Genetic Analysis as a Conservation Tool for Pet-trade Related Chelonian Threats

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March 23, 2014

Abstract

Fresh water and terrestrial turtle species are threatened worldwide by a rampant and seemingly insatiable pet trade. Rare and unusual species of turtles are taken in unsustainable numbers from the wild and sold illegally in Asia and Africa resulting in the placement of many of these species in a critically threatened status. While common species such as the red-eared slider (*T. scripta elegans*) is captive bred in the U.S. and sold all over the world only to be released into the wild after they are no-longer wanted creating invasive, feral populations in Asia, Europe and South America. The removal of turtles from the wild for food or medicine is not a modern problem; rather it has been going on since pre-historic times. The pet-trade itself has been an ongoing issue for turtles since its inception as turtle have always been popular pets as well as status symbols in some cultures such as China. However, today’s unsustainable level of removal and trade in rare and endangered species requires a more accelerated methodology for making informed decisions about how, when and where to act to save the most critically endangered of these species. The use of genetic analysis is a rapidly advancing tool which can help conservation scientists make these decisions in a much more efficient and timely manner.

Keywords: pet trade, chelonian, fresh-water turtle species, terrestrial species, tortoise, mtDNA, nDNA, anthropogenic threats, genetic analysis, conservation, phylogenetic

Introduction

Freshwater and terrestrial turtle species are under extreme pressure worldwide due largely to anthropogenic threats such as trade and habitat loss and fragmentation. Turtle trade in areas such as Asia is rampant and ranges from traditional medicinal usage to meat consumption to the pet trade (Spinks and Shaffer, 2007, Velo-Anton et al, 2011). In other areas such as Africa and the Americas, pet-trade for chelonian species ranges from black market trade in highly endangered species gathered from the wild and selling for top dollar to hobbyists, to ongoing captive breeding of more common species for sale as inexpensive pets to families (Parham et al, 2012). Turtles have long been a favorite pet as they are usually sold when young and small and appear to need little care; however, few potential pet owners are informed of their longevity, expected adult size or special dietary and husbandry needs. The result of this enormous pet-trade is the release of thousands of turtles into non-native, inappropriate habitats and countless other individuals living long, painful lives in captivity with inadequate food and space. The implication of this massive trade in live turtles, turtle meat, and turtle artifacts includes an ever expanding list of threatened and endangered species making chelonians one of the most imperiled taxon groups in the world (Turtle Conservation Coalition, 2011). According to leading experts, as many as half of the over 300 known species are currently threatened with extinction (Turtle Conservation Coalition, 2011). Trade implicates not only threats to

Figure 1 ***Trachemys scripta elegans***

diminishing wild populations due to poaching, but also competition for these species by invasive species such as the red-eared slider (*Trachemys scripta elegans*), sold throughout the world as a pet species and currently considered invasive in several European countries as well as within the United States in several states outside of its native range (IUCN, 2013, Parham et al, 2013).

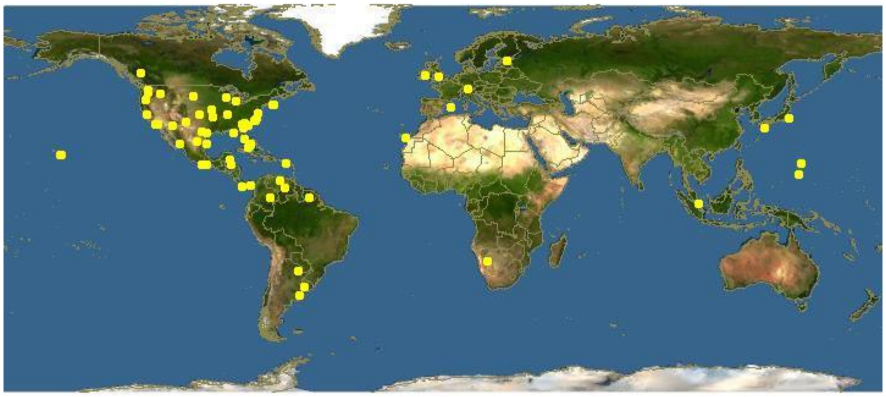

Figure 2 Distribution of Trachemys scripta elegans, including introduced populations in the Americas, Europe, Asia and Africa. Map courtesy of Discover Life (http://www.discoverlife.org/mp/20m?kind=Trachemys+scripta&flags=glean:&mobile=close)

This species is highly adaptable to new habitats and may out-compete native turtle species for basking and nesting habitats. Some evidence indicates that male red-eared sliders may even compete with males of native turtle species for mates (Cadi and Joly, 2003). This is further complicated by the fact that turtles are well known to hybridize amongst even distantly related species (Xia et al, 2011, Parham et al, 2013) and therefore increase the risk of outbreeding depression within endangered populations.

The combination of declining numbers of rare and endangered turtle species and the potential of those bred in captivity for the pet trade to be released into non-native regions ultimately becoming invasive, creates a near critical call for better understanding of the existing taxon, wild population dynamics and their habitat requirements. A critical tool for developing baseline information on terrestrial and freshwater species is the use of genetic analysis to study the phylogenetic relationships of wild populations determining taxonomic divisions amongst true species and hybrid mixes.

Additionally, genetics have been employed on various types of turtles found on the market and in breeding facilities, allowing the definition of new species and clarifying the lines amongst currently accepted species and variations. Focusing on the relationships amongst closely related taxa helps conservationists make the best decisions about continued efforts both ex-situ (captive breeding) and in-situ (control of invasive species, priority habitats). This paper presents a summary of genetic analysis, three exemplar phylogenetic studies of turtle taxa, and the conservation implications of each.

Discussion

Mitochondrial vs. Nuclear DNA

Despite the depth and longevity of the chelonian evolutionary tree, most experts agree that there is still a lot of unknown information on turtle phylogenetics. To determine the inter-relatedness of individual turtles within a closely related taxon of species, geneticists use a combination of mitochondrial DNA (mtDNA) analysis and nuclear DNA (nDNA) markers. The mtDNA is located within the mitochondria of each cell and is passed along to individuals via maternal lineage. It is haploid in nature (as opposed to diploid), meaning it is does not recombine during sexual reproduction. All offspring have the same mitochondrial DNA as their mother, while each individual's nuclear DNA is unique due to recombination (. In sea turtles, the mtDNA is used to determine natal homing tendency in females and helps to define genetically distinct populations even when the individuals being screened are on their feeding grounds and tend to mix freely with individuals from other regions (Bowen et al., 1992, Bowen and Karl, 2007).

However, the propensity for turtles to intermingle when placed in close proximity to other species, even if distantly related, and the ease at which they hybridize often requires a more sophisticated means of separating lineages (Spinks and Shaffer, 2007, Parham et al., 2013). Long term anthropogenic manipulation of freshwater and terrestrial turtle species distribution has created a complex situation with turtles being kept in captive situations and released in non-native regions. Therefore, the addition of nDNA markers to the mtDNA analysis makes for a stronger and more precise analysis when determining differences amongst species within the same genus or individuals which may be the result of past or ongoing hybridization events.

The Case of the European Pond Turtle (*Emys orbicularis)*

While the European pond turtle (*E. orbicularis*) is one of the widest-ranging of the extant fresh-water turtle species, its current distribution may be highly influenced by long-standing trade as both meat and pets (Velo-Anton et al., 2011). Due to this trade influence and because the species is currently in decline throughout much of its range, it is vital to understand the phylogenetics of this species and the origin of stocks currently held throughout the European Union (EU) as well as those occurring in the wild (Velo-Anton et al., 2011).

Due to an ever-increasing number of unwanted ex-pets, concerns about trans-located individuals as disease vectors for wild populations, increased likelihood of hybridization and the establishment of invasive populations of non-native species, a series of recovery centers have been established throughout Europe to house ex-pets creating an opportunity for studying the geographic origin of multiple genomes for *E. orbicularis* and comparing them to those of individuals captured from the wild (Velo-Anton et al., 2011). Previously established genome information was used to begin the study focusing on haplotype sequences within the mtDNA. The results of this study were the establishment of new haplotype information and the reconstruction of historic trade routes for this species. Several wild caught animals were established to be of a lineage non-native to the geographical region in which they were sampled, indicating anthropogenic translocation from one region of the *E. orbicularis'* range to another. For example, a lineage considered native to the Iberian Peninsula (Spain and Portugal) are found in areas of Germany where, due to written records, this species is known to have become extinct sometime in the early 1700's. It is believed that this re-established population is the direct result of the pet trade which began in the later part of the 19th century (Velo-Anton et al., 2011).

Spanish conservationists are working diligently to understand the current range for *E. orbicularis* as well as the influence of an invasive feral population of red-eared sliders within the Iberian Peninsula. Due to its classification as Near Threatened (NT) by the IUCN Red List and as Appendix II on the EU Habitats Directive, the Valencian government has partnered with the Research Center in Biodiversity and Genetic Resources to fulfill an EU funded LIFE Trachemys project which aims to reduce the negative influence of introduced *T. scripta elegans* through a diligent program of eradication, study the current population dynamics amongst *E. orbicularis* and *T. scripta elegans* where they occur together and reinforce the wild population of E. orbicularis through a captive breeding and release program (CIBIO, retrieved March 2014). Genetic studies such as the one described here have important implications for establishing sound protocols for determining appropriate breeding stock for captive breeding and head start programs for population reinforcement of *E. orbicularis* within the Iberian Peninsula. Further research into potential hybridization of native stocks with the invasive *T. scripta elegans* may be an important area of study as well.

The Case of Asian Box Turtles *(Cuora spp.)*

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Figure 3 ***Cuora trifasciata*** **or the Chinese three-striped box turtle is endangered due to the acclaimed medicinal qualities of its shell.**

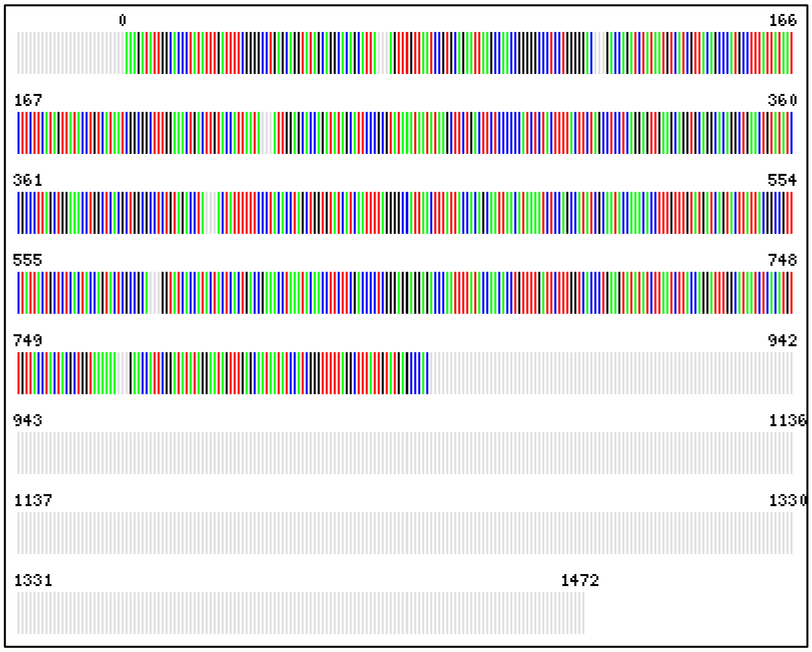

Figure 4 DNA barcodes available online for *Cuoara yunanensis*, a species with a second lease on life. This species was "rediscovered" in 2009 and up-listed from extinct to critically endangered on the IUCN Redlist of Species (Hagen, Hudson & Lau, 2011).

The majority of Asian fresh water turtle species are in grave peril due to the largely unsustainable trade for food, traditional Chinese medicine and pet markets (Spinks and Shaffer, 2007). The *Cuora* genus as a whole has been placed on the IUCN Red List, nine species of which are currently listed as Critically Endangered. The study performed by Spinks and Shaffer examined live animals kept in captivity in various zoos and captive breeding locations as well as wild caught individuals throughout the Hong Kong area. They examined all 12 known species using both mtDNA and nDNA analysis, providing a detailed and thoughtful analysis of the taxon. The results of their analysis indicate that some of the captive breeding stocks may, in fact, contain hybrid individuals rather than pure *C. trifasciata* or *C. pani* genotypes (Spinks and Shaffer, 2007). As many of these animals are kept in captivity to insure against their extinction and potentially reinforce wild populations, this kind of data is vital in planning for the conservation of true lineages.

Figure 5 Zhou's box turtle is only known from captive specimens from the pet trade.

Another important result of this study was the substantiation of the *C.zhoui* and *C.mccordi* lineages as true genealogical species

(Spinks and Shaffer, 2007). This is especially interesting to conservationists working with this critically endangered taxon because the only individuals of these species known are those in captivity as pets and have yet to be found in their native habitat (Spinks and Shaffer, 2007)! Therefore the founder population for the captive population is severely limited and the species is certainly rare if not already extinct in the wild. This fact is an overwhelming indication of the paucity of information available to scientists working to save these critically endangered animals.

The Case of Antillean Freshwater Sliders (*Trachemys spp.*)

In this study, researchers tackled the genus *Trachemys*, which includes the red-eared slider (*T. scripta elegans*), arguably the most transplanted turtle species in the history of the pet trade. One of the goals of this study was to determine the extent of hybridization between native Antillean *Trachemys* species and feral populations of *T. scripta elegans* (Parham et al., 2013). They also wanted to examine the occurrence of other hybrid mixes amongst geographically overlapping species or species that may have been trans-located due to pre-Colombian anthropogenic movements. Results of this study helped to define the diversity and inter-relatedness amongst island populations showing that there is little diversity in mtDNA and much evidence for repeated hybridization and backcrossing amongst different species (Parham et al., 2013).

Important findings from a conservation perspective include detrimental hybridization affects through the introduction of feral populations of *T. scripta elegans* in highly urbanized areas of Puerto Rico and to some extent the Cayman Islands (Parham et al., 2013). In other, areas such as Jamaica, this does not seem to be the case. However hybridization amongst the native *T. terrapin* may be seriously compromised through hybridization with *T. decussata* (a historically Cuban species) by causing an outbreeding depression where the offspring are less adapted or ecologically fit than either of the parent species or through the eventual extinction of the parent species due to a high degree of hybridization termed a hybrid swarm. Further studies are needed to determine if the contact between these two species was naturally occurring due to ocean currents or whether it was in fact the result of human activities such as food transfer during pre-Colombian times. Some natural hybridization does occur due to overlap in species range, but hybridization due to the introduction of invasive species and other anthropogenic affects are increasing worldwide. Solving this mystery may have important implications on conservation decisions for the *Trachemys* complex within Jamaica and therefore deserves more investigation (Parham et al., 2013).

With morphologically and phylogenetically similar species such as those within the *Trachemys* genus, it is difficult to work out all of the intricacies of species, subspecies and potential hybridization events through the use of only one technique. It is important to use a variety of tools to disentangle the web of natural overlap and anthropogenic influence to come up with the best strategy for conserving the diversity of freshwater species still surviving with the Antilles islands

Conclusions

As with any technology, it appears that even genetic analysis has limitations. For example, with this tool we can determine the legitimacy of *C. zhou* and *C. mccordi* as genealogical species, but we cannot determine from where exactly they originate geographically or in what habitat they occupy. We can also determine that there is hybridization amongst the Jamaican *T. terrapin* and the Cuban *T. decussata*, but not by what means the Cuban slider originally arrived in Jamaica. However, genetic analysis is clearly a powerful tool for defining parental stock for critically endangered species and for determining the historic movements of turtle species as they have been shaped by human influences throughout the ages. It is also an important indicator of feral population impacts on existing turtle biodiversity. With genetic analysis, biologists and decision makers can make more informed decisions, prioritize their efforts and help shape the future of turtle conservation.

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Appendix: Personal Photos of Interest

Fig. 1 & 2

<u>This lady is a *Cuora serrata*: a naturally occurring hybrid species</u> between *C. galbinifrons* and *C. mouhotii*. Opinions differ amongst experts about this Asian box turtle's conservation status due to its hybrid nature: some arguing that as a naturally occurring hybrid, it should be considered as a species while others dispute it's value (Chris Hagen, Director of Animal Management, Turtle Survival Center, personal communication, April 2014).

Fig. 3 McCord's box turtle (*C. mccordi*) are currently held in captivity as assurance colonies by institutions like the Turtle Survival Alliance (TSA) and the Muenster Zoo. These guys were once known only through captive individuals in the pet trade. They were first found in the wild in 2007 (http://www.boxturtles.com/mccords-box-turtle/).

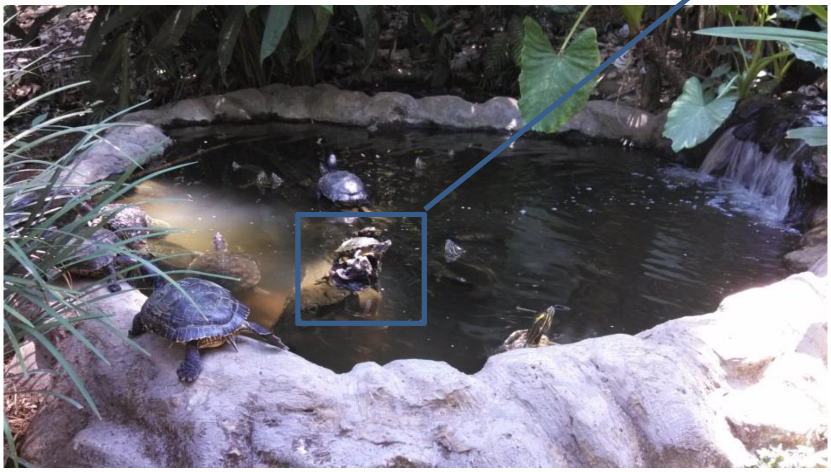

Fig. 4 & 5 These photos were taken at Zoo Ave in Alajuela, Costa Rica during my recent visit. Both *T. emolli* and *T. venusta* occur naturally in this area (not sure which one this attractive lady is), but you will notice that in the group shot, front and center is a red-eared slider (*T. scripta elegans*). Such evidence, indicate the far ranging effects of the pet trade really are. If all slider species are vulnerable to hybridization with this one subspecies, then we are in for an enormous loss of genetic biodiversity in our fresh water turtles!

Fig. 6 Okay, not the prettiest photo, but fascinating none the less: this little red-eared slider was found in the stomach of a Great horned owl upon necropsy at the Georgia Sea Turtle Center! I am not sure if it was the final straw for this animal, which came in severely emaciated and extremely weak. It may have picked this hatchling up as a last ditch effort to eat something and just couldn't pass or regurgitate it. But it definitely confirms breeding for this invasive species on Jekyll Island, GA.