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The mock turtle syndrome: genetic identification of turtle meat purchased in the south-eastern United States of America

Joseph Roman[1,†] and Brian W. Bowen[2,]

[1] Department of Wildlife Ecology and Conservation, University of Florida, 303 Newins-Ziegler Hall, Gainesville, FL 32611, USA
[2] Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL 32606, USA

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Abstract

Much of the demand for turtle meat in North America and Europe during the past four centuries has been met using green turtle (*Chelonia mydas*) and other marine turtles. As stocks of marine turtles dwindled, harvest of the alligator snapping turtle (*Macroclemys temminckii*), the largest freshwater turtle in North America, increased in the south-eastern USA. As a result, this species has declined and is now protected in every state of the USA except Louisiana. There is concern that the remaining legal trade in turtle products may serve as a cover for illegally harvested species. To assess the composition of species in commerce, we purchased 36 putative turtle meat products in Louisiana and Florida. Using cytochrome *b* and control region sequences of the mitochondrial genome, we identified 19 samples as common snapping turtle (*Chelydra serpentina*), three as Florida softshell (*Apalone ferox*), one provisionally as softshell turtle (*Apalone* sp.), one as alligator snapping turtle, and eight as American alligator (*Alligator mississippiensis*). It appears that *M. temminckii* is no longer the predominant species in markets of Louisiana. The presence of alligator meat in a quarter of the samples indicates that the trade in turtle products is not entirely legitimate. As is often the case for unsustainable wildlife harvests, large, esteemed species, such as green turtle and alligator snapper, have been replaced by smaller, more-abundant or mislabelled species, a phenomenon we refer to as the mock turtle syndrome.

> *'Once,' said the Mock Turtle at last,*
> *with a deep sigh, 'I was a real Turtle.'*
> Lewis Carroll
> *Alice's Adventures in Wonderland*

INTRODUCTION

Molecular genetics provides a powerful tool for resolving the origin of wildlife products and detecting commerce in protected species. Market surveys, using mitochondrial DNA sequence comparisons, have been conducted on lucrative wildlife products such as whale meat, pinniped penises and sturgeon roe (Baker & Palumbi, 1994; Malik *et al*., 1997; Birstein *et al*., 1998). The results indicate that legal trade in profitable wildlife species is often a cover for the sale of misrepresented or illegally harvested animals.

Marine turtles, especially green turtles (*Chelonia mydas*), were the predominant species in turtle trade for much of the last 400 years (Carr, 1954; Witzell, 1994). The green turtle was especially prized for its meat and calipee, which was often consumed in soup. More than 200 000 cases of canned turtle soup were processed yearly in Key West, Florida, in the 1880s, and as recently as 1957, approximately 465 000 kg of live marine turtles were imported into the USA (Parsons, 1962). After many populations declined or disappeared, government restrictions ended the legitimate U.S. trade in marine turtles in 1973. Freshwater turtles, however, remained largely unregulated.

As the trade in marine turtles was curtailed, the demand increased for the alligator snapping turtle (*Macroclemys temminckii*), the largest freshwater turtle in North America. This long-lived turtle was hunted extensively in Louisiana and the south-eastern USA (Pritchard, 1989; Ernst, Lovich & Barbour, 1994; Sloan & Lovich, 1995). As a result of declining stocks, most states in the region barred commercial trapping of this slow-growing species. The state of Louisiana, however, continues to permit trapping and trade in alligator snapping turtles. Anecdotal reports of poaching beyond Louisiana's borders to supply this market persist and

†All correspondence to: J. Roman. Current address: Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138, USA. Tel: 617-496-8667; Fax: 617-495-1958; E-mail: jroman@oeb.harvard.edu.

provided part of the motivation for this study. We used polymerase chain reaction (PCR) technology, which can recover genetic markers from small tissue samples, partially degraded meat and some cooked products, to determine species of origin for putative turtle products sold by an often secretive industry.

METHODS

To assess species exploited in the turtle trade, we purchased 36 putative turtle meat products in retail markets, wholesale outlets and restaurants in Louisiana and Florida. Most samples were bought directly by J. R. during two excursions to Louisiana, in 1995 and 1998. One sample, TM23, was purchased from Louisiana over the Internet.

DNA extractions were performed using Chelex (Bio-Rad, Hercules, CA, USA) according to the procedures described by Roman *et al*. (1999). For cooked products, visible pieces of meat were removed and washed gently with distilled water before Chelex extractions. We employed a hierarchical approach for each specimen: (1) control-region primers were used to identify common snapping turtles (*Chelydra serpentina*) and alligator snapping turtles, (2) cytochrome *b* primers were used to identify species for specimens that did not yield snapping turtle sequences from the control region. The control region of the mitochondrial genome was included because polymorphisms in this segment can indicate river of origin in alligator snappers (Roman *et al*., 1999), which we anticipated would be a staple in the Louisiana marketplace. A 480 base-pair (bp) fragment of the control region and adjacent $tRNA^{PRO}$ gene was amplified using primers designed for common and alligator snapping turtles (5' TCT TCC TAG AAT AAT CAA AAG 3' in the $tRNA^{PRO}$ gene; 5' ATG ACC CTG AAG AAA GAA CCA G 3' in the central control region). For samples that did not amplify with our snapping turtle primers, a 305 bp fragment in the cytochrome *b* gene was targeted using primers that work on a broad range of vertebrates, in case we encountered mislabelled products (5' AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA 3', 5' AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A 3': Kocher *et al*., 1989). All cooked meat products were amplified using cytochrome *b* primers, since the target sequence is shorter than our control-region sequence and more likely to amplify from a partially degraded template (Kohn *et al*., 1995). PCR reactions (50 μl) were performed using a Biometra Personal Cycler under the following conditions: 5 μl 10x buffer, 3 mM $MgCl_2$, 0.5 mM dNTPs, 1 μM of each primer and 0.5–1 unit Taq DNA polymerase (Fisher). Amplifications (35 cycles: 1 min 94 °C, 1 min 50 °C, 2 min 72 °C) were purified using Ultrafree-MC 30 000 filters (Millipore). PCR and extraction blanks (negative controls) were included in all isolations and amplifications to test for contamination.

Using the manufacturer's recommended conditions, single-stranded DNA-sequencing reactions were conducted using a robotic work station (Applied Biosystems model 800), and the labelled extension products were processed using an automated DNA sequencer (Applied Biosystems model 373A). Fragments were aligned and edited using Sequencher version 3.0 (Gene Codes Corporation). Samples that exactly matched our reference sequences (control region or cytochrome *b)* were considered diagnostic in terms of species identification. Reverse sequences were obtained for all DNA fragments that yielded ambiguous nucleotide designations.

Sample sequences that did not match our reference sequences were compared to entries in GenBank using the BLAST program (National Center for Biotechnology Information: NCBI) to identify DNA fragments of high similarity. We used the Kimura two-parameter model (Phylip 3.57: Felsenstein, 1993) to calculate genetic distances between DNA fragments from meat products, reference sequences and entries retrieved using the BLAST program.

A neighbour-joining analysis (Saitou & Nei, 1987) of cytochrome *b* sequences was used to infer phylogenetic affiliation and species of origin. We used bootstrapping (Felsenstein, 1985), a method of testing phylogenetic groupings, to assess the consistency of species assignments.

Our analysis included all species that we thought could be represented in the marketplace. These included three turtle species: the common snapping turtle, alligator snapping turtle and Florida softshell (*Apalone ferox*). Sequences from two regional species, the red-eared slider (*Trachemys scripta*) and the false map turtle (*Graptemys pseudogeographica*), were included in the analysis to provide a larger framework of local genotypes (see Shaffer, Meylan & McKnight, 1997). The American alligator (*Alligator mississippiensis*) was included because this reptile is sold in markets and might serve as a substitute for turtle. The only other native crocodilian, the American crocodile (*Crocodylus acutus*), was also included. All reference sequences are available in GenBank (accession numbers AF159026–AF159030).

RESULTS

Of 36 samples, 32 were successfully amplified: a 394 bp sequence was resolved for 17 samples using the control region primers designed for snapping turtles, and a 256 bp sequence was resolved using cytochrome *b* primers for 15 samples (Table 1). Except for two samples, sequences from all frozen products were identical to reference sequences. Two cooked and two canned products (TM7, TM9, TM20 and TM34) did not yield sequence information for either locus.

For cooked samples, we found Chelex isolations – after meat had been removed from the broth and washed – to be more effective than conventional DNA isolation using phenol and chloroform. As expected, amplifications from cooked products (soup and chili) were more successful using the shorter cytochrome *b* fragment. A few ambiguous nucleotide designations from these processed samples were resolved in favour of consensus sequences.

Table 1. Results from 36 meat samples purchased as turtle from markets in Louisiana and Florida: four samples did not yield DNA sequences

Sample	Location	Date	Condition	Region sequenced	Species diagnosis
TM1	Seafood market, Baton Rouge, LA	Nov. 1995	Frozen	CR	Common snapping turtle
TM2	Seafood market, Baton Rouge, LA	Nov. 1995	Frozen	CR	Common snapping turtle
TM3	Seafood market, Baton Rouge, LA	Nov. 1995	Frozen	CR	Common snapping turtle
TM4	Seafood market, Manchac, LA	Nov. 1995	Frozen	CR	Common snapping turtle
TM5	Seafood market, Jefferson, LA	Nov. 1995	Frozen	CYB	Florida softshell
TM6	Seafood market, New Orleans, LA	Nov. 1995	Frozen	CR	Common snapping turtle
TM8	Restaurant, New Orleans, LA	Nov. 1995	Chili meat	CYB	Florida softshell
TM10	Seafood market, Metairie, LA	Nov. 1995	Frozen	CR	Common snapping turtle
TM11	Seafood market, Metairie, LA	Nov. 1995	Frozen	CYB	American alligator
TM12	Seafood market, Metairie, LA	Nov. 1995	Frozen	CR	Common snapping turtle
TM13	Seafood market, Kenner, LA	Nov. 1995	Frozen	CR	Common snapping turtle
TM14	Seafood market, Slidell, LA	Nov. 1995	Frozen	CYB	American alligator
TM15	Seafood market, New Orleans, LA	June 1996	Frozen	CYB	American alligator
TM16	Food market, New Orleans, LA	June 1996	Frozen	CR	Common snapping turtle
TM17	Food market, New Orleans, LA	June 1996	Frozen	CYB	Florida softshell
TM18	Seafood processor, New Orleans, LA	June 1996	Frozen	CR	Common snapping turtle
TM19	Seafood market, Pensacola, FL	Oct. 1996	Turtle soup	CYB	Common snapping turtle
TM21	Internet order, Kenner, LA	Dec. 1997	Frozen	CR	Common snapping turtle
TM22	Food market, Gainesville, FL	Dec. 1997	Canned soup	CYB	Common snapping turtle
TM23	Seafood market New Orleans, LA	June 1998	Freshly butchered	CYB	American alligator
TM24	Seafood market, Metairie, LA	June 1998	Frozen	CR	Common snapping turtle
TM25	Seafood market, Metairie, LA	June 1998	Frozen	CR	Common snapping turtle
TM26	Seafood market, Metairie, LA	June 1998	Frozen	CR	Common snapping turtle
TM27	Seafood market, Metairie, LA	June 1998	Frozen	CR	Common snapping turtle
TM28	Seafood market, Marrero, LA	June 1998	Frozen	CR	Common snapping turtle
TM29	Seafood market, New Orleans, LA	June 1998	Frozen	CYB	Softshell†
TM30	Seafood market, Harvey, LA	June 1998	Frozen	CR	Alligator snapping turtle
TM31	Seafood market, Slidell, LA	June 1998	Frozen	CYB	American alligator
TM32	Seafood market, Slidell, LA	June 1998	Frozen	CYB	American alligator
TM33	Seafood market, Slidell, LA	June 1998	Frozen	CYB	American alligator
TM35	Turtle farm, Ponchatoula, LA	June 1998	Frozen	CYB	Common snapping turtle
TM36	Seafood processor, New Orleans, LA	June 1998	Frozen	CYB	American alligator

Data provided includes market location, condition of meat, mtDNA sequence (CR, control region; CYB, cytochrome *b*) and species identification.
†TM29 clusters with softshell turtles (genus *Apalone*), but not close enough for a species diagnosis.

Sixteen samples had sequences that were identical to the predominant control region haplotype reported in common snapping turtle (Walker *et al.,* 1998). The sequence from TM19, a sample of meat retrieved from canned turtle soup, was not completely readable, yet all resolvable nucleotides conformed to our cytochrome *b* sequence for the common snapping turtle. Despite the partly degraded state of this sample, the sequence included 14 diagnostic nucleotide positions unique to *C. serpentina* among our reference samples. In total, *Chelydra* was the most prevalent turtle on the market, found in 59% of samples analyzed.

We identified eight samples as alligator meat, representing 25% of the resolvable samples. Seven samples were exact matches to reference sequences from the American alligator. The eighth sample, TM14, differed by 1 bp from the American alligator reference sequence, but clustered with alligator, having 100% bootstrap support in the neighbour-joining trees of candidate reptiles.

The sequence for TM29, which did not closely match any of our reference sequences, was compared to sequences posted in GenBank using the BLAST program. This cytochrome *b* fragment was most similar to that of the spiny softshell (*Apalone spinifera*, Shaffer *et al.*, 1997), with sequence divergence of 7.4%, and differed from our reference Florida softshell by about 7.8%. In our neighbour-joining analysis, the sample clustered with the genus *Apalone* with a bootstrap value of 100%, indicating that this sample is probably from a softshell turtle (Fig. 1).

Alligator snapping turtle was identified in only one sample, from Louisiana. We compared this sequence to haplotypes from throughout the turtle's range (Roman *et al.*, 1999); it was an exact match for a haplotype found only in the Mississippi River and east Texas drainages.

DISCUSSION

From the time of the European colonization of the New World through to the twentieth century, marine turtles have provided the primary source of turtle meat in North American markets (Carr, 1954; Parsons, 1962). This fishery eventually declined in the USA as a result of depleted populations and government regulations (Witzell, 1994). Even after protective measures were taken in the 1970s, there continue to be isolated instances of marine turtle consumption and sales (Encalada, Eckert & Bowen, 1994).

The largest freshwater turtle in North America, the alligator snapper, was perhaps an inevitable choice to replace marine turtles in the marketplace. Alligator snapping turtles dominated the market in the south-eastern USA in the 1970s and 1980s (Pritchard, 1989; Sloan & Lovich, 1995). Yet our market survey indicates that

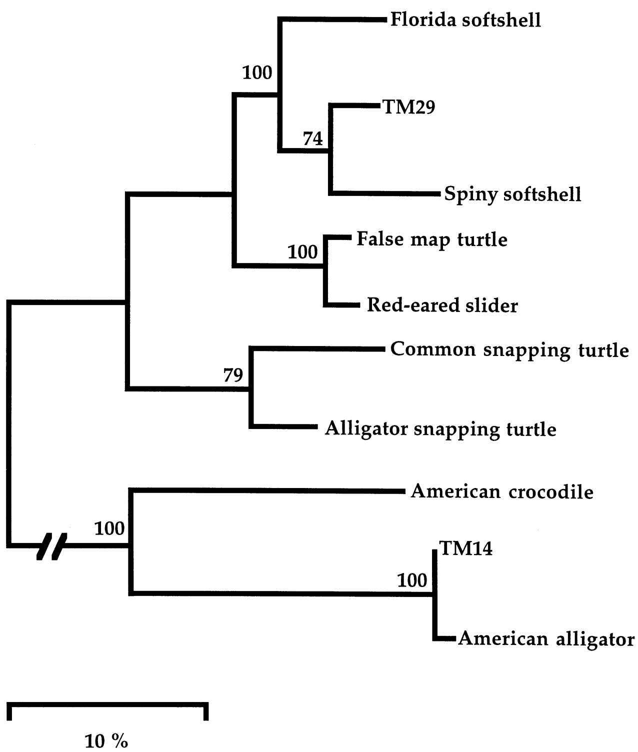

Fig. 1. Neighbour-joining tree of reference cytochrome *b* sequences and putative turtle-meat samples that were not exact sequence matches. Bootstrap values, based on 1000 replicates, are shown above relevant branches. Scale bar is for turtles only.

M. temminckii is no longer an important component in the turtle-meat trade. The presence of *M. temminckii* in only one Louisiana sample, containing a haplotype found throughout the Mississippi River and eastern Texas drainages, suggests that local trappers continue to harvest this species, perhaps opportunistically. Declining populations and the restrictions on commercial trade of this species have probably made full-time trapping unfeasible.

The surveys in 1995 and 1998 indicate that markets have been restocked with the smaller, more common and unregulated *C. serpentina*. Although this species has a wide range in North America and is found in many freshwater habitats, Congdon, Dunham & van Loben Sels (1994) noted that the sustained commercial harvest of this long-lived species in the wild is almost certain to cause population declines. One market we visited in Louisiana had more than 80 common snapping turtles in pens for sale. The salesman indicated that these had come from out of state, perhaps from as far away as South Carolina.

Like the common snapping turtle, the Florida softshell is still legally harvested throughout its range. This species was detected in three purchased samples. An additional sample (TM29) clustered with both softshell sequences, indicating that it is a member of the genus *Apalone*. Though we were unable to obtain a reference sample for this study, the smooth softshell *(Apalone mutica)* is found throughout much of the Mississippi and adjacent drainages; TM29 may be derived locally from this legally harvested species.

Despite the dwindling supply of turtle products, the demand in Louisiana remains strong: in our telephone calls to markets, many vendors indicated that they did not have turtle meat in stock but were eager to resupply. As populations and market demands fluctuate, the composition of turtle meat in trade will probably follow suit. Our results indicate that market surveys may be a good indicator of population trends in harvested species. A trade that began with marine turtles, weighing up to 200 kg, and proceeded to the smaller, though still formidable, alligator snapping turtles (up to 80 kg) has now shifted to the common snapping turtle (up to 30 kg). If market trends continue, subsequent harvests may put pressure on smaller freshwater species, including the softshell turtles (genus *Apalone*) and possibly emydid turtles, such as the river cooter (*Pseudemys concinna*).

We found American alligator in 22% of the samples. The American alligator is plentiful in the south-eastern USA; in the 1990s, Louisiana wild and farm harvests amounted to more than 500 000 kg of deboned meat per year (Louisiana Department of Wildlife and Fisheries, unpublished data). The mislabelling of these samples may indicate that turtle vendors, faced with depleted turtle populations and few trappers, are turning to a more reliable (if less esteemed) source of meat. Processors may be exploiting the shortage of turtle meat to market abundant alligator meat.

This pattern of species replacement in the turtle market bears a striking resemblance to trends in other harvested wildlife resources. In the markets for luxury products, molecular techniques have consistently revealed evidence of substitutions and fraudulent replacements: Palumbi & Cipriano (1998) detected dolphin and porpoise in 28% of their whale meat samples in a 1996 survey; Malik *et al.* (1997) found that a third of their pinniped purchases actually derived from land mammals such as domestic cattle (*Bos taurus*); and Birstein *et al.* (1998) noted that 23% of black caviar designations were mislabelled as to species or region of origin. We found about the same level of fraudulent replacements in turtle markets.

In nineteenth century England, green turtle soup 'provided the pièce de résistance of diplomatic dinners and ceremonial banquets' and became a symbol of Victorian opulence (Parsons, 1962). Unable to afford imported marine turtle, the middle and working classes consumed mock turtle soup, derived from veal. This culinary deception inspired Lewis Carroll to add the Mock Turtle, a character with a calf's head and a turtle's body, to the cast of *Alice's Adventures in Wonderland.* In recognition of this early allusion to species replacements in the market, we suggest the label *mock turtle syndrome* for the phenomenon of progressive substitutions of less-esteemed wildlife products.

In a study of Louisiana turtle markets in 1984 to 1986, Sloan & Lovich (1995) were alarmed by the large harvests of alligator snapping turtles. We are equally alarmed by the *lack* of alligator snapping turtles a decade later. Has this large, slow-growing species been hunted beyond commercially sustainable levels? Marine turtles were removed from the marketplace before they were commercially extinct, and it was reassuring that no marine turtle sequences were recovered from meat products in our survey. Protection at the national level seems to be effective in this regard. The alligator snapper, however, has not had federal protection and seems to have reached commercial extinction in Louisiana. To the extent that market trends may reflect population depletions or abundance, the absence of alligator snappers on the market may indicate dire trends in natural populations. While most states have done a commendable job of protecting alligator snappers in recent years, the primary marketplace, Louisiana, is not likely to implement sufficient conservation measures. Perhaps additional measures are warranted to protect this once-common river turtle.

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