WWF

REPORT

AUS

2019

THIS REPORT HAS BEEN PRODUCED IN COLLABORATION WITH

NOAA

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

U.S. DEPARTMENT OF COMMERCE

WORKING TOGETHER TO PROTECT THE HAWKSBILL TURTLE

Royal Caribbean INTERNATIONAL

CRACKING THE CODE

DEVELOPING A TORTOISESHELL DNA EXTRACTION AND SOURCE DETECTION METHOD

ACKNOWLEDGEMENTS

This report was written by Michael Jensen (Research Contractor to WWF), in collaboration with Erin LaCasella (NOAA) and Peter Dutton (NOAA), with contributions and review by Christine Madden Hof (WWF-Australia).

We appreciate the support of Christine Madden Hof, Dr Ian Bell, turtle conservation rangers of the Arnavon Community Marine Park, Dr Richard Hamilton, Dr Jeff Kinch and Rachel Groom with the collection of samples for this study.

Southwest Fisheries Science Center/NOAA

The SWFSC is the research arm of NOAA's National Marine Fisheries Service in the Southwest Region. Center scientists conduct marine biological, economic and oceanographic research, observations and monitoring of living marine resources and their environment.

As the NOAA-Fisheries' National Sea Turtle Genetics Lab, the SWFSC Marine Turtle Genetics Program (MTGP) has the lead responsibility for generating, analysing and interpreting genetic data to address the scientific and management needs for the agency. A priority for NOAA-SWFSC is to implement Recovery Plans for sea turtles under the U.S. Endangered Species Act (ESA) which includes engaging with international partners (such as WWF), to further the science of conservation of sea turtles throughout their range and life cycle.

WWF

WWF is one of the world's largest and most experienced independent conservation organizations, with over 5 million supporters and a global Network active in more than 100 countries.

WWF's mission is to stop the degradation of the planet's natural environment and to build a future in which humans live in harmony with nature, by: conserving the world's biological diversity, ensuring that the use of renewable natural resources is sustainable, and promoting the reduction of pollution and wasteful consumption.

WWF-Australia in coordinating Southeast Asia and Pacific (Asia-Pacific) efforts to address unsustainable use and illegal trade in marine turtles and turtle products. With a focus on hawksbill turtles, the program of work is multifaceted and draws on collaborations between a number of organisations. One of the initiative's objectives is to develop and apply new innovative approaches to effectively track marine turtles and turtle products along the trade chain from source to sale. To catalyse enforcement and aid conservation, traceability of hawksbill turtle stocks targeted in trade will rely on WWF's collaborative trans-Pacific forensic study characterising hawksbill turtle genetic origins and relationships between nesting and foraging populations.

Royal Caribbean Cruises: This project would not have been possible without the generous support of Royal Caribbean Cruises. Since 2018, Royal Caribbean Cruises and WWF-Australia have been working together to better protect the hawksbill turtle from the illegal turtle trade.

Suggested citation: Jensen MP, LaCasella EL, Dutton PH & Madden Hof CA. 2019. CRACKING THE CODE: Developing a tortoiseshell DNA extraction and source detection method. Report to WWF-Australia.

Cover image: Close-up of a hawksbill turtle being caught for monitoring, in Papua New Guinea. Hawksbill turtles are under threat and affected by illegal wildlife trade. WWF-Australia is working with partners to monitor populations and ensure the protection of hawksbill turtles that use the area as a route between Australia and Papua New Guinea. © Christine Madden Hof.

A WWF-Australia production.

Published in August 2019 by WWF-Australia (Sydney, Australia).

CONTENTS

BACKGROUND

Conservation Status of Hawksbill Turtles

The Asia-Pacific region is home to six of the seven species of marine turtles including green, hawksbill, loggerhead, flatback, olive ridley, and leatherback. All species have been assessed at risk of extinction in the IUCN's Red List and are all listed as Vulnerable to Critically Endangered, except for Australia's data deficient endemic flatback turtle (IUCN Red List 2015).

The Tortoiseshell Trade

Marine turtles have survived for 120 million years, yet marine turtles in the Asia-Pacific region are facing a range of threats, including harvesting for food, bycatch in commercial fisheries, loss of nesting beaches and habitats, and climate change. Also, the hawksbill turtle is highly prized for its beautiful carapace and for centuries this species has been exploited for its tortoiseshell (bekko) to make jewellery such as earrings, necklaces, bracelets etc. It has been recently estimated that the tortoiseshell trade network, concentrated in Southeast Asia, harvested approximately 9 million hawksbill turtles over 150 years, over six times previous estimates (Miller et al. 2019).

THE TORTOISESHELL TRADE NETWORK HAS HARVESTED **APPROXIMATELY 9 MILLION HAWKSBILL TURTLES** OVER 150 YEARS

The abundance of hawksbill turtles is estimated to be at least 80% lower than historical levels, with many populations still declining (Mortimer and Donnelly 2008). As such, it is listed as Critically Endangered (IUCN Red List, 2015). Hawksbills are still subject to a significant illegal trade in many countries of Southeast Asia and Polynesia (Miller et al. 2019), which is now a major threat thought to be driving populations to near extinction.

Although interventions by the Convention on International Trade in Endangered Species (CITES) resulted in a downward trend in the bekko industry, the tortoiseshell trade is alive and intact (CITES Secretariat, 2019), and is considered to be having long-lasting detrimental effects on hawksbill turtle populations in the Asia-Pacific region (IOSEA, 2014). Between 1950-1992, almost 500,000 hawksbills were exported from the region for the bekko industry (Mortimer and Donnelly 2008). Between 2000 and 2008, a trade volume of over 9,180 marine turtle

©WWF-TNC JOINT MARINE PROGRAM, BERAU / TURTLE FOUNDATION

[1] including the South east Asia, The Coral Triangle, Western Pacific and Australia

products, 2,062 whole marine turtle, 6,161 crafted products and over 1 tonne of raw shell (789 scutes and 919 kg) were seized in East Asia (IOSEA, 2014). More recently between January 2015 – August 2018, seizures from Indonesia, Malaysia and Vietnam recorded trade of 84,116 eggs, 1,836 live turtles, 300 dead, 174 stuffed/ taxidermied, 687kg of meat, 936 crafted products and around 1 tonne of raw shell (1,023 scutes and 739kg shell) (CITES Secretariat, 2019).

While we know the key nations poaching hawksbill turtles for shell, we also know poachers are encroaching the national waters and countries of the Coral Triangle and Western Pacific (Lam et al., 2011). The illegal trade is thought to range from small scale harvests where turtles are harvested to support local markets to large scale operations where turtles are poached and then transported (overland, shipped or airlifted) internationally (Miller et al., 2019; Madden Hof, 2018). But because turtles may be poached both on nesting beaches and at feeding grounds it can be difficult to estimate where turtles are being sourced, how many, which nesting population[s] (genetic stock[s]) are being impacted the most. This knowledge is critical for designing targeted interventions for conserving and recovering hawksbill turtle populations.

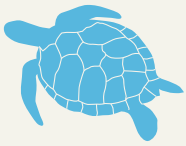
©ROGER LEGUEN / WWF

The Role of DNA

Because the marine turtle supply chain has changed significantly and tortoiseshell trade has seemingly shifted from open market to a more covert form (CITES Secretariat, 2019; Madden Hof, 2018) we need to use new technologies to track the source population of turtles poached. In addition to physical tagging and satellite transmitters, molecular (DNA) sampling is being used as a more time-efficient and cost-effective tracking tool to determine the geographical origin of individuals. Novel genetic approaches have been a useful tool in wildlife forensic investigations. These techniques have been successfully applied to illegal poaching of elephants, tigers, rhinoceros, birds etc. (Nishant et al. 2018). For example, DNA extracted from ivory has been used to identify the location of the poaching hotspots for African elephants, by statistically matching the genetic signature of confiscated ivory to geographic or population-specific genetic signatures (Ehman et al. 2015).

These methods have never been comprehensively applied to the tortoiseshell trade, but similar genetic techniques from turtle tissue have been extensively used to identify the nesting origin of marine turtles sampled at foraging areas, strandings and those caught as fishery by-catch (e.g. Jensen et al. 2013; LaCasella et al. 2013). There is one previous study that has extracted DNA from confiscated tortoiseshell products seized by the US Fish and Wildlife Services (Foran & Ray 2016). Through the sequencing of approximately 500 bp fragments of the mitochondrial DNA, they identified the Indo-Pacific region as a hotspot for illegal tortoiseshell products entering the United States but were not able to further pinpoint the geographical origin of the samples. This highlights the need for a concerted effort to further

develop and apply powerful genetic techniques to help identify poaching hotspots for this critically endangered species across Asia-Pacific.



A METHOD TO HELP IDENTIFY POACHING HOTSPOTS

Aims of this Study

Here, we build on these previous efforts by developing a reliable method and protocol for extracting and sequencing mtDNA from hawksbill turtle carapace, by:

1. Piloting DNA extraction and sequencing from both tissue and shell samples from the same individual to serve as a positive control for accurately assigning mtDNA haplotypes between individual turtle tissue and turtle shell.
2. Using shell product (donated from Papua New Guinea and the Solomon Islands) to refine the DNA extraction and sequencing method from processed shell products, using:
   a. modified, yet commercially available, kits that can be easily replicated and upscaled in tortoiseshell trade countries
   b. higher quality and longer fragment of mtDNA (>770bp) to match genetic nesting data
   c. updated techniques that are safer to use and do not rely on traditional potentially harmful chemicals such as phenol/chloroform
3. Sequencing additional tissues samples from nesting beaches of Milman Island, Queensland and Arnavon Islands, Solomon Islands and foraging grounds of the Howick Group of Islands to add to the Shell Bank baseline dataset being established across Asia-Pacific for hawksbill rookeries

This study forms part of a larger Initiative to address Marine Turtle Use and Trade in Asia-Pacific, coordinated by WWF-Australia. The overarching goal of this Initiative is to safeguard hawksbill turtle populations in the Asia-Pacific region, so they are no longer at risk of extinction, and no longer targeted for trade. This component of work aimed to develop a reliable method to extract DNA from turtle products to trace back which nesting turtle populations are being targeted along the use-trade chain from 'sale to source', and further our understanding of hawksbill turtle genetic stock boundaries for future management. This study provides the methodology and demonstrates the proof of concept that can be scaled up and trailed in other tortoiseshell demand and supply countries. Future iterations of this study, by Michael Jensen, NOAA collaborators and WWF include translating the DNA shell extraction and sequencing method into a scientific protocol detailing the use of this tool for future trials and use by decision-makers and forensic investigators.

©HAL BRINDLEY/TRAVEL FOR WILDLIFE.COM / TOO RARE TO WEAR

© WWF-AUS / MELISSA STAINES

HOW DOES IT WORK?

A brief introduction to hawksbill turtle population genetics

Marine turtles are known as the ancient mariners. They are highly migratory and often utilise foraging areas far (sometimes 1,000s of km) from where they were born. However, one of the most remarkable facts about marine turtles is their natal homing behaviour where female (and possibly male) turtles return to their region of birth to breed and lay eggs. The mitochondrial (mt) DNA is maternally inherited, meaning that it is only passed down from mothers to their offspring, making it the genetic marker of choice for detecting population structure of marine turtle nesting populations. Because female turtles are highly philopatric to their natal region, they generate a strong genetic similarity between turtles nesting within the same area and significant differences between regions (Jensen et al. 2013). As such, they essentially generate a characteristic genetic signature for each nesting region. These genetically distinct nesting populations are also referred to as genetic stocks or Management Units (MUs). When a comprehensive understanding of the genetic signature of all (or most) nesting populations is available, they can be used to identify the origin of samples collected away from the nesting beaches such as foraging areas, fisheries bycatch or illegal tortoiseshell products. This approach is known as mixed stock analysis and provides a rigorous statistical approach to estimating the geographical origin of samples. This analysis has been extensively used in marine turtles, including hawksbills to determine the rookery origin of turtles sampled at foraging areas (e.g. Gaos et al. 2017; Bell et al. 2018). However, the power of mixed stock analysis depends on the extent to which all the potentially contributing nesting populations have been sampled. For hawksbill turtles in the Asia-Pacific, our understanding of the population structure is limited. Nonetheless, recent studies have made significant advances identifying at least five distinct genetic stocks (Figure 1) (Nishizawa et al. 2016; Vargas et al. 2016,), and sampling is well underway by multiple organisations to characterise remaining gaps. Recently, a Shell Bank concept and hawksbill genetic working group were established by WWF to bring together these multiple organisations to collectively develop this trans-national genetic baseline database through partnership and capacity building.

DNA EXTRACTION METHOD

Sample Collection

Samples were collected from both live nesting and foraging turtles, as well as from tortoiseshell products, by WWF-Australia, The Nature Conservancy, and the Queensland Government.

A total of 23 tissue samples were collected from nesting females at Milman Island (Queensland, Australia) during the nesting season in 2017 and 28 tissue samples from Arnavons Islands, (Solomon Islands) in 2017/2018. A total of 16 samples were collected from eight live foraging turtles at the Howick Group of Islands (Queensland, Australia), two samples from each individual; one tissue sample and one carapace sample. These samples were used as positive controls by comparing a high-quality sample (soft tissue) and supposed low-quality sample (carapace). Finally, a total of 13 samples were donated from local markets in Papua New Guinea (Kokopo market, n=2; Nusa Island market, n=2; and 4 mile island market, n=2) and Solomon Islands (Mendana, n=2; Central market, n=3; and Ladies market, n=2) (Figure 2) and imported to NOAA under CITES scientific exchange permits AU062 and US057.

DNA Extraction

Laboratory work was conducted by scientists from the Southwest Fisheries Science Center (SWFSC) Marine Turtle Genetics Program (NOAA) in La Jolla, USA. As a global leader in generating, analysing and interpreting genetic marine turtle data, these scientists provided the necessary expertise to ensure the DNA shell extraction method developed was robust and could consistently and reliably be sequenced to match DNA extracted from turtle tissues.

Soft tissue samples from live turtles were extracted following a standard NucleoMag® 96 Tissue Kit extraction protocol (Macherey-Nagel, cat. No. 744300.4) and were performed in the Pre-PCR genetics laboratory at the SWFSC, La Jolla, USA.

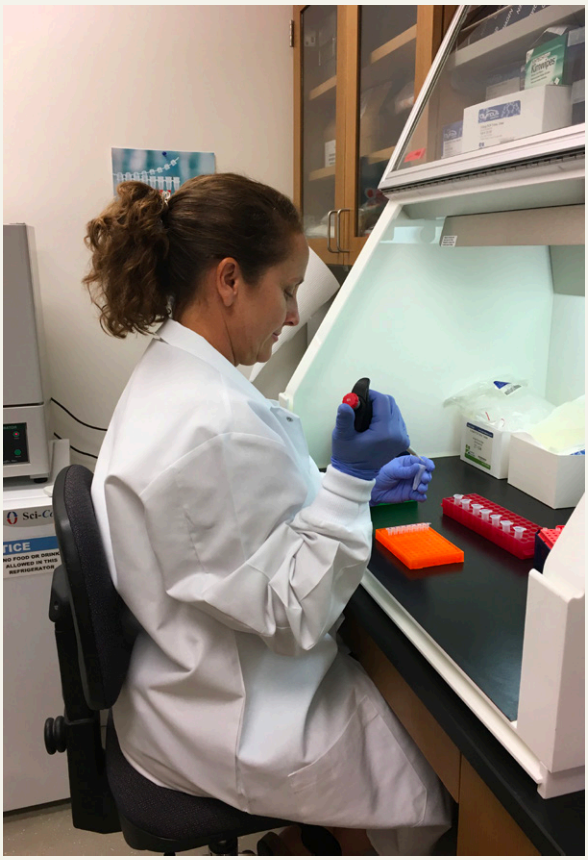
Due to the possible difficulties of obtaining high-quality DNA from turtle shells, the initial testing of DNA extraction was undertaken in the Ancient DNA Laboratory at the SWFSC. From the shell, a small amount (0.8-1.0 g) of powder was collected using a drill. The DNA is extracted following a modified protocol using the DNeasy® Blood & Tissue kit (cat. No. 69504. Qiagen, Germany) in a sterile environment to avoid contamination.

In both cases (tissue and shell), the DNA was sequenced by polymerase chain reaction (PCR) methodologies using the primers LCM-15382 and H950 g (Abreu-Grobois et al. 2006) to amplify approximately 800 base pairs (bp) of the mtDNA control region (or d-loop). The PCR products were then sequenced and the individual genetic variant (haplotype) determined.

A detailed laboratory protocol will be published separately.

Sequence Data

Forward and reverse sequences were aligned using Geneious v8.1 (www.geneious.com, Kearse et al. 2012). The quality of the sequences was assessed by eye. All sequences were aligned, and haplotype assigned by two independent people to assure accurate assignment. All sequences were named according to a standardised nomenclature for Indo-Pacific hawksbill turtles, using the prefix EiIP followed by the next sequential number. New haplotypes were submitted to the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).



© ERIN LACASELLA / NOAA

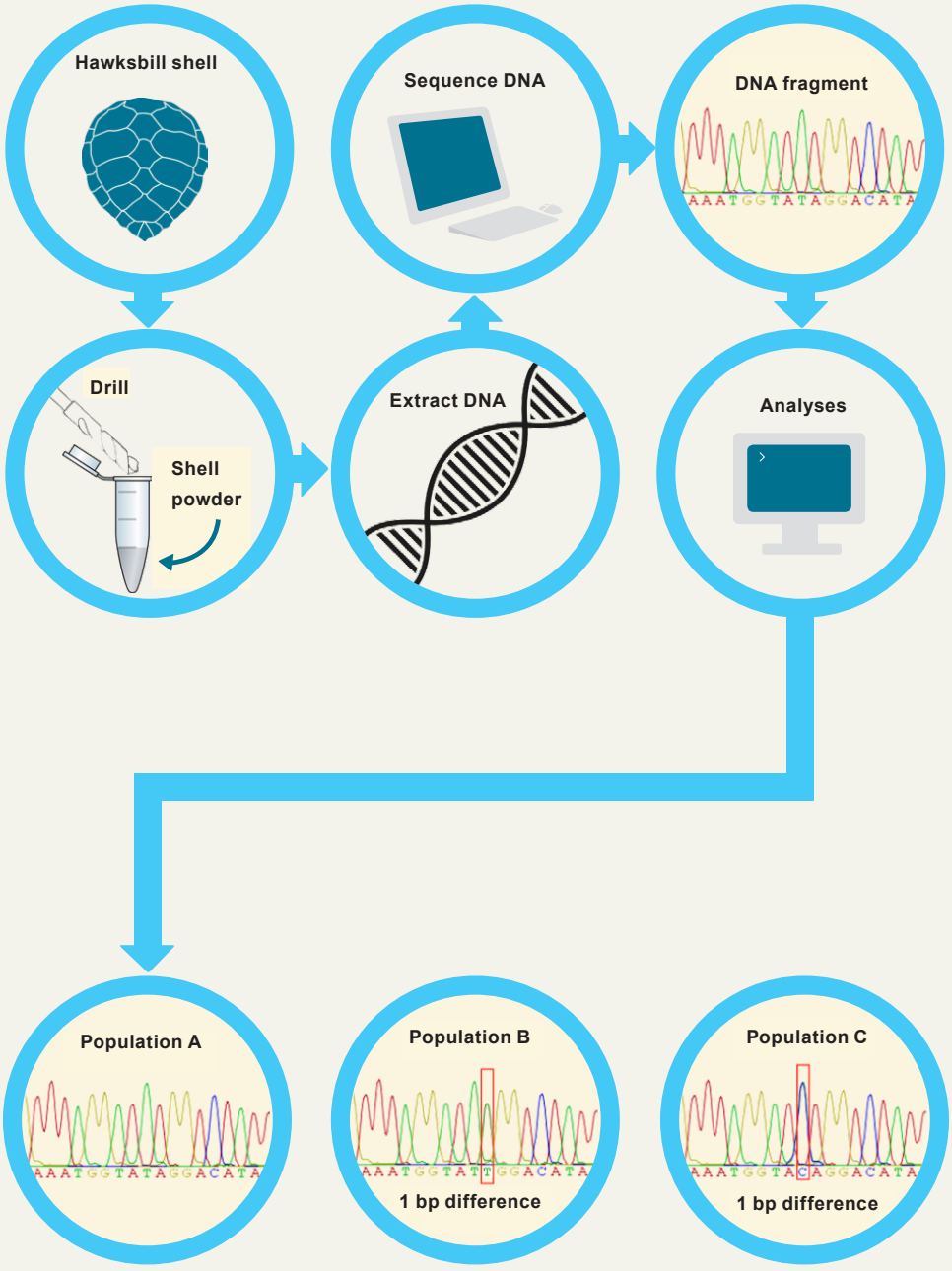
Nesting Data

The new nesting samples from Milman Island and Solomon Islands originated from rookeries previously published by Vargas et al. (2016). To test for temporal variability within each of those two rookeries and genetic differentiation among the two sampling locations we used two different measures of population differentiation; a conventional FST test based only on haplotype frequencies (Slatkin 1995), and the sequence-based φST test. Both the FST and φST tests were performed using Arlequin (v. 3.5) (data not shown).

Shell and Foraging Samples

The final step essentially compares the sample DNA to genetic signatures of possible source populations to determine the population of origin (refer Box 1). For this pilot study, the haplotypes identified in the shell samples were compared to surrounding rookeries for a basic assessment of nesting origin. When a larger sample size is available, a Bayesian statistical approach called mixed stock analysis will be applied (see discussion for more details). A detailed description of the DNA shell extraction method and analysis will be published in a scientific journal at a later stage.

DETECTION OF POPULATION ORIGIN USING DNA



Box 1: *Simplified workflow to determine the population origin of tortoiseshell.*

PRELIMINARY RESULTS

Expansion of the Nesting Baseline

The samples from Milman Island (n=22) and Solomon Islands (n=28) revealed three haplotypes from Milman Island and four haplotypes from the Solomon Islands with no haplotypes shared between the two rookeries. There were no significant differences in haplotype frequency between samples collected in this study and previously reported samples from Milman Island or Solomon Islands (Vargas et al. 2016) suggesting temporal stability in the haplotype frequencies over time. Therefore, samples from the two studies are combined to increase the sample size for these rookeries (Figure 1). When new and old data were combined, these two rookeries remain significantly differentiated and thereby support previous work that these two rookeries represent two demographically independent stocks (Vargas et al. 2016). These data were added to the regional database of rookeries throughout the region and will serve as part of the baseline dataset (Figure 1).

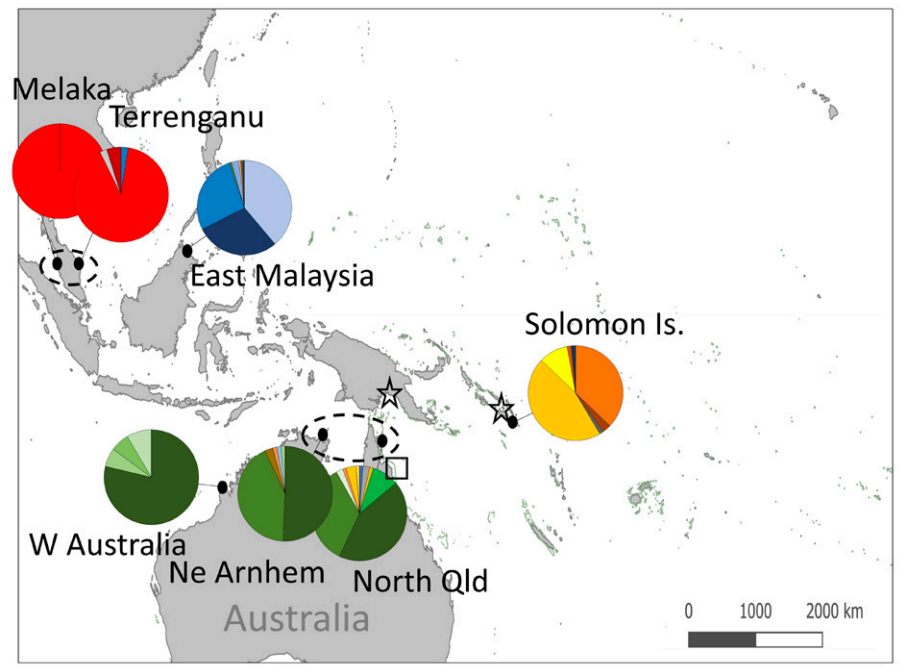


Figure 1. *Current rookery baseline for the western Pacific/SE Asia. Black circles show the locations of the sampled rookeries; Melaka (N=45), Terengganu (N=41), East Malaysia (N=157), Western Australia (N=47), Northeast Arnhem land (N=71), North Queensland(N=86) and Solomon Islands (N=70). Pie charts show the haplotype frequencies (genetic signature) of each rookery with different colours representing different haplotypes. Dotted circles group together rookeries that are not genetically differentiated and thereby represent the same genetic stock or management unit. Stars show the location of jewellery donated from markets in Papua New Guinea and the Solomon Islands, and square shows the location of the Howick Group foraging area.*

Validation of DNA Quality from Tortoiseshell

Two samples (one tissue and one carapace) were collected from the same individual for a total of eight animals collected from live turtles at the Howick Group foraging ground. The purpose of this was to compare the quality and haplotype assignment of

DNA sequences obtained from shell samples, which were assumed to be of low quality. The comparison between the tissue and carapace samples showed no difference in the overall quality of the sequences. All tissue and carapace samples from the same animal identified the same haplotype. One haplotype (EiIP64) was a previously unidentified "orphan" haplotype.

Market Product Samples

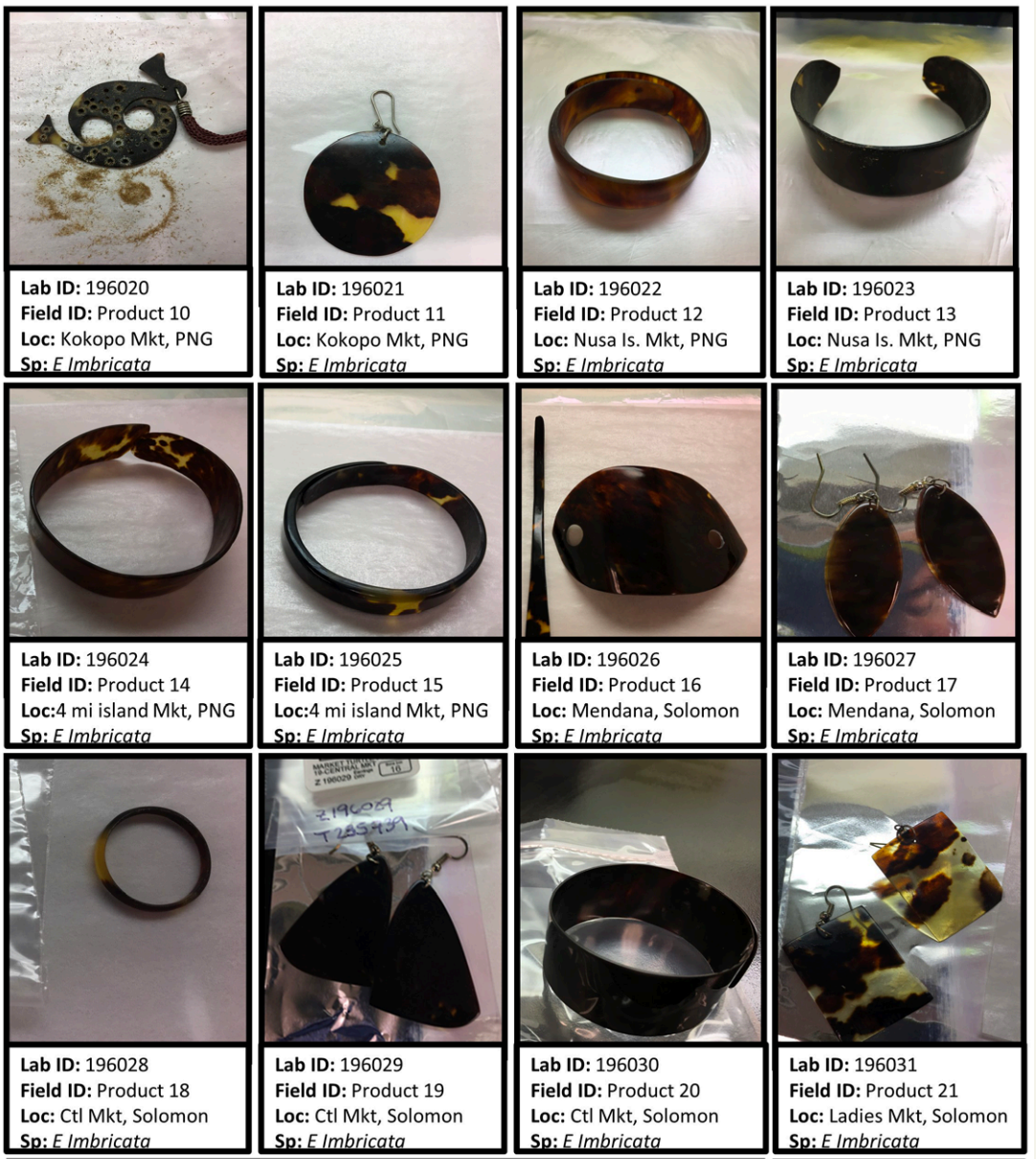
In the samples collected at markets in PNG we identified four different haplotypes, two of which have been identified at rookeries EiIP03 (N=1) and EiIP33 (N=2) and two (orphan) haplotypes, EiIP55 (N=1) and EiIP59 (N=1) (Figure 2). However, these "orphan" haplotypes have previously been identified in foraging turtles from the Howick Group (Bell et al. 2018). For the samples collected at markets in Solomon Islands, we identified three different haplotypes, EiIP33 (n=4), EiIP34 (n=1) and EiIP39 (n=2) of which the latter is also an orphan haplotype. A total of 12 samples (92%) amplified successfully for the approximately 800 bp fragment with only one sample not working (Lab-id 196025, a bracelet from PNG). Of the 12 samples, four (33%) were orphan with no known nesting origin yet. This highlights the urgent need for a comprehensive baseline dataset.

HAWKSBILL TURTLE HATCHLINGS © ANGE AMON / LISSENUNG ISLAND RESORT / WWF-AUS

JUVENILE HAWKSBILL TURTLE, PAPUA NEW GUINEA© JÜRGEN FREUND / WWF

HAWKSBILL TURTLE, HOWICK ISLANDS, JULY/AUGUST 2015. © WWF-AUS / CHRISTINE MADDEN HOF

It is the responsibility of all to ensure protection of turtles at all stages of their development.



Lab-ID	Location	Collection date	Haplo	Likely stock origin
196020	Rabaul, Kokopo market, PNG	06-04-16	EilP03	Solomon Stock
196021	Rabaul, Kokopo market, PNG	06/14/2016	EilP33	Solomon Stock
196022	Kavieng, Nusa Island markets, PNG	06/16/2018	EilP33	Solomon Stock
196023	Kavieng, Nusa Island markets,PNG	06/16/2018	EilP59	"Orphan"
196024	Port Moresby, 4 mile island market, PNG	06/13/2018	EilP55	"Orphan"
196025	Port Moresby, 4 mile island market, PNG	06/13/2018	Fail	-
196026	Mendana, Honiara, Solomon Is	04-03-17	EilP33	Solomon Stock
196027	Mendana, Honiara, Solomon Is	05-03-17	EilP33	Solomon Stock
196028	Central Market, Honiara, Solomon Is	03/28/2017	EilP33	Solomon Stock
196029	Central Market, Honiara, Solomon Is	03/28/2017	EilP39	"Orphan"
196030	Central Market, Honiara, Solomon Is	03/28/2017	EilP34	Solomon Stock
196031	Ladies Market, Honiara, Solomon Is	04-03-16	EilP39	"Orphan"
196032	Ladies Market, Honiara, Solomon Is	03/28/2017	EilP33	Solomon Stock

Figure 2. Photos of 13 E. imbricata products sampled in Papua New Guinea (n=6) and Solomon Islands (n=7). The table shows the Lab-ID, Location, collection date, mtDNA d-loop haplotype and likely origin for each sample (*the likely origin should be interpreted with caution until a more robust analysis can be completed, see Discussion).

DISCUSSION

The immediate aim of this project is to trace back the nesting origin of hawksbill turtles from tortoiseshell products sold throughout Asia-Pacific.

The ultimate aim is to help understand the genetic stock boundaries of turtle population for better national and regional conservation management. Whereby DNA extraction techniques exist for turtle tissue and are extensively available, tortoiseshell products can provide an additional means of assessing threats to specific populations, from which conservation strategies can be established.

The first step in this process - to extract high-quality DNA from turtle products - has been completed by successfully extracting and sequencing more than 770 base-pairs of the mitochondrial DNA d-loop of 20 turtle products. Of the 21 carapace samples sequenced only one did not sequence. To support the identification of haplotypes from carapace samples, all turtles for which both tissue and carapace was sequenced matched each other 100%. This shows that the extraction methods described in this report produce high-quality DNA sequences from tortoiseshell products.

HIGH-QUALITY DNA EXTRACTED FROM TORTOISESHELL PRODUCT

While the baseline sample of rookeries is still limited for hawksbill turtles in the region, all the samples matched known haplotypes (although some have only been found in foraging populations). However, of the samples collected from markets in PNG and Solomon Islands, 33% were “orphan” haplotypes, meaning that they have yet to be identified in a nesting population and their origin remains unknown at this point. The high percentage of orphan haplotypes is a sign that the turtles originated from a rookery with either a small sample size, or a yet to be sampled rookery. The remaining three samples from PNG were identical to haplotypes found primarily at Solomon Islands rookery. Likewise, two of the jewellery samples from the markets in the Solomon Islands matched common haplotypes from the Solomon Islands rookery while the rookery origin of one haplotype (EiIP39) remains unknown. While these initial tests give us high confidence in the feasibility of tracing back the nesting origin of tortoiseshell products, several challenges remain to be addressed to increase its efficiency, and before a protocol can be released for future use.

Future challenges

The main challenge for the success of accurately assigning the nesting origin is to build a comprehensive reference database. In recent years several genetic studies have improved our understanding of the population structure of hawksbill turtles across the Indo-Pacific (Vargas et al. 2016; Gaos et al. 2016; Nishizawa et al. 2016). Nonetheless, there are still gaps in our understanding of population genetic structure, mostly due to limited sampling (see Figure 3). Given the broad distribution and breeding ecology of hawksbill turtles, notably throughout the Pacific, many of the numerous and isolated archipelagos that might provide suitable nesting or foraging habitat to hawksbill turtles are rarely visited by researchers. These challenges are being actively addressed through WWF’ Shell Bank concept and formation of the hawksbill genetic working group (WWF/NOAA/Griffith University/ Queensland Government/The Nature Conservancy, and other regional partners). The primary purpose of this working group is to address these knowledge gaps by 1) identifying the nesting distribution of hawksbill turtles throughout Asia-Pacific, and 2) promote sample collection, analysis and sharing throughout the region so that a comprehensive rookery baseline can be available for future studies. The goal is to have available a rookery reference similar to that of green turtles, where the majority of key rookeries have been genetically characterised (Jensen et al. 2019).

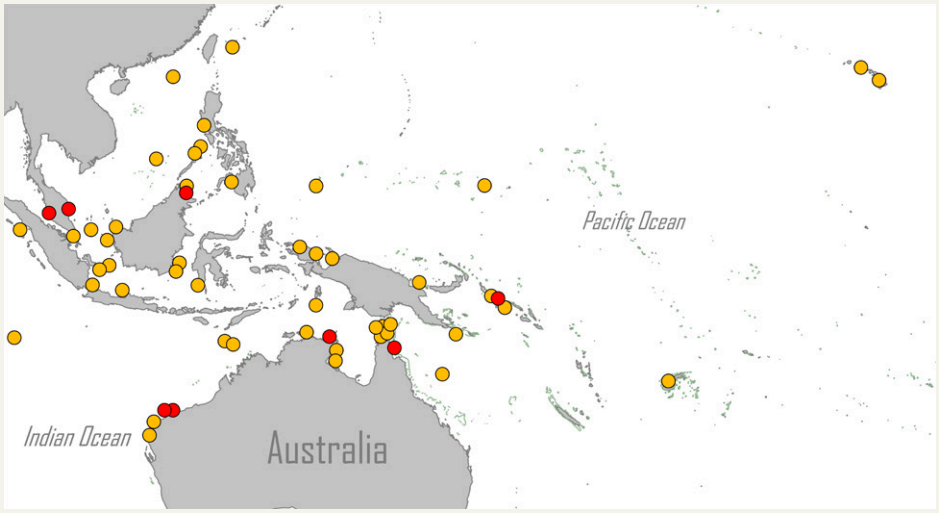


Figure 3. *Nesting distribution and current rookery baseline for hawksbill turtles in the western Pacific/SE Asia. Circles show known nesting locations for hawksbill turtles (adapted from FitzSimmons & Limpus 2014). Red circles show the locations of the sampled rookeries highlighting the need for further sampling.*

We must gain a better understanding of the geographical boundaries of genetic stocks. Often a genetic stock (i.e. nesting population) is only characterised by a single nesting beach when in reality the demographically independent unit that characterises the genetic stock is made up of multiple regional nesting beaches. This issue is highlighted by Bell et al. (2018) where genetic data suggest that hawksbill turtles foraging at the Howick Group of Islands in the northern Great Barrier Reef originated primarily from the Solomon Island stock rather than the local rookery at Milman Island. However, only one nesting site is used to characterise the Solomon Islands, and additional tagging data clearly shows that turtles that genetically assign to Solomon Islands, nested throughout the broader Bismarck-Solomon sea region. This suggests that the boundaries of the Solomon Islands nesting stock extend beyond Solomon Islands (Figure 4). Hence, the conclusions drawn from these types of studies need to be interpreted in light of the limitations or uncertainties of the data used. As the coverage of sampled hawksbill nesting beaches improves in the coming year(s), we will get a better understanding of the actual geographical boundaries of each nesting stock, and hence, be able to trace turtles back to their nesting population of origin more accurately.

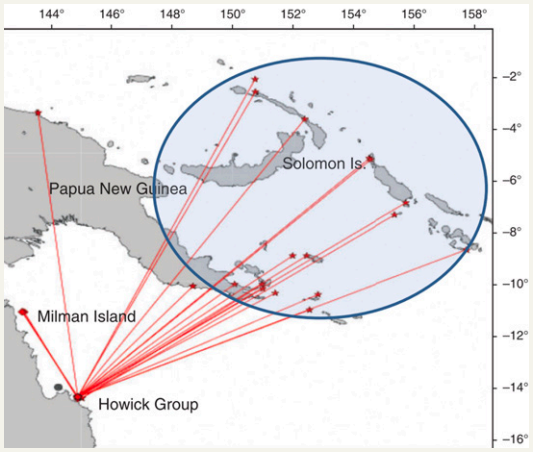
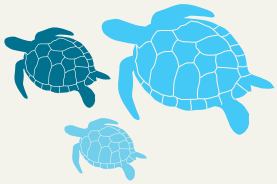


Figure 4. *Within-country and international tag recoveries from Eretmochelys imbricata originally tagged in the Howick Group and, subsequently, recaptured following a breeding migration to Bismarck-Solomon Sea region (shaded area). Foraging site capture (circles); international nesting beach (stars); within-country nesting beach (rectangles). Lines indicate connection between foraging and nesting locations. (Firgure 1. from Bell & Jensen 2018).*



CONCERTED EFFORTS SHOULD BE FOCUSED ACROSS ASIA-PACIFIC

Conclusion

With a small sample, we were able to extract and sequence high quality DNA from tortoiseshell products (for sale) and trace back to turtle populations (at source). The study showed that the products collected at markets in PNG and Solomon Islands most likely originated from the Solomon island stock and not from nesting populations in Australia or SE Asia. However, as discussed above, caution needs to be taken until a more comprehensive rookery baseline is available. Even so, this technique shows promise for the use of DNA to trace back the nesting origin of tortoiseshell products. Concerted efforts should be focused across Asia-Pacific to extend sampling of hawksbill nesting beaches to gain a comprehensive rookery baseline dataset. Also, efforts should focus on training and developing the necessary skills within countries to extract, sequence and analyse tortoiseshell samples, including for future use in forensic investigations.

REFERENCES

Abreu-Grobois FA, Horrocks JA, Formia A, Dutton PH, LeRoux RA, Velez-Zuazo X, Soares LS, Meylan AB. 2006. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analysis. In: *Proceedings of the 26th Annual Symposium on Sea Turtle Biology, Book of Abstracts*. Frick M, Panagopoulou A, Rees AF, Williams K (eds) ISTS, Island of Crete, Greece, p 179

Bell, IP and Jensen MP. 2018. Multinational Genetic Connectivity Identified in Western Pacific Hawksbill Turtles, *Eretmochelys imbricata*. *Wildlife Research* 45 (4): 307–15.

CITES Secretariat. 2019. Status, scope and trends of the legal and illegal international trade in marine turtles, its conservation impacts, management options and mitigation priorities. Eighteenth (18th) meeting of the CITES Conference of the Parties (Geneva, August 2019), Document CoP18 Inf. 18.

Ehman EC, Johnson GB, Villanueva-meyer JE, Cha S, Leynes AP, Eric P, Larson Z, and Hope TA. 2015. Genetic Assignment of Large Seizures of Elephant Ivory Reveals Africa's Major Poaching Hotspots. *Science* 46 (5): 1247–62.

Foran DR and Ray RL. 2016. Mitochondrial DNA Profiling of Illegal Tortoiseshell Products Derived from Hawksbill Sea Turtles. *Journal of Forensic Sciences* 61(4): 1062–1066.

Gaos AR, Lewison RL, Jensen MP, Liles MJ, Chavarria S, Mario C, Valle M, et al. 2017. Natal Foraging Philopatry in Eastern Pacific Hawksbill Turtles. *Royal Society Open Science* 4: 170153.

Gaos AR, Lewison RL, Liles MJ, Gadea V, Altamirano E, Henríquez AV, Torres P et al. 2016. Hawksbill Turtle Terra Incognita: Conservation Genetics of Eastern Pacific Rookeries. *Ecology and Evolution* 6(4): 1251–1264.

Jensen MP, FitzSimmons NN, Dutton PH and Michael P. 2013. Molecular Genetics of Sea Turtles. In: *The Biology of Sea Turtles, Volume 3*, edited by Wyneken J, Lohmann KJ and Musick JA. 14:135–54. CRC Press, Boca Raton, FL.

Jensen MP, FitzSimmons NN, Bourjea J, Hamabata T, Reece J and Dutton PH. 2019. The Evolutionary History and Global Phylogeography of the Green Turtle (*Chelonia mydas*). *Journal of Biogeography,* no. February 2017: jbi.13483.

Kearse, M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, et al. 2012. Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data. *Bioinformatics* 28 (12): 1647–49.

LaCasella EL, Epperly SP, Jensen MP, Stokes L and Dutton PH. 2013. Genetic Stock Composition of Loggerhead Turtles *Caretta Caretta* Bycaught in the Pelagic Waters of the North Atlantic. *Endangered Species Research* 22 (1): 73–84.

Lam T, Xu L, Takahashi S and Burgess EA. (2011). Market Forces: An Examination of Marine Turtle Trade in China and Japan. TRAFFIC East Asia, Hong Kong.

Madden Hof CA. 2018. WWF Marine Turtle Use and Trade in Asia-Pacific Program of Work." Initiative of WWF-Australia. Accessed 12 July 2019.

Miller EA, McClenachan L, Uni Y, Phocas G, Hagemann ME and Van Houtan KS. 2019. The Historical Development of Complex Global Trafficking Networks for Marine Wildlife. *Science Advances* 5 (3): eaav5948.

Mortimer JA and Donnelly M. 2008. Eretmochelys Imbricata. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1. <www.iucnredlist.org>. Downloaded on 14 August 2013.

Nishant K, Vrijesh KY and Ajay KR. 2018. Wildlife Forensic: Current Techniques and Their Limitations. *Journal of Forensic Science & Criminology 5* (4).

Nishizawa H, Joseph J and Chong YK. 2016. Spatio-Temporal Patterns of Mitochondrial DNA Variation in Hawksbill Turtles (*Eretmochelys imbricata*) in Southeast Asia. *Journal of Experimental Marine Biology and Ecology* 474: 164–70.

Slatkin M. (1995). A Measure of Population Subdivision Based on Microsatellite Allele Frequencies. *Genetics* 139:457–462.

Vargas SM, Jensen MP, Ho SYW, Mobaraki A, Broderick D, Mortimer JA and Whiting S, et al. 2016. Phylogeography, Genetic Diversity, and Management Units of Hawksbill Turtles in the Indo-Pacific. *Journal of Heredity,* no. 107: 199–213.

© NATUREPL.COM / INAKI RELANZON / WWF

PROTECT OUR OCEANS

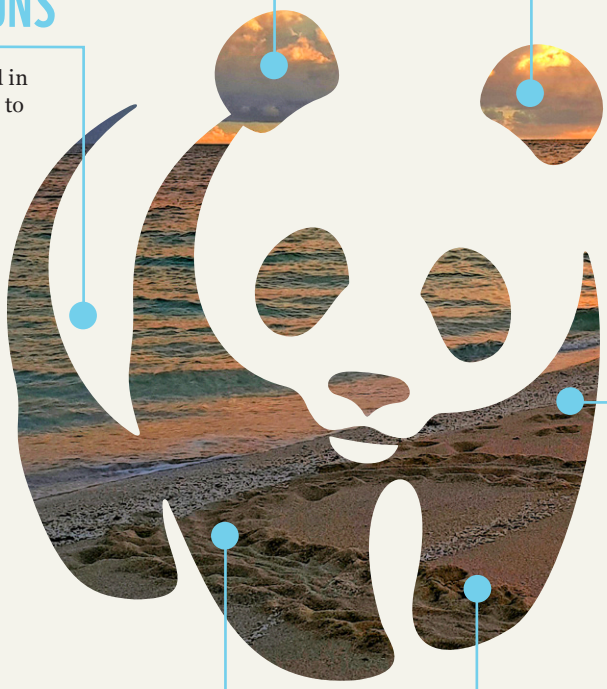
Ocean and reef ecosystems resilient, productive and contribute to food security.

SAVE THREATENED SPECIES

Priority ecosystems protected and restored and key species back from the brink of extinction.

ENGAGE MILLIONS

2 million Australians united in supporting WWF's mission, to invest in our natural world.



'PLANET-FRIENDLY' FOOD

More widely available than ever before and Australians making more sustainable food choices.

A STABLE CLIMATE

Australia on the way to net-zero emissions by 2050 and a more climate-resilient future.

CONSERVE NATURE WITH EQUITY

Delivering positive outcomes for people and nature across all WWF initiatives.

WWF

Why we are here

To stop the degradation of the planet's natural environment and to build a future in which humans live in harmony with nature.

wwf.org.au

WWF-Australia National Office

Level 1/1 Smail Street,
Ultimo NSW 2007
GPO Box 528
Sydney NSW 2001

Tel:+1800 032 551
enquiries@wwf.org.au
@WWF_Australia
wwf.org.au