Forensics, phylogeography and population genetics: a case study using the Australasian snake-necked turtle, *Chelodina rugosa*.

By

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Statement of contribution

Because this thesis is written as a series of chapters prepared for publication in peer-reviewed journals, several people other than myself have contributed to the work, and they deserve acknowledgement. These include:

- Arthur Georges (Institute for Applied Ecology, University of Canberra), who provided guidance and supervision for all aspects of the PhD study, and assisted in the preparation of manuscripts.
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These people are included as authors in the following chapters as well as the associated publications, in order of their contribution to the work. However, despite the collaborative nature of this thesis, the work within is my own, and I received no assistance other than that which is stated above.

I as primary supervisor agree with the above stated proportions of work undertaken for each of the published (or prepared for submission) peer-reviewed manuscripts contributing to this thesis:

Prof Arthur Georges  Date
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Abstract

Illegal trade of wildlife is a serious and growing crime. One of the greatest challenges in international efforts for policing of the illegal wildlife trade is the provision of evidence. DNA technologies are ideal for providing evidence for wildlife crime because they can be used on degraded and highly processed products to address a wide variety of forensic questions (i.e. species, regional and population-level identification). Theory, techniques and principles from phylogenetics, phylogeography and population genetics provide the fundamental genetic data required for forensic applications. This thesis demonstrates the benefits of merging the disciplines of phylogenetics, phylogeography and population genetics into wildlife DNA forensics – an emergent field that uses DNA technologies to provide evidence for wildlife crime. A DNA forensic identification system was developed using the freshwater turtle *Chelodina rugosa* Ogilby, 1890 as a case study. This species was chosen because a commercial industry is established to supply the pet shop trade.

Application of conservation genetics to freshwater turtles and tortoises was reviewed. General areas where genetic principles and empirical data can be profitably used in conservation planning are identified. Monitoring trade and directing enforcement to protect overexploited turtle populations was identified as one of three crucial future directions for conservation genetics of freshwater turtles and tortoises.

The extent of illegal wildlife trade in Australia was examined using case prosecution data from the Australian Customs service for the period of 1994 to 2007. Of cases prosecuted, 46% were for attempted export and 34% for attempted import. Reptiles were the most targeted (43%), then birds (26%), and native plants (11%). For the majority of prosecutions (70%) the sentence was a fine (70%) that was consistently only a fraction of the market value. I argue that tougher penalties are required to deter criminals from engaging in illegal wildlife trade and initiatives for improved policing (such as DNA technologies) are urgently required.

DNA technologies that have been used to provide evidence for wildlife cases are critically evaluated. Emphasis is placed on the science that is required to form the foundation for forensic applications. Baseline genetic data for species, regional and population level identification of wildlife seizures can be provided by phylogenetic, phylogeography and
population genetic studies, respectively. I advocate greater collaboration of forensic scientists with conservation geneticists to develop research programs that will jointly benefit conservation of traded species and policing of wildlife trade.

Seventeen microsatellite markers were developed specifically for *C. rugosa*. Sixteen of the loci were polymorphic but three of these loci had null alleles. These 17 microsatellite markers were tested for amplification in eight other species with varying success; 98% amplification in *C. burrengandjii*, 72% in *C. canni*, 38% in *C. expansa*, 58% in *C. longicollis*, 67% in *C. mecodii*, 73% in *C. oblonga*, 81% in *C. parkeri*, and 68% in *C. pritchardi*. These microsatellite markers will be useful for population assignment, gene flow, mating systems and hybridization studies in the genus *Chelodina*.

Phylogeography of the Australasian freshwater turtle *Chelodina rugosa* was investigated using 867 bp of the mitochondrial control and ND4 regions. There were two major haplotype lineages for *C. rugosa* consisting of (i) Northern Territory and (ii) New Guinea and northern Queensland extending east to the MacArthur River. The designation of the New Guinea form as a distinct taxon (formerly called *C. siebenrocki*) was refuted. Extensive hybridisation between *C. rugosa* and *C. burrengandjii* in Arnhem Land were found by the mitochondrial analysis and 17 microsatellite loci. A hybrid between *C. rugosa* and *C. canni* was also confirmed. The mitochondrial gene trees and nuclear R35 gene tree (898 bp) were incongruent with respect to the phylogenetic relationships between *Chelodina sp.* (Kimberley) and *C. canni*. Further research using a suite of nuclear markers is required to resolve these phylogenetic relationships and the taxonomic status of *Chelodina sp.* (Kimberley).

Population genetics of *C. rugosa* in the Blyth-Cadel drainages of Arnhem Land was investigated to provide recommendations for their sustainable harvesting. There were no detectable impacts from traditional harvesting. Genetic diversity estimates were similar for harvested and unharvested populations. Levels of genetic structure in the Blyth-Cadel region were low and populations functioned as a metapopulation. I recommend that sustainable harvesting can be conducted, provided that the impacts of pig predation are alleviated and gene flow between sites, through natural or artificial means, is maintained.
A DNA-based forensic identification system for *C. rugosa* was developed. An 898 bp region of the nuclear R35 intron discriminated *C. rugosa* from all other Australian chelid turtles. Individuals with recent hybrids origins between *C. rugosa* and *C. burrungandjii* were identified by 17 microsatellite loci. Geographic sources of specimens could be assigned to three distinct regions by sequencing 867 bp of the mitochondrial DNA: (i) Darwin (Finnis basin), (ii) Arnhem Land, and (iii) eastern Queensland including southern New Guinea. Specimens could not be identified to a source locality at the population-level (using 12 unlinked microsatellite loci) in the Blyth-Cadel basin of Arnhem Land where a commercial trade has been established. Given the isolation and inaccessibility of the Arnhem Land region, this level of identification may be adequate to verify the legality of specimens from the commercial industry.

This thesis merges the disciplines of phylogenetics, phylogeography and population genetics with the growing field of wildlife DNA forensics. It highlights issues for the development of forensic identification systems for wildlife. Emerging technologies on the horizon, such as single nucleotide polymorphisms (SNPs) and pyrosequencing will herald a new era for wildlife forensics. They will complement existing technologies enabling rapid discovery of molecular markers and screening of wildlife seizures. DNA technologies will be an increasingly important tool in international efforts to fight the burgeoning illegal wildlife trade.
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List of CoAuthorship

List of publications associated with this thesis:

