotypes varied greatly from H = 33 in PNG to H = 7 in Fiji. Nucleotide diversity also varied from $\pi = 0.014$ in PHTW to $\pi = 0.267$ in PNG (Table 4).

Table 4. Summary of various measures of genetic diversity (averages given) for mitochondrial DNA, microsatellites and SNP datasets in *S. lewini* across twelve sampling locations (see Green 2019). Samples shaded in grey represent the central Indo-Pacific. The number of individuals successfully amplified per marker and total (*n*), the observed (H_O) and expected heterozygosity (H_E), the number of polymorphic sites (S); for SNPs one site equals one locus, number of Haplotypes (H), haplotype diversity (*h*), nucleotide diversity (π), allelic richness (A_R), inbreeding coefficient (F_{IS}) and Hardy-Weinberg significance value (HWE_p).

			mtDNA (CR+ND4, 1 817bp)					Microsatellites (9 loci)						SNPs (5689 loci)					
Ocean	Site	Abbr.	*n*	S	H	*h*	π x 10^2	*n*	A_R	H_O	H_E	F_{IS}	HWE_p	*n*	S	A_R	H_O	H_E	F_{IS}
Indian	Seychelles	SEY	22	4	7	0.653	0.055	26	9.28	0.768	0.801	0.045	0.516	14	3,753	1.91	0.128	0.167	0.177
	Indonesia	IN	35	75	18	0.908	0.675	23	8.65	0.614	0.715	0.165	0.248	23	4,507	1.91	0.121	0.161	0.214
	West Australia	WA	10	17	6	0.780	0.478	27	9.13	0.612	0.761	0.220	0.173	21	4,491	1.92	0.130	0.166	0.178
Pacific	Philippines/Taiwan	PHTW	19	75	10	0.776	**1.419**	29	8.86	0.697	0.784	0.125	0.237	21	4,421	1.92	0.130	0.165	0.177
	North Australia	NT	54	27	20	0.882	0.233	33	9.13	0.743	0.783	0.048	0.204	28	4,812	1.92	0.126	0.165	0.206
	Papua New Guinea	PNG	77	82	33	0.912	0.267	37	9.57	0.746	0.800	0.073	0.416	67	5,315	1.93	0.126	0.166	0.228
	Princess Charlotte Bay	PCB	25	24	12	0.838	0.167	29	9.15	0.717	0.783	0.093	0.406	17	4,223	1.91	0.123	0.163	0.189
	Townsville	TSV	39	29	25	0.934	0.165	43	8.61	0.686	0.785	0.134	0.241	33	4,916	1.92	0.129	0.167	0.202
	New South Wales	NSW	25	23	13	0.774	0.135	30	9.09	0.738	0.788	0.076	0.311	26	4,686	1.92	0.126	0.165	0.196
	Fiji	FJ	21	5	7	0.712	0.064	22	9.43	0.770	0.792	0.027	0.383	19	4,263	1.92	0.126	0.165	0.192
	Hawaii	HAW	14	5	3	0.439	0.055	28	8.05	0.787	0.758	0.040	0.502	25	4,351	1.93	0.127	0.169	0.215
	Gulf of California	GoC	18	2	3	0.537	0.033	27	6.99	0.673	0.709	0.048	0.370	16	3,236	1.91	0.116	0.162	0.227
	Total *n*		359					354						310					

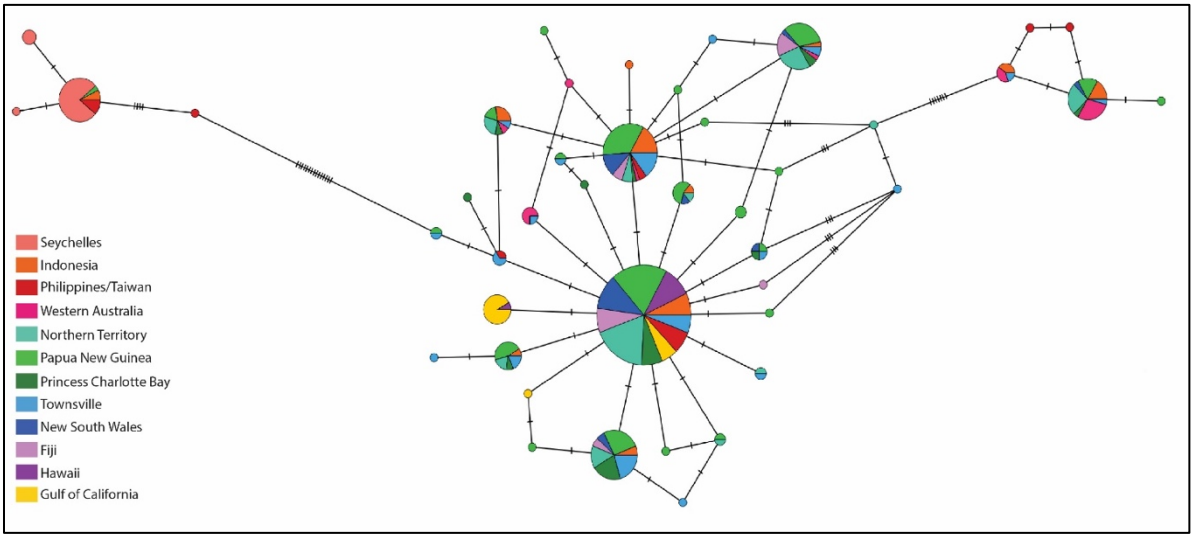


Figure 8. Figure taken from global analyses completed in Green (2019). Figure shows Mitochondrial DNA (CR and ND4) Median-Joining network analysis from POPart v1.7. *S. lewini* haplotype frequencies are relative to the size of the circles, colours represent sampling locations. Number of strokes joining nodes represents number of mutations between two haplotypes (across the concatenated 1 817 bp fragment).

A similar number of individuals were genotyped at the nine microsatellite loci. Allelic richness ranged from 8.86 in PHTW to a high of 9.57 in PNG. Individuals at the nine sampling locations were characterised by relatively high levels of observed heterozygosity (i.e. variation). A smaller number of individuals were genotyped at the SNP loci and unlike the Msat loci, allelic richness was a uniform 1.9 across the sampling locations albeit a much higher number of polymorphic SNP sites were observed (S = 4 223 in PCB – 5 315 in PNG).

Figure 8 shows individuals from the Indian and Pacific Ocean locations, and the central Indo-Pacific share similar genetic information in their mitochondrial genomes. This suggests matrilineal gene flow is strong across most locations. However, differences in individuals from sampling locations were separated by oceans. For example, the Gulf of California, Hawaii and Seychelles had unique haplotypes branching from the large central haplotype. This suggests the central Indo-Pacific was the centre of origin for *S. lewini* and colonization into other ocean basins occurred from movement out of the central Indo-Pacific.

Population connectivity based on nuclear markers

Population subdivision/connectivity identified based on three marker types (Figures 9, 10, 11) show little gene flow is occurring between the westernmost and easternmost populations of Seychelles, Hawaii and the Gulf of California and the central Indo-Pacific locations (Australia, Indonesia, Philippines, Taiwan, Papua New Guinea and Fiji). The large geographic separation between outer regions (SEY, HAW and GOC) likely explains the genetic structure identified in our study. Conversely, the continental shelves of Australia, Papua New Guinea and Indonesia provide well connected habitat enabling dispersive behaviours across the

central Indo-Pacific (with Fiji being the exception) and likely drive the high gene flow identified amongst individuals in the central Indo-Pacific region.

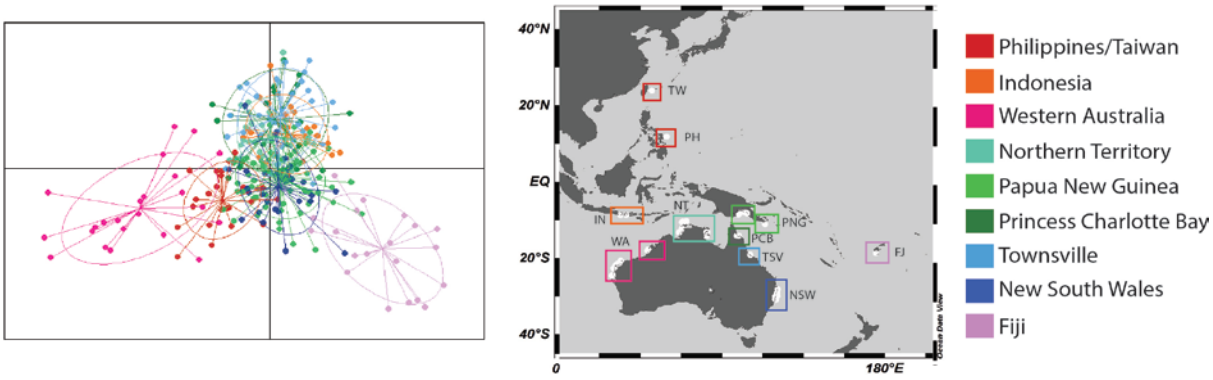


Figure 9. Scatterplot created using DAPC showing SNP variation between *S. lewini* individuals (dots) and populations (colours) with accompanying map of locations from the central Indo-Pacific.

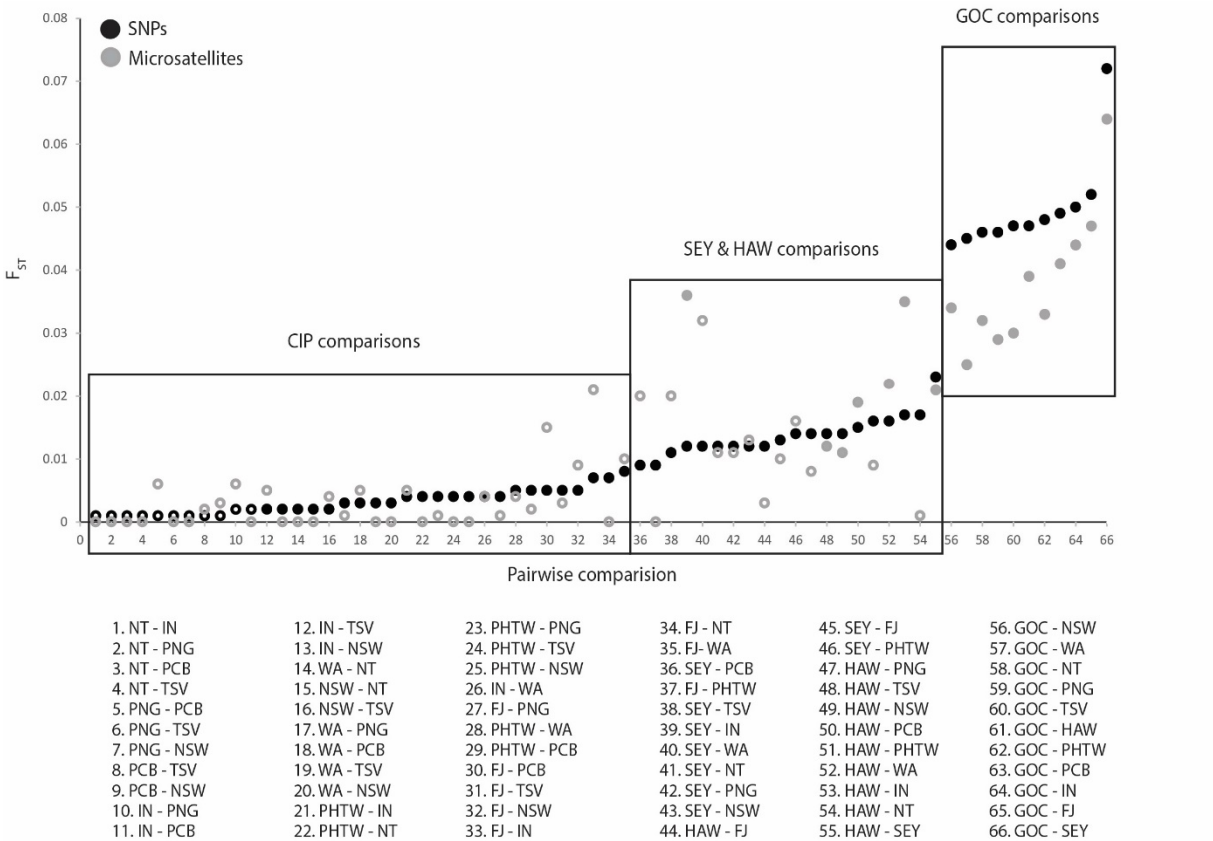


Figure 10. Estimates of pairwise genetic differentiation (F_{ST}) between all sampled locations for *S. lewini* using SNP (black) and microsatellite (grey) loci. Where CIP = central Indo-Pacific, SEY = Seychelles, HAW = Hawaii and GOC = Gulf of California (Green 2019). Comparisons are arranged in ascending order of SNP F_{ST} values (x-axis). Filled circles indicate significant *p*-values where $p = < 0.001$ and boxes represent pairwise comparisons between grouped locations (note 37 is the only CIP comparison within the SEY & HAW section).

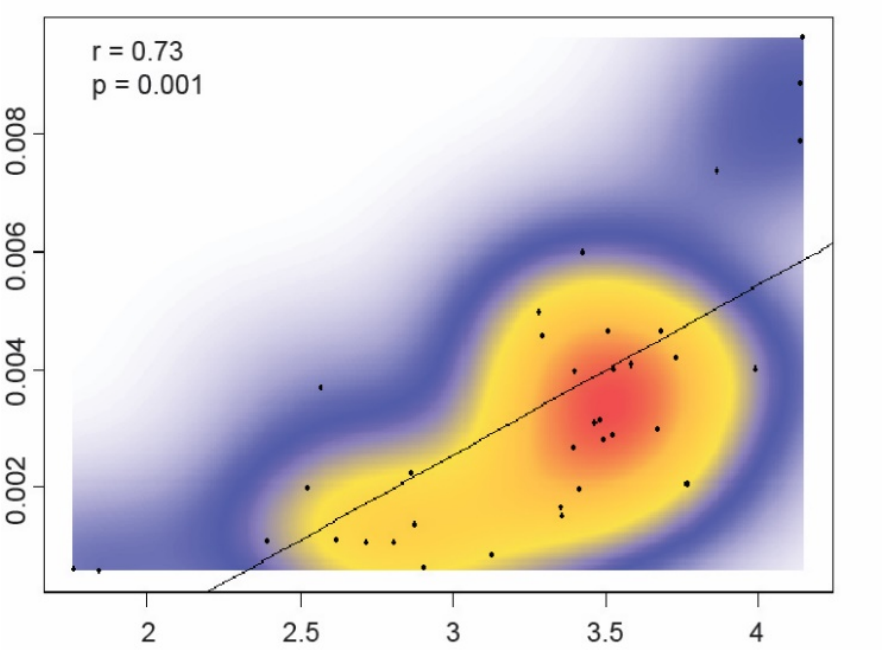


Figure 11. Isolation by Distance (IBD) plot showing the significant relationship between genetic distance (y-axis) and geographic distance (x-axis) as measured using SNPs. SNP IBD plots were generated using dartR package (Gruber et al 2018) where geographic distance is represented as the log of distance in meters.

Figure 9 shows Fiji (purple), Western Australia (pink) and Philippines/Taiwan (PHTW/red) as slightly removed from the central cluster of *S. lewini* individuals, based on SNPs. When formally tested, Fiji was significantly different from all other locations (Figure 10). There was also some level of differentiation for Western Australia and PHTW individuals. For *S. lewini*, distance appears to play a significant role in genetic connectivity; with isolation-by-distance plots detecting a significant correlation (r = 0.73) between genetic and geographic distance (Figure 11). This finding indicates that individuals from closely located regions such as Papua New Guinea, Indonesia, north and east Australia are genetically homogeneous and share genetic information. This observed genetic connectivity can be facilitated by a few adults effectively migrating to closely located regions. Thus, it does not necessarily mean large cohorts of *S. lewini* are moving between regions consistently.

Interestingly Fiji appears to be strongly separated from the other central Indo-Pacific locations indicating little to no movement of individuals occurs between these locations and no gene flow from the central Indo-Pacific to the eastern Pacific. *S. lewini* in Fiji are genetically distinct. Finally, limited gene flow to Western Australia could suggest very little exchange/ movement of *S. lewini* to or from Western Australia is occurring.

Conceptual models (that explain pattern of distribution) previously developed for *S. lewini* described four possible models of movement (Table 2) (Chin et al., 2017). Based on the genetic and genomic results of this current study in the central Indo-Pacific and more widely in Green (2019), we conclude Models 1 and 2 (that suggest panmixia and limited movement respectively) are unlikely explanations of connectivity in *S. lewini*. Instead, genetic data reported here support Model 3; continental shelf movement enabling connectivity between

Australia, Papua New Guinea and eastern Indonesia, but not east to the Pacific islands (i.e. Fiji). In addition to Model 3, the results suggest connectivity to Western Australia is limited.

SNPs better differentiated population structure than microsatellites

An additional objective was to assess whether differences in biological stock structure could be observed using a multi-marker approach. The work presented here indicates differences between Msats and SNPs do occur, with SNPs identifying more discrete population subdivision than Msats. The ability of genomic techniques to capture a large subset of highly differentiated markers provides a robust approach to identify population structure (Hohenlohe *et al.* 2019). These results suggest increased sampling regimes or loci are required if choosing to undertake population structure analyses exclusively with Msat markers. Therefore, undertaking a genomic approach using SNPs may be more suited for shark and ray population structure studies given the challenges faced (expense and accessibility) when obtaining adequate sample sizes.

3.2 Great hammerhead and winghead

A more detailed account of the genetic methods and results for great hammerhead and winghead sharks is provided in Appendix A and should be consulted for technical details of this research.

3.2.1 Methods

Sampling differed between the two species, with only samples from Australia and PNG were used in this study. The DNA extraction of tissues (per species) was the same and this was completed as outlined below (the standard method of DNA extraction was using the Promega Wizard SV 96 genomic kit). The three markers were each deployed in *S. mokarran* and *E. blochii* samples, and the same analysis pipelines were applied to data for both species.

Sampling locations for S. mokarran and E. blochii

A total of 215 *S. mokarran* samples were obtained from eleven locations from Australia and PNG, while 202 *E. blochii* samples were obtained from seven locations in Australia and PNG. Due to unplanned and opportunistic sampling, highly variable sample sizes, and as location metadata for some samples was missing, samples were arbitrarily placed into the following temporal and spatial ‘groupings’ (see Table 5 – known as ‘collections’ herein), with representative latitude and longitude points for these collections shown in Table 5.

Table 5. *Sphyrna mokarran* and *E. blochii* sampling locations and number of individuals sampled for current study.

Collection	Representative sampling date	Sample size	Representative latitude (°N)	Representative longitude (°E)	Collection designations
S. mokarran					
Papua New Guinea	2015-2016	14	-9.5376	146.7433	PNG15-16
Northern Territory	2015-2017	28	-12.4500	130.0600	NT15-17
	2012-2014	68	-12.4385	130.1589	NT12-14
	2009-2011	21	-11.0700	131.1100	NT09-11
	2006-2008	27	-13.4000	129.4100	NT06-08
	2003	7	-12.3476	130.3330	NT03
Gulf of Carpentaria	2005	3	-16.1320	138.7426	GoC05
Western Australia		12	-21.3730	114.5266	WA
Queensland	2012-2013	4	-18.6310	147.3010	QLD12-13
	2003-2004	9	-16.2921	145.5370	QLD03-04
New South Wales	2016-2017	22	-28.7870	153.6010	NSW16-17
E. blochii					
Papua New Guinea	2016-2017	35	-3.8500	144.5333	PNG16-17
	2014-2015	69	-8.0835	145.6506	PNG14-15
Northern Territory	2013-2014	6	-13.3200	129.4600	NT13-14
	2011-2012	18	-12.3100	130.1600	NT11-12
	2009-2010	19	-13.2000	129.5259	NT09-10
	2006-2007	43	-12.5100	130.1300	NT06-07
Queensland (QLD)	2004-2005	12	-14.1970	144.0020	QLD04-05

DNA extractions for S. mokarran and E. blochii

Please see Appendix A for details.

3.2.2 Results

DNA quality and quantity varied greatly amongst *S. mokarran* and *E. blochii* samples. As many of the samples were not recently collected, and tissues had been stored in sub-optimal conditions (e.g. long-term storage in DMSO), some extractions resulted in poorer quality DNA. As a result of the opportunistic sampling of *S. mokarran* and *E. blochii*, samples sizes per collection varied greatly (Table 6) and, in some instances, did not provide useful numbers of animals per location; to mitigate this, where appropriate, some collections were combined. Where the sample sizes per collection (per marker type) were N < 5, genetic diversity estimates are given but these collections were not part of the primary genetic homogeneity or connectivity assessments.

mtDNA diversity

On average, an 1120 bp portion of the CR and 810 bp of the ND4 gene regions were sequenced in *S. mokarran*. Smaller portions of the CR gene (523 bp) and ND4 (754 bp) were sequenced in *E. blochii*. Table 6 outlines the genetic diversity estimates for the two species, based on the two mtDNA genes.

Table 6. Summary of mtDNA genetic diversity (averages given) across eleven *S. mokarran* and seven *E. blochii* collections.

		mtDNA CR (1120 bp)					mtDNA ND4 (810 bp)				
Species	Collection	N	S	H[#]	Hd[##]	π JC[###]	N	S	H[#]	Hd[##]	π JC[###]
S. mokarran	PNG15-16	7	10	5	0.857	0.0032	8	0	1	0.000	0.0000
	NT15-17	22	21	19	0.983	0.0048	26	2	3	0.219	0.0002
	NT12-14	59	32	43	0.974	0.0047	54	1	2	0.107	0.0001
	NT09-11	14	23	14	1.000	0.0056	17	1	2	0.118	0.0001
	NT06-08	20	20	18	0.989	0.0046	22	1	2	0.173	0.0002
	NT03	1	0	1	0.000	0.0000	1	0	1	0.000	0.0000
	WA	4	10	4	1.000	0.0046	7	0	1	0.000	0.0000
	Goc05	3	9	3	1.000	0.0053	2	1	2	1.000	0.0012
	QLD12-13	2	3	2	1.000	0.0026	2	1	2	1.000	0.0012
	QLD03-04	4	12	4	1.000	0.0058	9	1	2	0.222	0.0002
	NSW16-17	15	20	12	0.943	0.0035	22	1	2	0.416	0.0005
		mtDNA CR (523 bp)					mtDNA ND4 (754 bp)				
E. blochii	PNG14-15	32	159	23	0.964	0.0615	50	4	4	0.479	0.0011
	PNG16-17	14	88	14	1.000	0.0362	28	0	1	0.000	0.0000
	NT13-14	0	0	0	0.000	0.0000	6	0	1	0.000	0.0000
	NT11-12	2	13	2	1.000	0.0298	16	1	2	0.125	0.0001
	NT09-10	5	2	3	0.800	0.0022	12	0	1	0.000	0.0000
	NT06-07	19	9	8	0.813	0.0032	31	1	2	0.064	0.0001
	QLD04-05	9	106	7	0.944	0.0647	9	0	1	0.000	0.0000

N = number of individuals screened per marker, S = segregating/polymorphic sites, H[#] = number of haplotypes (based on Nei 1987), Hd[##] = haplotype diversity (average number of nucleotide differences per site between two sequences, Nei 1987), πJC[###] = Jukes-Cantor nucleotide diversity. CR haplotype data from collections that were not subsequently used in haplotype homogeneity or connectivity analyses (due to small sample sizes) are shown in yellow; homogeneity analysis of the ND4 haplotypes are not presented in this report

Nuclear diversity

Microsatellite loci

The eight published usat loci from Nance et al. (2009) were polymorphic in 166 *S. mokarran* individuals across the 11 collections. Loci were checked for departures from HWE and linkage disequilibrium, however no single locus was out of HWE or in genotypic linkage in each of the 11 collections, therefore all loci were maintained. The number of microsatellite alleles ranged from 2.38 in the small NT03 collection to a high of 13.38 in the largest collection, NT12-14. Average observed heterozygosity (i.e. variation) was moderate at 0.544 across the eleven collections.

Eight of the nine newly developed microsatellite loci for *E. blochii* were polymorphic across the seven collections, albeit the number of alleles (across individuals, across loci) was not as large (maximum N_a = 4.00, in PNG14-15) as in *S. mokarran*. The *E. blochii* usat loci were also checked for conformation to HWE and genotypic linkage; all were retained. As with the number of individuals screened for mtDNA, there was a clear bias towards samples from PNG, with over 79 *E. blochii* individuals genotyped at the microsatellite loci, in comparison to 39 *E. blochii* individuals from five other Australian collections.

Average observed heterozygosity (0.400) (Table 6) across the seven *E. blochii* collections was lower than in the *S. mokarran* collections. A relatively high F_{IS} value was observed in the PNG16-17 collection – from 25 individuals, this value was 0.495. This within-population inbreeding coefficient indicates a deficiency of heterozygosity, possibly reflecting either allelic drop out and or the likely presence of related sibs (sharing the same usat alleles) in the collection.

SNP loci

Resulting species sequence data from the Illumina NextSeq was of a high quality (with > 87% of the 150 bases above Q30) (a quality control metric) across all *S. mokarran* samples and > 86% of the 150 base reads, above Q30 in the *E. blochii* samples. 129 *S. mokarran* individuals in eleven collections and 3 655 SNPs

Table 7 below outlines the summary of the nuclear genetic diversity in the *S. mokarran* and *E. blochii* collections at the microsatellite and SNP loci.

Table 7. Summary of genetic diversity (based on averages at microsatellite and SNP loci) across the eleven *S. mokarran* and seven *E. blochii* collections from Australia and Papua New Guinea. Diversity estimates are based on n_{usat} = 8 & n_{SNP} = 3 655 loci in *S. mokarran* and n_{usat} = 8 & n_{SNP} = 5 229 loci in *E. blochii.*

Species	Collection	N	N_a	Ar	H_O	H_E	HWEp	F_{IS}	N	A	%poly	Ar	H_O*	H_E*
S. mokarran	PNG15-16	7	5.38	2.88	0.599	0.735	0.279	0.233	5	5611	53.5	1.26	0.298	0.328
	NT15-17	21	8.38	3.00	0.523	0.650	0.385	0.160	22	6894	94.0	1.26	0.171	0.194
	NT12-14	58	13.38	3.34	0.525	0.767	0.040	0.311	54	7292	99.5	1.29	0.165	0.182
	NT09-11	12	6.00	2.81	0.556	0.686	0.227	0.202	11	6303	86.0	1.22	0.215	0.244
	NT06-08	15	7.38	2.80	0.622	0.660	0.458	0.043	8	5361	72.5	0.99	0.226	0.323
	NT03	3*	2.63	1.86	0.528	0.660	0.601	0.292	2	4552	26.0	1.12	0.581	0.626
	WA	8	5.13	2.67	0.561	0.707	0.411	0.226	2	4599	26.0	1.15	0.532	0.593
	Goc05	3*	3.63	2.75	0.542	0.641	0.550	0.309	3	4242	20.0	0.92	0.366	0.544
	QLD12-13	3*	2.88	2.22	0.438	0.588	0.701	0.375	3	5249	43.5	1.27	0.427	0.431
	QLD03-04	7	4.88	2.67	0.485	0.640	0.364	0.195	6	5819	59.0	1.27	0.280	0.300
	NSW16-17	20	8.00	3.15	0.603	0.704	0.080	0.194	13	6151	68.0	1.14	0.180	0.235
E. blochii	PNG16-17	25	2.22	1.49	0.167	0.309	0.341	0.495	23	6256	20.0	1.16	0.342	0.350
	PNG14-15	54	3.67	1.91	0.309	0.367	0.107	0.298	49	10037	92.0	1.48	0.174	0.182
	NT13-14	4	1.55	1.47	0.479	0.528	0.764	0.325	6	8261	58.0	1.44	0.289	0.292
	NT11-12	6	1.55	1.39	0.556	0.573	0.683	0.121	12	8949	71.0	1.45	0.217	0.232
	NT09-10	4*	1.67	1.46	0.556	0.571	1.000	0.002	7	7959	52.0	1.36	0.244	0.293
	NT06-07	16	2.11	1.59	0.322	0.349	0.789	0.062	17	8777	68.0	1.33	0.181	0.224
	QLD04-05	6	1.67	1.49	0.410	0.551	0.503	0.288	5	7653	46.5	1.36	0.310	0.342

N = number of individuals per collection genotyped; N_a = average number of alleles; Ar = allelic richness across loci; A = total number of alleles observed per collection (where total number of SNP alleles = $2n_{SNP}$); %poly = percentage of polymorphic loci (SNPs); H_O = average observed heterozygosity per locus; H_E = average expected heterozygosity per locus; HEW_P = probability as calculated in genpop after 10 000 Markov chains, averages across all loci given, bolded if significant; F_{IS} = inbreeding coefficient (Weir and Cockerham 1984); *based on polymorphic SNP loci within each collection. Collections that were not subsequently used in haplotype or homogeneity/structure tests (due to small sample sizes and likely presence of multiple sibs) are shown in yellow and green respectively.

Collection homogeneity testing and genetic structure outcomes

Based on the variable CR mtDNA gene region, overall tests of haplotypic homogeneity showed no significant differentiation ($P > 0.05$) across collections in either species. Similarly, F_{ST} comparisons (based on Weir and Cockerham's (1984) genetic distances) demonstrated no significant pair-wise haplotype differences among any of the *S. mokarran* collections (Table 8). A single F_{ST} comparison between *E. blochii* collections PNG16-17 and NT06-07 was significant after Bonferroni correction (Table 9).

Table 8. *S. mokarran* pair-wise mtDNA CR F_{ST}^{*} comparisons among collections where N > 5. Where significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collections	PNG15-16	NT15-17	NT12-14	NT09-11	NT06-08	NSW16-17
PNG15-16	*****					
NT15-17	-0.011	*****				
NT12-14	0.032	0.006	*****			
NT09-11	-0.005	0.021	0.002	*****		
NT06-08	0.014	0.016	-0.003	-0.013	*****	
NSW16-17	0.012	0.027	0.023	0.023	0.041	*****

Table 9. *E. blochii* pair-wise mtDNA CR F_{ST}^{*} comparisons among collections where N > 5. Significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collections	PNG16-17	PNG14-15	NT09-10	NT06-07	QLD04-05
PNG16-17	*****				
PNG14-15	0.023	*****			
NT09-10	-0.053	-0.058	*****		
NT06-07	**0.089**	0.037	-0.055	*****	
QLD04-05	0.006	-0.047	-0.071	0.087	*****

The ND4 gene displayed much lower relative levels of haplotypic variation in both species; there were no significant pairwise F_{ST} comparisons amongst collections observed in either species (F_{ST} data not shown).

There was no significant overall differentiation based on microsatellite genotype frequencies observed amongst the collections (exact test, $P > 0.05$) for either the *S. mokarran* or *E. blochii*. Based on the Weir and Cockerham (1984) distance method, F_{ST} pair-wise comparisons for the microsatellite data are shown in Tables 10 (*S. mokarran*) and 11 (*E. blochii*).

Table 10. *S. mokarran* pair-wise microsatellite F_{ST}^{*} comparisons based on genetic distances calculated among collections where N > 5. Significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collection	PNG15-16	NT15-17	NT12-14	NT09-11	NT06-08	WA	QLD03-04	NSW16-17
PNG15-16	*****							
NT15-17	0.031	*****						
NT12-14	-0.008	**0.095**	*****					
NT09-11	-0.015	**0.064**	-0.004	*****				
NT06-08	-0.001	**0.076**	-0.007	-0.010	*****			
WA	0.001	**0.085**	-0.024	0.006	0.010	*****		
QLD03-04	-0.005	**0.121**	0.020	0.013	0.018	-0.013	*****	
NSW16-17	0.015	0.008	**0.061**	**0.053**	**0.064**	**0.062**	**0.096**	*****

*negative F_{ST} values represent no differentiation (i.e. F_{ST} = 0.000)

Table 11. *E. blochii* pair-wise microsatellite F_{ST}^{*} comparisons among collections where N > 5. Significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collections	PNG16-17	PNG14-15	NT11-12	NT06-07	QLD04-05
PNG16-17	*****				
PNG14-15	**0.076**	*****			
NT11-12	**0.184**	-0.038	*****		
NT06-07	**0.144**	-0.006	0.031	*****	
QLD04-05	**0.199**	-0.013	0.011	-0.004	*****

*negative F_{ST} values represent no differentiation (i.e. F_{ST} = 0.000)

Based on the F_{ST} values (from the usat allele frequencies), the *S. mokarran* NT15-17 collection was significantly different to all collections aside from NSW16-17 and similarly, NSW16-17 was shown to be different to all other temporal collections from 2003 – 2012 (Table 10). This maybe a temporal effect. These two collections, sampled in Australia during 2015 – 2017 were not differentiated, displaying a low pair-wise F_{ST} value of 0.008 (representing unrestricted gene flow between the two collections) although they were shown to be different to the other collections (albeit, while the F_{ST} values were significant, F_{ST} ranges from 0.000 to 1.000 with 1.000 indicating complete differentiation). The three other NT collections were not different to each other, nor to the WA, QLD or PNG collections. The samples from PNG in 2015 – 2016 were also not different to any other samples, although this collection was represented by only seven individuals.

In contrast, the only collection that was differentiated (based on F_{ST}) to all others in the *E. blochii* comparisons was PNG16-17 (Table 11). This collection from the Sepik River, in the northern region of PNG (the Sepik River feeds into the Bismark Sea) was also different to the other PNG collection (PNG14-15). Contrastingly, the largest *E. blochii* collection, PNG14-15 was not shown to be significantly different to the other Australian collections.

A more robust analysis of the overall genomic diversity observed in these two hammerhead species was provided by the nuclear SNPs. The thousands of SNP loci (> 3600) detected relative genomic homogeneity among all *S. mokarran* collections. A global exact test of non-differentiation (in SNP allele frequencies) was non-significant (P > 0.05), a low G''_{ST} = 0.034 was observed from the *S. mokarran* collections and a non-significant global F_{ST} of 0.003 (P = 1.000) was recorded. In contrast to the usat data (based on eight non-species-specific loci),

all pairwise F_{ST} values (based on the SNP data) were low and non-significant; the negative values represent no differentiation (Table 12).

Table 12. *S. mokarran* pair-wise SNP genetic differentiation F_{ST}^{*} comparisons among collections where N > 5. Where significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collections	PNG15-16	NT15-17	NT12-14	NT09-11	NT06-08	QLD03-04	NSW16-17
PNG15-16	*****						
NT15-17	0.006	*****					
NT12-14	0.004	0.000	*****				
NT09-11	0.001	-0.004	-0.005	*****			
NT06-08	-0.083	-0.077	-0.078	-0.049	*****		
QLD03-04	0.005	0.008	0.008	0.002	-0.085	*****	
NSW16-17	-0.017	-0.088	-0.027	-0.010	-0.039	-0.012	*****

*negative F_{ST} values represent no differentiation (i.e. F_{ST} = 0.000)

Likewise, when the *E. blochii* PNG16-17 collection was not included (SNPs detected a high inbreeding value for this collection, which may represent either inclusion of sibs or poor genotyping), there was no strong genetic heterogeneity detected in the six *E. blochii* collections. A global exact test of non-differentiation (in SNP allele frequencies) was non-significant (P > 0.05), a low G''_{ST} = 0.011 was observed; a non-significant global F_{ST} of 0.004 (P > 0.01) was recorded. All pair-wise comparisons (Table 13) showed homogeneity, indicating no strong barriers to gene flow among the Australian and PNG collections and locations. However, the caveat on this data is the number of collections and sample sizes in the *E. blochii* were smaller and biased towards PNG (i.e. > 51% of individuals were from the PNG15-16 collection, this is an important as the data is clearly biased towards individuals not sampled within Australian waters). When the PNG16-17 collection was included, all pairwise comparisons with the other six collections were significant (ranging from F_{ST} = 0.411 – 0.535, including with PNG14-15 (F_{ST} = 0.411)), data not shown here.

Table 13. *E. blochii* pair-wise SNP genetic differentiation F_{ST}* comparisons among collections where N > 5. Where significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction

Collections	PNG14-15	NT13-14	NT11-12	NT09-10	NT06-07	QLD04-05
PNG14-15	*****					
NT13-14	0.003	*****				
NT11-12	0.004	0.003	*****			
NT09-10	-0.012	-0.021	-0.007	*****		
NT06-07	-0.055	-0.084	-0.050	-0.016	*****	
QLD04-05	-0.001	-0.009	0.001	-0.001	-0.014	*****

The final genetic treatment of the nuclear marker data was spatial analysis of principal components. Provided in Figures 12-15 are the alpha corrected DAPC outcomes for the genetic (i.e. microsatellite) and genomic (i.e. SNP) data in *S. mokarran* and *E. blochii* as

outlined in Table 6. As can be seen in Figures 12 and 13, there was one central genetic group for *S. mokarran*; there was no clear separation of collections based on the usat data; individuals and collections were clustered over each other, driven by one main discriminant function. In slight contrast, based on SNPS, the small number of QLD03-04 individuals were slightly offset to the central cluster of *S. mokarran*, however the pairwise F_{ST} comparisons between this and the other *S. mokarran* collections were all less than 0.009 and all non-significant.

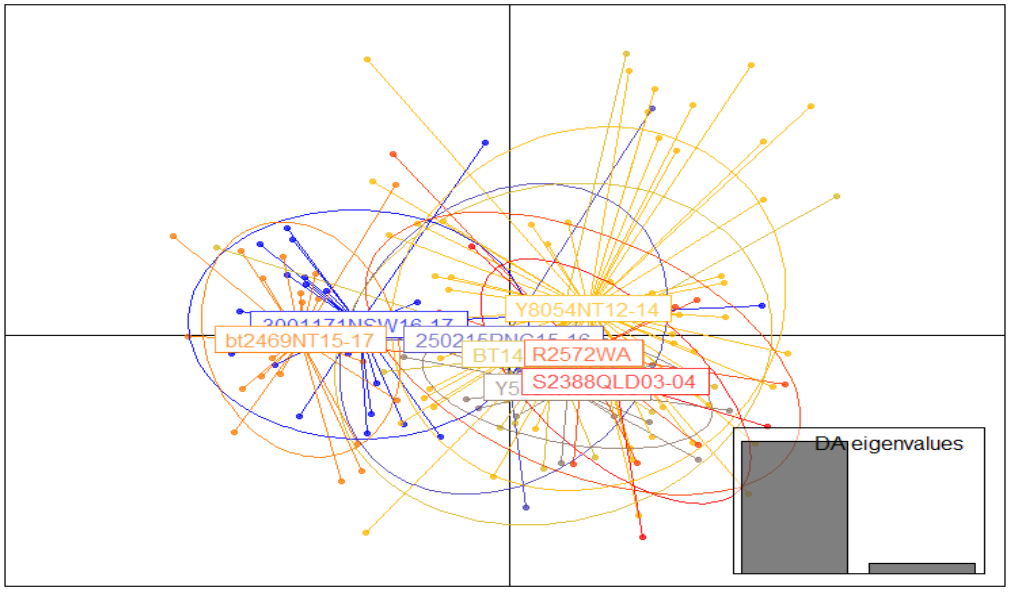


Figure 12. Discriminant analysis of principal components with priori grouping corresponding to the sample collections of *S. mokarran*. Scatter plot of based on 8 microsatellite loci, where collection sample sizes N > 5.

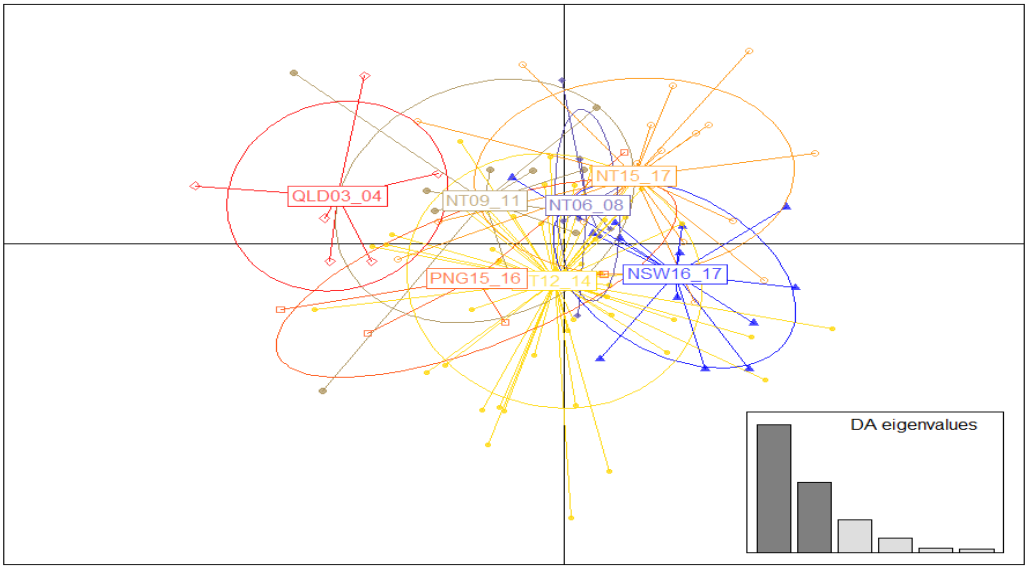


Figure 13. Discriminant analysis of principal components with priori grouping corresponding to the sample collections of *S. mokarran*. Scatter plot of based on 3 655 SNP loci, where collection sample sizes N > 5

A tighter, central cluster of *E. blochii* individuals (based on the usat data) is shown in Figure 14 - the PNG16-17 collection was located to the right of the central area with the other smaller *E. blochii* collections overlapping each other. Several individuals of the PNG16-17 collection extend into the RHS quadrant; this spatial clustering was reflected in the significant pair-wise F_{ST} comparisons.

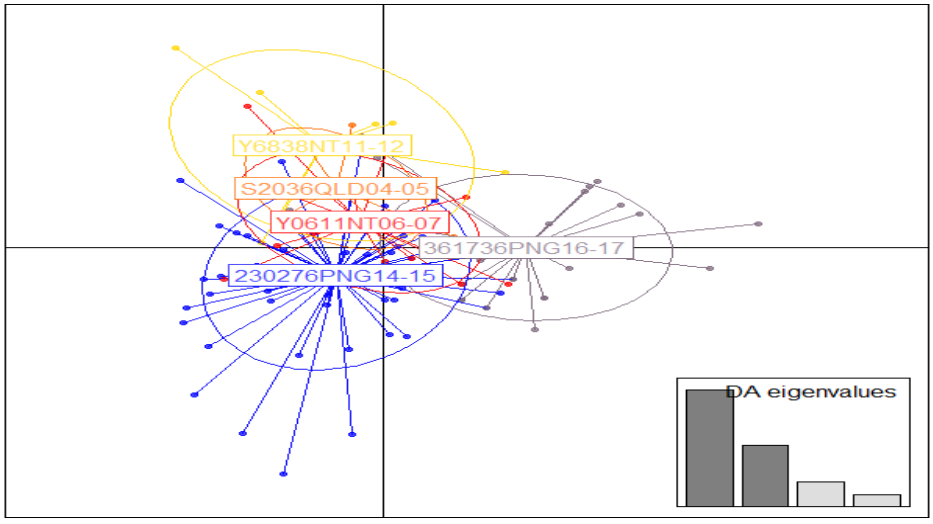


Figure 14. Discriminant analysis of principal components with priori grouping corresponding to the sample collections of *E. blochii*. Scatter plot of based on 8 microsatellite loci, where collection sample sizes N > 5.

Figure 15 shows the *E. blochii* DAPC based on the SNPs. Most of the variation between the *E. blochii* collections was described by two discriminant functions and no collection clustered well outside of the central grouping. This contrasts with the resulting DAPC when all *E. blochii* collections (including PNG16-17) was undertaken.

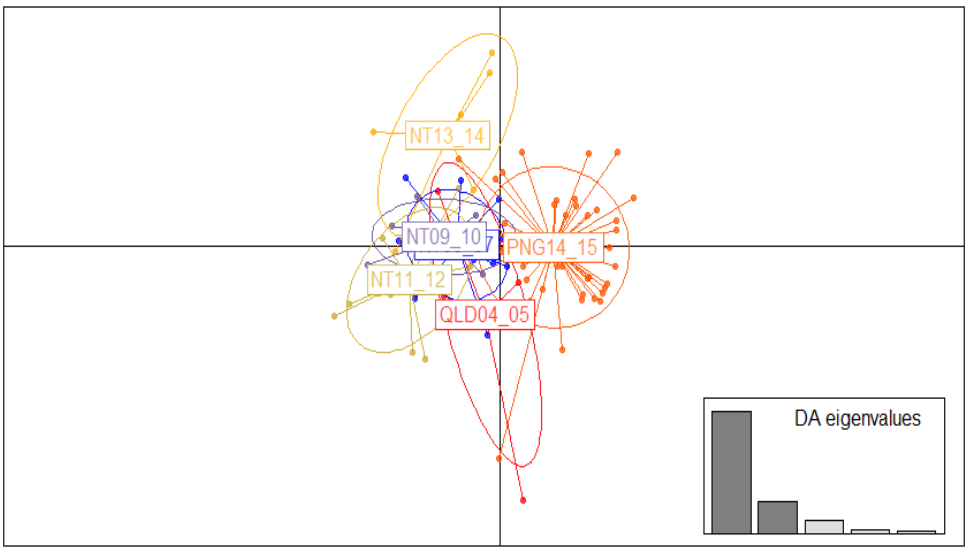


Figure 15. Discriminant analysis of principal components with priori grouping corresponding to the sample collections of *E. blochii*. Scatter plot of based on 5 229 SNP loci, where collection sample sizes N > 5 and PNG16-17 not include.

Overall, the non-model clustering in the DAPCs reflected the observations from the *F-statistics* and the genetic homogeneity among collections of both hammerhead and hammerhead-like species in Australian waters.

3.2.3 Discussion

This is the first instance of population genetics/genomics analyses undertaken in *S. mokarran* and *E. blochii* from multiple locations in Australia and Papua New Guinea. The genetic diversity, collection homogeneity and structure analyses were based on three different genetic markers. Importantly, this study provides new information on the connectivity of the two species in Australian waters. In the absence of extensive physical tagging or parasite information, the genetic knowledge outlined here provides the only connectivity assessment for *S. mokarran* and *E. blochii*, with spatial collections of individuals from both species considered part of wider northern Australian populations. Despite limited spatial and depauperate temporal collections, gene flow was detected in each species within the known ranges of northern Australia and Papua New Guinea.

The three classes of genetic markers reflected different levels of variation and modes of inheritance. The level of genetic diversity and connectivity was also directly correlated with sample size. Despite multiple mtDNA genes being screened in both species, as mtDNA is a circular genome inherited maternally, essentially only one marker was haplotyped in *S. mokarran* and *E. blochii*. Bi-parentally inherited genetic variation (detected as microsatellite alleles) were a magnitude greater than the mtDNA marker, while nuclear wide genomic variation, at thousands of SNP loci, provided the most stringent test of diversity and connectivity. As shown by Green (2019) in *S. lewini*, and while collections from only two countries were available for testing, the thousands of SNPs deployed in *S. mokarran* and *E. blochii* identified genomic wide diversity and connectivity across spatial scales that microsatellites did not (i.e. the genomic differentiation of the PNG16-17 *E. blochii* collection). Furthermore, as other shark studies have highlighted (Green 2019; Junge *et al.* 2019), it was important to undertake these diversity and connectivity analyses in the two species, rather than rely on or infer connectivity patterns from closely related species (such as *S. lewini*).

Analysis of mitochondrial genetic variation, based on the hyper-variable CR gene among collections of *S. mokarran* and *E. blochii*, showed that individuals sampled within Australian waters (primarily from Northern Territory, Queensland and New South Wales) were similar (i.e. not differentiated) from individuals in Papua New Guinea (the same outcome was observed in *S. lewini* from Indo-Pacific, Green (2019)). *S. mokarran* from the Gulf of Carpentaria and Western Australia were also included in the study, however these collections were represented by less than five individuals. Despite this, all *S. mokarran* individuals shared similar mtDNA genetics. Similarly, all *E. blochii* collections from Australia and Papua New Guinea were shown to be genetically similar at the CR gene – no significant structuring was detected, indicating maternal gene flow among the collections was relatively strong (both spatially and temporally). There was no evidence of structuring or collection differentiation across the northern locations of Australia and into Papua New Guinea.

The microsatellite data from *S. mokarran* suggested that alleles observed in individuals from NT15-17 and NSW16-17 were at different frequencies (thereby resulting in significant pair-

wise F_{ST} comparisons) to those observed in the other *S. mokarran* collections. However, these *F-statistics* are based on the assumptions of HWE in larger sample sizes. In NSW16-17 (average N = 20), 3/8 loci did not meet HWE and several loci in the NT15-17 collection (average N = 21) did not meet HWE assumptions. Non-model based DAPC on the microsatellite data however demonstrated that individuals from the *S. mokarran* collections formed a single cluster of Australian and PNG membership, with some radiations from the central cluster. In contrast, all F_{ST} pair-wise comparisons based on over 3 600 SNP loci demonstrated genomic homogeneity across the *S. mokarran* collections from Australia and Papua New Guinea. The DAPC of the 3 655 SNP loci showed individuals clustered to a central group; all AMOVA testing demonstrated negative *F*–statistics components, indicating the majority of SNP variance was observed within individuals rather than among collections.

Aside from comparison with the PNG16-17, the *E. blochii* microsatellite data showed a single significant pair-wise comparison between NT11-12 and QLD04-05 collections; however, these two collections were each represented by only six individuals – this is not a robust comparison of the individuals at these sampling locations. The F_{ST} comparisons based on the microsatellite data between all other collections was small or negative (and all non-significant). The SNP data showed no evidence of genetic heterogeneity among the *E. blochii* collections, indicating contemporary gene flow among the locations (aside from the Sepik River in PNG). *E. blochii* from the Sepik River (PNG16-17) show moderate genetic heterogeneity to the other collections, however this outcome requires confirmation. All collections clustered together in the DAPC.

This study found no evidence for consistent population sub-structuring across northern Australia (from Western Australia to New South Wales) and the Gulf of Papua. There was some support for a separation of *E. blochii* individuals from the Sepik River, Bismark Sea (based on neutral SNPs) albeit based on a smaller sample size of possibly related individuals. Given this, there was no strong evidence to suggest isolation by distance or that individuals in these areas should be considered part of separately managed stocks. The lack of structuring, particularly in *S. mokarran*, suggests these sharks are characterised by substantial gene flow (i.e. F_{ST} of 0.004) as detected by both classic and non-model statistical approaches.

The three genetic markers deployed in each species reflected relatively homogenous or panmictic collections both spatially and temporally (at least with respect to the *S. mokarran* individuals). As documented for *S. lewini* (Green 2019), the continental shelves of Australia and Papua New Guinea (connections to Indonesia were not tested in these two species) likely provide suitable habitat supporting dispersive behaviours across eastern, northern and western tropical-temperate areas resulting in gene flow amongst individuals.

Given the caveats and sample size restrictions outlined above and based on the conceptual models (that putatively explain patterns of distribution) of Chin et al. (2017), Models 1 and or 3 could describe the most likely explanation of movements of *S. mokarran* and *E. blochii* in the region. Chin et al. (2017) describe four hypothetical models of movement; 1. panmictic population throughout region; 2. limited movement; 3. continental shelf movement; 4. east-west Australian stock divide and continental shelf movements. Based on the multi-marker approach and data from this study, and as no Indonesian samples were considered, Model 3

(which suggests continental shelf movement enabling connectivity between Australia and Papua New Guinea) would be the preferred explanation for *S. mokarran* and *E. blochii*. If the differentiation of the *E. blochii* PNG16-17 collection is unequivocal (irrespective of the level of kinship in this collection) and the individuals here are significantly different (at least with respect to SNP alleles), this adds further support to Model 3.

These population genetic components provide a better understanding of the connectivity between individuals of two under described, yet biologically important hammerhead and hammerhead like species from the Australian region. This information is crucial for Australian conservation and biodiversity managers. However, further directed sampling and increased sample sizes are required to more fully understand population connectivity in these species.

4. THE USE OF PARASITES FOR DETERMINING HAMMERHEAD POPULATION STRUCTURE

Parasites have been used as biological 'tags' for identifying discrete populations in fishes since the 1930s (Catalano *et al.* 2014). Parasites can be used in this manner as certain parasites may only occur in specific areas, and thus, the composition and nature of parasites found in a fish can be used as proxy markers of different populations. Furthermore, the parasites found on fishes can themselves can be analysed further, for example, by examining their genetics, to discriminate different populations (Catalano *et al.* 2014). This part of the project involved examining parasites from hammerhead sharks collected by collaborators from NSW, QLD, and the NT, giving broad coverage across Australia.

4.1 Methods

4.1.1 Shark collection

Sharks were collected as part of various other projects and made available for parasite examination (Figure 16). Sharks collected from northern NSW were collected as part of the shark net trial program. These sharks were caught in gill nets set off beaches at Ballina and Evans Head over the summer of 2016/2017. Moribund and dead sharks were removed from the nets and frozen at the NSW DPI facilities. Sharks were defrosted; heads were removed, placed in bags and refrozen; the intestinal system (stomach, intestine and spiral valve) was removed, placed in bags and refrozen. Samples were transported frozen to Townsville where they were dissected. At the time of dissection, a small number of shark heads could not be dissected as they were fly blown (4 *S. mokarran*).

Sharks collected from North Queensland (NQ: Ayr and Cleveland Bay) and Far North Queensland (FNQ: Cairns) were collected as by-catch of commercial fishers in the region. Sharks were frozen whole and dissected at the James Cook University facilities, Townsville.

Sharks collected from the Northern Territory were collected as by-catch of commercial fishers in the various NT fishery zones (trawlers, net and line). Collection locations were grouped as: Gulf of Carpentaria (GoC), Arafura Sea (AS), North NT (offshore locations between the Tiwi Islands and the Wessel Islands), Darwin (DWN), Joseph Bonaparte Gulf (JBG) and the Timor Reef Fishery (TRF). Sharks were frozen whole. Some specimens were defrosted and processed with the heads and intestinal system bagged and refrozen; a number of sharks collected in the Darwin area (19 *S. mokarran* and 3 *S. lewini*) did not have their heads retained, thus only the intestinal system was dissected. Other specimens were processed at the time of dissection. Dissections occurred at the NT DPIR facilities in Darwin.

Sharks collected from Lombok, Indonesia, were collected by local fishers from areas to the south east of Lombok and landed at the Tanjung Luar fish market on the east coast of Lombok. Samples were collected from fishers at markets by staff from the Wildlife Conservation Fund (WCF). Approximately 30 *S. lewini* were collected and placed in a freezer for transport to the WCF Office in Mataram in preparation for dissection. Unfortunately, at the time of dissection, it was apparent that the sharks were not as fresh as they could have been; a number of these sharks were only examined for intestinal parasites as the gills

disintegrated on contact. About 10 sharks could not be examined at all. However, a further 10 sharks were obtained fresh from the Tanjung Luar fish market; for these sharks, gills and intestinal system were examined. Nasal fossae could not be examined for any of these sharks as fishers usually cut off the “hammer” at point of capture.

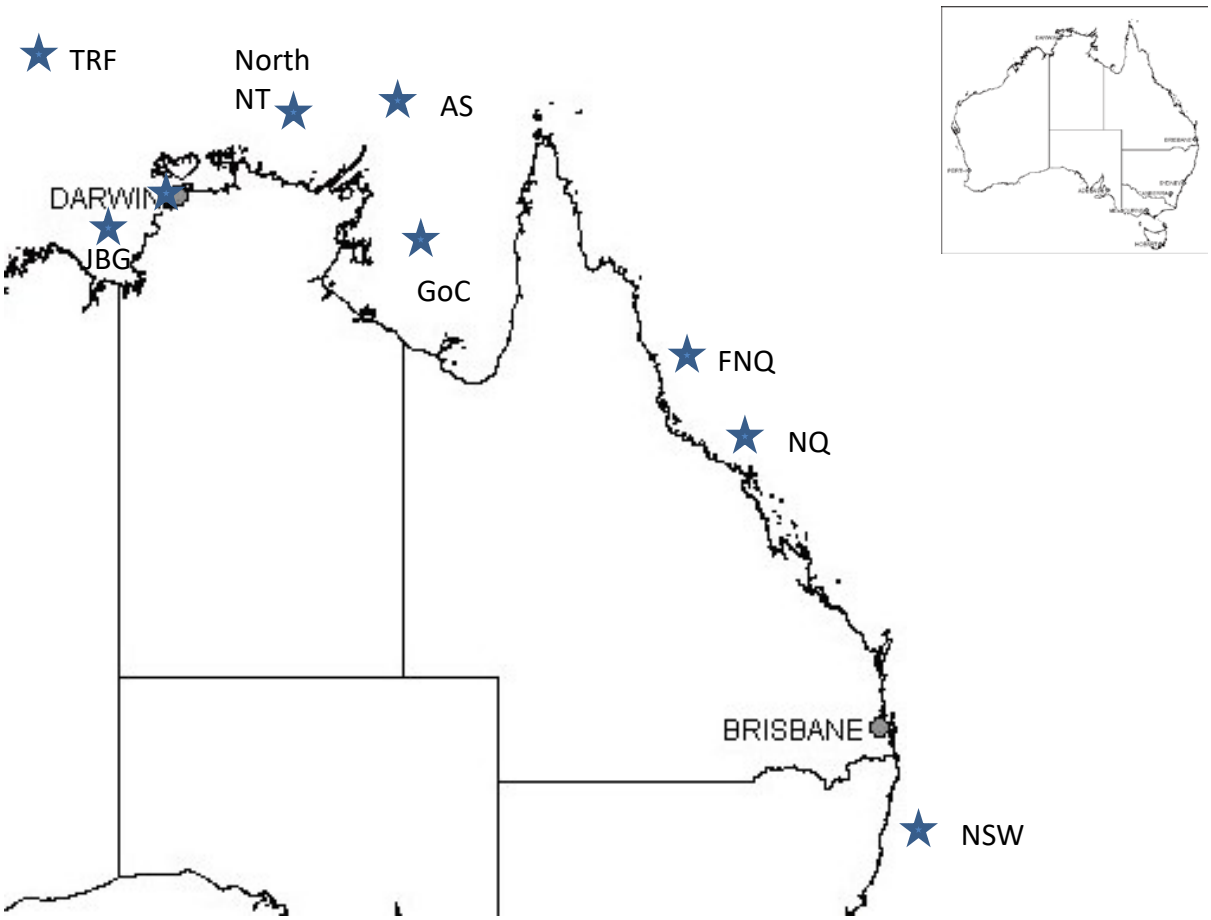


Figure 16. Map of collection locations for *Sphyrna mokarran* and S. *lewini* examined in this study. Locations in the Northern Territory include Gulf of Carpentaria (GoC), Arafura Sea (AS), North NT (offshore locations between the Tiwi Islands and the Wessel Islands), Darwin (DWN), Joseph Bonaparte Gulf (JBG) and the Timor Reef Fishery (TRF). Locations in Queensland include Ayr and Cleveland Bay (NQ) and Cairns (FNQ).

4.1.2 Parasite collection

Independent of the source of the sharks, processing of specimens was the same. The nasal fossae were dissected from the tips of the wings of the hammerhead sharks, opened along their length and placed into a jar of water. The jar was vigorously shaken and the nasal fossae removed and discarded (the nasal fossae of the first 20 sharks were examined under a microscope following the shaking but were not found to have any additional parasites, so this step was removed to aid in the speed of the dissection process). The washings were allowed to settle in the jar, the supernatant was carefully poured off and the remaining liquid

was then searched under a dissector microscope for parasites. Any parasites encountered were collected, identified to type and counted; parasites were preserved in 70% ethanol.

The 4 whole gills were individually dissected from each side (the anterior-most half gill was not dissected out); all gills were placed into a jar of water and processed as above. The intestinal system was separated at the junction of the stomach and intestine. The stomach was examined for dietary components and stomach contents were also searched for parasites. In stomachs with a large quantity of food or liquid, the stomach and its contents were placed into a jar and washed as above. In a number of sharks, a nodule was found in the stomach wall; these were usually infected with nematodes. For 8 sharks from the NT, a section of the stomach wall containing the nodule was excised and nematodes were removed, counted and preserved. The nodules were then placed into 10% BNF for future histopathology. The intestine and spiral valve were opened and placed into a jar to recover parasites as above.

4.1.3 Parasite identification

Parasites were separated into types based on morphological characters at the time of dissection and subsequently identified to as low a taxonomic unit as possible, including to genus or species. Parasites were sent to relevant experts to confirm identifications. A number of these parasites are new species and will be subsequently identified and described in collaboration with these experts. Parasites collected in Indonesia were retained by WCF staff for future deposition into a relevant museum collection.

4.1.4 Statistical analyses

Summary statistics of parasite data were compiled for each collection location, as well as for all sharks examined, by host species. This included mean abundance (total number of individuals of a particular parasite per collection location divided by the total number of hosts from that location examined, including uninfected hosts), mean intensity (total number of individuals of a particular parasite per collection location divided by the total number of infected hosts from that location examined), and prevalence (number of hosts infected with a particular parasite divided by the number of hosts examined per collection location, expressed as a percentage) for each parasite type, following the terminology of (Bush *et al.* 1997). Only parasites with prevalence greater than 10% in at least one of the locations were used in the analyses (Bush *et al.* 1990). All data analysis was done using R (R Core Team, 2017). Data was transformed (ln (x+1)).

Hosts are known to accumulate longer-lived parasite species with age. All parasite species that were selected on prevalence were examined for their correlation with host length (as an indicator of age) using linear models. Where length was significantly correlated with abundance, numbers greater than zero were adjusted to the mean host size (total length) using the methods described in (Moore *et al.* 2003). No adjustment was made if the parasite abundance was zero.

Within Australian samples, the dataset was grouped by geographical location. For *S. mokarran*, the locations were Northern NSW, NQ, Northern Territory waters (combined samples for JBG, TRF, North NT, AS, and GoC). As the majority of Darwin samples were

only the intestinal system, Darwin was not included in the analyses. A separate analysis of all locations (including Darwin) was conducted, using only intestinal parasites, but the results were not very clear. For *S. mokarran*, the locations were NQ, FNQ, GoC, AS, North NT, TRF, and JBG. A pregnant female was collected in the NQ samples; she was excluded from the analyses due to the potential effect of pregnancy on the acquisition of parasites via a reduced dietary intake. Additionally, as three of the five *S. mokarran* from Darwin had their heads discarded, all of the Darwin great hammerheads were removed from the analyses.

Spatial variation in parasite assemblages among regions and locations within regions were investigated using single-factor multivariate analysis of variance (MANOVA). Linear discriminant function analysis (LDFA) was conducted to examine variation in parasite assemblages across all locations and among locations within each region. Classification success for the LDFA was calculated by jack-knife cross-validation matrices. Classification success rates and an associated proportional chance criterion (the expected proportion of correct classification by chance alone) (Poulin and Kamiya 2015) was calculated for comparison with calculated reclassification success. To show the separation achieved for the groups in the analysis, the first two discriminant functions for each individual were plotted and the 95% confidence ellipses around the centroid means of the first two discriminant functions for each group in the sample using the ellipse package in R.

To test the effect of distance between locations on parasite assemblages, similarity in parasite communities was analysed using the Jaccard Index, which was calculated as c÷(a+b-c) where a and b are the species richness in the two communities being compared and c is the number of parasite species they have in common (Poulin and Morand 1999). Jaccard indices were then compared against distance between locations by Pearson correlation (see Poulin and Morand 1999).

The individual assignment of sharks was tested through a Bayesian approach across all fish. Classes and posterior probabilities were calculated by jack-knife cross-validation to assign individuals to most probable locations within each region and determine individual mis-classifications.

Only *S. mokarran* were collected in Indonesia. Initially, analyses for *S. mokarran* were conducted for Australian only samples. Then the Australian samples were compared against the results for the Indonesian sharks. Due to the issues with the collection of parasites from Indonesian sharks, all nasal fossae and gill parasites were removed from these analyses; thus, all results incorporating Indonesian sharks is only for parasites found in the intestinal system.

4.2 Results

4.2.1 Great hammerhead, *Sphyrna mokarran*

A total of 57 *S. mokarran* (mean TL 1584.3 mm (range 546-3838 mm) were examined. There were more male sharks dissected than females: 33 males (1849.7 (546-3838) mm) and 24 females (1219.5 (554-3255) mm). The sharks that did not include heads in their dissections were removed from the data set for future analyses; this left 34 *S. mokarran* (1526 (546-

3838) mm TL) (Table 14). The resulting sampling included more female sharks than males: 18 female (1111.8 (560-2620) mm) and 16 male sharks (1992.6 (546-3838) mm).

Overall, 32 of the 34 (94.1%) sharks were infected with at least one parasite. A total of 24 types of parasites were collected. Mean intensity of infection was 123.9 (4-2089) with a mean species richness of 6.2 (1-11). A total of 15/16 (93.8%) of male sharks were infected with a parasite; with a mean intensity of 80.1 (4-368) and mean species richness of 6.1 (3-9). A total of 17/18 (94.4%) of female sharks were infected with a parasite; with a mean intensity of 162.5 (5-2089) and mean species richness of 6.3 (1-11).

Low numbers of sharks were collected from a number of locations in the Northern Territory (GoC, AS, TRF, and JBG all had less than 5 sharks collected from each). The data for all NT locations were combined; analyses were then conducted at a State level (NSW, Qld, NT).

Of the 24 parasite types collected, three were removed from the analyses based on a prevalence of infection less than 10% at all locations (being State level) (Table 14). This had minimal impact on overall mean intensity (123.8 (4-2088)) and species richness (6.1 (1-11)) with prevalence staying the same. Male and female sharks showed a similar result: female sharks 94.4% prevalence, mean intensity 162.4 (5-2088) and mean species richness 6.2 (1-11); male sharks 93.8% prevalence, mean intensity 80.1 (4-368) and mean species richness 6.0 (3-9).

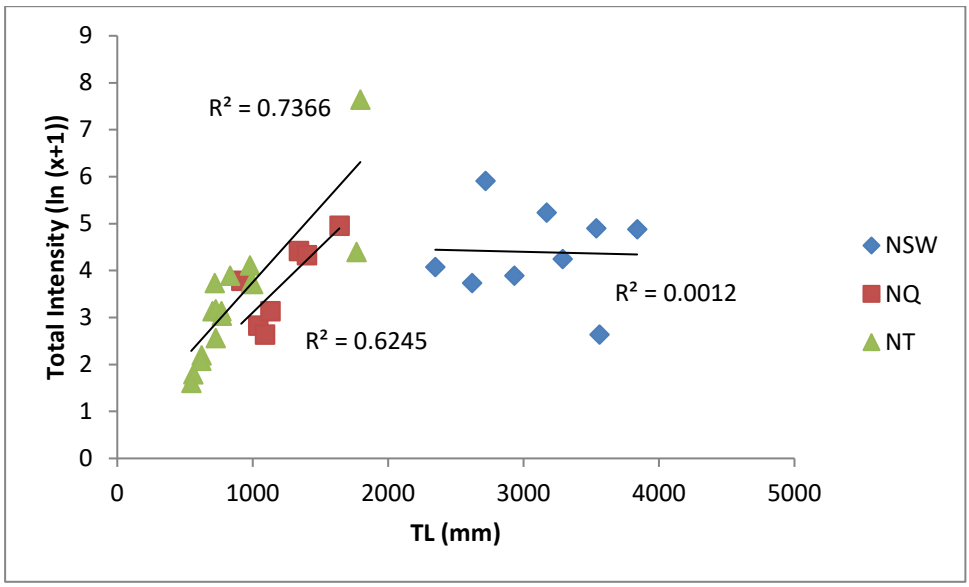
There was a significant relationship between TL of shark and total intensity of parasite infection overall (r^2=0.2651, df=30, p<0.001) and by sex (males r^2=0.3824, df=13, p<0.01; females r^r=0.3542, df=15, p<0.01). When examined by state, both north Queensland (r^2=0.6245, df=5, p<0.05) and the NT (r^2=0.7366, df=14, p<0.001) had a significant relationship between TL and total intensity of infection; NSW did not have a significant relationship (r^2=0.0012, df=7, p>0.05) (Figure 17).

Table 14. Parasites found infecting the 34 *Sphyrna mokarran* analysed in this study. Data is presented for individual locations within the Northern Territory (AS, North NT, JBG and TRF) although this data is combined for analyses within the study. Data is presented as mean abundance with prevalence in parentheses. Data presented in untransformed. Only parasites used in analyses are included.

	Parasite	NSW	NQ	AS	North NT	JBG	TRF	Overall
Gills	*Eudactylina* sp.	4.9 (78)	1.0 (14)					1.5 (22)
	Nemesis sp.	1.8 (67)			2.1 (33)	1.0 (50)	1.3 (67)	1.3 (36)
	Nessipus sp.	2.9 (78)	20.4 (100)		0.7 (11)	2.0 (25)	8.0 (67)	6.1 (50)
	Kroyeria sp.	1.8 (22)	0.6 (29)	6.0 (100)	3.2 (78)	2.8 (100)	2.0 (67)	2.1 (50)
	Irodes sp.		3.0 (71)	1.0 (100)	1.4 (67)	6.0 (50)	0.3 (33)	1.8 (42)
	Erpocotyle sp.	28.7 (33.3)	2.3 (86)		3.3 (33)	0.5 (25)	37.3 (67)	12.3 (42)
Nasal Fossae	*Kroeyerina* sp.	4.2 (78)	2.7 (86)	4.0 (100)	1.4 (33)	0.5 (25)	1.7 (67)	2.4 (56)
Intestinal System	Anisakid Nematodes	23.9 (89)	1.4 (29)	3.0 (100)	0.8 (33)	1.0 (25)	647.3 (67)	64.1 (47)
	Capillarid Nematode		0.1 (14)		0.1 (11)	0.8 (50)	3.0 (33)	0.4 (14)
	Corynosoma sp.*	0.3 (11)						0.1 (3)
	*Serrasentis sagittifer**		0.1 (14)		0.1 (11)	4.3 (75)		0.6 (14)
	Mixonybelinia edwinlintoni	42.3 (100)	7.4 (86)		3.3 (11)	9.3 (50)	7.0 (100)	15.3 (58)
	Paratobothrium balli	5.4 (44.4)	0.3 (14)					1.5 (14)
	Bombicyrrhynchus sphaerenaicus	0.1 (11)	0.1 (14)					0.1 (6)
	Dasyrhynchus pacificus	0.1 (11)			0.2 (22)			0.1 (8)
	Dasyrhynchus sp.		2.1 (29)				7.7 (33)	1.1 (8)
	Nybelinia sphyrnae		2.0 (57)	8.0 (100)	0.4 (22)	4.5 (75)	1.7 (67)	1.4 (33)
	Otobothrium sp.				0.2 (11)		11.7 (67)	1.1 (8)
	Trypanorhynch Cestode 1					0.3 (25)	0.3 (33)	0.1 (6)
	Tetraphyllid Cestode		12.3 (43)		1.9 (33)	1.5 (50)		3.2 (22)
	Opecoelid Digenean	0.1 (11)						0.03 (3)

*Larval stage

a)



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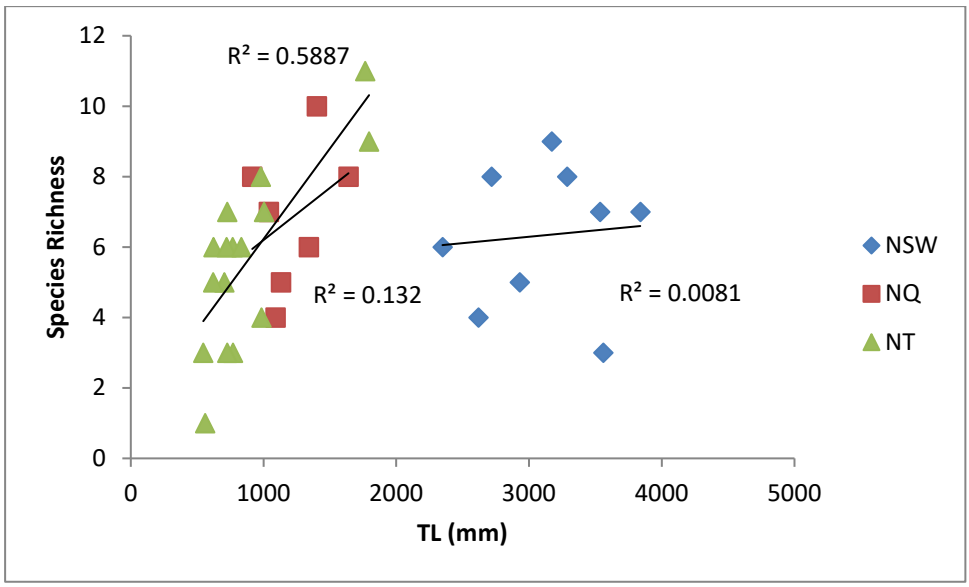


Figure 17. Pearson's correlation between Total Length of *Sphyrna mokarran* and a) total intensity (ln(x+1)) and b) species richness between the States as analysed.

Jaccard indices for the overall similarity in parasite communities between different states ranged from 0.4 (NSW v NT) to 0.63 (NQ v NT). When looking at the original collection locations within the NT, North NT had a strong similarity to JBG (0.8), and the TRF (0.69). JBG also had a strong similarity with the TRF (0.73). NQ had a strong similarity to all NT locations (JBG 0.65, North NT 0.61 and TRF 0.56) except AS (0.33). AS had low levels of similarity to all NT locations (all under 0.4). All NSW levels of similarity were below 0.5 with the exception on NQ (0.53). There was no significant relationship with Jaccard Index and

distance (r^2=0.4179, df=13, p>0.1), although the relationship was negative showing a decline in similarity with increasing distance.

The parasite assemblages differed significantly between the three states (MANOVA, Pillai's Trace=1.7443, df=40,26, p<0.001). LDFA of the overall parasite assemblage data successfully reclassified 71% of fish back to their collection location, which was almost double that expected by chance (39%). Graphically, all states were separate from each other (Figure 18). Reclassification success within state was 55.6% for NSW, 71.4% NQ and 77.8% NT (Table 15). Examination of individual mis-assignments showed no clear pattern; sharks from each state being mis-assigned to the other states across the range of lengths of sharks collected. A total of 10 of the 34 sharks were mis-assigned, with most (5) being mis-assigned to NT (mean TL 2949.2 (1130-3838)), then NQ (4; 1220.8 (620-1795)) and NSW (1; 1040).

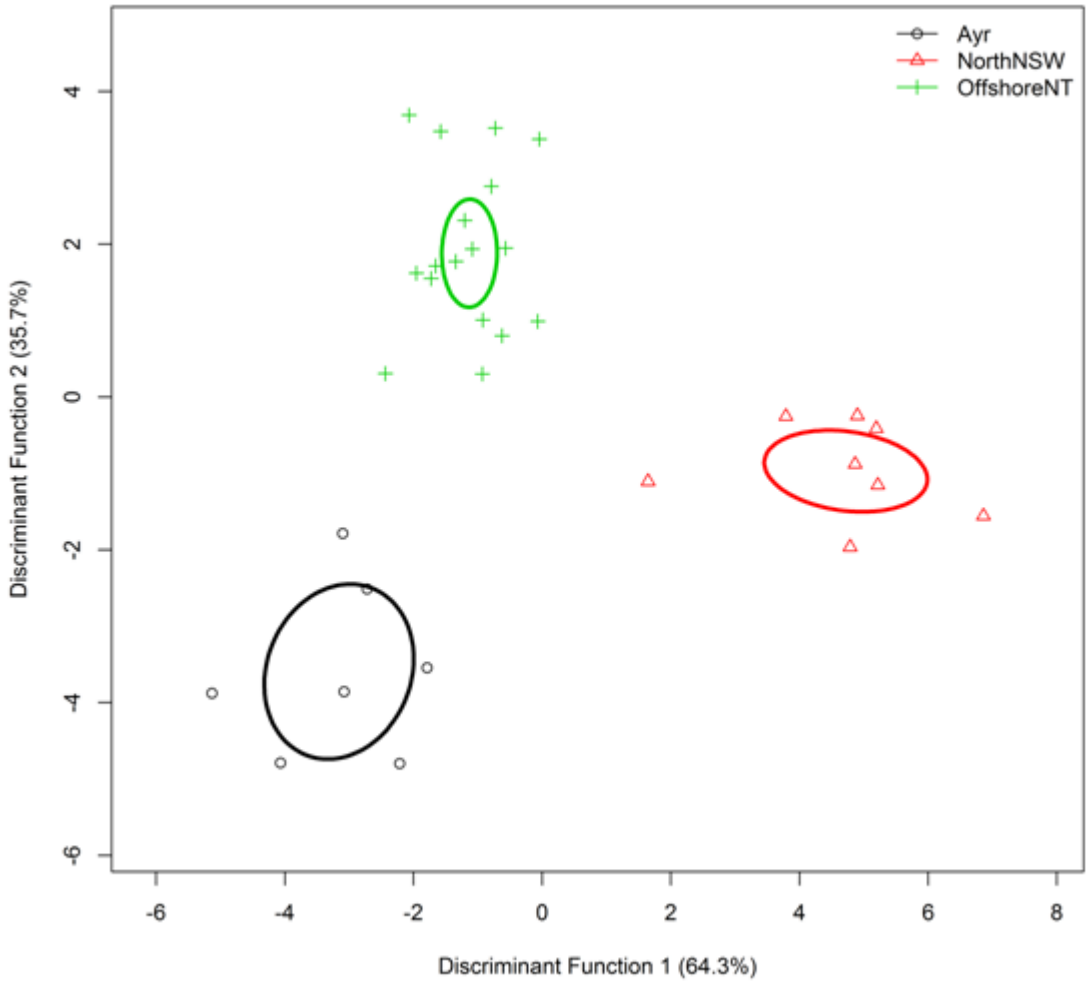


Figure 18. Plot of the first two discriminant function scores showing spatial variation in the parasite assemblage of *Sphyrna mokarran* collected from NSW, NQ and the NT. Ellipses are 95% confidence intervals around the group centroid for each region and data points represent individual sharks.

Table 15. Jack-knife reclassification success of the linear discriminant function analysis (DFA) for the overall parasite assemblage of *Sphyrna mokarran*. Samples from the Northern Territory were combined into a single set due to small numbers of sharks from a number of locations.

	Parasites
Location	% Correct*
NSW	55.6
NQ	71.4
NT	77.8
Total	70.6 (39.3)

*Poulin and Kamiya's (2015) proportional chance criterion is shown in bracket after the total classification success.

4.2.2 Scalloped hammerhead, *Sphyrna lewini*

A total of 209 great hammerheads (mean TL 776.7 mm (range 325-2630 mm) were examined in this study. There were more male sharks dissected than females: 125 male (815.8 (325-1860) mm) and 84 female (718.5 (443-2630) mm). Five sharks, collected from Darwin, were excluded from analyses as the heads were not collected and the pregnant female collected in NQ were removed from the data set for future analyses; this left 203 scalloped hammerheads (754.6 (325-1860) mm TL): 122 male (805.8 (325-1860) mm) and 81 female (677.5 (443-1320) mm) (Table 16).

Table 16. Host data for the *Sphyrna lewini* collected from Australian waters and Indonesian waters analysed in this study. Data is presented for male sharks, female sharks and overall for all locations examined. Total Length (TL) is given as the mean TL in mm, with the range in parentheses.

	Males		Females		Overall	
Location	No.	TL	No.	TL	No.	TL
NQ	11	131.4 (662-1860)	4	754 (490-858)	15	1165.1 (490-1860)
FNQ	8	1015 (640-1340)	4	1039.8 (790-1320)	12	1023.3 (640-1340)
GoC	39	836.8 (455-1065)	10	696 (456-856)	49	808.0 (455-1065)
AS	12	517.4)452-632)	15	498.5 (443-609)	27	506.9 (443-632)
North NT	29	699.6 (424-1434)	30	669.2 (446-1111)	59	684.2 (424-1434)
JBG	6	700.5 (551-1006)	10	728.5 (454-952)	16	731.7 (325-1026)
TRF	17	728.9 (325-1026)	8	736.3 (471-1012)	25	716.5 (454-1006)
LBK	9	1453.3 (1330-1670)	18	1949.4 (1230-2820)	27	1784.1 (1230-2820)

Overall, 186 of the 203 (91.6%) sharks were infected with at least one parasite. A total of 25 types of parasites were collected. Mean intensity of infection was 48.7 (1-445) with mean species richness of 4.5 (1-10). A total of 115/122 (94.3%) of male sharks were infected with a parasite with a mean intensity of 57.6 (1-445) and mean species richness of 4.8 (1-10). A total of 71/81 (87.7%) of female sharks were infected with a parasite with a mean intensity of 34.1 (1-165) and mean species richness of 4.1 (1-8).

Of the 25 parasite types collected, eight were removed from the analyses based on a prevalence of infection less than 10% at all locations (Table 17). This had minimal impact on overall mean intensity (48.6 (1-445)) with species richness and prevalence staying the same. Male and female sharks showed a similar result: male sharks mean intensity 57.5 (1-445) and female sharks mean intensity 34.0 (1-165).

There was a significant relationship between TL of shark and total intensity of parasite infection overall (r^2=0.4636, df=201, p<0.001) and by sex (males r^2=0.4843, df=120, p<0.001; females r^r=0.3993, df=79, p<0.001). When examined by location, all locations, except FNQ, had a significant relationship between TL and total intensity of infection (Figure 19; Table 18). Species richness had a significant relationship with TL at all locations except FNQ and GoC (Figure 19; Table 18).

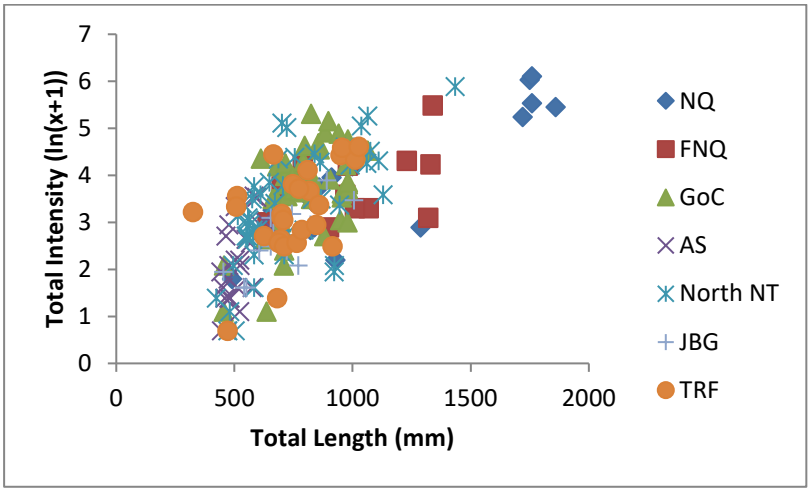
Table 17. Parasites found infecting the 203 *Sphyrna lewini* from Australia analysed in this study. Data is presented as mean abundance with prevalence in parentheses. Data presented in untransformed. Only parasites used in analyses are included.

	Parasite	NQ	FNQ	GoC	AS	North NT	JBG	TRF	Overall
Gills	*Eudactylina* sp.	0.53 (27)	0.17 (8)						0.05 (3)
	Nemesis sp.	2.87 (7)	0.25 (25)	0.73 (33)	0.04 (4)	0.46 (22)		0.52 (33)	0.61 (21)
	Nessipus sp.	8.07 (67)	1.25 (50)	0.39 (25)		0.56 (22)	0.21 (14)	0.41 (15)	1.0 (23)
	Kroyeria sp.	0.93 (13)	0.58 (17)	0.61 (14)	3.0 (67)	1.02 (25)	1.21 (36)	0.78 (26)	1.13 (28)
	Irodes sp.	8.07 (73)	5.50 (83)	0.84 (53)	0.96 (48)	0.42 (24)	0.29 (14)	1.11 (44)	1.54 (43)
	Erpocotyle sp.	12.6 (73)	3.25 (67)	4.33 (80)	0.30 (19)	4.61 (66)	3.21 (64)	7.26 (82)	4.73 (66)
Nasal Fossae	*Kroeyerina* sp.	0.27 (27)	0.25 (17)	0.10 (6)	0.44 (26)	0.17 (14)			0.17 (12)
Intestinal System	Anisakid Nematodes	71.07 (93)	42.25 (100)	42.27 (94)	2.85 (67)	23.49 (71)	12.43 (86)	15.96 (85)	28.14 (82)
	Capillarid Nematode	5.53 (47)		3.39 (69)	0.04 (4)	1.95 (41)	0.71 (43)	3.48 (63)	2.31 (44)
	Mixonybelinia edwinlintoni	0.27 (27)	3.33 (58)	0.10 (6)	0.04 (4)		0.43 (14)	0.04 (4)	0.28 (9)
	Dasyrhynchus pacificus	0.07 (7)		0.08 (6)		0.19 (7)	0.29 (29)		0.10 (6)
	Dasyrhynchus sp.	0.33 (13)				0.02 (2)			0.03 (2)
	Nybelinia sphyrnae	2.73 (67)	1.5 (75)	0.65 (29)	0.63 (33)	0.34 (25)	0.14 (14)	0.48 (30)	0.70 (33)
	Otobothrium sp.			0.51 (16)		2.56 (14)		0.52 (11)	0.94 (9)
	Tetraphyllid Cestode	6.67 (27)		1.73 (29)	1.0 (15)	4.05 (27)	1.64 (21)	2.70 (19)	2.69 (23)
	Unknown Cestode	0.13 (13)							0.01 (1)
	Caryophyllid Cestode			0.06 (6)		0.08 (7)		0.15 (11)	0.06 (5)

Table 18. Summary statistics of the Pearson's correlation for total length and both total intensity and species richness of the parasite assemblage for Australian *Sphyrna lewini* by location.

Location	Total Intensity			Species Richness		
	R^2	df	p value	R^2	df	p value
NQ	0.7823	13	P<0.001	0.6858	13	P<0.001
FNQ	0.1899	12	p>0.05	0.0266	12	p>0.05
GoC	0.2870	47	P<0.001	0.0269	47	p>0.05
AS	0.3389	21	P<0.01	0.2860	21	P<0.01
North NT	0.4309	45	P<0.001	0.1445	45	P<0.01
JBG	0.6796	12	P<0.001	0.2969	12	P<0.05
TRF	0.2410	25	P<0.02	0.3808	25	P<0.001

a)



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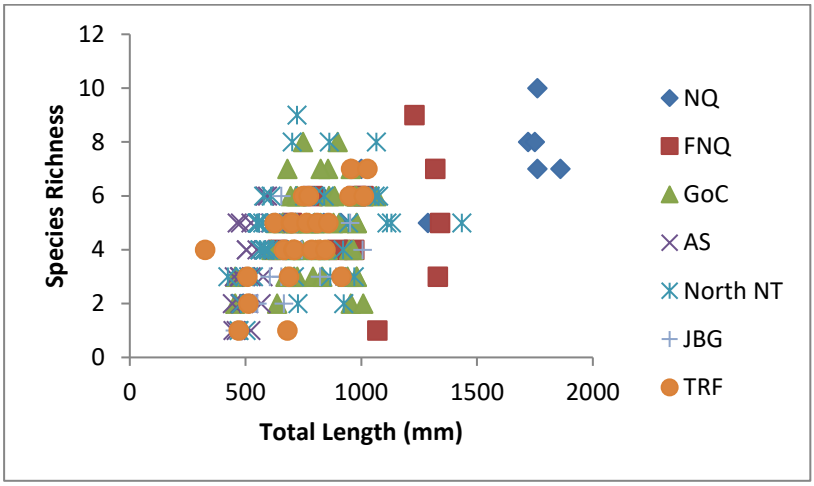


Figure 19. Pearson's correlation between Total Length of *Sphyrna lewini* and a) total intensity (ln(x+1)) and b) species richness between the 7 locations analysed.

Jaccard indices for the overall similarity in parasite communities between different locations ranged from 0.4 (FNQ v TRF) to 0.68 (GoC v North NT). Apart from two instances (GoC v North NT and GoC v TRF), all locations had a strong similarity over 0.5. Despite this, there was a significant negative relationship with Jaccard Index and distance (r^2=0.3661, df=19, p<0.01).

The parasite assemblages differed significantly between the three states (MANOVA, Pillai's Trace=1.4115, df=96,1116, p<0.001). LDFA of the overall parasite assemblage data successfully reclassified 42% of fish back to their collection location, which was double that expected by chance (19%). Graphically, results were mixed: NQ and AS sharks were separate from all other locations, with no overlap; GoC and TRF overlapped, as did FNQ and JBG with both these groups overlapped by North NT (Figure 20). Reclassification success within location ranged from 0% (JBG) to 59% (AS). Examination of individual mis-assignments showed some general trends: of the smaller sharks that were mis-assigned, the majority were mis-assigned back to North NT from AS, GoC, TRF and JBG. Interestingly the larger mis-assigned sharks from North NT were mis-assigned as GoC, JBG and TRF. There was no obvious pattern among the mis-assignments for the Qld locations, although there was more mis-assignments to the NT locations than to either of the Qld locations (Table 19).

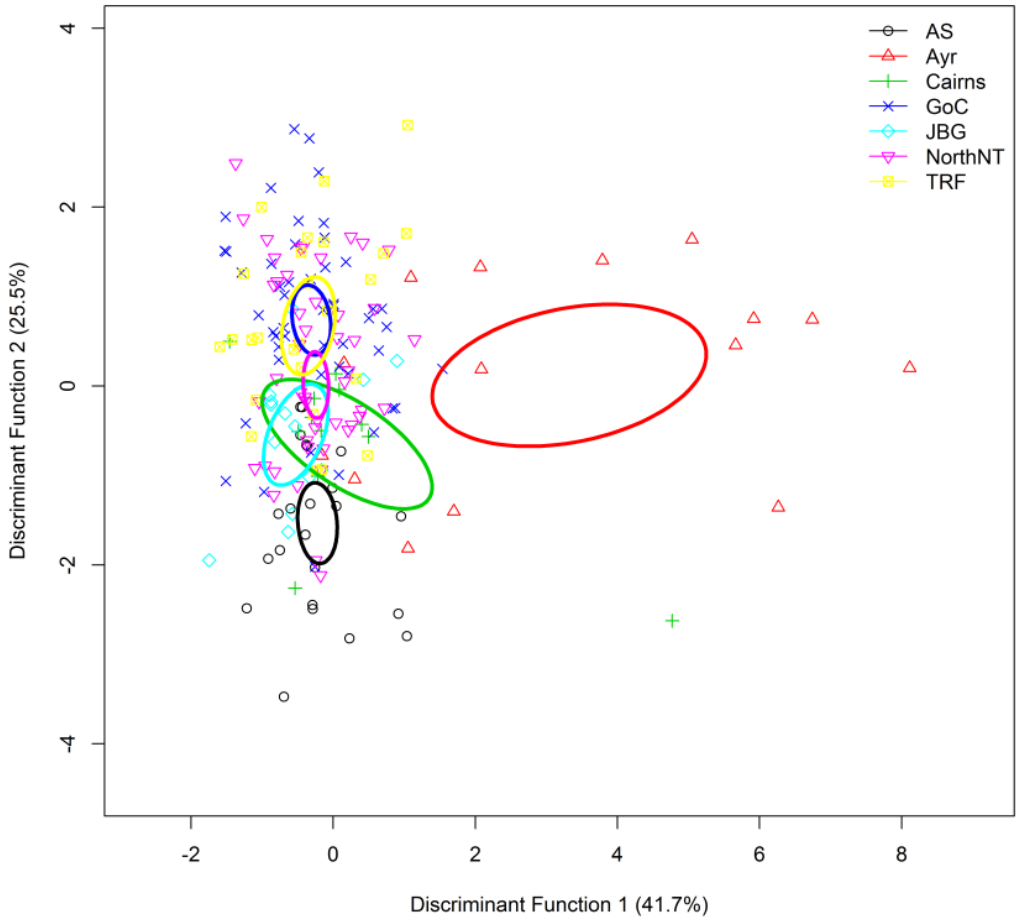


Figure 20. Plot of the first two discriminant function scores showing spatial variation in the parasite assemblage of *Sphyrna lewini* collected from 7 locations across the NT and Qld. Ellipses are 95% confidence intervals around the group centroid for each region and data points represent individual sharks.

Table 19. Jack-knife reclassification success of the linear discriminant function analysis (DFA) for the overall parasite assemblage of Australian *Sphyrna lewini*.

Location	Parasites % Correct*
NQ	46.7
FNQ	33.3
GoC	53.1
AS	59.3
North NT	49.2
JBG	0.0
TRF	11.1
Total	41.9 (19.2)

*Poulin and Kamiya's (2015) proportional chance criterion is shown in bracket after the total classification success.

4.2.3 Scalloped hammerhead - Indonesia

A total of 27 scalloped hammerheads (mean TL 1784.1 mm (range 1230-2820 mm) were examined in this study (Table 20). There were more female sharks dissected than males: 18 female (1949.4 (1230-2820) mm) and 9 male (1453.3 (1330-1670) mm). Overall, 21 of the 27 (77.8%) sharks were infected with at least one parasite. A total of 20 types of parasites were collected (Table 20). However, due to the issues outlined above, the gill parasites had to be discounted from all analyses.

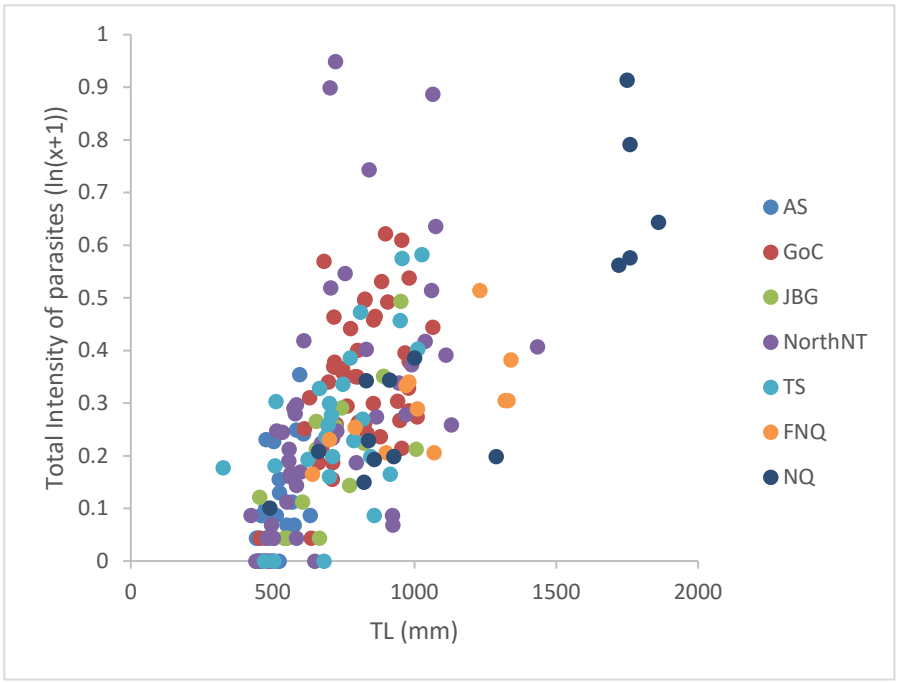
Table 20. Parasites found infecting the 27 *Sphyrna lewini* from Indonesia analysed in this study. Data is presented as mean abundance with prevalence in parentheses. Data presented in untransformed. Gill parasites are recorded but were not used in analyses. Parasites marked with an asterix were the parasites used in analyses.

	Parasite	LBK
Gills	Copepod type 1	0.04 (4)
	Gnathidae Praniza larva	0.07 (7)
	Adult Isopod	1.3 (15)
	Digenea Metacercaria type 1	0.04 (4)
	Erpocotyle sp.	0.56 (26)
	Monogenea type 1	0.04 (4)
Intestinal System	Anisakid Nematodes*	0.96 (33)
	Capillarid Nematode	0.26 (4)
	Hooded Nematode	0.04 (4)
	*Mixonybelinia edwinlintoni**	0.33 (11)
	Dasyrhynchus sp.*	0.07 (7.4)
	*Nybelinia sphyrnae**	0.89 (26)
	Trypanorhynch Type 3	0.07 (7)
	Trypanorhynch Type 4	0.07 (7)
	Trypanorhynch Type 5	0.04 (4)
	Tetraphyllid Cestode Type 2	0.19 (4)
	Procercoid cysts*	2.93 (26)
	Digenea Metacercaria Type 2	0.11 (4)
	Gorgorhynchoides sp.	0.04 (4)
	Rhadinorhynchidae	0.04 (4)

Of the 20 parasite types collected, six were removed from the analyses based on the issues with parasite collection outlined above. This meant that only 19 of the 27 sharks (70.4%) of the sharks were infected with at least one intestinal system parasite. Overall mean intensity of infection of the intestinal system parasites was 8.6 (1-36) with mean species richness of 2 (1-5). A total of 5 of the 9 (55.6%) male sharks were infected with intestinal system parasites with a mean intensity of 7.2 (1-22) and species richness 2.4 (1-5). A total of 14 of the 18 (77.8%) of female sharks were infected with intestinal system parasites with a mean intensity of 9.1 (1-36) and species richness 1.9 (1-5).

There was no significant relationship between TL of shark and total intensity of parasite infection overall (r^2=0.0169, df=25) or TL of shark and species richness (r^2=0.0256, df=25) (Figure 21); due to the low numbers of sharks examined and the high number of uninfected sharks, analyses were not separated by sex.

a)



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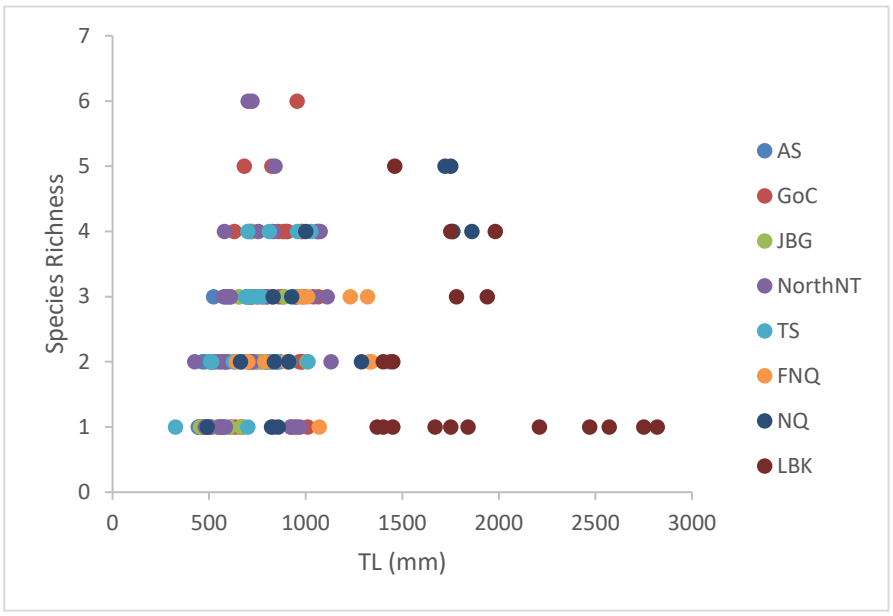


Figure 21. Pearson's correlation between Total Length of *Sphyrna lewini* and a) total intensity (ln(x+1)) and b) species richness between the 7 Australian and 1 Indonesian locations analysed.

Parasite assemblages differed significantly between the Indonesian and Australian samples (MANOVA, Pillai's Trace=1.1069, df=63, 1498, p<0.001) (Figure 22). LDFA of the overall parasite assemblage data successfully reclassified 35.7% of sharks back to their collection location, which was double that expected by chance (16%) (Table 21). Graphically, results

were very different to the analyses of Australian sites alone. LBK was separate from all other locations, however, the Australian sites now all had varying levels of overlap with each other (Figure 22). Reclassification success within location ranged from 0% (JBG) to 60% (GoC). Examination of individual mis-assignments showed that no Australian shark was mis-assigned to LBK, and the levels of mis-assignment between the Australian sites was higher. Of the LBK sharks, the greatest majority (16 of the 27) were mis-assigned to AS, with a few others mis-assigned to NNT (3) and CNS (1). All of the sharks reassigned to LBK were infected with procercoid cysts, which were unique to the LBK sharks

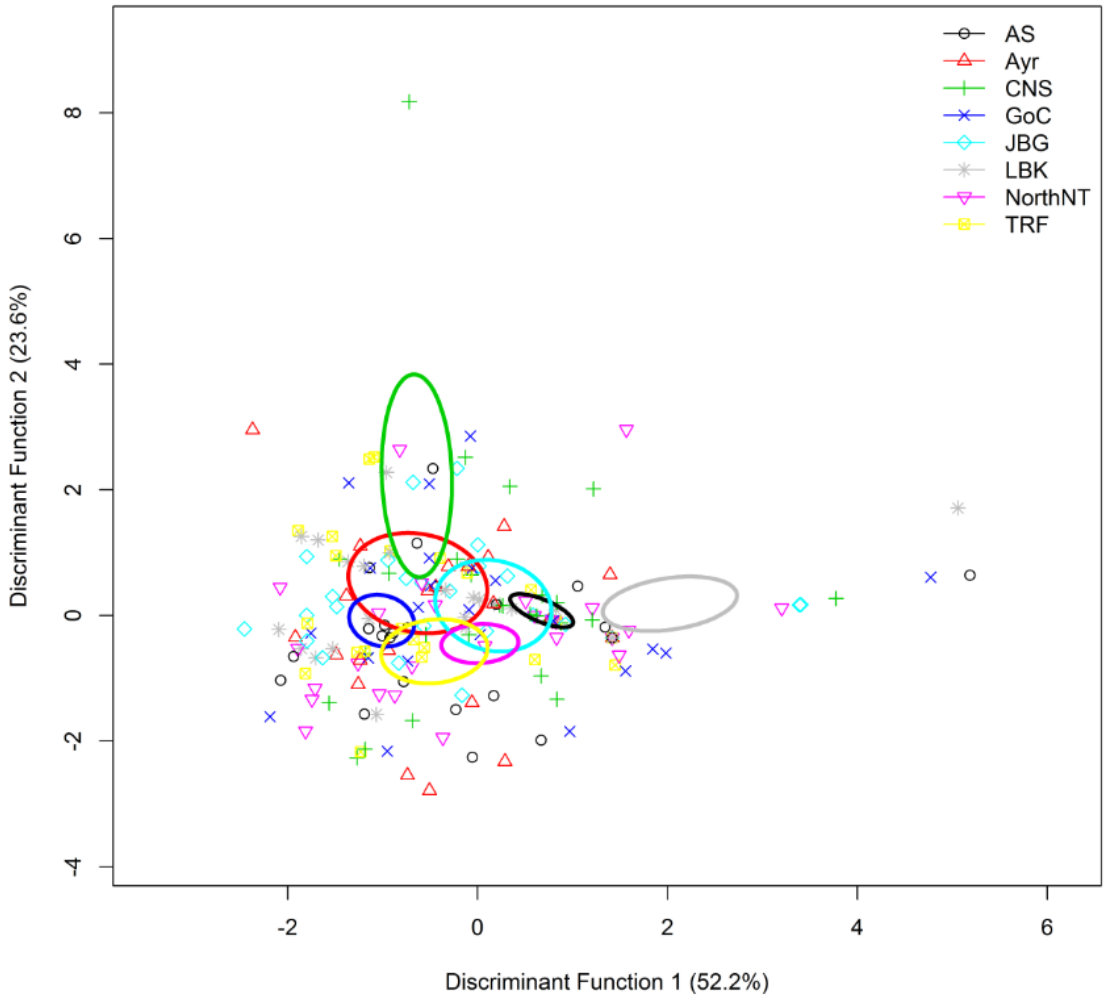


Figure 22. Plot of the first two discriminant function scores showing spatial variation in the parasite assemblage of *Sphyrna lewini* collected from 9 locations across the NT and Qld and 1 location in Indonesia. Ellipses are 95% confidence intervals around the group centroid for each region and data points represent individual sharks.

Table 21. Jack-knife reclassification success of the linear discriminant function analysis (DFA) for the intestinal system parasite assemblage of *Sphyrna lewini* from Australia and Indonesia.

Location	Parasites % Correct*
NQ	20.0
FNQ	58.3
GoC	59.6
AS	44.4
North NT	32.1
JBG	0.00
TRF	19.2
LBK	25.9
Total	35.7 (16.0)

*Poulin and Kamiya's (2015) proportional chance criterion is shown in bracket after the total classification success.

4.3 Discussion

4.3.1 Australian results

Analysis of the parasite assemblages of both *S. mokarran* and *S. lewini* showed some level of population structuring. Analysis of *S. lewini* was able to be conducted at the level of collection locations, which gave a more mixed result than for *S. mokarran* which could only be analysed at a State level. Although TL of sharks was taken into account in analyses determining population structure, it is obvious that length (or age as a factor of length) is playing a role in the development and maintenance of parasite assemblages in these sharks. As discussed below, there are appears to be considerable change in the parasite fauna of sharks over 200 cm TL, for both species, which roughly coincides with sexual maturity.

Parasites with a similar life span to their hosts are the best candidates for use as biological tags (MacKenzie and Abaunza 1998). "Permanent" parasites (encysted larval stages), a group of parasites often used in population structure studies of teleosts, can have a life span of years, with dead cysts able to be counted as an indication of previous infection; this group of parasites, however, was not present in any of the sharks dissected. For the parasites collected, they can be separated into external and internal parasites. Although the exact life cycles of these parasites remain unknown, the external parasites (copepods and monogeneans found on the gills and nasal fossae) are usually direct, requiring no other intermediate or paratenic hosts, whereas the internal parasites (found in the intestinal system) usually require a number of intermediate or paratenic hosts. Thus, external parasites are preferred as biological tags as they are directly related to the presence/absence of the host, whereas the internal parasite's distribution is reliant on the presence/absence of animals other than the host in question (MacKenzie and Abaunza 1998). Recent examination of fish parasite assemblages (Taillebois *et al.* 2017; Barton *et al.* 2018) have shown that the

use of the entire parasite community is able to provide high levels of population structuring. The majority of life spans of marine parasites remain unknown, but it is highly unlikely that any would have a comparable life span to their hosts, which have been estimated at 30-55 years (Piercy *et al.* 2007; Piercy *et al.* 2010).

Population structuring of great hammerheads

- There was high level support for the separation of *S. mokarran* into three populations based on the LDFA, with results obtained twice that expected by chance alone, showing that there was a significant relationship.
    - Although length of sharks was taken into account in the analysis, there was an obvious difference in biology with increasing shark length in the results.
    - Unfortunately, NSW sharks were all significantly larger than sharks collected in NQ and the NT. This provides a confounding factor in the interpretation of the results – are the differences due to a different location or to the different sizes of the sharks?
- The level of mis-assignment of NSW sharks to the NT may be due to similarities in their parasite assemblages based on the "offshore" or deep water locations of collection. However, three of the four NT sharks mis-assigned to NQ came from deep water locations (AS and TRF), with the fourth shark collected in the JBG.

Population structuring of scalloped hammerheads

Although the results were not as obvious as for *S. mokarran*, there was still evidence of separation of populations of *S. lewini* based upon their parasites.

- The scalloped hammerheads from NQ (Ayr & Cleveland Bay) were separate from all other populations
- The scalloped hammerheads from AS were separate from all other populations
- GoC and TRF formed a grouping, with almost identical parasite communities.
- FNQ and JBG formed a grouping, with some overlap of parasite communities.
- As for scalloped hammerheads, there were significant relationships between TL and total intensity of infection and species richness. Fortunately, there was overlap in lengths of sharks among all locations, except NQ which had generally larger sharks, although all sharks were under 200 cm TL. Thus all sharks examined were under approximately 10 years of age (Piercy *et al.* 2007). FNQ, with the larger sharks, was the only location to have a non-significant relationship between TL and parasite intensity which may indicate that there is a change in biology, as with the *S. mokarran*, at the point where the sharks reach 150 cm TL.

4.3.2 Indonesian results

Scalloped hammerheads - Indonesia

The sample of sharks from Lombok provided some interesting results but there are a number of issues that make the results difficult to interpret. The sharks were obviously not fresh. This

impacts the parasite samples in a number of ways – most of the external parasites will drop off the sharks and internal parasites will also start to leave and/or become degraded by the decomposition process of the host. Many of the shark parasites are soft bodied, so are rapidly degraded. This is noticeable in the much lower infections of the sharks and the high number of uninfected sharks, in comparison to Australian sharks of similar sizes (at least at the lower end). However, a number of parasites not previously encountered in Australian sharks were still found in the sharks from LBK – most noticeably the procercoid cestodes which were encysted in the stomach wall. Thus, degradation of the host could not have had an impact on their presence due to their encysted state.

A number of parasites were reported in LBK that were also reported in Australian sharks. This is not surprising as many parasite species have distributions reported to cover almost the entire geographical distribution of the host. Molecular analyses would help determine if there are geographical variation in the species across the range. However, the LBK samples were not able to be returned to Australia and the Indonesian partners do not possess the capabilities to conduct the genetic research.

Population structuring of scalloped hammerheads– Australia & Indonesia

As all external parasites had to be removed from analyses, this impacted the robustness of the overall population discrimination analysis due to lower numbers of species and the variability in the infection levels of the LBK sharks. However, we were able to show some separation of the LBK sharks from the Australian sharks.

- All Australian sites overlapped each other to varying degrees and the pattern of overlap changed from the Australian only analyses
- The amount of mis-assignment back to the North NT was reduced and the GoC increased in mis-assignments.
- All sharks that were reassigned to LBK were infected with the procercoid cysts in the stomach wall. Those without were mis-assigned elsewhere.

4.4 Conclusion

Of the size classes examined in this study, parasite data suggest limited connectivity among sampled locations. However, data did suggest a strong element of size in these results with parasite fauna related to size (and age). Therefore, the samples examined here could be indicative of limited movement in smaller size classes prior to broader movements and greater accumulation and mixing of parasite fauna as individuals reach sexual maturity.

Comparing the results collected in this study with the conceptual population structure models presented in Chin *et al.* (2017) (see Table 2) for *S. lewini*, parasitology results would support Model 3 (Continental Shelf Movement with some northward movement into eastern Indonesia). Prior to the collection of the LBK samples, the high level of reclassification of smaller *S. lewini* to North NT and GoC suggests that these areas could be the primary nursery areas in northern waters and there was a division between the eastern coast and northern waters by the Torres Strait land bridge (Model 3). Although there was overlap between sharks from northern Australia and Indonesia, there are a number of parasites that

do not overlap in these regions. If there is movement of sharks between Australia and Indonesia, it is minimal and potentially unidirectional and LBK specific parasites (like the procercoid cysts) have not been reported in Australian individuals.

If the same models were applied to *S. mokarran*, parasite data indicate Model 4 as the best fit with elements of Model 3 (continental shelf movement). Although there is clear separation of populations by State, it is possible that there is large-scale movement, over the lifespan of individuals from northern Australia to southern waters. However, the numbers are too low to make any definitive conclusions.

5. DISCUSSION

5.1 Scalloped hammerhead

The majority of this report was devoted to improving the understanding of the stock structure and connectivity of *S. lewini* within Australian waters and between Australian waters and adjacent nations. Three approaches were used – satellite tracking, genetics and parasites. The use of these different approaches provided different types of evidence about stock structure, differing mostly in the time scales over which differences are manifested. Tracking and parasites provided information indicative of short time scales (within a generation), while genetics provide information at evolutionary time scales. Based on the results of the three approaches (Table 22) there were different conclusions regarding stock structure. Satellite tracking and parasite analysis showed little or no evidence of cross-jurisdictional movements (either domestically or internationally), indicating that in the short-term *S. lewini* move over relatively small spatial scales. In addition, neither approach supported the hypothesis of a single well mixed Australian stock. In contrast, genetic analysis showed strong support for connectivity between *S. lewini* in northern and eastern Australia and both Papua New Guinea and Indonesia. The genetic data did not support the hypothesis of a single well-mixed Australian stock, with samples from Western Australia being significantly different from those from the rest of Australia, Indonesia and Papua New Guinea. Genetic samples also suggested that *S. lewini* from Fiji were significantly different from all Australian, Indonesian and Papua New Guinean samples. Overall, these results suggest limited movements of *S. lewini* between Australia and its regional neighbours, but that there are sufficient movements to maintain genetic mixing. The exception being Western Australian samples which appear separate from the rest of the region. Thus it is possible to examine the hypothesised connections posed in Chin *et al.* (2017) with this updated information (Figure 23). However, these results should be interpreted with caution due to the lack of large individuals in satellite tracking and parasite sampling.

Data generated during this project did not identify any use of Australian Marine Parks and did not provide any new information on biologically important areas for this species. Future research should attempt to locate and sample these size classes, especially large mature females, and include areas such as Australian Marine Parks to identify potential benefits of use of these protected areas.

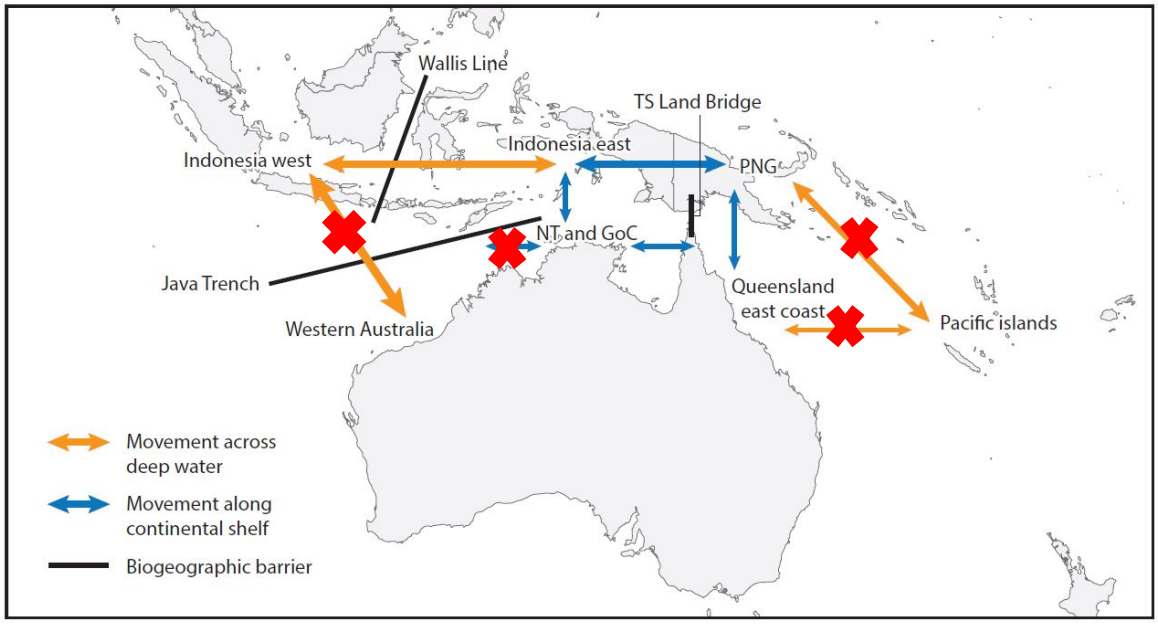
While the use of multiple methods helps better define stock structure and connectivity in populations, there are still limitations of these methods. Conclusions drawn from tracking and parasite approaches were limited by the size classes that were available to researchers. Both approaches were unable to sample the largest size classes, which have been shown to have the greatest likelihood of moving over long distances (Ketchum *et al.* 2014b). As such this project was not able to discount the hypothesis that larger size classes undertake regular movements between jurisdictions (domestic and international) that support the genetic evidence of population connectivity. In contrast, the evolutionary time scales which genetic data represent combined with the conserved nature of shark genetic structure can result in movement of a limited number of individuals producing a population level connection. Therefore, conclusions about connectivity of Australian hammerheads with regional neighbours should be considered using a precautionary lens.

Given the listing of *S. lewini* as Conservation Dependent under the Environment Protection and Biodiversity Conservation Act in 2018 the results of this project indicate that while the actions of international jurisdictions may have some effect on Australian stocks, it is likely that these effects are limited. As such management actions within Australian jurisdictions are essential to rebuilding scalloped hammerhead populations.

Table 22. Support for four stock structure hypotheses for hammerhead sharks (from Table 2) as proposed by Chin et al. (2017) from three different approaches.

Method	Stock structure hypothesis				Comments
	Panmictic across region	Limited movement	Continental shelf movements	East-west Australia stock divide and continental shelf movements	
Scalloped hammerhead					
Satellite tracking	No	Yes	Yes	Yes	No large adults tagged, still a question as to location of adult females in Australian waters
Genetics	No	No	Partial (WA, Pacific differs from rest of region)	Partial (WA differs from rest of Australia)	
Parasites	No	Partial	Yes	Partial	Differences in sizes between areas sampled
Great hammerhead					
Satellite tracking	No	Yes	Yes	Yes	No large adults tagged
Genetics	Partial	No	Yes	No	Best support for Model 3 (continental shelf movements)
Parasites	Not tested	No	Possibly	Not tested	Low sample sizes
Winghead					
Genetics	Partial (Some evidence northern PNG separate)	No	Yes	No	Best support for Model 3 (continental shelf movements)

Figure 23. Revised population connections for scalloped hammerheads based on the results in this report from hypothesised connections Chin et al. (2017).



5.2 Great hammerhead and winghead

Satellite tracking, genetics and parasite analysis provided data on stock structure and connectivity of *S. mokarran*. Genetics data and satellite tracking were limited to Australian samples, while parasite data was limited in quantity. Tracking and parasite data supported stock structure hypotheses that involved limited movement of individuals over short timeframes (months to years), while genetic data supported mixing across the extent of their distribution within Australian waters over longer timeframes (Table 22). Data generated during this project did not identify any use of Australian Marine Parks and did not provide any new information on biologically important areas for this species. Further research is needed to determine if the Australian stock is connected to our regional neighbours.

Only genetic data was available to assess the stock structure of winghead sharks. These results could not be used to distinguish any pattern beyond panmixia. The status and connectivity of this species in the region requires further study applying both genetic and non-genetic methods.

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Satellite tagging and tracking

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Parasite analysis

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8. APPENDIX A: DETAILED GENETIC METHODS AND RESULTS FOR GREAT HAMMERHEAD AND WINGHEAD SHARKS

8.1 Methods

The analyses and presentation of results/discussion for the *S. mokarran* and *E. blochii* are presented independently as outlined below. Sampling differed across the two species, but only samples from Australia and PNG were used in this study. A large proportion of the samples for both species were archival (the oldest were *S. mokarran* samples from Northern Territory, collected in 2003). The most recent samples were collected in 2016-2017 for *E. blochii* from PNG via collaboration with the CSIRO/ACIAR/PNG-NFA ACIAR, FIS/2012/102 study and *S. mokarran* samples sourced through the NSW shark netting program. Samples from Northern Territory and Western Australia were collected as part of professional fishing operations, samples from Queensland and the Gulf of Carpentaria were provided by Jenny Ovenden (UQ).

Despite contributions from collaborators, we did not achieve optimal sample sizes (either spatially or temporally) of 30 - 40 individuals per collection since most samples were obtained opportunistically. It was therefore not possible to rigorously test temporal stability or spatial connectiveness across all sampling locations. As outlined below, where collection sample sizes were less than five, diversity estimates are presented, however these are not included in connectivity or structure analyses. Samples were not evenly distributed across the species' ranges and for future studies, more reflective sampling through dedicated tagging and or fishing events are required. Additionally, as with the sampling and data analyses of *S. lewini* (see Green 2019), there was no paired physical tagging or parasite faunal interrogation for any of the individuals outlined below.

The DNA extraction of tissues (per species) was the same and this was completed as outlined below (the standard method of DNA extraction was using the Promega Wizard SV 96 genomic kit). The three markers were each deployed in *S. mokarran* and *E. blochii* samples, and the same analysis pipelines were applied to data for both species.

8.1.1 Sampling locations for *S. mokarr*an and *E. blochii*

A total of 215 *S. mokarran* samples were obtained from eleven locations from Australia and PNG, while 202 *E. blochii* samples were obtained from seven locations in Australia and PNG. Due to unplanned and opportunistic sampling, highly variable sample sizes, and as location metadata for some samples was missing, samples were arbitrarily placed into the following temporal and spatial 'groupings' (see Table 23 and Figures 24, 25 – known as 'collections' herein), with representative latitude and longitude points for these collections shown in Table 23.

Table 23. *Sphyrna mokarran* and *E. blochii* sampling locations and number of individuals sampled for current study.

Collection	Representative sampling date	Sample size	Representative latitude (°N)	Representative longitude (°E)	Collection designations
S. mokarran					
Papua New Guinea	2015-2016	14	-9.5376	146.7433	PNG15-16
Northern Territory	2015-2017	28	-12.4500	130.0600	NT15-17
	2012-2014	68	-12.4385	130.1589	NT12-14
	2009-2011	21	-11.0700	131.1100	NT09-11
	2006-2008	27	-13.4000	129.4100	NT06-08
	2003	7	-12.3476	130.3330	NT03
Gulf of Carpentaria	2005	3	-16.1320	138.7426	GoC05
Western Australia		12	-21.3730	114.5266	WA
Queensland	2012-2013	4	-18.6310	147.3010	QLD12-13
	2003-2004	9	-16.2921	145.5370	QLD03-04
New South Wales	2016-2017	22	-28.7870	153.6010	NSW16-17
E. blochii					
Papua New Guinea	2016-2017	35	-3.8500	144.5333	PNG16-17
	2014-2015	69	-8.0835	145.6506	PNG14-15
Northern Territory	2013-2014	6	-13.3200	129.4600	NT13-14
	2011-2012	18	-12.3100	130.1600	NT11-12
	2009-2010	19	-13.2000	129.5259	NT09-10
	2006-2007	43	-12.5100	130.1300	NT06-07
Queensland (QLD)	2004-2005	12	-14.1970	144.0020	QLD04-05

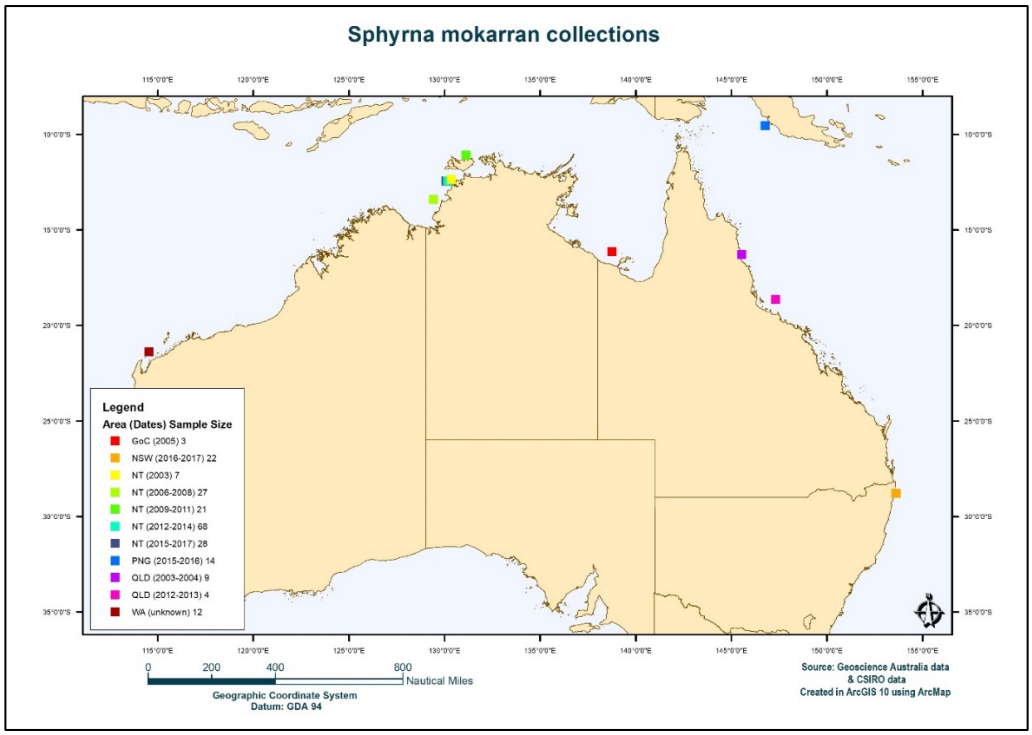

Figure 24. Sample collections for *S. mokarran* in Australian and Papua New Guinea waters. Legend shows locations and sample sizes.

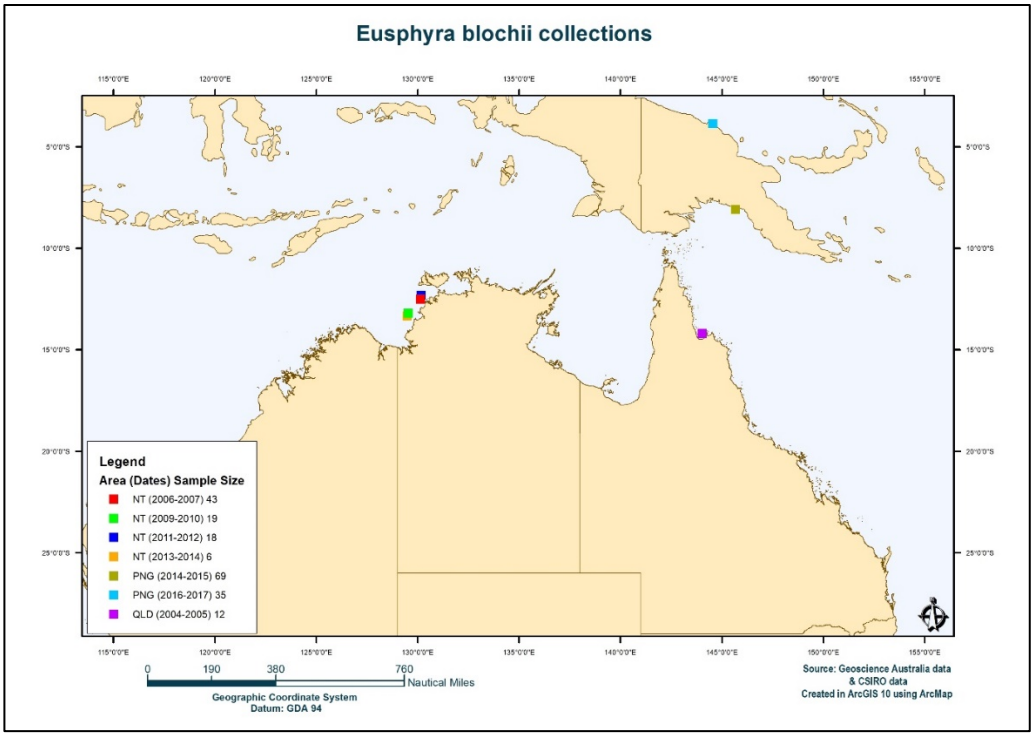

Figure 25. Sample collections for *E blochii* in Australian and Papua New Guinea waters. Legend shows locations and sample sizes.

DNA extractions for S. mokarran and E. blochii

Approximately 25 mg of tissue (fin clips/vertebrae/muscle) from samples outlined in Table 23, were DNA extracted according to a slightly modified Promega Wizard SV 96 well extraction protocol (https://au.promega.com/products/dna-and-rna-purification/genomic-dna-purification-kits/wizard-sv-96-genomic-dna-purification-system/; https://au.promega.com/resources/protocols/technical-bulletins/101/wizard-sv-96-genomic-dna-purification-system-protocol/).

The Wizard SV extraction protocol was based on a spin column format – the columns contained a silica resin that selectively binds DNA depending on salt conditions. Tissues were digested overnight with Proteinase K and total DNA was eluted in DNAse free water. DNA was quantified (ng/μL) on a Nanodrop 8000 (Thermo Fisher Scientific, Australia) with A260:A280 ratios reflecting DNA quality. Working stocks of DNA (at approximately 10 ng/μL) were stored at 4°C and undiluted stocks of archival DNA have been plated into 96 well plates and frozen in -80°C ultra-freezers at the CSIRO marine laboratories.

The extracted DNA was used in several downstream processes such as PCR, mtDNA gene sequencing, nuclear usat genotyping and nuclear SNP genotyping. Barcoding of the mtDNA cytochrome oxidase subunit I (COI) gene was also deployed when the genetic species identity of tissues was required or needed verification. The same two maternally inherited mtDNA genes were amplified and sequenced in *S. mokarran* and *E. blochii* samples. Prior to the commencement of this study, it was not known which gene fragment, if either, would provide enough sequence variation for homogeneity studies. Importantly, for the genetic analyses on *E. blochii*, new novel usat markers were developed. For *S. mokarran*, usat primers and loci developed for *S. lewini* (Nance et al., 2009) were used rather than developing species specific loci. Furthermore, GBS (for SNPs) was undertaken for the first time in these two hammerhead-like species.

For both species, mtDNA sequencing and usat genotyping were undertaken at the CSIRO marine laboratories, while the development and genotyping of SNPs was undertaken at the AGRF in Melbourne. All raw data processing and quality controls, and subsequent population genetics/genomics analyses were undertaken at CSIRO. Although the estimation of effective population size was not undertaken, the comparison of outcomes from the three marker types was a major focus. Mitochondrial DNA haplotype and microsatellite genotype frequencies will be deposited on the CSIRO DAP along with the raw and filtered SNP variant call files and associated strata data files in December 2019.

S. mokarran and E. blochii mtDNA screening

Two maternally inherited mtDNA gene regions were sequenced in *S. mokarran* and *E. blochii*. The CR and NADH dehydrogenase subunit 4 (ND4) genes were amplified and sequenced separately. For the CR gene, Pro-L and 12Sr primers were used (Palumbi *et al.* 2002), while the ND4 (Arèvalo *et al.* 1994) and H12293-Leu (Inoue *et al.* 2001) primers were used for amplification of the ND4 gene in both species.

mtDNA haplotype (CR and ND4) sequencing

PCR amplifications were performed in a 25 μL volume reaction mix and as per the amplification method in Appleyard *et al.* (2018) except with the primers outlined above, and annealing temperatures of 60°C for both pairs of primers. Amplified products were bi-directionally sequenced at the CSIRO marine laboratories on a 16 capillary ABI 3130XL DNA Autosequencer (Applied Biosystems™, USA) (as per amplification and sequencing protocols outlined in Appleyard et al., (2018) using BigDye® Terminator v3.1 Cycle sequencing kit (Life Technologies, USA)). Sequences were analysed in Geneious vers R8.1.4 (Biomatters Ltd, New Zealand). Removal of ambiguities in the beginning and end of each sequence was undertaken. Consensus sequences for each sample were assembled in Geneious. Subsets of sequences from each gene were compared with the NCBI databases (http://www.ncbi.nlm.nih.gov/, all GenBank+EMBL+DDBJ+PDB sequences) using the basic local alignment search tool (BLASTn) feature to ensure that the correct gene fragment in the target species had been amplified.

mtDNA haplotype statistical analyses

Consensus sequences per mtDNA gene and per species were analysed in MEGA v6.0 (Tamura *et al.* 2013) for best-fit substitutional models. Gene regions were subsequently analysed separately (i.e. not concatenated) as the two genes were described by different substitutional models, and not all individuals were sequenced successfully at both gene regions. Molecular diversity summary indices for each collection included: the number of haplotypes, the unbiased haplotype diversity corrected for sample size (h) and nucleotide diversity (π) (the mean number of differences between all pairs of haplotypes in a population) and were calculated in DNASP vers 6.12.01 (Rozas *et al.* 2017) and Arlequin vers 3.5. (Excoffier and Lischer 2010). Mitochondrial DNA haplotype frequencies for the two species are listed in Appendix A (Tables 1 & 3 – *S. mokarran*; Tables 2 & 4 – *E. blochii*).

To visualize haplotype networks among the collections, median-joining network analysis was undertaken in POPart v1.7 (http://popart.otago.ac.nz) (Bandelt et al., 1999). Following this, several methods for testing collection homogeneity and structure were undertaken. Global F_{ST} (for estimation of genetic differentiation overall) and among collections was calculated using pairwise F_{ST} in Arlequin. Each analysis consisted of 10 000 bootstraps, with *P* values corrected for multiple comparisons using sequential Bonferroni (Holm 1979; Rice 1989).

An Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) based on Euclidean distances was undertaken in Arlequin to assess hierarchical structure of the *S. mokarran* and *E. blochii* collections. In panmictic collections, most of the genetic variance is expected to arise from within samples; therefore, structure is assumed if most of the variance occurs among samples within collections or among collections.

S. mokarran and E. blochii nuclear marker screening

Species appropriate microsatellite and SNP loci were screened independently in *S. mokarran* and *E. blochii* individuals.

Nuclear microsatellite typing

There are no published *S. mokarran* usat primers or loci. Given the relative phylogenetic closeness of *S. lewini* and *S. mokarran*, and as this study had limited resources, it was decided that *S. lewini* usat primers would be used to screen for loci and alleles in *S. mokarran* samples. As part of the cross species testing of *S. lewini* usat primers in *S. mokarran*, two sets of multiplex reactions were developed here for *S. mokarran* individuals.

The *S. mokarran* individuals were genotyped using eight polymorphic usat loci described in Nance et al. (2009). After several rounds of optimisation and testing in a small panel of *S. mokarran* individuals, two master mixes were developed for the amplification of these published loci (MM1 GHH = SLE027; SLE018; SLE089; SLE086; annealing temperature 58°C; MM2 GHH = SLE038; SLE054; SLE081; SLE071; SLE077; annealing temperature 58°C) (see Nance et al., 2009 for primer details).

Additionally, prior to this study, there were no primers (i.e. usat loci) available for *E. blochii* nor were there any published usat primers from other closely related species. Therefore, during the current study, next generation sequencing was employed to develop a novel usat loci suite for the winghead sharks. The sequencing was undertaken at AGRF, with the resultant primer library screened *in-silico* at CSIRO.

The *E. blochii* individuals were genotyped using nine loci developed from the *in-silico* usat library. After several rounds of optimisation, testing for polymorphism and preliminary amplification consistency testing in a small panel of winghead shark individuals, the following unpublished loci were multiplexed together (MM1 WHS = Ebl02; Ebl03; Ebl05; Ebl06; Ebl14; annealing temperature 50°C. MM2 WHS = Ebl07; Ebl12; Ebl13; Ebl04; annealing temperature 50°C) (S. Appleyard pers. Obs.).

All PCR amplifications were undertaken in multiplex reactions with forward primers for the loci labelled with proprietary fluorophore dyes; 6-FAM, VIC, NED, PET (Applied Biosystems, USA). PCRs consisted of GoTaq® Colourless Master Mix (Promega), BSA, 10 μM of each individual F and R primer, and 20 ng/μL of template DNA in 25 μL reaction volumes. Each multiplex was undertaken in an ABI 9700 thermocycler (as used for the mtDNA gene amplification); PCR cycling conditions were as follows: initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 1 min, annealing temperature for 1 min 30 s (depending on master mix, see above), and 72°C for 1 min followed by a final extension cycle of 72°C for 10 min.

Three microlitres of the amplified products were then diluted in a mix of HiDi Formamide (Applied Biosystems) and water and denatured at 94°C for 2 min. Samples were run on an ABI 3130XL DNA Autosequencer (at the CSIRO marine labs) against an internal GeneScan™ 600 LIZ Size Standard (Applied Biosystems). Genotypes (per locus, per species) were visualised and scored using the *Microsatellite* plug-in program in Geneious. Allele frequencies for each locus per species were binned in Excel, and PGD Spider vers 2.1.1.5 (Lischer and Excoffier 2011) was used to produce input files for various downstream analyses.

Nuclear SNP typing

The commercial GBS service that AGRF offers was employed to develop ddRAD-based libraries per species (Peterson *et al.* 2012) leading to SNP detection. Based on preliminary investigations into appropriate restriction enzymes for SNP development (using 24 samples per species in the library establishment phase), ddRAD libraries were independently prepared at AGRF from submitted genomic DNA for *S. mokarran* (using *EcoRI* and *MseI*) and *E. blochii* (*PstI* and *HpyCH4IV*). AGRF then undertook independent batch GBS processing based on its' in-house/proprietary protocol which included:

- DNA digestions with restriction enzymes (see above)
- ligation of barcoded adapters (compatible with the restriction enzyme overhangs)
- size selection of pooled digested-ligated fragments
- amplification of libraries via PCR using indexed primers
- sequencing on an Illumina© NextSeq platform flow cell (Illumina Inc, USA) with 150 cycles in MID-output mode according to their in-house GBS methodology

AGRF then processed the raw reads from the NextSeq platform using their in-house bioinformatic pipeline and Stacks software v1.47, (http://catchenlab.life.illinois.edu/stacks/) (Catchen *et al.* 2013). Briefly this included:

- raw sequences were demultiplexed, checked for read quality and restriction site presence and trimmed; RAD-tags were analysed in Stacks (resulting in a separate FASTQ file for each sample) (using 'process_radtags' in Stacks)
- sequence reads were aligned into matching stacks/tags from which loci were formed and SNPs were detected ('ustacks','cstacks' 'sstacks' in Stacks); parameters used to define a 'stack' and resulting subsequent SNPs for each individual from the catalogue included: a minimum depth coverage of two to create a stack; disabling haplotype calls from secondary reads; one mismatch allowed between sample tags when generating the catalogue; a minimum of five reads to call a homozygous genotype and a heterozygote was called when the frequency of the minor allele in a stack was 0.05 - 0.1 across the entire dataset

AGRF provided the post processed data (following the Stacks pipeline) as raw and unfiltered SNP output in a variant call format (VCF) file. A VCF for each species was downloaded from the AGRF secure file transfer portal.

Individuals in the VCF file were renamed (taking out the indexing information from AGRF) using bcftools reheader (Li *et al.* 2009) and filtered initially using VCFtools v0.1.14 (Danecek *et al.* 2011) in CSIRO's Galaxy instance (version release 18.01), with initial high-level filtering undertaken by treating all individuals as arbitrarily belonging to one group. Filtering removed sites whose minor allele frequency (maf) was too low (as a result of sequencing or alignment errors) and kept variants that had been successfully genotyped in at least 50% of individuals. When multiple SNPs were detected on the same fragment, a single SNP was randomly chosen for analyses to avoid linkage disequilibrium between loci. Bi-allelic SNPs were

retained. The renamed and filtered VCF files, along with the *S. mokarran* and *E. blochii* strata/collection data and original VCF files from AGRF can be found at the DAP.

The resulting species VCF files were further filtered and converted (i.e. prior to population genomic analyses) using R-Packages (R version 3.5.1 and R-Studio vers 1.1.463 (R Core Team 2018); vcfR and dartR (Knaus and Grünwald 2017; Gruber *et al.* 2018) with an emphasis on filtering the SNPs for population structure when a limited number of individuals were sampled (i.e. maintaining individuals per population vs reducing the number of loci; see dartR manual, Gruber et al., 2018). For *S. mokarran*, this consisted of a). filtering out monomorphic loci; b). using one SNP per tag; c). filtering on call rate per individual and population > 0.70; d). ensuring loci with a maf > 0.025 were used; e). using loci in Hardy-Weinberg Equilibrium. For *E. blochii*, the same parameters were used except for filtering on call rate per individual was > 0.85.

Nuclear marker statistical analyses

The treatment and statistical analyses of the usat and SNP nuclear loci was the same; the only difference was in the number of loci. For microsatellites, up to nine loci per species were screened whereas in the SNP sets, there were thousands more loci. As outlined below, the statistical and graphical capabilities of R (including various analysis packages) were used to reformat input files, check for duplicates and undertake the post processing (following filtering) analysis of genomic data (i.e. R packages utilised were - pegas (Paradis 2010); adegenet (Jombart 2008; Jombart and Ahmed 2011); diveRsity (Keenan *et al.* 2013); radiator (https://thierrygosselin.github.io/radiator/authors.html); hierfstat (https://CRAN.R-project.org/package=hierfstat); mmod (Winter 2012); ade4 (Bougeard and Dray 2018); strataG (Archer *et al.* 2017) and genepop (Rousset 2008)). Additionally, PGDSpider and Arlequin were also used for file conversions, genetic/genomic diversity and connectivity analyses.

The resulting usat and SNP collection data sets were used to calculate population genomic diversity estimates (including allele frequencies, allelic richness, percentage of polymorphic loci, mean observed heterozygosity and mean expected heterozygosity (H_O and H_E respectively)) and deviations from Hardy-Weinberg Equilibrium (HWE) and linkage equilibrium (for usats) in diveRsity, hierfstat and Arlequin. An estimate of inbreeding across the collections (F_{IS}) at all usat loci was undertaken in GenePop.

Genetic assessments also determined if the collections were structured/differentiated into clusters or groups of closely related individuals. An assessment of genetic diversity/differentiation (G''_{ST})(Meirmans and Hedrick 2011) across the collections was undertaken in mmod (where G''_{ST} ranges from 0 to 1 - with 0 indicating no differentiation and 1 indicates that collections are segregating for differing alleles; with G''_{ST} corrected to account for the average within collection heterozygosity and the number of collections). Classic pair-wise population genetic differentiation estimates (based on F_{ST}) (Wright 1949) and the (Weir and Cockerham 1984) implementation)) were undertaken in Arlequin; values range from 0 to 1 with high F_{ST} implying considerable differentiation among collections. Exact tests in GenePop were then undertaken to find the collections that were significantly

different. The *F*-statistics describe the expected level of heterozygosity in a collection and are a measure of the correlation between genes drawn at different hierarchical levels in collections (Wright 1949). Significance for all tests was assessed following 10 000 permutations and *P*-values for each pair-wise comparison were corrected for multiple comparisons with a sequential procedure (sequential *P* = conventional 0.05 divided by number of tests per marker type) (Rice 1989). SNPs presented here were considered neutral markers.

An AMOVA based on Euclidean distances was undertaken in Arlequin to assess hierarchical structure of the collections. Analyses of variance were based on designated spatial and temporal groupings.

The above model-based methods of assignment for individuals are based on multi-locus genotypes and the expected probability of genotypes occurring in various collections. Assumptions behind these models include collections conforming to HWE and linkage equilibrium. However, often these assumptions may be invalidated particularly in small collections such as in the *S. mokarran* and *E. blochii* collections of this study. Therefore, a non-model-based method, Discriminant Analysis of Principal Components (DAPC), was used with the *S. mokarran and E. blochii* genotype data to assess the number of genetic groups.

As a robust alternate to Bayesian clustering methods like STRUCTURE (Pritchard *et al.* 2000), DAPC in adegenet (with the optimal number of clusters based on Bayesian Information Criterion (BIC)) was undertaken in the collections; utilising both the usat and SNP data sets. DAPC is a multi-variate, sequential method that identifies clusters of genetic variation maximised between clusters of individuals and minimised within clusters (Pritchard *et al.* 2000; Jombart *et al.* 2010). DAPC provides a determination of genetic clusters using synthetic variables (i.e. the discriminant functions) and derives probabilities of membership (i.e. the genetic proximity of individuals to the different clusters) into different groups. As outlined by Jombart *et al.* (2010), one third of the Principle Components were retained in the current DAPC analysis (i.e. α-corrected) so that discriminant functions were not overfitted.

8.1.2 Results

DNA quality and quantity varied greatly amongst *S. mokarran*and *E. blochii* samples. As many of the samples were not recently collected, and tissues had been stored in sub-optimal conditions (e.g. long-term storage in DMSO), some extractions resulted in poorer quality DNA. As a result of the opportunistic sampling of *S. mokarran* and *E. blochii*, samples sizes per collection varied greatly (Table 23) and, in some instances, did not provide useful numbers of animals per location; to mitigate this, where appropriate, some collections were combined. Where the sample sizes per collection (per marker type) were N < 5, genetic diversity estimates are given but these collections were not part of the primary genetic homogeneity or connectivity assessments.

Mitochondrial DNA cytochrome oxidase subunit I (COI) barcoding confirmed species identification where required. Several tissue samples from the Northern Territory, which had been labelled as *S. mokarran* were found to be incorrectly identified, and tissues from two presumptive *E. blochii* individuals were also incorrect.

mtDNA diversity in S. mokarran and E. blochii

On average, an 1120 bp portion of the CR and 810 bp of the ND4 gene regions were sequenced in *S. mokarran*. Smaller portions of the CR gene (523 bp) and ND4 (754 bp) were sequenced in *E. blochii*. The CR primers did not amplify and sequence as successfully in *E. blochii* samples compared to *S. mokarran*. Generally, the CR gene was more difficult to sequence in both species and required manual editing for consensus sequence generation.

Table 24 outlines the genetic diversity estimates for the two species, based on the two mtDNA genes. An average of 161 *S. mokarran* from 11 collections were successfully sequenced at the mtDNA genes, and 117 *E. blochii* from 7 collections were sequenced. Over a hundred CR haplotypes were observed in *S. mokarran* but many of these haplotypes differed by only one base pair. A less conservative approach of binning mtDNA haplotypes may be warranted here; this would reduce the number of haplotypes but not the distribution or frequency of the haplotypes. Future CR analyses will consider this treatment, but for the current report, haplotype frequencies are as per Appendix A. Half this number of CR haplotypes were observed in *E. blochii*. The number of haplotypes at the CR gene varied greatly in *S. mokarran* collections from H = 1 to H = 43 (observed in the smallest (NT03) and largest (NT12-14) collections respectively), while the number of CR haplotypes (with a smaller number of base pairs) in *E. blochii* collections varied from H = 2 to H = 23. The total number of ND4 haplotypes in *S. mokarran* and *E. blochii* was magnitudes smaller than that observed in the CR gene, at 4 and 5 haplotypes respectively. Overall, the number of observed mtDNA haplotypes depended on the collection sample size.

Table 24. Summary of mtDNA genetic diversity (averages given) across eleven *S. mokarran* and seven *E. blochii* collections.

		mtDNA CR (1120 bp)					mtDNA ND4 (810 bp)				
Species	Collection	N	S	H#	Hd##	π JC###	N	S	H#	Hd##	π JC###
S. mokarran	PNG15-16	7	10	5	0.857	0.0032	8	0	1	0.000	0.0000
	NT15-17	22	21	19	0.983	0.0048	26	2	3	0.219	0.0002
	NT12-14	59	32	43	0.974	0.0047	54	1	2	0.107	0.0001
	NT09-11	14	23	14	1.000	0.0056	17	1	2	0.118	0.0001
	NT06-08	20	20	18	0.989	0.0046	22	1	2	0.173	0.0002
	NT03	1	0	1	0.000	0.0000	1	0	1	0.000	0.0000
	WA	4	10	4	1.000	0.0046	7	0	1	0.000	0.0000
	Goc05	3	9	3	1.000	0.0053	2	1	2	1.000	0.0012
	QLD12-13	2	3	2	1.000	0.0026	2	1	2	1.000	0.0012
	QLD03-04	4	12	4	1.000	0.0058	9	1	2	0.222	0.0002
	NSW16-17	15	20	12	0.943	0.0035	22	1	2	0.416	0.0005
		mtDNA CR (523 bp)					mtDNA ND4 (754 bp)				
E. blochii	PNG14-15	32	159	23	0.964	0.0615	50	4	4	0.479	0.0011
	PNG16-17	14	88	14	1.000	0.0362	28	0	1	0.000	0.0000
	NT13-14	0	0	0	0.000	0.0000	6	0	1	0.000	0.0000
	NT11-12	2	13	2	1.000	0.0298	16	1	2	0.125	0.0001
	NT09-10	5	2	3	0.800	0.0022	12	0	1	0.000	0.0000
	NT06-07	19	9	8	0.813	0.0032	31	1	2	0.064	0.0001
	QLD04-05	9	106	7	0.944	0.0647	9	0	1	0.000	0.0000

N = number of individuals screened per marker, S = segregating/polymorphic sites, H# = number of haplotypes (based on Nei 1987), Hd## = haplotype diversity (average number of nucleotide differences per site between two sequences, Nei 1987), πJC### = Jukes-Cantor nucleotide diversity. CR haplotype data from collections that were not subsequently used in haplotype homogeneity or connectivity analyses (due to small sample sizes) are shown in yellow; homogeneity analysis of the ND4 haplotypes are not presented in this report

Figures 26 and 27 show the mtDNA CR gene median joining network analyses in *S. mokarran* and *E. blochii*, respectively. The large number of haplotypes observed in *S. mokarran* individuals is reflected in the branching nature of the network. The smaller number of haplotypes observed in both species at the ND4 gene can be seen in Appendix A, Figures 1 and 2. As the CR was the more variable of the two gene regions, genetic homogeneity and structure analyses of the mtDNA in the individuals and collections were based on the CR haplotypes.

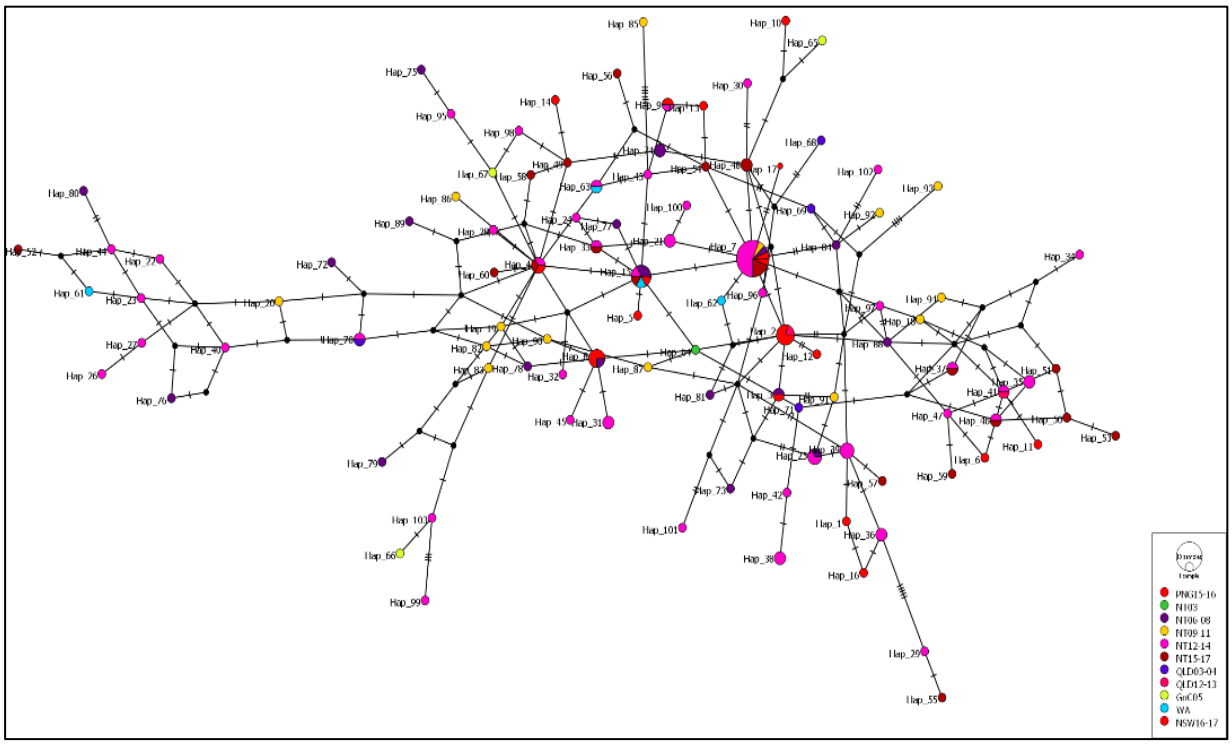

Figure 26. mtDNA CR median joining network analyses in *S. mokarran*. Haplotype frequencies are represented by the size of circles, sampling locations represented by colours; number of strokes joining nodes represents the number of mutations between two haplotypes across the gene fragment.

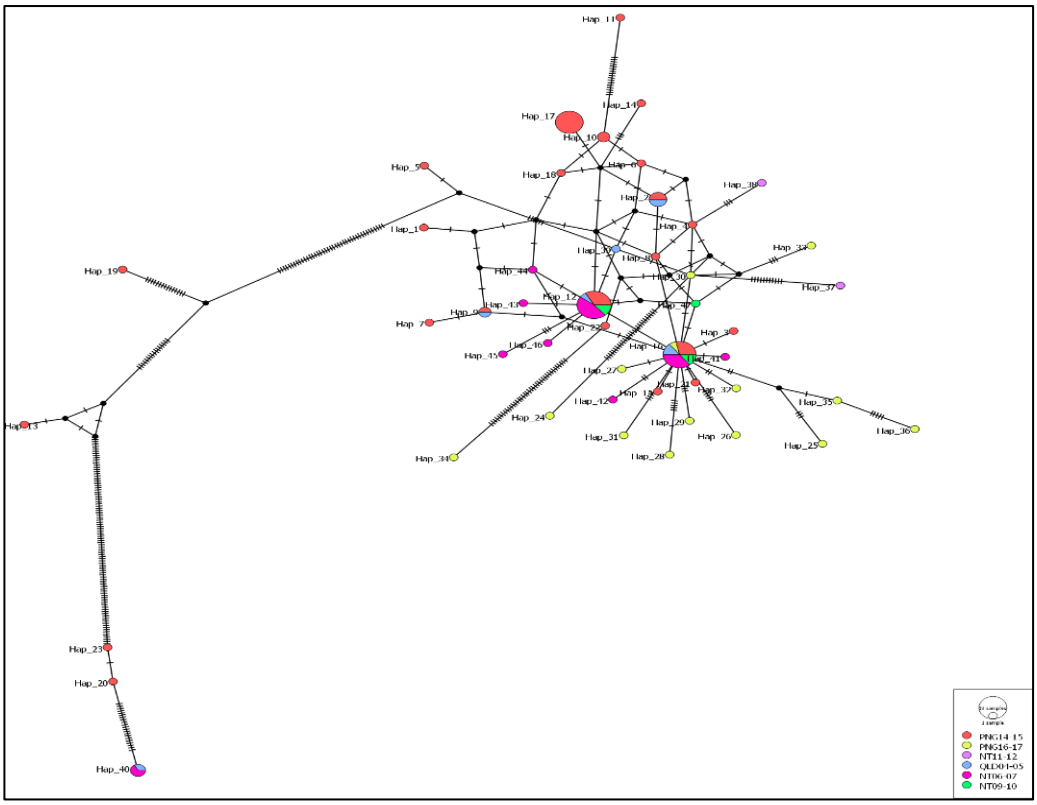

Figure 27. mtDNA CR median joining network analyses in *E. blochii*. Haplotype frequencies are represented by the size of circles, sampling locations represented by colours; number of strokes joining nodes represents the number of mutations between two haplotypes across the gene fragment.

Nuclear diversity in S. mokarran and E. blochii

Microsatellite loci

The eight published usat loci from Nance et al. (2009) were polymorphic in 166 *S. mokarran* individuals across the 11 collections. Loci were checked for departures from HWE and linkage disequilibrium, however no single locus was out of HWE or in genotypic linkage in each of the 11 collections, therefore all loci were maintained. The number of microsatellite alleles ranged from 2.38 in the small NT03 collection to a high of 13.38 in the largest collection, NT12-14. Average observed heterozygosity (i.e. variation) was moderate at 0.544 across the eleven collections.

Eight of the nine newly developed microsatellite loci for *E. blochii* were shown to be polymorphic across the seven collections, albeit the number of alleles (across individuals, across loci) was not as large (maximum N_a = 4.00, in PNG14-15) as in *S. mokarran*. The *E. blochii* usat loci were also checked for conformation to HWE and genotypic linkage; all were retained. As with the number of individuals screened for mtDNA, there was a clear bias towards samples from PNG, with over 79 *E. blochii* individuals genotyped at the microsatellite loci, in comparison to 39 *E. blochii* individuals from five other Australian collections.

Average observed heterozygosity (0.400) (Table 24) across the seven *E. blochii* collections was lower than in the *S. mokarran* collections; likely a result of smaller sample sizes and the deployment of variable, but not widely screened or tested newly developed microsatellite loci. Resourcing did not enable more extensive usat loci testing in *E. blochii*, however the *in-silico* primer library could be further screened at a later date if additional usat loci are required. Additionally, a relatively high F_{IS} value was observed in the PNG16-17 collection – from 25 individuals, this value was 0.495. This within-population inbreeding coefficient indicates a deficiency of heterozygosity, possibly reflecting either allelic drop out and or the likely presence of related sibs (sharing the same usat alleles) in the collection.

SNP loci

Resulting species sequence data from the Illumina NextSeq was of a high quality (with > 87% of the 150 bases above Q30) (a quality control metric) across all *S. mokarran* samples and > 86% of the 150 base reads, above Q30 in the *E. blochii* samples. *S. mokarran* GBS sequencing produced 50.2 GB of data and 332 183 366 single reads. The average number of RAD-tags per individual in the *S. mokarran* catalogue was 152 689 with average tag coverage (i.e. tag depth) per individual of seven. *E. blochii* GBS sequencing produced a larger amount of data (59.56 GB) and 394 420 006 single reads with an average of 162 811 RAD-tags per individual in the catalogue and a tag depth twice that of *S. mokarran*, at thirteen.

Following analysis in Stacks (as per parameters outlined above, with duplicate individuals removed) and filtering (for the most informative SNPs across individuals and filtering out individuals with low numbers of RAD-tags and depth), the genlight files for R contained:

- 184 *S. mokarran* individuals in eleven collections; 16 725 loci (SNPs)
- 142 *E. blochii* individuals in seven collections; 22 977 loci (SNPs)

Following additional filtering according to the dartR pipeline (see above), genomic population analyses per species were then based on final datasets of:

- 129 *S. mokarran* individuals in eleven collections and 3 655 SNPs (see Figure 28 for sample sizes per collection)

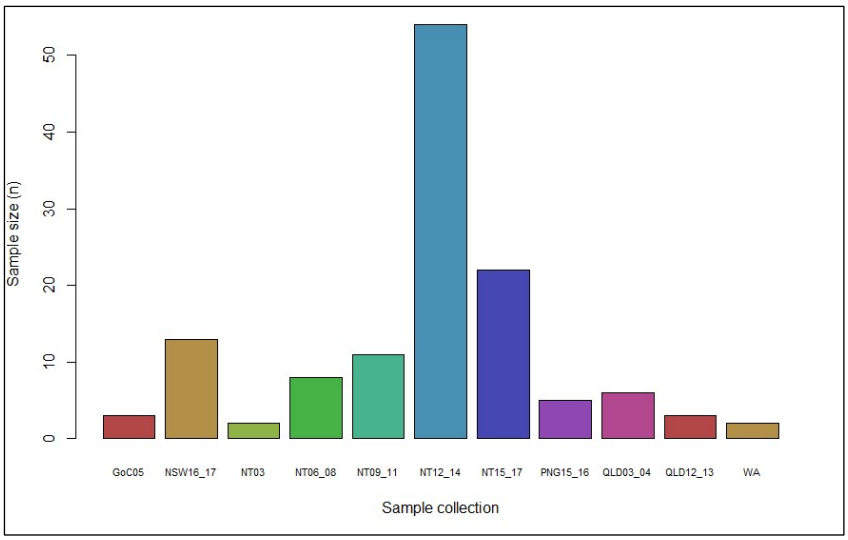

Figure 28 *S.mokarran* samples analysed for SNPs following filtering steps in Galaxy and R

- and 119 *E. blochii* individuals in seven collections and 5 229 SNPs (see Figure 29 for sample sizes per collection).

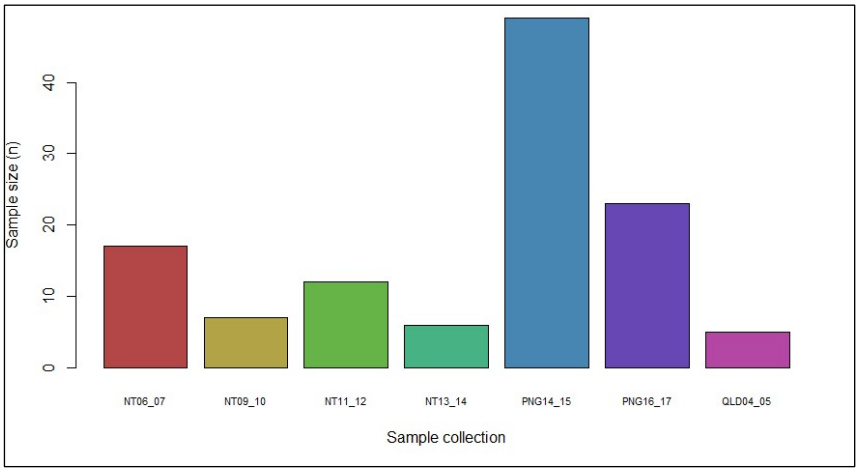

Figure 29 *E. blochii* samples analysed for SNPs following filtering steps in Galaxy and R

The reduction in the number of individuals and SNPs per species throughout the filtering pipeline was a result of stringent quality control, removing monomorphic loci across all individuals and collections, filtering on threshold levels per individual and loci, and filtering for loci considered to be in HWE.

Table 25 below outlines the summary of the nuclear genetic diversity in the *S. mokarran* and *E. blochii* collections at the microsatellite and SNP loci.

Table 25. Summary of genetic diversity (based on averages at microsatellite and SNP loci) across the eleven *S. mokarran* and seven *E. blochii* collections from Australia and Papua New Guinea. Diversity estimates are based on n_{usat} = 8 & n_{SNP} = 3 655 loci in *S. mokarran* and n_{usat} = 8 & n_{SNP} = 5 229 loci in *E. blochii.*

Species	Collection	N	N_a	Ar	H_O	H_E	HWEp	F_{IS}	N	A	%poly	Ar	H_O*	H_E*
S. mokarran	PNG15-16	7	5.38	2.88	0.599	0.735	0.279	0.233	5	5611	53.5	1.26	0.298	0.328
	NT15-17	21	8.38	3.00	0.523	0.650	0.385	0.160	22	6894	94.0	1.26	0.171	0.194
	NT12-14	58	13.38	3.34	0.525	0.767	0.040	0.311	54	7292	99.5	1.29	0.165	0.182
	NT09-11	12	6.00	2.81	0.556	0.686	0.227	0.202	11	6303	86.0	1.22	0.215	0.244
	NT06-08	15	7.38	2.80	0.622	0.660	0.458	0.043	8	5361	72.5	0.99	0.226	0.323
	NT03	3*	2.63	1.86	0.528	0.660	0.601	0.292	2	4552	26.0	1.12	0.581	0.626
	WA	8	5.13	2.67	0.561	0.707	0.411	0.226	2	4599	26.0	1.15	0.532	0.593
	Goc05	3*	3.63	2.75	0.542	0.641	0.550	0.309	3	4242	20.0	0.92	0.366	0.544
	QLD12-13	3*	2.88	2.22	0.438	0.588	0.701	0.375	3	5249	43.5	1.27	0.427	0.431
	QLD03-04	7	4.88	2.67	0.485	0.640	0.364	0.195	6	5819	59.0	1.27	0.280	0.300
	NSW16-17	20	8.00	3.15	0.603	0.704	0.080	0.194	13	6151	68.0	1.14	0.180	0.235
E. blochii	PNG16-17	25	2.22	1.49	0.167	0.309	0.341	0.495	23	6256	20.0	1.16	0.342	0.350
	PNG14-15	54	3.67	1.91	0.309	0.367	0.107	0.298	49	10037	92.0	1.48	0.174	0.182
	NT13-14	4	1.55	1.47	0.479	0.528	0.764	0.325	6	8261	58.0	1.44	0.289	0.292
	NT11-12	6	1.55	1.39	0.556	0.573	0.683	0.121	12	8949	71.0	1.45	0.217	0.232
	NT09-10	4*	1.67	1.46	0.556	0.571	1.000	0.002	7	7959	52.0	1.36	0.244	0.293
	NT06-07	16	2.11	1.59	0.322	0.349	0.789	0.062	17	8777	68.0	1.33	0.181	0.224
	QLD04-05	6	1.67	1.49	0.410	0.551	0.503	0.288	5	7653	46.5	1.36	0.310	0.342

N = number of individuals per collection genotyped; N_a = average number of alleles; Ar = allelic richness across loci; A = total number of alleles observed per collection (where total number of SNP alleles = $2n_{SNP}$); %poly = percentage of polymorphic loci (SNPs); H_O = average observed heterozygosity per locus; H_E = average expected heterozygosity per locus; HEW_P = probability as calculated in genpop after 10 000 Markov chains, averages across all loci given, bolded if significant; F_{IS} = inbreeding coefficient (Weir and Cockerham 1984); *based on polymorphic SNP loci within each collection. Collections that were not subsequently used in haplotype or homogeneity/structure tests (due to small sample sizes and likely presence of multiple sibs) are shown in yellow and green respectively.

As Table 25 shows, several collections were represented by a small number of individuals. These collections were not used for homogeneity or structure analyses due to the bias in genetic diversity – as shown, the H_E values in smaller sample sizes are inflated. Compared to the usat markers, many SNP alleles were detected across individuals and collections of both species. While the average number of usat alleles (per locus) observed in the species ranged from approximately 2 to 14, over 10 000 SNP alleles were observed in the *E. blochii* collection from PNG14-15. In contrast to the *S. lewini* microsatellite alleles (when species specific loci from Nance et al., 2009 were screened), the same *S. lewini* loci screened in *S. mokarran* resulted in much lower allelic richness (*S. lewini* Ar ranged from 8.86 to 9.57 in Indo-Pacific collections (Green 2019); while Ar in *S. mokarran* from Australia and PNG ranged from 1.86 to 3.37). This is a known outcome of deploying cross-species microsatellites – often the loci are less variable in the non-target species.

Providing a more robust data set for testing homogeneity across the collections, in *S. mokarran*, the SNP % polymorphic loci per collection ranged from a low of 53% in PNG15-16 (five individuals were typed) to a high of 99% in NT12-14. Similarly, the % polymorphic loci per collection in *E. blochii* was lowest in the smallest collection (NT09-10) and highest (92%) in the largest collection (PNG14-15, N = 49).

The lower total number SNP alleles (as compared to the other *E. blochii* collections) and reduced % polymorphic loci in PNG16-17 reflects the likely occurrence of siblings in this collection. While not undertaken here, this collection will be examined further for the likelihood of individual relatedness amongst these 24 individuals – all *E. blochii* samples from this collection were from the Sepik River in PNG.

Collection homogeneity testing and genetic structure outcomes

Based on the variable CR mtDNA gene region, overall tests of haplotypic homogeneity showed no significant differentiation ($P > 0.05$) across collections in either species. Similarly, F_{ST} comparisons (based on Weir and Cockerham's (1984) genetic distances) demonstrated no significant pair-wise haplotype differences among any of the *S. mokarran* collections (Table 26). A single F_{ST} comparison between *E. blochii* collections PNG16-17 and NT06-07 was significant after Bonferroni correction (Table 27). These collections were sampled at least ten years apart.

Table 26. *S. mokarran* pair-wise mtDNA CR F_{ST}^* comparisons among collections where N > 5. Where significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collections	PNG15-16	NT15-17	NT12-14	NT09-11	NT06-08	NSW16-17
PNG15-16	*****					
NT15-17	-0.011	*****				
NT12-14	0.032	0.006	*****			
NT09-11	-0.005	0.021	0.002	*****		
NT06-08	0.014	0.016	-0.003	-0.013	*****	
NSW16-17	0.012	0.027	0.023	0.023	0.041	*****

Table 27. *E. blochii* pair-wise mtDNA CR F_{ST}^{*} comparisons among collections where N > 5. Significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collections	PNG16-17	PNG14-15	NT09-10	NT06-07	QLD04-05
PNG16-17	*****				
PNG14-15	0.023	*****			
NT09-10	-0.053	-0.058	*****		
NT06-07	**0.089**	0.037	-0.055	*****	
QLD04-05	0.006	-0.047	-0.071	0.087	*****

The ND4 gene displayed much lower relative levels of haplotypic variation in both species; there were no significant pairwise F_{ST} comparisons amongst collections observed in either species (F_{ST} data not shown).

There was no significant overall differentiation (based on microsatellite genotype frequencies) observed amongst the collections (exact test, $P > 0.05$) for either the *S. mokarran* or *E. blochii* individuals. Based on the Weir and Cockerham (1984) distance method, F_{ST} pair-wise comparisons for the microsatellite data are shown in Tables 28 (*S. mokarran*) and 29 (*E. blochii*).

Table 28. *S. mokarran* pair-wise microsatellite F_{ST}^{*} comparisons based on genetic distances calculated among collections where N > 5. Significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collection	PNG15-16	NT15-17	NT12-14	NT09-11	NT06-08	WA	QLD03-04	NSW16-17
PNG15-16	*****							
NT15-17	0.031	*****						
NT12-14	-0.008	**0.095**	*****					
NT09-11	-0.015	**0.064**	-0.004	*****				
NT06-08	-0.001	**0.076**	-0.007	-0.010	*****			
WA	0.001	**0.085**	-0.024	0.006	0.010	*****		
QLD03-04	-0.005	**0.121**	0.020	0.013	0.018	-0.013	*****	
NSW16-17	0.015	0.008	**0.061**	**0.053**	**0.064**	**0.062**	**0.096**	*****

*negative F_{ST} values represent no differentiation (i.e. $F_{ST} = 0.000$)

Table 29. *E. blochii* pair-wise microsatellite F_{ST}^{*} comparisons among collections where N > 5. Significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collections	PNG16-17	PNG14-15	NT11-12	NT06-07	QLD04-05
PNG16-17	*****				
PNG14-15	**0.076**	*****			
NT11-12	**0.184**	-0.038	*****		
NT06-07	**0.144**	-0.006	0.031	*****	
QLD04-05	**0.199**	-0.013	0.011	-0.004	*****

*negative F_{ST} values represent no differentiation (i.e. $F_{ST} = 0.000$)

Based on the F_{ST} values (from the usat allele frequencies), the *S. mokarran* NT15-17 collection was significantly different to all collections aside from NSW16-17 and similarly,

NSW16-17 was shown to be different to all other temporal collections from 2003 – 2012 (Table 28). This maybe a temporal effect. These two collections, sampled in Australia during 2015 – 2017 were not differentiated, displaying a low pair-wise F_{ST} value of 0.008 (representing unrestricted gene flow between the two collections) although they were shown to be different to the other collections (albeit, while the F_{ST} values were significant, F_{ST} ranges from 0.000 to 1.000 with 1.000 indicating complete differentiation). The three other NT collections were not different to each other, nor to the WA, QLD or PNG collections. The samples from PNG in 2015 – 2016 were also not different to any other samples, although this collection was represented by only seven individuals.

In contrast, the only collection that was differentiated (based on F_{ST}) to all others in the *E. blochii* comparisons was PNG16-17 (Table 30). This collection from the Sepik River, in the northern region of PNG (the Sepik River feeds into the Bismark Sea) was also different to the other PNG collection (PNG14-15). Contrastingly, the largest *E. blochii* collection, PNG14-15 was not shown to be significantly different to the other Australian collections.

A more robust analysis of the overall genomic diversity observed in these two hammerhead species was provided by the nuclear SNPs. The thousands of SNP loci (> 3600) detected relative genomic homogeneity among all *S. mokarran* collections. A global exact test of non-differentiation (in SNP allele frequencies) was non-significant ($P > 0.05$), a low $G''_{ST} = 0.034$ was observed from the *S. mokarran* collections and a non-significant global F_{ST} of 0.003 ($P = 1.000$) was recorded. In contrast to the usat data (based on eight non-species-specific loci), all pairwise F_{ST} values (based on the SNP data) were low and non-significant; the negative values represent no differentiation (Table 30).

Table 30. *S. mokarran* pair-wise SNP genetic differentiation F_{ST}^{*} comparisons among collections where N > 5. Where significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction.

Collections	PNG15-16	NT15-17	NT12-14	NT09-11	NT06-08	QLD03-04	NSW16-17
PNG15-16	*****						
NT15-17	0.006	*****					
NT12-14	0.004	0.000	*****				
NT09-11	0.001	-0.004	-0.005	*****			
NT06-08	-0.083	-0.077	-0.078	-0.049	*****		
QLD03-04	0.005	0.008	0.008	0.002	-0.085	*****	
NSW16-17	-0.017	-0.088	-0.027	-0.010	-0.039	-0.012	*****

*negative F_{ST} values represent no differentiation (i.e. $F_{ST} = 0.000$)

Likewise, when the *E. blochii* PNG16-17 collection was not included (SNPs detected a high inbreeding value for this collection, which may represent either inclusion of sibs or poor genotyping), there was no strong genetic heterogeneity detected in the six *E. blochii* collections. A global exact test of non-differentiation (in SNP allele frequencies) was non-significant ($P > 0.05$), a low $G''_{ST} = 0.011$ was observed; a non-significant global F_{ST} of 0.004 ($P > 0.01$) was recorded. All pair-wise comparisons (Table 31) showed homogeneity, indicating no strong barriers to gene flow among the Australian and PNG collections and

locations. However, the caveat on this data is the number of collections and sample sizes in the *E. blochii* were smaller and biased towards PNG (i.e. > 51% of individuals were from the PNG15-16 collection, this is an important as the data is clearly biased towards individuals not sampled within Australian waters). When the PNG16-17 collection was included, all pairwise comparisons with the other six collections were significant (ranging from F_{ST} = 0.411 – 0.535, including with PNG14-15 (F_{ST} = 0.411)), data not shown here.

Table 31. *E. blochii* pair-wise SNP genetic differentiation F_{ST}* comparisons among collections where N > 5. Where significant (based on 10 000 permutations) F_{ST} values are shown in bold, following sequential Bonferroni correction

Collections	PNG14-15	NT13-14	NT11-12	NT09-10	NT06-07	QLD04-05
PNG14-15	*****					
NT13-14	0.003	*****				
NT11-12	0.004	0.003	*****			
NT09-10	-0.012	-0.021	-0.007	*****		
NT06-07	-0.055	-0.084	-0.050	-0.016	*****	
QLD04-05	-0.001	-0.009	0.001	-0.001	-0.014	*****

The molecular variance in haplotypic and allelic frequencies in both the mitochondrial and nuclear markers was then tested using hierarchical AMOVA. Tables 32 and 33 show the *F–statistic* outcomes of the designated testing in the collections.

Table 32. *S. mokarran* variance hierarchical AMOVA testing, with significance tested following 10 000 permutations. Based on collections where N > 5.

Tested groupings	F_{ST}*	F_{SC}*	F_{CT}*
S. mokarran (panmixia; Aust & PNG collections) mtDNA CR	0.011 (*P* = 0.127)	NA	NA
S. mokarran (panmixia; Aust & PNG collections) usats	0.037 (*P* = 0.000)	NA	NA
S. mokarran (panmixia; Aust & PNG collections) SNPs	-0.015 (*P* = 0.999)	NA	NA
S. mokarran (spatial; Aust collections) mtDNA CR	0.010 (*P* = 0.132)	NA	NA
S. mokarran (spatial; Aust collections) usats	0.042 (*P* = 0.000)	NA	NA
S. mokarran (spatial; Aust collections) SNPs	-0.017 (*P* = 0.999)	NA	NA
S. mokarran (spatial: E v W collections) mtDNA CR	0.024 (*P* = 0.124)	0.003 (*P* = 0.299)	0.021 (*P* = 0.066)
S. mokarran (spatial: E v W collections) usats	0.036 (*P* = 0.000)	0.037 (*P* = 0.000)	-0.002 (*P* = 0.444)
S. mokarran (spatial: E v W collections) SNPs	-0.012 (*P* = 0.999)	-0.018 (*P* = 0.999)	0.006 (*P* = 0.423)
S. mokarran (temporal: 06-08 v 09-11 v 12-14 v 15-17 collections) mtDNA CR	0.011 (*P* = 0.128)	0.005 (*P* = 0.235)	0.006 (*P* = 0.202)
S. mokarran (temporal: 03-04 v 06-08 v 09-11 v 12-14 v 15-17 collections) usats	0.043 (*P* = 0.000)	0.013 (*P* = 0.105)	0.030 (*P* = 0.304)
S. mokarran (temporal: 03-04 v 06-08 v 09-11 v 12-14 v 15-17 collections) SNPs	-0.016 (*P* = 1.000)	-0.008 (*P* = 0.886)	-0.008 (*P* = 0.628)

*F_{ST} = the variance among sub collections relative to the total variance; F_{SC} = the variance among sub collections within groups; F_{CT} = the variance among groups relative to the total variance; significance of *P* values given in brackets. NA – not applicable

Reflecting the outcomes from the mtDNA pair-wise F_{ST} comparisons, there was no evidence of significant mtDNA haplotype differences among the Australian collections (F_{ST} = 0.010) or among all *S. mokarran* collections (F_{ST} = 0.011) (F_{ST} ranges from 0 – 1, with 1 representing considerable degree of differentiation, as expected between different species). Similarly, hierarchical AMOVA on east and west collections and temporal collections also demonstrated that most of the genetic (i.e. haplotypic) variance was observed within individuals.

The SNP data reflected genetic homogeneity in all comparisons with no indication of spatial structuring in the SNP alleles. In contrast to the mtDNA and SNP results, the eight microsatellite primers developed in *S. lewini* and deployed in *S. mokarran*, detected significant ($P < 0.001$), but low variance components; albeit the *F*-statistic in the panmixia testing was 0.037 (presumably driven by the variance in usat allele frequencies from the two Australian 2015 - 2017 *S. mokarran* collections). Compared to pair-wise F_{ST} comparisons in *S. lewini* (see Green 2019) the relatively higher F_{ST} values here maybe due to smaller sample sizes in some collections and less variable microsatellite loci (i.e. fewer alleles were detected by the *S. lewini* usat primers when deployed in *S. mokarran*).

Similarly, no significant variances (reflecting differentiation among collections) were found in *E. blochii* when the PNG16-17 collection was not considered. The small number of *E. blochii* samples and a lack of consistent temporal sampling meant that only hierarchical spatial

testing was undertaken. All AMOVA testing, across the three marker types resulted in low and non-significant F-statistics (see Table 33).

Table 33. *E. blochii* variance hierarchical AMOVA testing, with significance tested following 10 000 permutations. Based on collections where N > 5.

Tested groupings	F_{ST}*	F_{SC}*	F_{CT}*
E. blochii (panmixia; Aust & PNG collections) mtDNA CR	0.009 (*P* = 0.294)	NA	NA
E. blochii (panmixia; Aust & PNG collections) usats	0.036 (*P* = 0.005)	NA	NA
E. blochii (panmixia; Aust & PNG collections) SNPs	0.212 (P = 0.000)	NA	NA
E. blochii (panmixia; Aust & PNG+ collections) mtDNA CR	-0.002 (*P* = 0.333)	NA	NA
E. blochii (panmixia; Aust & PNG+ collections) usats	-0.025 (*P* = 0.989)	NA	NA
E. blochii (panmixia; Aust & PNG+ collections) SNPs	-0.020 (*P* = 0.996)	NA	NA
E. blochii (spatial; Aust collections) mtDNA CR	0.074 (*P* = 0.198)	NA	NA
E. blochii (spatial; Aust collections) usats	0.016 (*P* = 0.261)	NA	NA
E. blochii (spatial; Aust collections) SNPs	-0.024 (*P* = 0.968)	NA	NA
E. blochii (spatial: E v W collections+) mtDNA CR	0.019 (*P* = 0.332)	-0.059 (*P* = 0.404)	0.073 (*P* = 0.098)
E. blochii (spatial: E v W collections+) usats	-0.033 (*P* = 0.987)	-0.005 (*P* = 0.419)	-0.027 (*P* = 0.729)
E. blochii (spatial: E v W collections+) SNPs	-0.018 (*P* = 0.995)	-0.024 (*P* = 0.977)	0.006 (*P* = 0.605)

*F_{ST} = the variance among sub collections relative to the total variance; F_{SC} = the variance among sub collections within groups; F_{CT} = the variance among groups relative to the total variance; significance of *P* values given in brackets. +PNG16-17 not included. NA – not applicable

When the PNG16-17 collection was included (and this collection should be treated with caution until further testing has been completed; the smallest number of SNP alleles was recorded in this collection – 6 256 SNP alleles cf. 10 037 SNP alleles in PNG14-15), the SNP data (from over 5 200 screened loci) demonstrated significant and strong variance components were attributed to genomic differences in this collection (F_{ST} = 0.212, *P* = 0.000). Likewise, the usat data (bi-parentally inherited, as in SNPs) detected a similar outcome whereas the maternally inherited CR haplotypes did not detect significant variance components (F_{ST} = 0.009, *P* = 0.294). Such differentiation could be a result of higher sibling relationships within this collection, or the most northly *E. blochii* collection in this study was significantly different to the other *E. blochii* collections. Indeed, as the pairwise F_{ST} values showed, PNG16-17 was also different to PNG14-15.

The final genetic treatment of the nuclear marker data was spatial analysis of principal components. Provided in Figures 30-33 are the alpha corrected DAPC outcomes for the genetic (i.e. microsatellite) and genomic (i.e. SNP) data in *S. mokarran* and *E. blochii* as outlined in Table 24.

As can be seen in Figures 30 and 31, there was one central genetic group for *S. mokarran*; there was no clear separation of collections based on the usat data; individuals and collections were clustered over each other, driven by one main discriminant function. In slight contrast, based on SNPS, the small number of QLD03-04 individuals were slightly offset to the central cluster of *S. mokarran*, however the pairwise F_{ST} comparisons between this and the other *S. mokarran* collections were all less than 0.009 and all non-significant.

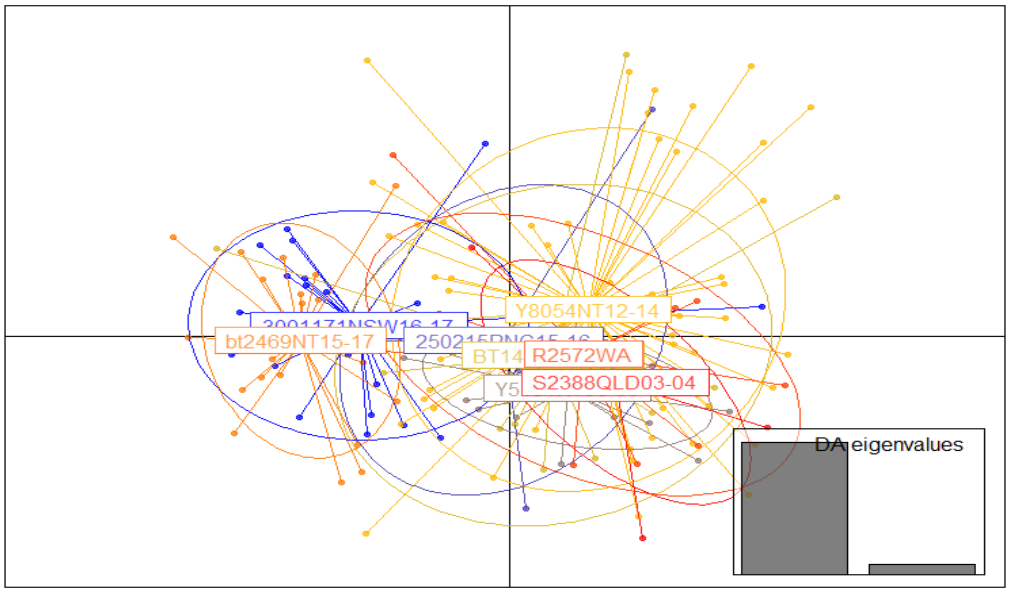

Figure 30. Discriminant analysis of principal components with priori grouping corresponding to the sample collections of *S. mokarran*. Scatter plot of based on 8 microsatellite loci, where collection sample sizes N > 5.

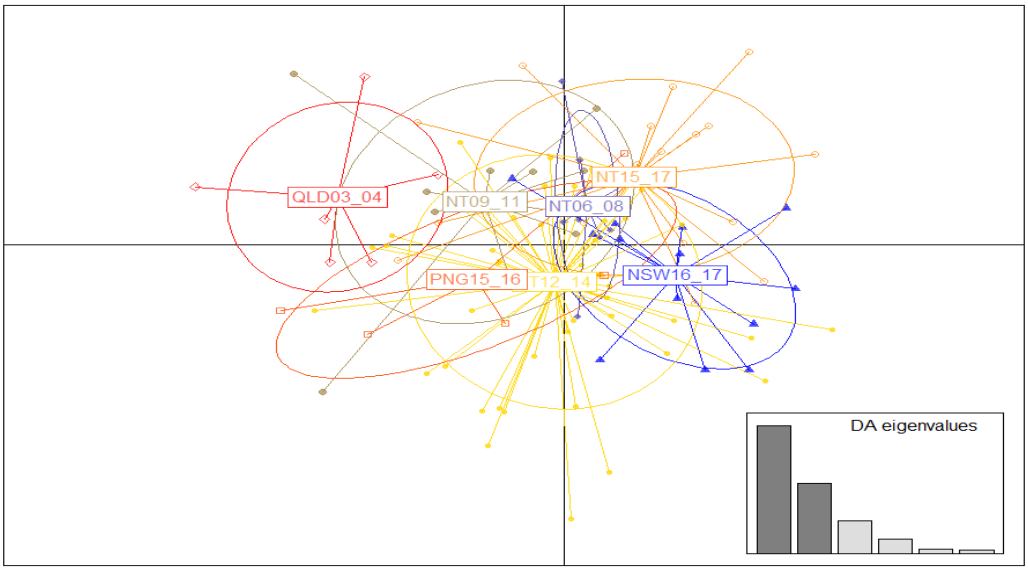

Figure 31. Discriminant analysis of principal components with priori grouping corresponding to the sample collections of *S. mokarran*. Scatter plot of based on 3 655 SNP loci, where collection sample sizes N > 5

A tighter, central cluster of *E. blochii* individuals (based on the usat data) is shown in Figure 32 - the PNG16-17 collection was located to the right of the central area with the other smaller *E. blochii* collections overlapping each other. Several individuals of the PNG16-17 collection extend into the RHS quadrant; this spatial clustering was reflected in the significant pair-wise F_{ST} comparisons.

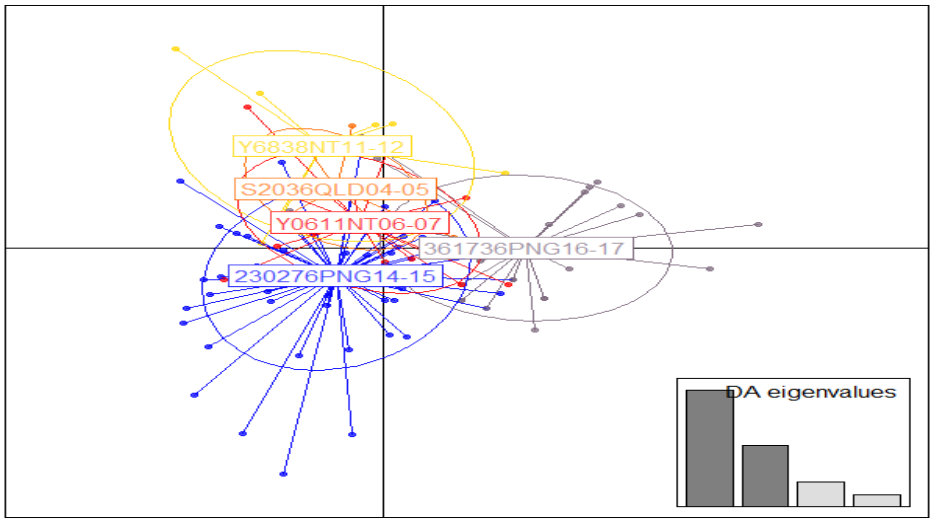

Figure 32. Discriminant analysis of principal components with priori grouping corresponding to the sample collections of *E. blochii*. Scatter plot of based on 8 microsatellite loci, where collection sample sizes N > 5.

Figure 33 shows the *E. blochii* DAPC based on the SNPs. Most of the variation between the *E. blochii* collections was described by two discriminant functions and no collection clustered well outside of the central grouping. This contrasts with the resulting DAPC when all *E. blochii* collections (including PNG16-17) was undertaken.

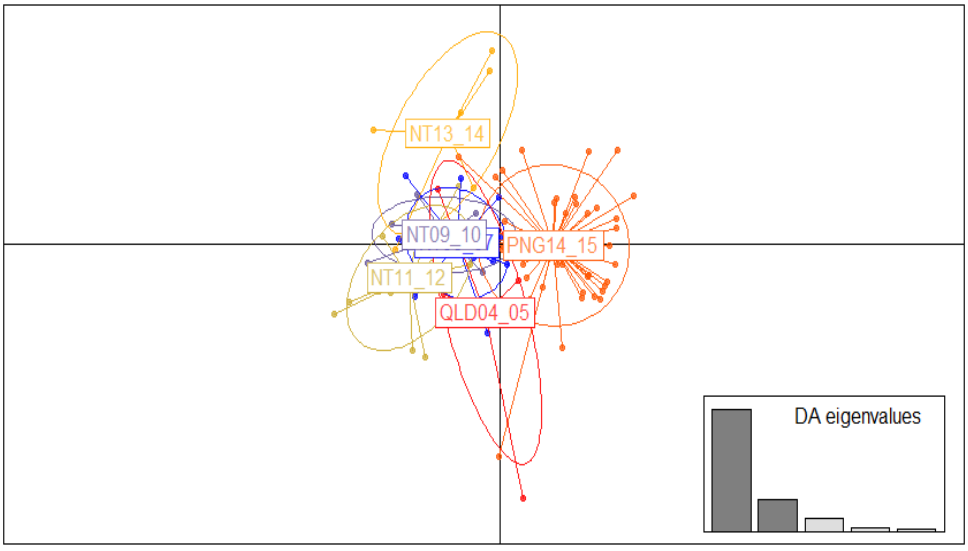

Figure 33. Discriminant analysis of principal components with priori grouping corresponding to the sample collections of *E. blochii*. Scatter plot of based on 5 229 SNP loci, where collection sample sizes N > 5 and PNG16-17 not include.

Overall, the non-model clustering in the DAPCs reflected the observations from the *F-statistics* and the genetic homogeneity among collections of both hammerhead and hammerhead-like species in Australian waters.

8.1.3 Discussion

This is the first instance of population genetics/genomics analyses undertaken in *S. mokarran* and *E. blochii* from multiple locations in Australia and Papua New Guinea. The genetic diversity, collection homogeneity and structure analyses were based on three different genetic markers. Importantly, this study provides new information on the connectivity of the two species in Australian waters. In the absence of extensive physical tagging or parasite information, the genetic knowledge outlined here provides the only connectivity assessment for *S. mokarran* and *E. blochii*, with spatial collections of individuals from both species considered part of wider northern Australian populations. Despite limited spatial and depauperate temporal collections, gene flow was detected in each species within the known ranges of northern Australia and Papua New Guinea.

The three classes of genetic markers reflected different levels of variation and modes of inheritance. The level of genetic diversity and connectivity was also directly correlated with sample size. Despite multiple mtDNA genes being screened in both species, as mtDNA is a circular genome inherited maternally, essentially only one marker was haplotyped in *S. mokarran* and *E. blochii*. Bi-parentally inherited genetic variation (detected as microsatellite alleles) were a magnitude greater than the mtDNA marker, while nuclear wide genomic variation, at thousands of SNP loci, provided the most stringent test of diversity and connectivity. As shown by Green (2019) in *S. lewini*, and while collections from only two countries were available for testing, the thousands of SNPs deployed in *S. mokarran* and *E. blochii* identified genomic wide diversity and connectivity across spatial scales that microsatellites did not (i.e. the genomic differentiation of the PNG16-17 *E. blochii* collection). Furthermore, as other shark studies have highlighted (Green 2019; Junge *et al.* 2019), it was important to undertake these diversity and connectivity analyses in the two species, rather than rely on or infer connectivity patterns from closely related species (such as *S. lewini*).

Analysis of mitochondrial genetic variation, based on the hyper-variable CR gene among collections of *S. mokarran* and *E. blochii*, showed that individuals sampled within Australian waters (primarily from Northern Territory, Queensland and New South Wales) were similar (i.e. not differentiated) from individuals in Papua New Guinea (the same outcome was observed in *S. lewini* from Indo-Pacific, Green (2019)). *S. mokarran* from the Gulf of Carpentaria and Western Australia were also included in the study, however these collections were represented by less than five individuals. Despite this, all *S. mokarran* individuals shared similar mtDNA genetics. Similarly, all *E. blochii* collections from Australia and Papua New Guinea were shown to be genetically similar at the CR gene – no significant structuring was detected, indicating maternal gene flow among the collections was relatively strong (both spatially and temporally). There was no evidence of structuring or collection differentiation across the northern locations of Australia and into Papua New Guinea.

The microsatellite data from *S. mokarran* suggested that alleles observed in individuals from NT15-17 and NSW16-17 were at different frequencies (thereby resulting in significant pair-

wise F_{ST} comparisons) to those observed in the other *S. mokarran* collections. However, these *F-statistics* are based on the assumptions of HWE in larger sample sizes. In NSW16-17 (average N = 20), 3/8 loci did not meet HWE and several loci in the NT15-17 collection (average N = 21) did not meet HWE assumptions. Non-model based DAPC on the microsatellite data however demonstrated that individuals from the *S. mokarran* collections formed a single cluster of Australian and PNG membership, with some radiations from the central cluster. In contrast, all F_{ST} pair-wise comparisons based on over 3 600 SNP loci demonstrated genomic homogeneity across the *S. mokarran* collections from Australia and Papua New Guinea. The DAPC of the 3 655 SNP loci showed individuals clustered to a central group; all AMOVA testing demonstrated negative *F*–statistics components, indicating the majority of SNP variance was observed within individuals rather than among collections.

Aside from comparison with the PNG16-17, the *E. blochii* microsatellite data showed a single significant pair-wise comparison between NT11-12 and QLD04-05 collections; however, these two collections were each represented by only six individuals – this is not a robust comparison of the individuals at these sampling locations. The F_{ST} comparisons based on the microsatellite data between all other collections was small or negative (and all non-significant). The SNP data showed no evidence of genetic heterogeneity among the *E. blochii* collections, indicating contemporary gene flow among the locations (aside from the Sepik River in PNG). *E. blochii* from the Sepik River (PNG16-17) show moderate genetic heterogeneity to the other collections, however this outcome requires confirmation. All collections clustered together in the DAPC.

This study found no evidence for consistent population sub-structuring across northern Australia (from Western Australia to New South Wales) and the Gulf of Papua. There was some support for a separation of *E. blochii* individuals from the Sepik River, Bismark Sea (based on neutral SNPs) albeit based on a smaller sample size of possibly related individuals. Given this, there was no strong evidence to suggest isolation by distance or that individuals in these areas should be considered part of separately managed stocks. The lack of structuring, particularly in *S. mokarran*, suggests these sharks are characterised by substantial gene flow (i.e. F_{ST} of 0.004) as detected by both classic and non-model statistical approaches.

The three genetic markers deployed in each species reflected relatively homogenous or panmictic collections both spatially and temporally (at least with respect to the *S. mokarran* individuals). As documented for *S. lewini* (Green 2019), the continental shelves of Australia and Papua New Guinea (connections to Indonesia were not tested in these two species) likely provide suitable habitat supporting dispersive behaviours across eastern, northern and western tropical-temperate areas resulting in gene flow amongst individuals.

Given the caveats and sample size restrictions outlined above and based on the conceptual models (that putatively explain patterns of distribution) of Chin et al. (2017), Models 1 and or 3 could describe the most likely explanation of movements of *S. mokarran* and *E. blochii* in the region. Chin et al. (2017) describe four hypothetical models of movement; 1. panmictic population throughout region; 2. limited movement; 3. continental shelf movement; 4. east-west Australian stock divide and continental shelf movements. Based on the multi-marker

approach and data from this study, and as no Indonesian samples were considered, Model 3 (which suggests continental shelf movement enabling connectivity between Australia and Papua New Guinea) would be the preferred explanation for *S. mokarran* and *E. blochii*. If the differentiation of the *E. blochii* PNG16-17 collection is unequivocal (irrespective of the level of kinship in this collection) and the individuals here are significantly different (at least with respect to SNP alleles), this adds further support to Model 3.

These population genetic components provide a better understanding of the connectivity between individuals of two under described, yet biologically important hammerhead and hammerhead-like species from the Australian region. This information is crucial for Australian conservation and biodiversity managers. However, further directed sampling and increased sample sizes are required to more fully understand population connectivity in these species.

9. APPENDIX B – MITOCHONDRIAL DNA HAPLOTYPE FREQUENCIES

Table 1 CR (1120 bp) mtDNA haplotype frequencies in *S. mokarran* from Australia and Papua New Guinea sampling locations.

Haplotype	PNG15-16	NT03	NT06-08	NT09-11	NT12-14	NT15-17	QLD03-04	QLD12-13	GoC05	WA	NSW16-17
CR_Hap_1	0	0	0	0	0	0	0	0	0	0	1
CR_Hap_2	0	0	0	0	0	0	0	1	0	0	4
CR_Hap_3	0	0	1	0	0	0	0	0	0	0	1
CR_Hap_4	0	0	0	0	1	1	0	0	0	0	1
CR_Hap_5	0	0	0	0	0	0	0	0	0	0	1
CR_Hap_6	1	0	0	0	0	0	0	0	0	0	0
CR_Hap_7	1	0	1	1	9	3	0	0	0	0	1
CR_Hap_8	3	0	1	0	0	0	0	0	0	0	0
CR_Hap_9	1	0	0	0	1	0	0	0	0	0	0
CR_Hap_10	1	0	0	0	0	0	0	0	0	0	0
CR_Hap_11	0	0	0	0	0	0	0	0	0	0	1
CR_Hap_12	0	0	0	0	0	0	0	0	0	0	1
CR_Hap_13	0	0	0	0	0	0	0	0	0	0	1
CR_Hap_14	0	0	0	0	0	0	0	0	0	0	1
CR_Hap_15	0	0	2	0	1	1	0	0	0	1	1
CR_Hap_16	0	0	0	0	0	0	0	0	0	0	1
CR_Hap_17	0	0	0	0	0	0	0	0	0	0	0
CR_Hap_18	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_19	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_20	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_21	0	0	0	0	2	0	0	0	0	0	0
CR_Hap_22	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_23	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_24	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_25	0	0	1	0	2	0	0	0	0	0	0
CR_Hap_26	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_27	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_28	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_29	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_30	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_31	0	0	0	0	2	0	0	0	0	0	0
CR_Hap_32	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_33	0	0	0	0	1	1	0	0	0	0	0
CR_Hap_34	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_35	0	0	0	0	2	0	0	0	0	0	0
CR_Hap_36	0	0	0	0	2	0	0	0	0	0	0
CR_Hap_37	0	0	0	0	1	1	0	0	0	0	0
CR_Hap_38	0	0	0	0	2	0	0	0	0	0	0

CR_Hap_39	0	0	0	0	3	0	0	0	0	0	0
CR_Hap_40	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_41	0	0	0	0	1	0	0	1	0	0	0
CR_Hap_42	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_43	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_44	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_45	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_46	0	0	0	0	1	1	0	0	0	0	0
CR_Hap_47	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_48	0	0	0	0	0	2	0	0	0	0	0
CR_Hap_49	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_50	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_51	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_52	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_53	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_54	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_55	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_56	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_57	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_58	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_59	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_60	0	0	0	0	0	1	0	0	0	0	0
CR_Hap_61	0	0	0	0	0	0	0	0	0	1	0
CR_Hap_62	0	0	0	0	0	0	0	0	0	1	0
CR_Hap_63	0	0	0	0	1	0	0	0	0	1	0
CR_Hap_64	0	1	0	0	0	0	0	0	0	0	0
CR_Hap_65	0	0	0	0	0	0	0	0	1	0	0
CR_Hap_66	0	0	0	0	0	0	0	0	1	0	0
CR_Hap_67	0	0	0	0	0	0	0	0	1	0	0
CR_Hap_68	0	0	0	0	0	0	1	0	0	0	0
CR_Hap_69	0	0	0	0	0	0	1	0	0	0	0
CR_Hap_70	0	0	0	0	1	0	1	0	0	0	0
CR_Hap_71	0	0	0	0	0	0	1	0	0	0	0
CR_Hap_72	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_73	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_74	0	0	2	0	0	0	0	0	0	0	0
CR_Hap_75	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_76	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_77	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_78	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_79	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_80	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_81	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_82	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_83	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_84	0	0	1	0	0	0	0	0	0	0	0

CR_Hap_85	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_86	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_87	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_88	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_89	0	0	1	0	0	0	0	0	0	0	0
CR_Hap_90	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_91	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_92	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_93	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_94	0	0	0	1	0	0	0	0	0	0	0
CR_Hap_95	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_96	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_97	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_98	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_99	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_100	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_101	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_102	0	0	0	0	1	0	0	0	0	0	0
CR_Hap_103	0	0	0	0	1	0	0	0	0	0	0
Sample size	7	1	20	14	59	22	4	2	3	4	15

Table 2 CR (523 bp) mtDNA haplotype frequencies in *E. blochii* from Australia and Papua New Guinea sampling locations.

Haplotype	PNG14-15	PNG16-17	NT11-12	QLD04-05	NT06-07	NT09-10
CR_Hap_1	1	0	0	0	0	0
CR_Hap_2	2	0	0	2	0	0
CR_Hap_3	1	0	0	0	0	0
CR_Hap_4	1	0	0	0	0	0
CR_Hap_5	1	0	0	0	0	0
CR_Hap_6	1	0	0	0	0	0
CR_Hap_7	1	0	0	0	0	0
CR_Hap_8	1	0	0	0	0	0
CR_Hap_9	1	0	0	1	0	0
CR_Hap_10	2	0	0	0	0	0
CR_Hap_11	1	0	0	0	0	0
CR_Hap_12	5	0	0	1	7	2
CR_Hap_13	1	0	0	0	0	0
CR_Hap_14	1	0	0	0	0	0
CR_Hap_15	1	0	0	0	0	0
CR_Hap_16	4	1	0	2	5	2
CR_Hap_17	10	0	0	0	0	0
CR_Hap_18	1	0	0	0	0	0
CR_Hap_19	1	0	0	0	0	0
CR_Hap_20	1	0	0	0	0	0
CR_Hap_21	1	0	0	0	0	0
CR_Hap_22	1	0	0	0	0	0
CR_Hap_23	1	0	0	0	0	0
CR_Hap_24	0	1	0	0	0	0
CR_Hap_25	0	1	0	0	0	0
CR_Hap_26	0	1	0	0	0	0
CR_Hap_27	0	1	0	0	0	0
CR_Hap_28	0	1	0	0	0	0
CR_Hap_29	0	1	0	0	0	0
CR_Hap_30	0	1	0	0	0	0
CR_Hap_31	0	1	0	0	0	0
CR_Hap_32	0	1	0	0	0	0
CR_Hap_33	0	1	0	0	0	0
CR_Hap_34	0	1	0	0	0	0
CR_Hap_35	0	1	0	0	0	0
CR_Hap_36	0	1	0	0	0	0
CR_Hap_37	0	0	1	0	0	0
CR_Hap_38	0	0	1	0	0	0
CR_Hap_39	0	0	0	1	0	0
CR_Hap_40	0	0	0	1	2	0
CR_Hap_41	0	0	0	0	1	0
CR_Hap_42	0	0	0	0	1	0
CR_Hap_43	0	0	0	0	1	0

CR_Hap_44	0	0	0	0	1	0
CR_Hap_45	0	0	0	0	1	0
CR_Hap_46	0	0	0	0	1	0
CR_Hap_47	0	0	0	0	0	1
Sample size	32	14	2	9	19	5

Table 3 ND4 (810 bp) mtDNA haplotype frequencies in *S. mokarran* from Australia and Papua New Guinea sampling locations.

Haplotype	NSW16-17	PNG15-16	NT03	NT06-08	NT09-11	NT12-14	NT15-17	WA	QLD03-04	QLD12-13	Goc05
ND4_ Hap_1	16	8	1	20	16	51	23	7	8	1	1
ND4_ Hap_2	6	0	0	2	1	3	0	0	1	1	1
ND4_Hap_3	0	0	0	0	0	0	1	0	0	0	0
ND4_Hap_4	0	0	0	0	0	0	2	0	0	0	0
Sample size	22	8	1	22	17	54	26	7	9	2	2

Table 4 ND4 (754 bp) mtDNA haplotype frequencies in *E. blochii* from Australia and Papua New Guinea sampling locations.

Haplotype	PNG14-15	PNG16-17	NT11-12	QLD04-05	NT06-07	NT09-10	NT13-14
ND4_Hap_1	35	28	15	9	30	12	6
ND4_ Hap_2	9	0	0	0	0	0	0
ND4_Hap_3	4	0	0	0	0	0	0
ND4_Hap_4	2	0	0	0	0	0	0
ND4_Hap_5	0	0	1	0	1	0	0
Sample size	50	28	16	9	31	12	6

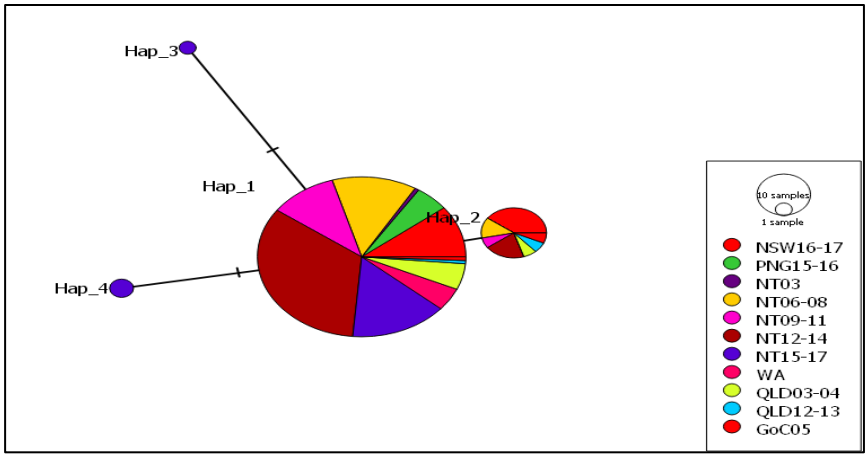

Figure 1 mtDNA ND4 median joining network analyses in *S. mokarran*. Haplotype frequencies are represented by the size of circles, sampling locations represented by colours; number of strokes joining nodes represents the number of mutations between two haplotypes across the gene fragment.

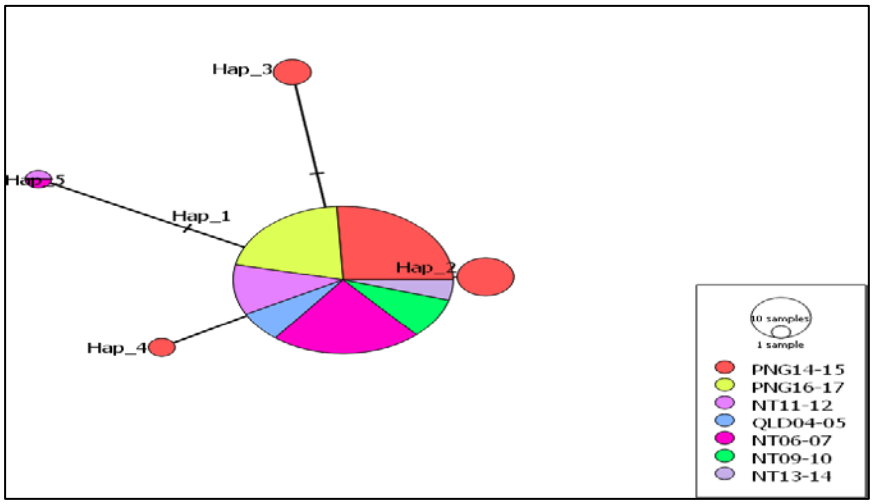

Figure 2 mtDNA ND4 median joining network analyses in *E. blochii*. Haplotype frequencies are represented by the size of circles, sampling locations represented by colours; number of strokes joining nodes represents the number of mutations between two haplotypes across the gene fragment.

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