

## TECHNICAL NOTE

## CRIMINALISTICS

David R. Foran,<sup>1</sup> Ph.D.; and Rebecca L. Ray,<sup>2</sup> M.S.

## Mitochondrial DNA Profiling of Illegal Tortoiseshell Products Derived from Hawksbill Sea Turtles

**ABSTRACT:** The hawksbill sea turtle (*Eretmochelys imbricata*) is a highly endangered species, commonly poached for its ornate shell. “Tortoiseshell” products made from the shell are widely, although illegally, available in many countries. Hawksbills have a circumglobal distribution; thus, determining their origin is difficult, although genetic differences exist geographically. In the research presented, a procedure was developed to extract and amplify mitochondrial DNA from tortoiseshell items, in an effort to better understand where the species is being poached. Confiscated tortoiseshell items were obtained from the U.S. Fish and Wildlife Service, and DNA from 56 of them was analyzed. Multiple mitochondrial haplotypes were identified, including five not previously reported. Only one tortoiseshell item proved to be of Atlantic origin, while all others corresponded to genetic stocks in the Indo-Pacific region. The developed methodology allows for unique, and previously unattainable, genetic information on the illegal poaching of sea turtles for the decorative tortoiseshell trade.

**KEYWORDS:** forensic science, tortoiseshell, poaching, mitochondrial haplotypes, *Eretmochelys imbricata*, hawksbill sea turtle

An estimated 350 billion plants, animals, and their parts are bought or sold annually on the worldwide black market, representing a monetary value in the tens or hundreds of billions of dollars (1). Wildlife poaching has also been directly linked to the illicit drug trade, human trafficking, and the funding of terror organizations (1). Marine organisms represent one of the largest poaching targets, be it fish for basic human consumption, exotic species for the aquarium trade, or marine mammals (1). Sea turtles, all species of which are threatened or endangered, are also hard hit, even though they are listed in Appendix 1 of the Convention on International Trade in Endangered Species (CITES), meaning trade between signatory countries is forbidden, and within most countries possession or taking of sea turtles or sea turtle products is illegal. In spite of this protection, however, sea turtle numbers continue to decline as their nesting beach habitats are destroyed, their eggs are collected for food, and the animals themselves are taken for meat.

One species of sea turtle, the hawksbill (*Eretmochelys imbricata*), faces yet another peril, as its carapace, or shell, is covered with colorful and ornate overlapping scutes. Turtles’ shells are composed of the protein keratin, meaning they are both easy to cut and, like hair under a curling iron, can readily be molded into different shapes through the application of heat. The scutes are formed into a variety of decorative “tortoiseshell” items such

as jewelry, boxes, ornaments, or accent pieces (e.g., Fig. 1). Use and trade in tortoiseshell dates back thousands of years and continues today, which has led to harvesting throughout their circumglobal equatorial range (2).

Once hawksbills are poached (or taken legally, depending on the country) either on beaches or at sea, their carapaces can be processed and sold locally, they can be illegally shipped to other



FIG. 1—Hawksbill sea turtle “tortoiseshell” items confiscated at U.S. ports of entry, sent to Michigan State University’s Forensic Biology Laboratory by the U.S. Fish and Wildlife Service. Shown clockwise from top left are a violin bow, pins, box, hair pieces/comb, bracelet, guitar picks, rings, earrings, bookmarks, small boxes, and a decorative ukulele.

<sup>1</sup>Forensic Science Program, School of Criminal Justice and Department of Integrative Biology, Michigan State University, 655 Auditorium Road, 560 Baker Hall, East Lansing, MI 48824.

<sup>2</sup>Forensic Science Program, School of Criminal Justice, Michigan State University, 655 Auditorium Road, 560 Baker Hall, East Lansing, MI 48824.

Received 19 May 2015; and in revised form 11 Aug. 2015; accepted 22 Aug. 2015.

countries to be made into decorative items, or the items can be made locally and then illegally exported. However, the workings of this black market are not well understood, even though it is very robust. For instance, the Dominican Republic's hawksbill sea turtle population has been reduced to a small fraction of its former size, yet in 2006, the World Wildlife Fund and International Union for Conservation of Nature's TRAFFIC documented over 23,000 decorative tortoiseshell items for sale in markets in that country, indicating active illicit trade (although owing to political pressure the number of items in the Dominican Republic has dropped substantially) (3). Tortoiseshell items can also be found for sale in many other countries throughout the Americas, Africa, and Asia, as can whole stuffed specimens; thus, the overall level of illegal trade is unknown. When hawksbill products are purchased unwittingly by tourists or are otherwise transported into the United States and identified by customs agents, they are confiscated. This contraband is turned over to the U.S. Fish and Wildlife Service (USFWS), which houses them at their National Wildlife Property Repository in Commerce City, Colorado.

Owing to the global distribution of hawksbill sea turtles, understanding where and how they are being poached has been problematic, as there is no way to visually determine the geographic origin of a tortoiseshell item. However, the breeding habits of sea turtles are well understood, and extensive genetic testing has taken place (e.g., 4,5). Hawksbills spend most of their lives in tropical and subtropical waters, where they feed and, once reaching sexual maturity (20–40 years), mate (6). Females then home to the area where they hatched, often the same beach, to lay eggs in the nests they create in the sand. The result of this homing means that maternally derived DNA markers, particularly mitochondrial DNA (mtDNA), may become geographically fixed through generations, and thus have the potential to reveal the geographic origin of a hawksbill sea turtle (7). In the research described here, the feasibility of obtaining sequenceable mtDNA from hawksbill sea turtle derived tortoiseshell items was examined, with a goal of better understanding the geographic foundation of their illegal trade.

## Methods

### *Sources of Tortoiseshell Items*

Obtaining genetic information from turtle carapaces has been demonstrated (8,9); however, the feasibility of reliably generating it from processed tortoiseshell items was unknown. We hypothesized that the lack of chemical treatment carapaces undergo during tortoiseshell item production means forensic techniques may be viable for DNA isolation and analysis, similar to what are undertaken in hair examinations. Preliminary tests were conducted on a hawksbill carapace loaned from the Michigan State University Museum of Natural History. Samples were taken from an inconspicuous portion of the carapace, and DNA was isolated using a standard hair shaft protocol, amplified, and sequenced (detailed below).

Next, the USFWS was contacted regarding obtaining confiscated hawksbill items. The USFWS does not keep specific records regarding when and where each confiscation occurs, but instead gives the confiscation a unique number and keeps items from a confiscation combined. Therefore, the U.S. port of entry of the tortoiseshell items was not known, although multiple items that were confiscated together, and were thus likely purchased at a common venue and could have a common biological

origin, were available. A trial set of 15 mostly dissimilar items was sent to the Forensic Biology Laboratory at Michigan State University, which was followed by a larger set of samples that consisted not only of unique items (e.g., a violin bow) but also of highly similar items, such as guitar picks and earrings. The majority of items consisted of jewelry pieces (earrings, pins, bracelets, hair combs, rings, necklaces), guitar picks, and boxes and other decorative items (exemplified in Fig. 1). Some of the objects had been confiscated individually and had their own unique USFWS identification number, while others were confiscated as a group and shared an identification number. Items tested are listed in Table 1.

### *DNA Isolation, Amplification, and Sequencing*

More extensive details of this study, including optimizations of many of the procedures below, are available in Shattuck (10). Tortoiseshell items were processed individually in a Labconco PCR hood (Labconco Corporation, Kansas City, MO) that was wiped with 10% bleach, followed by 70% ethanol, and then UV irradiated for a minimum of 10 min. A 7/64th inch drill bit and drill parts were soaked in 10% bleach, then wiped with 70% ethanol. A Dremel Multipro rotary tool (Robert Bosch Tool Corporation, Mount Prospect, IL) was wiped with 10% bleach followed by 70% ethanol. Once dry, the drilling materials, along with a plastic weigh boat and a 1.5 mL microcentrifuge tube, were UV irradiated in a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation, Westbury, NY) for 10 min (approximately 5 J/cm<sup>2</sup>).

Each tortoiseshell item was cleaned briefly with 10% bleach, and a powder was created by drilling an indentation in the item approximately one millimeter deep. The powder, ranging from 0.013 to 0.040 g, was collected in a weigh boat and transferred to a 1.5 mL tube, and 400  $\mu$ L of digestion buffer (20 mM Tris pH 8, 50 mM EDTA, 0.5% SDS), 5  $\mu$ L of 20 mg/mL proteinase K, and 10  $\mu$ L of 1M dithiothreitol were added. A reagent blank was initiated with each set of DNA extractions. The tube was vortexed and incubated overnight at 55°C. Four hundred microliters of phenol was added and the tube was vortexed and centrifuged at 14,000 rpm (20,817  $\times$  g) for 5 min. The aqueous layer was transferred to a new tube containing 400  $\mu$ L of chloroform. The tube was vortexed and centrifuged at 14,000 rpm for 5 min. The aqueous layer was transferred to a Microcon YM-30 or Amicon Ultra 30K spin column (Millipore, Billerica, MA) and centrifuged at 14,000  $\times$  g for 15 min. The eluent was removed, and the DNA was washed three times with 300  $\mu$ L of TE (10 mM Tris, 1 mM EDTA, pH 7.5) and centrifuged at 14,000  $\times$  g for 10 min. DNA was recovered by adding 30  $\mu$ L of TE to the Microcon column, or from what remained in the retentate of the Amicon column (ca. 20  $\mu$ L). The column was inverted into a new tube and centrifuged at 1000  $\times$  g for 2 min. DNA was stored at –20°C.

Multiple PCR and sequencing primers in or flanking the *E. imbricata* mtDNA control region were designed and tested. Subsequently, a forward primer of 5'-GTGCCAGAACACCA TAGC-3' in the proline tRNA gene and a reverse primer of 5'-GTTTCATCAATTCGGCAGGT-3' in the control region were utilized in 30  $\mu$ L PCR amplifications containing 3  $\mu$ L 20  $\mu$ M forward and reverse primer, 3  $\mu$ L 25 mM MgCl<sub>2</sub>, 3  $\mu$ L 10X PCR Buffer II, 3  $\mu$ L 0.2 mM dNTPs, 3  $\mu$ L 100  $\mu$ g/ $\mu$ L BSA, 11  $\mu$ L distilled water, 1 U AmpliTaq Gold (Applied Biosystems, Carlsbad, CA), and 1  $\mu$ L of sample DNA, reagent blank, or water for a negative control. Optimized PCR parameters were as

TABLE 1—Tortoiseshell items tested.

| Sample* | Item type     | Haplotype† |
|---------|---------------|------------|
| 1-1     | Earring       | Ei-1       |
| 1-2     | Earring       | Ei-1       |
| 1-3     | Earring       | Ei-1       |
| 1-4     | Earring       | Ei-1       |
| 1-5     | Earring       | Ei-1       |
| 2-1     | Hair Comb     | Santos‡    |
| 2-2     | Hair Comb     | Ei-9       |
| 2-3     | Hair Comb     | Santos‡    |
| 2-4     | Hair Comb     | TS2        |
| 3       | Hair Comb     | Ei-1       |
| 4       | Hair Comb     | TS3        |
| 5       | Hair Comb     | TS3        |
| 6       | Hair Comb     | Ei-1       |
| 7       | Hair Comb     | TS3        |
| 8       | Hair Comb     | TS3        |
| 9       | Hair Comb     | Ei-1       |
| 10-1    | Box           | Ei-1       |
| 10-2    | Box           | TS1        |
| 11-1    | Box           | Ei-1       |
| 11-2    | Box           | Ei-1       |
| 12      | Box           | NA§        |
| 13      | Box           | Ei-1       |
| 14      | Violin Bow    | Santos‡    |
| 15      | Necklace      | Ei-1       |
| 16-1    | Pin           | Ei-1       |
| 16-2    | Pin           | Ei-1       |
| 17      | Bracelet      | Ei-1       |
| 18      | Bracelet      | Ei-1       |
| 19      | Bracelet      | Ei-1       |
| 20      | Bracelet      | Ei-1       |
| 21      | Bracelet      | EIJ5       |
| 22      | Bracelet      | G          |
| 23      | Bracelet      | NA¶        |
| 24      | Bracelet      | NA¶        |
| 25      | Ring          | Ei-1       |
| 26      | Jewelry Piece | Ei-1       |
| 27-1    | Guitar Pick   | Ei-1       |
| 27-2    | Guitar Pick   | Ei-1       |
| 27-3    | Guitar Pick   | Ei-1       |
| 27-4    | Guitar Pick   | Ei-1       |
| 27-5    | Guitar Pick   | Ei-1       |
| 27-6    | Guitar Pick   | Ei-1       |
| 27-7    | Guitar Pick   | Ei-1       |
| 27-8    | Guitar Pick   | Ei-1       |
| 27-9    | Guitar Pick   | Ei-1       |
| 27-10   | Guitar Pick   | TS4        |
| 27-11   | Guitar Pick   | Ei-1       |
| 27-12   | Guitar Pick   | Ei-1       |
| 27-13   | Guitar Pick   | TS5        |
| 27-14   | Guitar Pick   | EIJ10      |
| 27-15   | Guitar Pick   | Ei-1       |
| 27-16   | Guitar Pick   | TS5        |
| 27-17   | Guitar Pick   | Ei-13      |
| 27-18   | Guitar Pick   | Ei-1       |
| 27-19   | Guitar Pick   | Ei-1       |
| 27-20   | Guitar Pick   | Ei-1       |

\*Similar objects that were confiscated together are denoted by hyphenated sample numbers (e.g., 10-1 and 10-2 resulted from one confiscation).

†Nomenclature for haplotypes is from GenBank.

‡“Santos” was not named in GenBank, and thus refers to the first author.

§No DNA was recovered.

¶Item was plastic.

follows: a 10 min 94°C denaturation step followed by 38 cycles of 30 sec at 94°C, 1 min at 50°C, and 1 min at 72°C. Five microliters of PCR products was electrophoresed on a 2% agarose gel to ensure amplification, and PCR products were purified using Diffinity Rapid Tips (Diffinity Genomics, Rochester, NY).

PCR products were sequenced in 10 µL reactions using a CEQ DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA)

containing 4 µL Quick Start mix, 50 – 100 fM DNA template, and 1 µL of 20 µM forward or reverse primer. Sequencing reactions and sequencing product purification were conducted according to the manufacturers' instructions. DNAs were loaded onto a 96 well plate and were separated using a CEQ 8000 Genetic Analysis System (Beckman Coulter) using the LFR-1 program (50°C capillary temperature, denature at 90°C for 120 sec, inject at 2 kV for 15 sec, and separate at 4.2 kV for 85 min).

Forward and reverse sequences from each item were aligned and edited using BIOEDIT 1.2 software (11), and sequences were aligned using MEGA 5.2 software (12). *E. imbricata* control region mtDNA sequences in GenBank were downloaded from the Basic Local Search Alignment Tool (BLAST) database (13); accession numbers are listed in Table S1. Haplotypes were assigned using COLLAPSE 1.2 software (14) and were compared to geographic information from the BLAST database and to data in the literature when available. A phylogenetic tree was constructed with the sequences obtained from this study and all GenBank sequences. jModeltest was used to infer the mode of nucleotide substitution (15), and the Tamura–Nei plus gamma model (16) was used in the construction of the tree. Phylogenetic relationships between these sequences were determined by the neighbor-joining method (17) using MEGA with 1000 bootstrap replications (12). The green sea turtle (*Chelonia mydas*) was used as an outgroup, as the taxon Cheloniidae is distantly related to the hawksbill (18).

## Results

Original testing of the hawksbill carapace loaned from the MSU Museum showed that mtDNA could be obtained from the scutes, thus testing of tortoiseshell items commenced. Overall, 56 pieces made up of several general item types (Table 1) were processed. Two pieces (items 23 and 24, both bracelets) proved to be plastic, and one other (item 12) did not yield a usable sequence. The remaining 53 items generated an average of 506 bp of controls region sequence. No amplification occurred in the negative controls or reagent blanks.

Twelve different mtDNA haplotypes were obtained (Table 1). Seven of these, Ei-1, Ei-9, Ei-13 (19), EIJ5, EIJ10 (20), Santos (21), and G (22), were present in GenBank, while the remaining five were not previously reported (denoted TS1 – 5 for tortoiseshell; GenBank accession numbers KM068143, KM068144, KM068145, KM068146, and KM068147, respectively). Ei-1 was by far the most common haplotype found in this study, occurring in 37 of the sequenced items (70%). The other previously observed haplotypes occurred once, except for Santos which occurred three times. TS3 and 5 occurred four and two times respectively, while the remaining three TS haplotypes each occurred once. TS3 contained a transversion at position 236 (relative to the Ei-1 reference sequence), while the remaining TS haplotypes differed by one or more transitions.

Phylogenetic analysis of published *E. imbricata* haplotypes, along with the TS haplotypes from this study, produced the tree shown in Fig. S1. This resulted in a distinct separation between turtles of Atlantic and Indo-Pacific origin. Of the 53 tortoiseshell items tested in this study, only item 22, a bracelet, yielded an Atlantic haplotype (published haplotype G; Table S1). The remaining 52 items grouped with known haplotypes of Indo-Pacific origin. For the most part the Indo-Pacific haplotypes from this study, both the TS ones and those previously observed, were distributed broadly along the Indo-Pacific clade.

## Discussion

Poaching of plants and animals is one of the most widespread illegal activities in the world, yet it receives relatively little publicity. This is likely for several reasons, such as the perception that poaching does relatively little human harm, or that it is primarily carried out by local people for their sustenance. In fact, wildlife poaching ranks second only to drug trafficking in generating illegal revenue and is closely tied to other forms of international organized crime (1). Further, the market for plants and animals and their parts often envelops noncriminals, when they purchase an aquarium fish, mahogany furniture, or as is the case here, a readily available piece of tortoiseshell jewelry or other decoration. The ability to objectively identify poached plants and animals has obvious legal implications, a novel form of which was explored in this research.

The complete hawksbill sea turtle shell tested in a preliminary study produced a clean mtDNA sequence, demonstrating the viability of the proposed strategy for testing items made from hawksbill scutes. In subsequent work, all but one of the 54 tortoiseshell items tested (excluding the two made of plastic) generated DNA data. DNA extracts were often dark in color, indicating that a contaminant, probably melanin, coextracted with the DNA. These regularly displayed PCR inhibition; however, this was easily overcome through addition of BSA. In the long run, a different purification procedure may be advisable (e.g., 23), particularly if long-term storage of DNAs is desired, as the level to which such impurities might affect DNA integrity is not known.

Once mtDNA sequences were obtained, phylogenetic analysis readily identified their oceanic origin. Published hawksbill mtDNA control region sequences were differentiated into two distinct geographic groups: those originating from Atlantic beaches/waters and those originating from the Indo-Pacific. Inclusion of the tortoiseshell item haplotypes into the phylogenetic analysis showed that only one (1.9%) of the tested items, a bracelet, was Atlantic in origin. Its haplotype, G, has been recorded six times at a nesting beach in Tortugero, Costa Rica and once at a nesting beach in Gale's Point, Belize (24), while it has not been found in other heavily sampled Atlantic nesting locations such as Cuba, Puerto Rico, Mexico, Brazil, Venezuela, or the US Virgin Islands.

Conversely, 98.1% of the tortoiseshell items tested produced haplotypes that grouped with hawksbills whose known origins were the Indian and Pacific Oceans, indicating either the items were produced in the Indo-Pacific, or the animals were harvested there and the raw materials illegally transported to other markets. Known locations for the Indo-Pacific hawksbill haplotypes range from Eastern Africa to the Pacific coast of South America. The most common haplotype, Ei-1, which was obtained from 70% of the items, has been documented in turtles as distant as coastal Iran, East Asia, and the Pacific coast of Columbia. The other Indo-Pacific haplotypes are less common. Nishizawa et al. (20) reported haplotypes EIJ5 and EIJ10 in hawksbill feeding aggregates near the coast of Japan. The authors analyzed 44 hawksbill samples, 32 of which were caught, and reportedly released, by fishermen within an 8-year time span. These two haplotypes have not been recorded elsewhere, and thus, it is likely these tortoiseshell items originated from nesting populations in the Northwest Pacific. Based on data in GenBank, haplotypes Ei-9 and Ei-13 have only been recorded from nesting beaches in East Asia, while haplotype Santos origin is described as "North of Mozambique Channel". These types of data reveal the broad

geographical range over which hawksbill sea turtles are being poached, information on which was previously unavailable.

The data produced also have the potential to provide information on the number of hawksbills being poached for the tortoiseshell trade. For instance, 20 of the tortoiseshell items tested were guitar pics from a single confiscation, which produced five different Indo-Pacific haplotypes. Clearly these did not originate from a single animal, and most likely were not from a single nesting location. However, it is possible they originated from a single production source that obtained/imported turtles from several locations, or from aggregates of turtles at sea. In contrast, the five earrings from one confiscation shared the Ei-1 haplotype; thus, it is possible that they originated from a single animal or nesting location, although the commonness of the Ei-1 haplotype must be considered. Not confiscated together were four hair combs that shared the TS3 haplotype. Given that this haplotype has not been seen before, it is quite feasible that these items had the same origin. Once again, this represents information that has not previously been available.

The methods and results presented here show that forensic techniques can be creatively applied to nonstandard evidence in a very successful manner. Not only were genetic data obtained from the tortoiseshell items at a very high rate, they were conclusive in establishing the region of the world from which this endangered species is being poached. MtDNA analysis shows that the vast majority of hawksbill sea turtle poaching for tortoiseshell production and trade originates from diverse regions of the Indo-Pacific. These novel genetic data have clear implications regarding antipoaching and smuggling efforts, helping to focus legal efforts where they are needed most.

## Acknowledgments

The authors wish to fully acknowledge and thank Elizabeth Shattuck for all of her hard work and efforts, as her Michigan State University Masters research and thesis (Shattuck, 2011) formed the foundation for most of the work presented here. They also thank Michigan State University's Dr. Meredith Gore for all her helpful insights into this project, and Laura Abraczinskas of the MSU Museum for providing a hawksbill carapace for the preliminary portions of this work. Dr. Kim Scribner's efforts in developing the phylogenetic analyses were invaluable. Thank you to Crawford Allen and Adrian Reuter of World Wildlife Fund for sharing their ideas regarding tortoiseshell item origins. And finally many thanks to Doni Sprague, USFWS repository specialist, for taking the time to sort and supply the large number of tortoiseshell items made available.

## References

- Gosling J, Reitano T, Shaw M. The global response to transnational organized environmental crime. Switzerland: Global Initiative against Transnational Organized Crime, 2014.
- <http://www.iucnredlist.org/details/8005/0> (accessed February 14, 2016).
- <http://www.traffic.org/home/2009/3/25/turtles-no-longer-turn-to-souvenirs-in-dominican-republic.html> (accessed February 14, 2016).
- Bowen BW, Bass AL, Garcia-Rodriguez A, Diez CE, Van Dam R, Bolten A, et al. Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. *Ecol Appl* 1996;6(2):566-72.
- Bowen BW, Karl SA. Population genetics and phylogeography of sea turtles. *Mol Ecol* 2007;16(23):4886-907.
- Bjorndal KA. Conservation of hawksbill sea turtles: perceptions and realities. *Chelonian Conserv Bi* 1999;3(2):174-6.
- Avisé JC, Bowen BW. Investigating sea turtle migration using DNA markers. *Curr Opin Genet Dev* 1994;4(6):882-6.

8. Hsieh HM, Huang LH, Tsai LC, Liu CL, Kuo YC, Hsiao CT, et al. Species identification of *Kachuga tecta* using the cytochrome b gene. *J Forensic Sci* 2006;51(1):52–6.
9. Lee JCI, Tsai LC, Liao SP, Linacre A, Hsieh HM. Species identification using the cytochrome b gene of commercial turtle shells. *Forensic Sci Int Genet* 2009;3(2):67–73.
10. Shattuck E. Geographical origins of illegally harvested hawksbill sea turtle products [thesis]. East Lansing, MI: Michigan State University, 2011.
11. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acid S* 1999;41:95–8.
12. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731–9.
13. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;25(17):3389–402.
14. <http://collapse.sharewarejunction.com/> (accessed February 14, 2016).
15. Posada D, Buckley TR. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Syst Biol* 2004;53(5):793–808.
16. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1992;10(3):512–26.
17. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4(4):406–25.
18. Naro-Maciel E, Le M, FitzSimmons NN, Amato G. Evolutionary relationships of marine turtles: a molecular phylogeny based on nuclear and mitochondrial genes. *Mol Phylogenet Evol* 2008;49(2):659–62.
19. [http://www.ncbi.nlm.nih.gov/nucleotide/295983964?report=genbank&log=nuclalign&blast\\_rank=1&RID=W4F2VCGM014](http://www.ncbi.nlm.nih.gov/nucleotide/295983964?report=genbank&log=nuclalign&blast_rank=1&RID=W4F2VCGM014) (accessed February 14, 2016).
20. Nishizawa H, Okuyama J, Kobayashi M, Abe O, Arai N. Comparative phylogeny and historical perspectives on population genetics of the pacific hawksbill (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*), inferred from feeding populations in the Yaeyama Islands, Japan. *Zool Sci* 2010;27(1):14–8.
21. [http://www.ncbi.nlm.nih.gov/nucleotide/385247070?report=genbank&log=nuclalign&blast\\_rank=1&RID=W1UN6HWJ014](http://www.ncbi.nlm.nih.gov/nucleotide/385247070?report=genbank&log=nuclalign&blast_rank=1&RID=W1UN6HWJ014) (accessed February 14, 2016).
22. Bowen BW, Grant WS, Hillis-Starr Z, Shaver DJ, Bjorndal KA, Bolten AB, et al. Mixed-stock analysis reveals the migrations of juvenile hawksbill turtles (*Eretmochelys imbricata*) in the Caribbean Sea. *Mol Ecol* 2007;16(1):49–60.
23. Graffy EA, Foran DR. A simplified method for mitochondrial DNA extraction from head hair shafts. *J Forensic Sci* 2005;50(5):1119–22.
24. Monzon-Arguello C, Rico C, Marco A, Lopez P, Lopez-Jurado LF. Genetic characterization of Eastern Atlantic hawksbill turtles at a foraging group indicates major undiscovered nesting populations in the region. *J Exp Mar Biol Ecol* 2010;387(2):9–14.

Additional information and reprint requests:

David Foran, Ph.D.  
 School of Criminal Justice and Department of Integrative Biology  
 560 Baker Hall  
 655 Auditorium Road  
 Michigan State University  
 E. Lansing, MI 48824  
 U.S.A.  
 E-mail: foran@msu.edu

### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Phylogenetic tree developed from available *Eretmochelys imbricata* GenBank sequences, along with the new tortoiseshell (TS) haplotypes found in this study. The horizontal dashed line denotes the division between Atlantic and Indo-Pacific haplogroups. Haplotypes from tortoiseshell items obtained in the current study are denoted by an \*, only one of which was Atlantic in origin. Phylogenetic relationships between these sequences were determined by the neighbor-joining method in Mega 5.2, with 1000 bootstrap replications. *Chelonia* refers to *Chelonia mydas*, which was used as an outgroup for phylogenetic analysis.

**Table S1.** GenBank accession numbers for *E. imbricata* mtDNA sequences used to produce the phylogenetic tree (Figure S1).