



# SURRENDER YOUR SHELL

DETECTING THE ORIGIN OF TORTOISESHELL PRODUCTS  
JUNE 2022



**Acknowledgements**  
This report was written by Christine Madden Hof (WWF-Australia), Michael Jensen (Aalborg University/research contractor to WWF-Australia) and Greta Frankham (Australian Museum), with contributions and review by Francesca Roncolato (WWF-Australia).

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**Australian Museum**  
The Australian Museum (AM) was founded in 1827 and is the nation's first museum. It is internationally recognised as a natural science and culture institution focused on Australia and the Pacific. The AM's mission is to ignite wonder, inspire debate and drive change. The AM's vision is to be a leading voice for the richness of life, the Earth and culture in Australia and the Pacific. The AM commits to transforming the conversation around climate change, the environment and wildlife conservation; to being a strong advocate for First Nations cultures; and to continuing to develop world-leading science, collections, exhibitions and education programs. With more than 21.9 million objects and specimens and the Australian Museum Research Institute (AMRI), the AM is not only a dynamic source of reliable scientific information on some of the most pressing environmental and social challenges facing our region, but also an important site of cultural exchange and learning.

Within AMRI, the Australian Centre for Wildlife Genomics (ACWG) is a key example of the scientific and research infrastructure underpinning AMRI research and houses the genomic laboratories and a frozen tissue collection. ACWG staff provide both research and molecular diagnostic capabilities to AMRI staff, as well as external stakeholders and collaborators. The research carried out by ACWG and AMRI scientists focuses on species discovery, evolution, practical conservation applications for Australia's threatened species, and improved wildlife forensics testing capacity for species caught in the illegal wildlife trade. The ACWG is an ISO 17025 accredited facility offering wildlife forensics services (DNA and morphology) to assist Australian Government agencies in achieving improved enforcement and compliance outcomes in wildlife crime and biosecurity infringements.

**WWF**  
WWF is one of the world's largest and most experienced independent conservation organisations, 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 is 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 involves collaboration 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, the traceability of hawksbill turtle stocks targeted in trade will rely on WWF's collaborative trans-Pacific forensic study characterising hawksbill turtle genetics origins and relationships between nesting and foraging populations.

**Royal Caribbean Group**  
This project would not have been possible without the generous support of Royal Caribbean Group. Since 2018, Royal Caribbean Group and WWF-Australia have been working together to better protect the hawksbill turtle from the illegal turtle trade. At Royal Caribbean Group, respect for the oceans and the environment is not a choice; it's a way of life. Oceans make up 71% of the planet and are 100% critical to our business and our right to operate. That's why we are intensely focused on using the best technology to advance our efforts to ensure a sustainable future for all of us.



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# BACKGROUND

## CONSERVATION STATUS OF HAWKSBILL TURTLES

Hawksbill turtles (*Eretmochelys imbricata*) are well known for their beautiful shells and the essential role they play in maintaining the health of coral reef ecosystems.

However, steep declines in many hawksbill turtle populations mean they are now listed as Critically Endangered internationally (IUCN, 2019), with only ~15–25,000 adult females left in the world (Mortimer and Donnelly, 2008). Many factors have contributed to this decline, including bycatch in fisheries, pollution, degradation of nesting and foraging habitats, and climate change, but the main threat driving hawksbills towards extinction in the Asia-Pacific region is legal and illegal unsustainable take (hunting and use of turtles and turtle parts) and trade. Scientists are concerned that continuing pressures on already depleted populations is rapidly pushing some hawksbill populations towards extinction (Bell et al., 2020; Hamann et al., 2022; Madden Hof et al., 2022).

## THE TORTOISESHELL TRADE

Hawksbill turtles were once abundant across most tropical regions of the world, but populations have been reduced by more than 75% in the Pacific Ocean when compared to historic levels. Only ~4,800 adult females are thought to remain (Mortimer and Donnelly, 2008). The south-western Pacific region was highly exploited to meet demand from the tortoiseshell trade throughout the nineteenth and twentieth centuries (Hamilton et al., 2015; Kinch and Burgess, 2009; Limpus, 2009), with much of this effort concentrated in north-eastern Australia, where hawksbill turtles were once abundant. From the early 1900s to the 1970s, items made from tortoiseshell were fashionable and demand for accessories made from tortoiseshell was high. Large numbers of hawksbills were also harvested in countries neighbouring Australia to meet this demand (Limpus, 2009). Laid in jewellery boxes and ornamental masks, used as guitar picks, hairpins, combs, earrings and sunglass frames, the tortoiseshell pattern remains as iconic as it was in the past, and demand for it continues to devastate hawksbill turtle populations. Recent estimates suggest the tortoiseshell trade network, concentrated in Southeast Asia, harvested approximately 9 million hawksbill turtles over 148 years from 1844–1992 (Miller et al., 2019).

*“Approximately 9 million hawksbill turtles were harvested over 148 years from 1844–1992.”*



The international trade of hawksbill turtles (and their parts) was banned globally in 1977 under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), with Japan removing its exemption to the CITES trade ban in 1992. But, even today, hawksbill turtles are still subject to a significant illegal trade in many countries globally (CITES Secretariat, 2019; Nahill et al., 2020). Demand is high through new and re-emerging black markets in Southeast Asia, including China, Japan, Vietnam, Taiwan and Hong Kong (Kitade et al., 2021; Lam et al., 2012). The online trade has also grown, courtesy of the internet. Unfortunately, the marine turtle supply chain has become more fragmented and opaque, making policy responses and enforcement increasingly difficult. In Australia, it is assumed that most tortoiseshell items were brought into the country prior to the ban (1977), however confiscation records show Australians, either knowingly or unknowingly, continue to buy tortoiseshell products made from this critically endangered species (Jo Beath, pers. communication).

It remains unclear where turtle take and trade is most prevalent today. The marine turtle supply chain has also changed significantly, and seemingly shifted in some countries from open to more covert markets. The illegal trade in hawksbill turtles now ranges from domestic, small-scale hunting to support local markets, through to small-scale or commercial fisheries supplying larger illegal trade networks, to large-scale international operations directly harvesting and trafficking large quantities of turtles and turtle products (CITES Secretariat, 2019; Miller et al., 2019; Vuto et al., 2019).

A lack of investigation, indictment, compliance and enforcement capacity across the region enables the illegal hawksbill trade to continue. It cannot be dismantled without knowing where and how the tortoiseshell is being sold or, more importantly, data and evidence of where and what turtles are being illegally harvested. This is why, with the help of marine turtle genetics and DNA-based wildlife forensic science, WWF and its partners are building a toolkit to help governments, researchers and conservation managers track the turtle trade from ‘sale’ back to ‘source’.



# THE ROLE OF WILDLIFE FORENSIC SCIENCE

During the 21st Century, wildlife and environmental crime has emerged as one of the most pervasive and lucrative transnational crimes, only surpassed by drugs, weapons and human trafficking (Nellemann et al., 2016).

*“Wildlife forensics uses similar methods to investigating human-victim crime but focuses on IF a crime has taken place, WHAT species the suspect sample or item is, and WHERE it came from.”*



Combined estimates place the monetary value of all global, transnational organised environmental crime at between US\$70 billion and \$213bn per year, with Southeast Asia accounting for up to one-quarter of global demand for illegal wildlife (OECD, 2020). Like other transnational crimes, the direct and indirect consequences of wildlife crimes are vast, and include pushing many threatened species towards extinction. As with any criminal activity, dedicated enforcement, and investigative and forensic science capability is required to secure evidence that is admissible in court. Wildlife forensic science uses many of the same foundational techniques and methods used by forensic practitioners investigating human-victim crime, but within the context of wildlife crime the testing requirements often differ. DNA-based human-victim forensics is most commonly employed after a crime has taken place, with the purpose of determining who a sample belonged to (victim, perpetrators, witnesses, etc). In contrast, due to the varied nature of species protection at both a domestic and international level, wildlife forensic science is most commonly employed to determine *if* a crime has taken place, and usually focuses on determining *what* species a suspect sample or item comes from. Where sufficient species-level databases exist (which is rare), determining *where* an item came from (source location), or *who* the individual samples belong to (for example, matching a confiscated rhino horn to a poached carcass), and *how* they are related (individualisation, pedigree/parentage test, etc) is also becoming more relevant to a wildlife crime investigation.



*Being able to identify the location of populations targeted by the illegal trade is not only a vital traceability tool to aid law enforcement but a vital step in focussing protection and conservation efforts on the most vulnerable populations.*



**LIMITED  
TO VISUAL  
EXAMINATION**  
DNA-BASED IDENTIFICATION  
IS OFTEN LACKING IN MARINE  
TURTLE FORENSICS

Tortoiseshell products (and thus species) can often be identified visually, however, due to their global distribution, determining the geographical origin of the item suspected to have been illegally traded is particularly relevant to an investigation. Being able to identify the location of populations targeted by the illegal trade is not only a vital traceability tool to aid law enforcement but a vital step in focussing protection and conservation efforts on the most vulnerable populations.

Currently, there are only a handful of wildlife forensic laboratories around the world that are accredited (i.e. ISO 17025) or working to appropriate standards (Moore et al., 2021) to carry out wildlife forensic testing to produce court-admissible evidence. The Australian Centre for Wildlife Genomics (ACWG) at the Australian Museum is one such laboratory. It regularly carries out wildlife forensic casework for a range of state and federal agencies tasked with overseeing environment and biosecurity enforcement and compliance in Australia. Along with

forensic casework, the ACWG conducts research to improve scientific tools for investigating the illegal wildlife trade (eg. Ahlers et al., 2017; Brown et al., 2021; Ewart et al., 2018; Hogg et al., 2018; Summerell et al., 2019). While the ACWG has the capacity and resources to test for a range of species, including tortoiseshell products, many countries on the frontline of the illegal wildlife trade are developing economies, and resources for costly DNA-based wildlife forensic testing is often focused on more high-profile species and high-volume products, such as elephant ivory, rhino horn and pangolin scales. In many of these countries, marine turtle forensics is limited to visual examination, and rarely includes DNA-based species identification testing.



# TRACING THE POPULATION ORIGIN OF HAWKSBILL TURTLES

*Hawksbill turtles from different regions are genetically distinct, so we can trace tortoiseshell back to a turtle’s original nesting population, meaning we can identify those populations that have been targeted in the past and may still be targeted today in the tortoiseshell trade.*

Marine turtles are highly migratory and are known to travel hundreds and sometimes thousands of kilometres between nesting and foraging areas. However, one of the key life-history traits that make tracing the population origin of hawksbill turtles possible is their natal homing (philopatric) behaviour – females return to their region of birth to breed and lay eggs. The mitochondrial (mt) DNA is only passed down from mothers to their offspring. Because female turtles are highly philopatric to their natal region, those nesting within the same area are genetically similar, and genetically dissimilar from those nesting in other regions (Jensen et al., 2013). As such, turtles from each nesting region have a distinctive genetic signature. These genetic signatures can be used as a roadmap to trace the nesting origin of turtles, simply by comparing the DNA sequences of turtles from known nesting populations. Such stock identification methods can be used to estimate the geographical origin (nesting population) of samples and are routinely used to determine the origin of turtles at foraging areas (Jensen et al., 2016; Read et al., 2015; Velez-Zuazo et al., 2008), as well as those caught as fisheries bycatch or stranded (LaCasella et al., 2013; Rankin-Baransky et al., 2001; Stewart et al., 2016). But this technique has only recently been applied to the tortoiseshell trade (LaCasella et al., 2021).

The success of these methods depends on the extent to which all the potentially contributing nesting populations have been sampled. Our understanding of the population structure of hawksbill turtles in the Asia-Pacific remains limited. Recent studies have made significant advances in identifying at least five distinct genetic stocks (Nishizawa et al., 2016; Vargas et al., 2016), and sampling is well underway by multiple organisations to characterise remaining gaps.

WWF established the ShellBank program and Indo-Pacific Hawksbill Genetic Working Group (IPHWG) to bring together these multiple organisations to collectively develop a transnational genetic baseline database through partnership and capacity building.

The ShellBank Genetic Database is a global repository for hawksbill mtDNA haplotype (genetic variant) data that contains published and unpublished (though the IPHWG) genetic data in three categories: Rookery Baseline, In-Water and Tortoiseshell Products. The Rookery Baseline contains genetic data from samples collected from known hawksbill nesting populations. This is essential to tracing the origin of tortoiseshell products. The In-Water category primarily contains genetic data from juvenile and adult hawksbill turtles sampled at foraging areas, but also from stranded turtles or those caught as fisheries bycatch. While not directly used to trace the origin of tortoiseshell, this data allows for the geographical boundaries of nesting populations to be assessed (and, in turn, informs the ‘boundaries’ of criminal activity). The final category, Tortoiseshell Products, contains genetic information on tortoiseshell collected from warehouse stockpiles, seizures and the Surrender Your Shell (SYS) project. Initially, this database will help inform what Rookery Baseline data is missing. In time, it will grow into a unique resource for better understanding the population structure, connectivity and tortoiseshell trade of hawksbill turtles.



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*WWF established ShellBank and, as part of it, Surrender Your Shell, which provided Australians with an opportunity to contribute to marine turtle conservation in the Asia-Pacific region.*



## BUILDING SHELL BANK HELPS TO SUPPORT ACTION AGAINST ILLEGAL TURTLE TRADE

### SURRENDER YOUR SHELL

Subsequent to the establishment of a reliable method of DNA extraction from tortoiseshell products (LaCasella et al., 2021), a public-facing campaign called Surrender Your Shell (SYS) was launched to help advance knowledge of the hawksbill trade through citizen science tortoiseshell donations and surveys. SYS encouraged Australians to surrender historically purchased tortoiseshell to assist with the development of the Tortoiseshell Product Database through the identification of hawksbill populations directly targeted by the illegal tortoiseshell trade.

SYS was also established to trial WWF’s ShellBank in practice – to test the ShellBank toolkit to track turtle trade throughout Southeast Asia (and globally) by applying the DNA extraction method and further scrutinising its effectiveness on a greater variety of historical and newly donated tortoiseshell items. This was the first opportunity to improve the process of sample collection, sample handling, chain

of custody (a documented process of item handling and transportation between institutions) and laboratory procedures. Ultimately, our goal is to see ShellBank become a vital resource for law enforcement, allowing routine identification of populations most impacted by the illegal turtle trade.

This project is part of WWF’s Global Protecting Turtles for Tomorrow Strategy and forms part of a larger Marine Turtle Use and Trade Initiative (“Cracking the Code for Recovery”) to address marine turtle use and trade in the Asia-Pacific, coordinated by the WWF-Coral Triangle Programme. 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.





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## AIMS OF SURRENDER YOUR SHELL

To support the SYS initiative, the Australian Government adopted a six-month policy (3 December 2020–3 June 2021) that allowed Australians to send historically purchased tortoiseshell products to WWF-Australia, along with details of where and when the items were purchased, without risk of prosecution. With the Australian Government, and in partnership with Australia's only accredited wildlife forensics laboratory – the ACWG at the Australian Museum – our goal for SYS was to gain a snapshot of Australia's contribution to the tortoiseshell trade, including its geographical scale and impact. We aimed to:

- test methods developed by LaCasella et al., 2021 on a wider range of sample types;
- add new data (mtDNA results from surrendered tortoiseshell items) to the Tortoiseshell Product Database to inform the Asia-Pacific genetic 'bank'; and
- demonstrate the utility of the ShellBank database to law enforcement agencies.

In doing so, we aimed to:

- catalogue the type and location (city/country) where turtle products were sourced from;
- identify the location (nesting population/country/region) where illegal hawksbill harvesting has or may still be occurring; and
- increase awareness of hawksbill conservation and the illegal trade in tortoiseshell by informing Australians and regional travellers that buying and importing CITES-listed turtle products is illegal.

*Building the DNA database – ShellBank – enables us to identify those hawksbills most at risk, so we can improve turtle protection.*

# HOW DOES IT WORK?

## SYS STRUCTURE

The SYS public-facing campaign called for donations from 3 December 2020 to 3 June 2021. It aimed to raise awareness that tortoiseshell can be made from the critically endangered hawksbill turtles and highlight how citizen scientists can help to protect hawksbills.

Australians were encouraged to donate items made from tortoiseshell (such as jewellery, combs, sunglasses and trinkets) they had bought, been gifted or inherited. In addition, formal letters were sent to auction houses and major opportunity shops (e.g., the Salvation Army), seeking their support and donations. All donations were sent to the WWF-Australia office in Sydney. There, they were curated into the SYS database, then transported to the Australian Museum (Sydney) for genetic sampling (DNA extraction and sequencing) and storage, and the sequencing data analysed by SYS turtle geneticists (Figure 1).

Donations were surrendered, received and curated by:

1. Visiting [www.wwf.org.au/surrenderyourshell](http://www.wwf.org.au/surrenderyourshell) to enter survey details about the tortoiseshell product on a web form, including when and where it was purchased, entering a photo of the donation, and retrieving a unique identification number.
2. Attaching the unique identification number to the tortoiseshell product and either posting it through Australia Post or having it collected by approved couriers.
3. Storing the product securely at a dedicated post office box for delivery to WWF-Australia's head office in Sydney for curation. All curated products were stored temporarily at WWF under lock-and-key and monitored by CCTV security cameras 24/7.
4. Following the SYS 'chain of custody' procedure, whereby each surrendered tortoiseshell item was recorded as a separate entry on the digital database (at time of generating survey web form entry) using its unique identification number. All entries were stored securely on a WWF server, with access given only to SYS partners.

5. Receiving products at the WWF office, whereby each donation was physically opened to:
  - a. check whether the product was real or fake;
  - b. cross-reference the initial survey data entry to the donated item, and check that photos taken of the item using the survey form were adequate; and
  - c. match, mark the items with the same unique identifier as the database entry (as generated upon survey submission) and take an additional photo showing the unique identifier, before returning the item to the locked cabinet. Note, if multiple items were received, a new database entry was made with a new unique identifier number for each item.
6. Choosing and transporting subsets of donated items (depending on quantity received and timing) for sampling at the Australian Museum. Once samples were chosen, these were marked on the secure database, and notifications were sent to the Australian Government to provide information on the quantity, packaging and movement of the products by an approved courier, until all samples were secured at the Australian Museum.
7. Confirming delivery and assigning each item a unique laboratory number (as per ACWG sample handling and labelling standard operating procedures).
8. DNA extraction and sequencing of selected items to identify mtDNA variants (haplotypes). All relevant laboratory results were added to the secure database.

Any tortoiseshell items that the Australian Museum Herpetology Collection does not accession into their collection will be returned to the Australian Government for destruction, except for the DNA extractions, which will be kept for long-term storage at the Australian Museum.

Alongside WWF-Australia, SYS was supported by the Australian Museum and Royal Carribbean Group marketing and engagement teams.

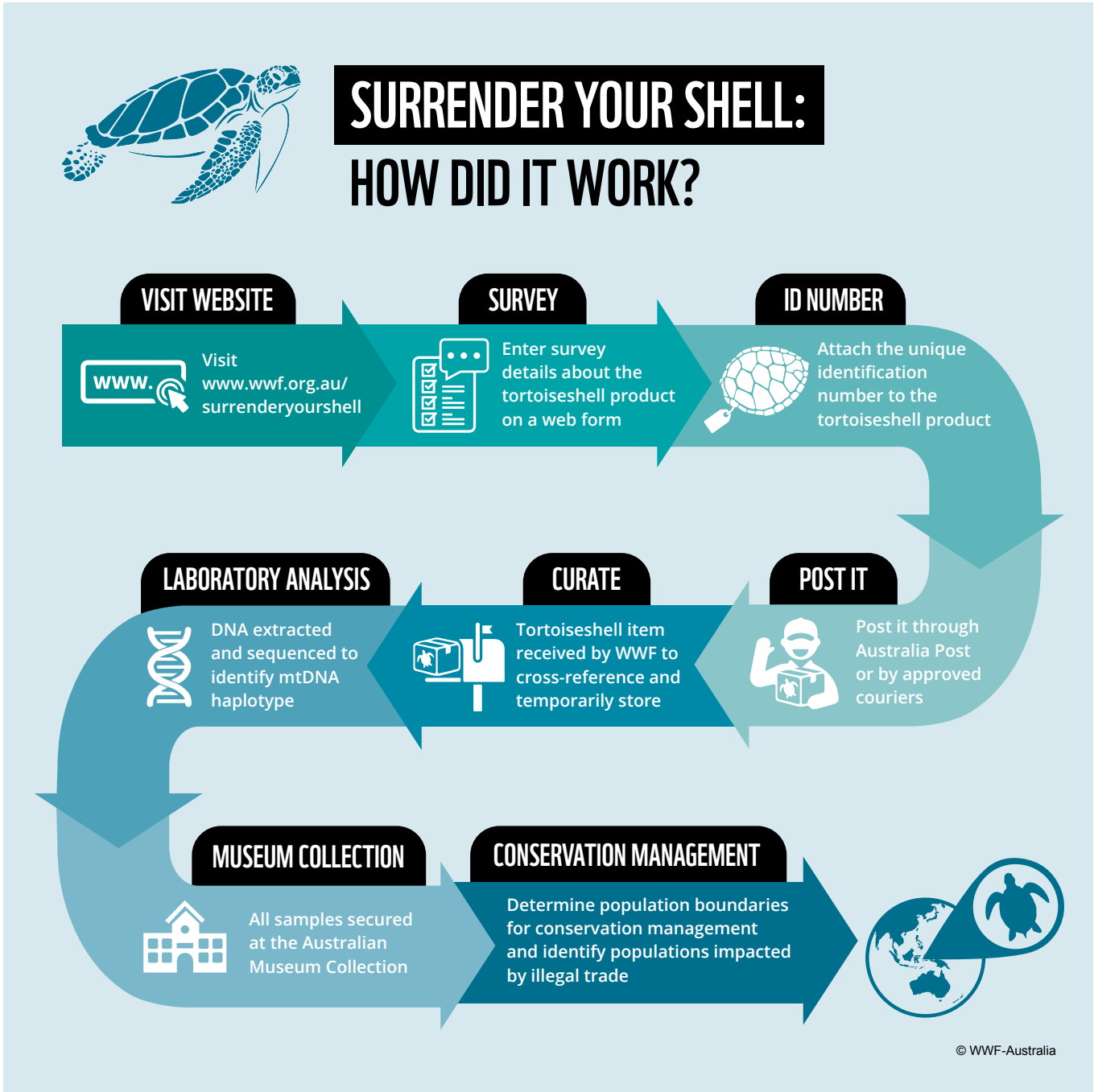


Figure 1: Flowchart of SYS in practice.



# EXTRACTING DNA FROM TORTOISESHELL

## DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA extraction of the surrendered items was carried out in the ultra-clean laboratory facilities of the Australian Centre for Wildlife Genomics at the Australian Museum. This is a designated, low-template DNA laboratory with positive air pressure and HEPA-filtered air-handling to minimise contamination of samples being processed.

## THE DNA EXTRACTION PROCESS FOLLOWED THESE STEPS:

### Breaking down the tortoiseshell:

To break down that hard surface of the tortoiseshell, a Dremel 8220 Cordless Rotary Tool 12V with a high-speed cutter was used. The surface layer of the tortoiseshell item was removed and discarded. The Dremel and high-speed cutter were then cleaned using a compressed air duster and used to collect shell shavings from the now cleaned area for DNA extraction.

### DNA extractions:

DNA was extracted using a Qiagen DNEasy Blood and Tissue Kit following the modifications outlined by LaCasella et al. (2021).

### DNA amplification:

Due to DNA degradation in the older surrendered items (up to 100 years old), three primer pairs were used to amplify the mtDNA control region. The primer pair LCM-15382 and H950g (Abreu-Grobois et al., 2006), were used to amplify approximately 770 base pairs (bp) of the mtDNA control region.

If this longer fragment was unsuccessful due to DNA degradation, a shorter 384 bp region (within the 770 bp region) was targeted using TCR5 and TCR6 (Norman et al., 1994). Two additional primers were designed during SYS to pair with TCR5 and TCR6 to amplify this region as two overlapping fragments for those samples that were too degraded to amplify using TCR5/TCR6. These two fragments

were a 194 bp region amplified by pairing TCR5 with the new primer TCR5aR (5' AGGGTTGCTTATTTCTCGTG 3'), and a 219 bp region amplified by pairing the new primer TCR6aF (5' CATGAATATCGTCACAGTA 3') with TCR6.

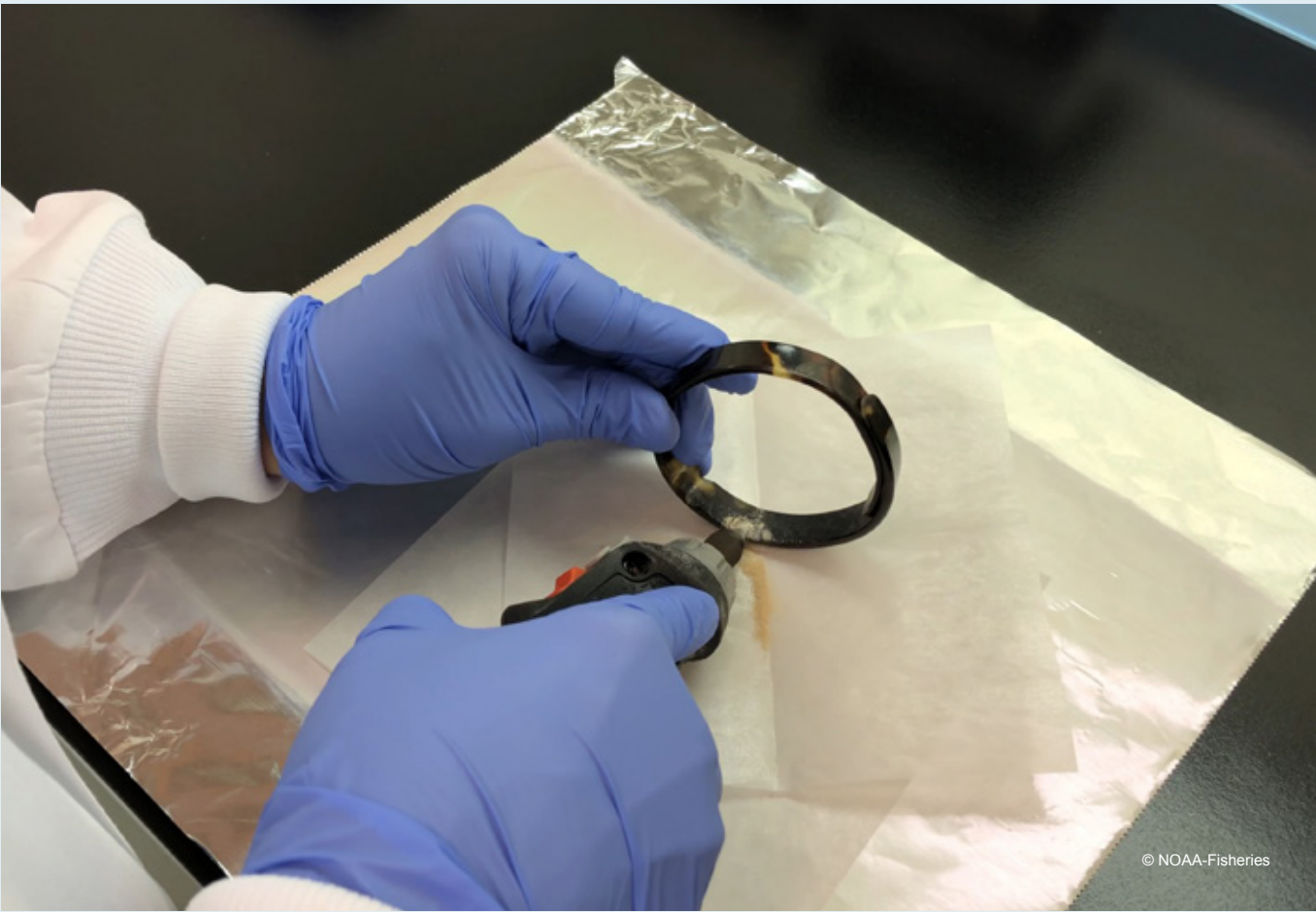
### DNA sequencing:

Amplified sequences were cleaned using ExoSAP-IT (ThermoFisher Scientific), and sequencing was carried out by AGRF (Sydney) using an AB 3730xl. Sequences were quality assessed via inspection of chromatograms using Sequencer version 5.2.4 (Gene Codes Corporation).

### Assigning haplotype:

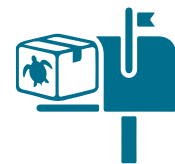
All sequences were then compared against published and unpublished hawksbill sequences in the Rookery Baseline and In-Water databases. All samples were subsequently assigned a haplotype (specific genetic variant), based on either short (~384 bp, SYS prefix) or long (~770 bp, EiIP/ EiA-prefix) that were used for further analysis.

The detailed extraction, amplification and sequencing protocol will be available in training material currently being developed.





# SURRENDERED SHELL AND SYS SURVEY



328 individual items were received during SYS.

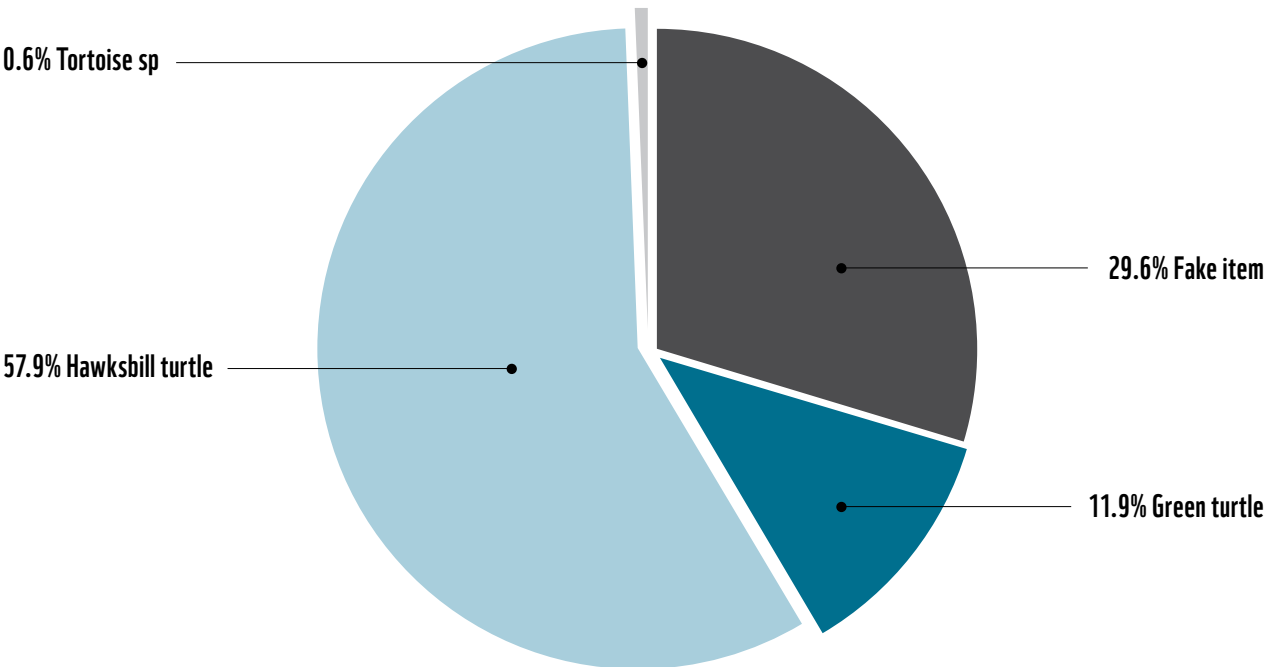
## HOW MANY ITEMS WERE DONATED?

During the six-month period, 73 survey web forms were completed by 55 members of the public who donated one or multiple items, with a total of 220 individual items received. In addition, seven suspected hawksbill items were received from the Department of Agriculture, Water and Environment, and 101 items from Trade Records Analysis of Flora and Fauna in Commerce (TRAFFIC). These were also entered into the web form, totalling 166 submissions completed and 328 individual items received. *(Note: paired items, e.g., earrings, salad servers, etc, were counted as two separate items, as tortoiseshell from more than one turtle may have been used to manufacture each piece.)*

## REAL OR FAKE TORTOISESHELL?

The first step in the curation process was to assess if the donated items were made from real tortoiseshell or fake (e.g, plastic). Results showed that donations consisted of both real tortoiseshell (hawksbill turtles) (190 items) and fake items (97 items). Donations also included items made from green turtles (*Chelonia mydas*) (39 items) and two shells from tortoise species – one identified (via shell morphology) as the South American species the yellow-footed tortoise (*Chelonoidis denticulatus*) and the other identified (via shell morphology) as belonging to the genus *Testudo* – all of which are CITES listed (Figure 2).

## REAL OR FAKE?



**Figure 2:** Pie chart showing the proportion of real and fake tortoiseshell received during the Surrender Your Shell campaign, including items made from green turtles and tortoises.



# RESULTS



WHAT TYPE OF ITEMS WERE SURRENDERED?

The items surrendered ranged from small rings no more than a few millimetres thick to whole taxidermied turtles and dried shells, including one shell measuring more than 90 cm in length (Figure 3). Items received included:

- knitting needles (82);
- hair accessories (82): hair pins (56), hair clips (21), hair combs (5);
- loose (unprocessed) scutes (48);
- jewellery (46): bracelets (25), earrings (6 pairs), rings (4), pendants (3), necklace (1);
- bookmarks (29);
- whole shell/stuffed (19): whole shell (14), bellows (1), whole stuffed turtles (4);
- beauty accessories (6): brooches (3), buttons (2), belt (1);
- cutlery (3): salad servers (2), spoon (1);
- other beauty items (3): mirrors (2), brush (1); and
- a random assortment (10): boxes (3), masks (2), a decorative item (1), guitar (1), letter opener (1), keyring (1) and shorthorn (1) (Figures 3 and 4).

ITEM TYPE

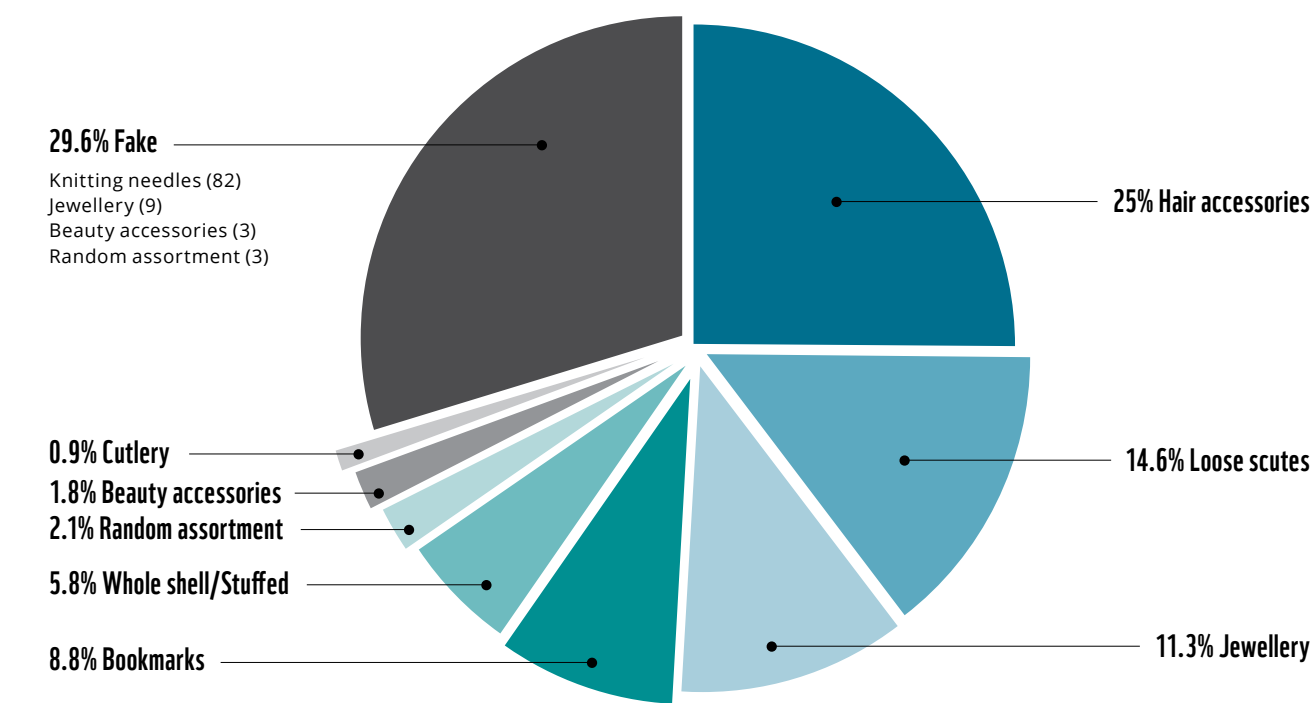


Figure 3: Types of items received during the Surrender Your Shell campaign.

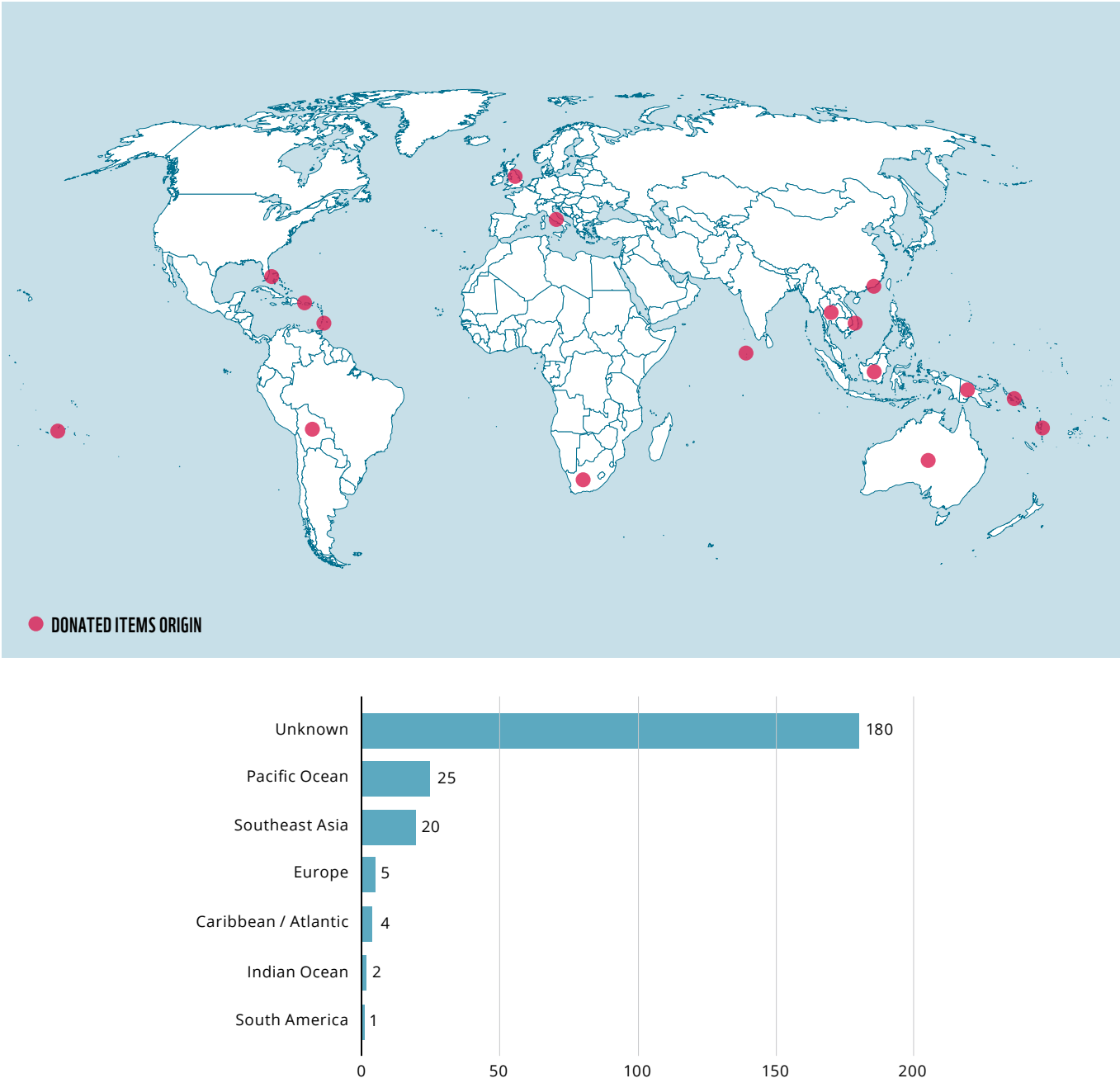


Figure 4: A selection of the items received as part of the Surrender Your Shell campaign. Items depicted include those made from hawksbill turtle, green turtle, land tortoises and plastic, considered fake tortoiseshell.



WHERE WERE THE ITEMS INITIALLY PURCHASED?

Information about the surrendered items was provided through the survey web form hosted by WWF. People were asked to add as much information about the items as possible, based on the best available knowledge. Excluding the fake (97) items, 33% of received items came with information on original origin (Figure 5). These items showed a remarkable geographical spread, including items purchased in Europe (Italy), the Caribbean (Bahamas, St. Lucia, Puerto Rico), South America (Bolivia), Western Pacific (Australia, Fiji, Solomon Islands and other unspecified South Pacific islands), Africa (South Africa), Indian Ocean (Maldives) and Southeast Asia (Thailand, Vietnam, Timor Leste, Papua New Guinea, Indonesia, Hong Kong).



**Figure 5:** Map showing the countries (pink dots) where donated items were originally purchased/obtained (excluding plastic items). The graph shows the number of items from each region (77% of items had unknown origin).

WHEN WERE ITEMS INITIALLY PURCHASED?

Of the 231 real items, only 52 (22%) had information about when they were purchased (or estimated to have been purchased). Based on the information provided on the web form, 32 items were purchased before CITES regulations (1977) were introduced. The time period ranged from the beginning of the century to the mid-1970s. Twenty items were purchased post CITES, between 1977 and 2021. Unfortunately, most items (183) had no information that indicated when they were purchased. Given the low sample size, there were no clear patterns of where items were purchased pre- versus post CITES (Figure 6). Nonetheless, it seems that tortoiseshell items are still entering Australia (and most likely from Southeast Asia), despite regulations banning the importation of tortoiseshell into Australia.



**Figure 6:** Bar graphs showing the number of real items entering Australia from different regions pre- and post implementation of cites regulations in 1977.



# DNA EXTRACTION AND SEQUENCING

## HOW MANY GENETIC VARIANTS WERE FOUND?

### HAWKSBILLS

Of the 190 items suspected to be real tortoiseshell based on an initial visual inspection, 104 were processed for DNA extraction. A total of 62 hawksbill samples (60%) were successfully sequenced for the short control region fragment (~384 bp) and, of those, nine samples were successfully amplified using the long (~770 bp) fragment of the control region (refer to Extracting DNA From Tortoiseshell box).

Each genetic variant (called a haplotype) was assigned an SYS-prefix (e.g., SYS-01, SYS-02, etc) for the short fragments, while standardised nomenclature (EiIP-prefix for Indo-Pacific haplotypes and EiA-prefix for Atlantic haplotypes) were used for the long fragments. Twenty haplotypes were identified using the shorter mtDNA fragment. The most common haplotype (SYS-01) was found in 53% of items, while all remaining haplotypes were found at low frequency (2-3%) (Figure 7a). Among the long fragments, seven haplotypes were identified, including two new haplotypes and five known haplotypes: EiIP33 (3), EiIP47, EiIP03, EiIP04

### HAWKSBILL TURTLE

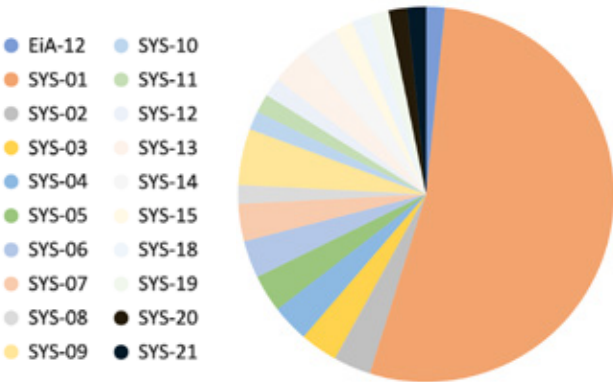


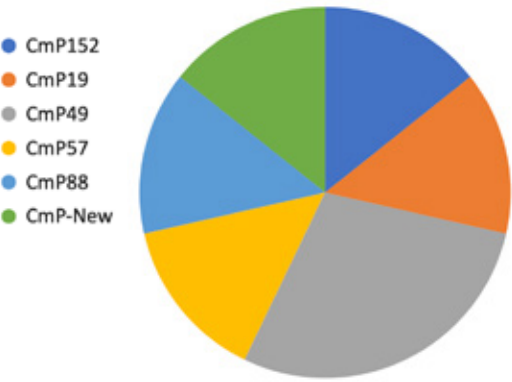
Figure 7A - B

and EiIP08. Combined, these included 11 haplotypes from 48 items that had previously been identified in nesting (Rookery) populations of the Eastern Pacific, Persian Gulf, Solomon Islands, Australia, Malaysia and Caribbean. Four haplotypes from seven items had not yet been identified at any nesting population, but have been observed in foraging (In-Water) populations (mostly in Australia but also in Japan) and seven haplotypes from seven items were described for the first time as “orphan” (Table 1).

### GREEN TURTLES

In addition to the hawksbill items, seven items were determined to be green turtles based on sequencing results. Six haplotypes were recovered from these items using short haplotypes (Figure 7b), five of which were previously identified in nesting populations and one new haplotype. All five known haplotypes have been identified in rookeries centred in Southeast Asia and foraging areas across the region (Table 1).

### GREEN TURTLE



The following table shows the haplotypes for the short (384 bp) and long (770 bp) control region fragment, the sample size (n), matches found in the Rookery and In-Water databases and the likely geographical origin for 69 products.

SHELLBANK DATABASE MATCHES					
SHORT	LONG	N	ROOKERY DATABASE	IN-WATER DATABASE	LIKELY REGION OF ORIGIN
HAWKSBILL TURTLES					
SYS-01	–	28	Indian/Pacific oceans	Indian/Pacific oceans	Indian/Pacific oceans
SYS-01	EiIP33	3	Indian/Pacific oceans	Indian/Pacific oceans	Indian/Pacific oceans
SYS-01	EiIP47	1	Sabah TI, Malaysia	SE Asia	East Malaysia
SYS-01	New1	1	Orphan	Orphan	?
SYS-02	–	2	Orphan	Queensland, Australia	SW Pacific
SYS-03	–	2	Orphan	Yaeyama Islands, Japan	SE Asia/W Pacific
SYS-04	–	2	Eastern Pacific	Queensland, Australia	SW Pacific
SYS-05	–	2	Solomon Islands	Queensland, Australia	SW Pacific
SYS-06	–	2	Orphan	Queensland, Australia	SW Pacific
SYS-07	–	1	Orphan	Queensland, Australia	SW Pacific
SYS-07	New2	1	Orphan	Orphan	?
SYS-08	–	1	Orphan	Orphan	?
SYS-09	–	2	neQld/NT/WA, AUS	Queensland (nGBR)	SW Pacific
SYS-09	EiIP08	1	neQld/NT/WA, AUS	Queensland (nGBR)	SW Pacific
SYS-10	–	1	Orphan	Orphan	?
SYS-11	–	1	Orphan	Orphan	?
SYS-12	–	1	Orphan	Orphan	?
SYS-13	–	1	Solomon Islands	Queensland (nGBR)	SW Pacific
SYS-13	EiIP03	1	Solomon Islands	Queensland (nGBR)	SW Pacific
SYS-14	–	2	Sabah TI, Malaysia	–	East Malaysia
SYS-15	–	1	Malaysia	Queensland (nGBR)	East Malaysia
SYS-18	–	1	Caribbean	?	Caribbean
SYS-19	–	1	Orphan	Orphan	?
SYS-20	–	1	Sabah TI, Malaysia	–	East Malaysia
SYS-21	EiIP04	1	Milman Island	Queensland (nGBR)	SW Pacific
EiA-12	–	1	Costa Rica	–	Costa Rica, Caribbean
Total Ei		62			
GREEN TURTLES					
CmP-new	–	1	Orphan	–	?
CmP49	–	2	Multiple - SE Asia	Multiple - SE Asia	SE Asia
CmP152	–	1	West Java, Indonesia	–	Indonesia
CmP19	–	1	GoC, Taiwan	Malaysia	Se Asia/Australia
CmP57	–	1	Borneo	Malaysia	SE Asia
CmP88	–	1	Multiple SE Asia	–	SE Asia
Total Cm		7			

Key: Southeast Asia (SE Asia), Gulf of Carpentaria (GoC), Sabah Turtle Islands (Sabah TI), north-east Queensland (neQLD), Northern Territory (NT), Western Australia (WA) and northern Great Barrier Reef (nGBR).

Table 1: Haplotype frequency and likely origin of sea turtle products from hawksbill (EI; *Eretmochelys Imbricata*) and green (CM; *Chelonia Mydas*) turtles.





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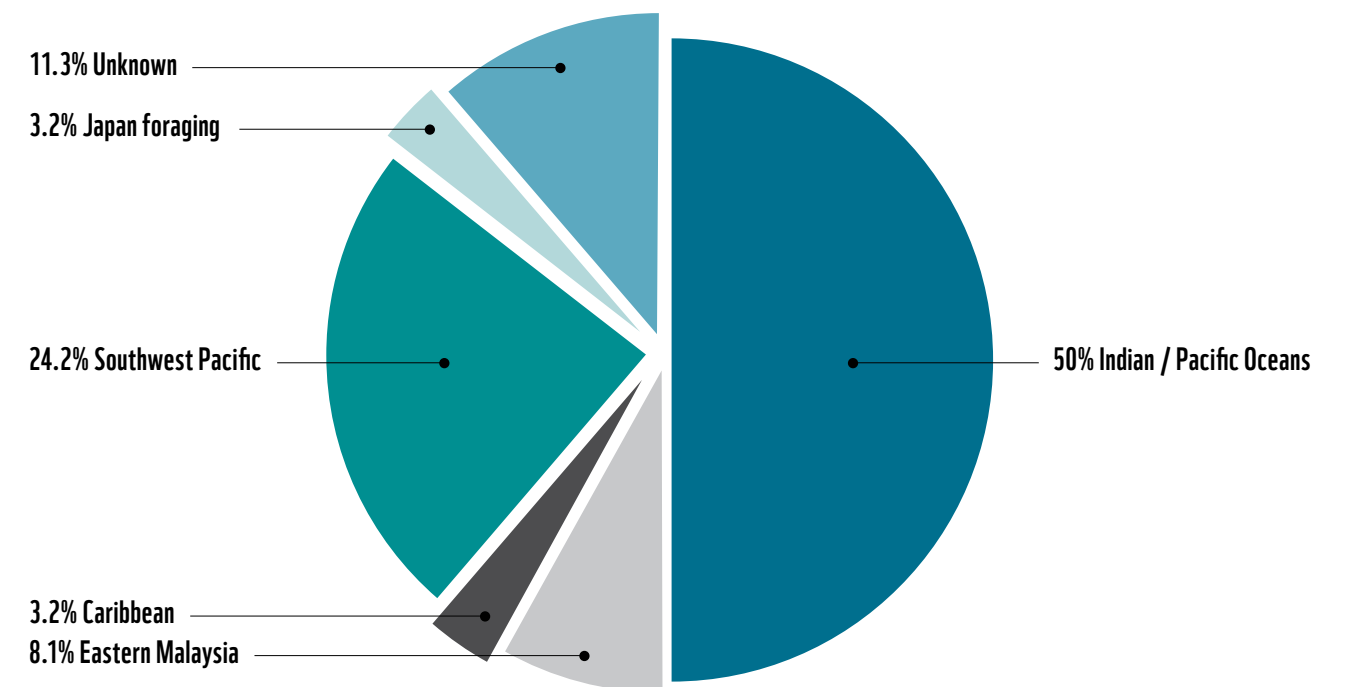
## LIKELY ORIGIN, BASED ON GENETICS

Tracing the nesting origin of tortoiseshell with reasonable confidence relies on two critical factors. Firstly, having a comprehensive rookery baseline representing hawksbill turtle nesting populations globally (Rookery Baseline Database) and, secondly, having the resolution in genetic markers to differentiate between distinct populations. Currently, a comprehensive Rookery Baseline Database is lacking for hawksbill turtles, but multiple efforts are underway to rectify this (see Discussion). Furthermore, the age of many of the items surrendered resulted in degraded DNA, and only the shorter DNA fragment/haplotype could be recovered, resulting in a slight loss of resolution in the data generated. Most notable was haplotype SYS-01, which was found to be common and widespread across the Indian and Pacific oceans. Nonetheless, a simplified approach to assessing

the origin of the items was made by directly comparing haplotypes from the surrendered items to the current Rookery Baseline Database for a basic assessment of nesting origin. Comparisons with the In-Water Database also reveals information on the possible origin, although at a broader regional scale. As such, qualified inferences about the likely geographical origin of the items analysed were made (Table 1 and Figure 8).

It is important to point out that these results should be interpreted with caution. A more advanced stock identification approach (i.e. mixed stock analysis) should be applied when a more comprehensive database is available.

## REGIONAL ORIGIN



**Figure 8:** Likely regional origin of 62 hawksbill products.



# DISCUSSION

The general Australian public who contributed to the ShellBank database by surrendering their historically purchased tortoiseshell products has provided some of the first direct evidence of which hawksbill turtle populations have been impacted by this trade.



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SYS has successfully built on the previous phases of ShellBank by incorporating a public-facing campaign to raise awareness of the plight of the hawksbill turtle and facilitate the collection of a wider variety of tortoiseshell products. The information gathered, including genetic data, has helped inform hawksbill conservation and trade knowledge across the Asia-Pacific. WWF-Australia's purpose-built survey (hosted by its website) helped capture information from people surrendering their shell, supported by a suite of content and stories to generate awareness of the project. The high rate of follow-through – only two completed surveys did not result in an item being submitted – illustrates an engaged and motivated Australian audience keen to participate in efforts to conserve hawksbill turtles. In addition to this, the Supporter Relations team at WWF-Australia received an additional 61 inquiries about the program, a testament to the eagerness of people to get involved and make a difference in protecting the hawksbill turtle.

While the 220 individual tortoiseshell items surrendered by the public is only likely to represent a fraction of what Australian citizens have in their possession, the geographical spread of places of purchase was diverse. Most were purchased in the Pacific (including all the South Pacific islands, Fiji, Solomon Islands and Australia) and Asia-Pacific (including Hong Kong, Indonesia, Papua New Guinea, Thailand, Timor Leste and Vietnam). Although information was limited on post-CITES items, continued imports from Asia-Pacific seem likely.

Given its close proximity to Australia, and the fact that the Pacific is one of Australians' most popular travel destinations (Australian Bureau of Statistics, 2020), it was no surprise that many tortoiseshell items were sourced from this region. However, it does raise concern that these hawksbill populations, and neighbouring Southeast Asia, continues to be targeted for trade to support travellers' interests, among other reasons. Genetic analyses supported this pattern, showing (to the best of the current Rookery Baseline Database's accuracy) that most sequenced items likely originated from somewhere in the Indo-Asia-Pacific, including the eastern Pacific, Solomon Islands, Malaysia and Australia. The fact that just over half of the short haplotypes could be defined by the Rookery Baseline Database (55%) or In-Water Database (20%), with only 36% new haplotypes, demonstrates that ShellBank is well on its way to becoming a successful toolkit for tracking the tortoiseshell trade.

While knitting needles (82) were the most common items donated to SYS, and real tortoiseshell knitting needles were manufactured historically, those donated were determined to be made from celluloid (shellonite) rather than real

tortoiseshell. As individual items, the top four most common items donated that were either all or predominantly real tortoiseshell were: hair pins (56), scutes (48), bracelets (25) and hair clips (21), which represented a variety of manufactured and raw tortoiseshell (i.e. scute) item types. A hawksbill turtle has 13 carapace scutes, so based on the number of raw scutes donated, it was not surprising that the genetic analysis confirmed these came from more than one turtle. While the scutes received as part of SYS were bought either in the 1970s or before, trading in raw scutes continues today in Pacific countries, including the Solomon Islands (Vuto et al., 2019) and Papua New Guinea (Kinch and Burgess, 2009), where it has recently been documented that raw scutes are being purchased by Asian buyers who pay higher prices than local manufacturers or carvers can afford (Vuto et al., 2019). Whether there is a transition to trading in pre-manufactured raw scutes requires investigation, but is aligned to more recent large-scale seizures in the USA and Vietnam (e.g. large-scale seizure of mostly hawksbill turtle scutes in Miami, Florida, in [Miami Herald, 2020](#)).

Interestingly, two different item types (bookmarks (29) and a shoehorn (1)) visually identified as tortoiseshell were later identified as green turtles via DNA sequencing. Although the donation of whole shell/taxidermied green turtles was not unexpected – these are known to be sold in the tourist trade – identifying manufactured green turtle products was more of a surprise. It is generally presumed the scutes of green turtles are unsuitable (too thin) for the commercial production of tortoiseshell. However, SYS has uncovered that these products can also be handcrafted from green turtle shells. This raises alarm bells and fears that the hawksbill tortoiseshell industry could be replaced by another threatened species if and when hawksbills become extinct.

The number of whole shell and whole taxidermied green turtles, hawksbill turtles and tortoise sp. (19) suggests these remain a popular tourist trinket, both historically and today, and that many more are likely to exist in homes around Australia. It was evident that the general public may not know the species of the whole shell they own, and even shells donated by law enforcement were incorrectly identified. With proper training, it is relatively easy to distinguish between green and hawksbill turtles, and between marine turtles and tortoise species based on shell morphology. ShellBank could be further developed to include this morphology training, to ensure more accurate identification and reporting, especially in countries that rely only on visual identification.



# CURRENT LIMITATIONS AND FUTURE CHALLENGES

The results gained from the analysed items provided the first opportunity to test the utility of the ShellBank project with a law enforcement agency.

The project also showed that DNA can be extracted from tortoiseshell items of variable age and quality, and then compared to a (at present incomplete) Rookery Baseline Database to trace from shell to source. However, it did highlight some limitations to ShellBank, as outlined below.

## AGE OF THE SURRENDERED ITEMS

Australia was a suitable country to roll out SYS due to the government support and the Australian Museum's wildlife forensics capacity. However, a large number of products surrendered were obtained pre-CITES, with several items thought to be over 100 years old. This presented a range of unforeseen challenges, but also new opportunities to enhance the ShellBank toolkit for tracing this trade.

DNA in any biological material degrades and breaks down over time, so it is harder to recover from older items. Recovering DNA from these items required further modification of the protocols developed by La Casella et al., 2021, which targeted a ~770 bp region of DNA. In the older, degraded items, a shorter fragment of DNA had to be targeted (~384 bp) for amplification, and this was done using either previously published primers (Norman et al., 1994) or two new primers that were designed during the SYS program that paired with the Norman primers to amplify the same region in two short, overlapping fragments. For most items tested, only the short 384 bp region of DNA could be amplified, reducing the resolution of the genetic marker. For example, the most common haplotype in this study (SYS-01) was found to be common and widespread in rookeries across the Indian and Pacific oceans, affecting the precision to which SYS-01 individuals could be traced back to rookery origin. When longer fragments are used, this haplotype splits into 10 new haplotypes, adding resolution to the stock origin.

While the shorter fragment of DNA limited our ability to identify populations being impacted by this trade, the upside of working with this older material was the expansion of the ShellBank toolkit to include amplification methods that can



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now be used on a range of degraded sample types that might be encountered in countries with a long history (or older stockpiles, like Japan) in the tortoiseshell trade. In the future, these methods can be further improved to generate longer DNA fragments from degraded items.

For the nine items where the longer fragment of DNA was amplified from the SYS products, three items with the common short haplotype SYS-01 were split into three new haplotypes: one common (EiIP33), one new, and one haplotype (EiIP47) that is only found in the Sabah Turtle Islands, Malaysia. Another three haplotypes identified from the longer fragments are only found across Australian rookeries (EiIP08), Milman Island, Australia (EiIP04), and the Solomon Islands (EiIP03). This highlights the advantages of using ShellBank when processing contemporary seizures or confiscations, which are likely to make up the bulk of the items encountered by law enforcers across the Asia-Pacific. As demonstrated by La Casella (2021), reliable amplification of the longer control region fragment should be possible from modern items.

## INCOMPLETE ROOKERY BASELINE DATABASE

Currently, the Rookery Baseline Database holds 31 different global genetic stocks. However, the majority are identified in the Atlantic Ocean and only five genetic stocks have been identified for the Asia-Pacific. Almost half (42%) of all the hawksbill haplotypes (short and long fragments) identified in the SYS items were either new or have only previously been identified in foraging animals (In-Water Database), highlighting the incomplete rookery baseline for the Asia-Pacific region.

A core element of ShellBank is to expand the current knowledge of hawksbill populations' genetic stock structure and connectivity in the Asia-Pacific region. WWF has been

working with many local communities, universities and government partners to coordinate access to and expand the 'bank' of hawksbill genetic data from nesting populations to continue to build this Rookery Baseline Database. In addition, it is still crucial to continue to collect genetic data from other wild hawksbills (e.g. fisheries bycatch, foraging and stranded animals), to build the In-Water Database, as well as historically purchased tortoiseshell products (Tortoiseshell Products Database), like those surrendered as part of SYS or seized by law enforcement, to further inform the overall genetic 'bank'. Data from the In-Water and Tortoiseshell Product databases will provide a more robust understanding of the geographic boundaries of hawksbill genetic stocks, identify regions that are missing from the Rookery Database and inform future collection efforts. To date, the Hawksbill Genetic Working Group (IPHWG) has doubled the Rookery Database, but the data remains unpublished and could not be included in this report. In addition, with several new sampling locations identified and current sampling underway, we anticipate tripling the database by the end of 2022. This milestone will greatly expand the coverage of key rookeries across the region and significantly improve the precision of stock assignments.

## COVID-19

Of the 190 items surrendered that were suspected of being real tortoiseshell after initial visual inspection, only 104 were able to be processed for DNA extraction due to the COVID-19 lockdown of Sydney (from 25 June–11 October 2021), which restricted and limited access to the Australian Museum site and ACWG labs.



# WHAT THE AUSTRALIAN GOVERNMENT IS DOING TO TRACK THE HAWKSBILL TRADE

The Department of Agriculture, Water and the Environment is the Australian Government's Department responsible for wildlife conservation and CITES matters.

The [Australian Border Force](#) regulates movements of certain specimens across Australia's international borders and seizes specimens that do not accompany the required permits for lawful entry. Australian Border Force officers have delegation under the *Environment Protection and Biodiversity Conservation Act 1999* to seize illegally imported flora and fauna specimens at the Australian border on behalf of the [Department of Agriculture, Water and the Environment](#). The Australian Government is constantly working to seize illegally traded specimens, identify suspects and prosecute offenders of wildlife trafficking using their powers to monitor and search passengers, cargo and premises. Until very recently, there wasn't an optimised DNA extraction method for tortoiseshell products or traceability tool available for marine turtles; nor is there a dedicated forensic database program in place (like the Elephant Trade Information System or Rhino DNA Indexing System) for marine turtles.



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What seems to be a much more covert illegal marine turtle trade operation nowadays where detection is not as obvious and confiscations in Australia have in recent years been limited, as a result of SYS, the Australian Government has agreed to continue to:

- share seized turtle specimens for forensic investigations, research and education purposes where possible. The genetic results will be added to the ShellBank database and used to inform prosecutions where needed; and
- support neighbouring countries willing to implement ShellBank, to help bolster [CITES Turtle Decisions \(18.210 - 18.217\)](#) and continue to show leadership in CITES-related matters concerning marine turtles.

## LINKS TO OTHER COUNTRIES (THEIR LAWS) AND OTHER INITIATIVES

The marine turtle trade is well regulated in Australia. This is not the case in many neighbouring countries. For example, hawksbill turtles are not a protected species in Papua New Guinea, meaning there is no monitoring of their use and domestic trade or protection. In the Solomon Islands, hawksbill turtles can be harvested for subsistence purposes, but trade (including that of all turtle parts and products) is banned. In Indonesia, hawksbill turtles are protected under government regulations, whereby any catch, trade, import, export, possession and transfer are prohibited. Yet, like other countries including Malaysia, provincial legislation may not be harmonised across the country or with different pieces of legislation. As a result, the sale of turtle products continues in all these countries and many beyond (Gomez and Krishnasamy, 2019; Kinch and Burgess, 2009; Lam et al., 2012; Vuto et al., 2019).

The inconsistencies in marine turtle conservation, management, and

protection policy and legislation is compounded by the fact that hawksbill turtles are a shared resource. Scientists have shown that hawksbill turtle populations often nest and feed in different countries, including throughout the western Pacific Ocean (Bell and Jensen, 2018; Hamilton et al., 2015; Limpus, 2009, Madden Hof in prep.). Yet, to this day, there remain many gaps in our understanding of the status, distribution and stocks of hawksbill populations (Madden Hof et al., 2022) and many others throughout the Indian and Pacific ocean basins (Hamann et al., 2022). Many countries across the globe have adopted a variety of regional and international agreements aimed at protecting hawksbill turtles and their habitats, or to mitigate threats that may directly or indirectly affect hawksbills and the illegal trade (Madden Hof et al., 2022). However, coordinated efforts between countries and the exchange of information at the national, regional and global levels is needed if hawksbill populations are to recover. These efforts need to be supported by:

- improved monitoring, detection and law enforcement activities related to marine turtles in coastal areas and at transaction points (e.g. in marketplaces, online, maritime areas, and at airports and seaports);
- the collection of marine turtle samples for DNA analysis (including from seized specimens) to determine origin in support of research, investigations and prosecutions;
- the ascertaining of key trade routes, methods, volumes and trade 'hotspots' using available technologies; and
- the enforcement of national and international regulations that apply to marine turtle take and trade.

These are all activities that countries (Party's) that are signatories to CITES have agreed to implement. For many, these are actions repeated in a number of other legally and non-legally binding instruments (refer to the Convention on the Conservation of Migratory Species of Wild Animals, 2022).



# WHERE TO NEXT

As an engaging, public-facing campaign, SYS had multiple benefits in helping to inform the Tortoiseshell Product Database, identify trade routes into Australia and create awareness of the illegal marine turtle trade.

ShellBank has shown that, with complete Rookery and In-Water databases, it is possible to accurately trace the population origin, and to advance hawksbill conservation at national and regional levels, as well as enhance a country's enforcement efforts against illegal traders. This takes a coordinated effort and requires many countries to participate.

Aligned to regional and international policy and mandates, and with greater national, regional and international involvement, we can together further ShellBank to:

- build on the open-source transnational genetic 'bank' (including the Rookery/In-Water/Tortoiseshell Product databases) through genetic sample collection, analysis and data sharing throughout the region;
- advance knowledge and reporting of turtle product sourced, transiting or sold at transaction points through citizen science donations and surveys; and
- build in-country capacity for genetic sample collection and analysis by conducting workshops and providing expert advice that can be applied to all species of marine turtles.

Participation in ShellBank offers a coordinated approach – with standardised tools, databases, guides, capacity building and some funding (in priority locations and as advised by the IPHGWG). It also offers support and data to help deliver on national, regional and international commitments (e.g. CITES, The Convention on Migratory Species (CMS), The Coral Triangle Initiative on Coral Reefs, Fisheries and Food Security (CTI-CFF), and The Indian Ocean–South-East Asian Marine Turtle Memorandum of Understanding (IOSEA MOU)), as well as enhancing each country's enforcement efforts against illegal traders, and highlighting responsibilities to recover critically endangered hawksbill populations for people and nature.

Although initially focussed on the Asia-Pacific region, ShellBank can be applied in other regions (e.g. the Caribbean and Western Indian Ocean) and to other species where the exploitations, and, and unsustainable use and trade remain drivers of turtle population declines.



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# CONCLUSION

The SYS campaign proved an effective model to engage with the public about the plight of the hawksbill turtle. The message appeared to resonate, and donations of surrendered items increased each time the campaign was featured in the mainstream media (e.g. nightly news stories or newspaper articles). Despite challenges dealing with the variety of older items that were donated, and a limited baseline, SYS demonstrated the applicability and effectiveness of using DNA methods to address the tortoiseshell trade, as donated items enabled us to generate useful information about the regions that were targeted. The results of SYS highlight the benefits of implementing ShellBank as a traceability tool across the Asia-Pacific region and beyond, with a key priority to continue to build a strong genetic database.

Concerted efforts should now focus on extending collaborations, to enable increased sampling of hawksbill nesting beaches to expand and improve the Rookery Baseline Database; the In-Water Database of foraging, stranded and bycatch animals; and the Tortoiseshell Product Database of surrendered and confiscated products. Continued growth of these databases will refine geographic stock boundaries and provide useful intelligence about where marine turtle trade is most lucrative, to aid in criminal investigations, and help decipher which populations are being targeted and therefore require further protection. This will make ShellBank fit for purpose as a traceability tool to track and dismantle this reprehensible marine turtle trade.



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WE MAKE TODAY WILL  
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