

Development of a SNP-based genetic analysis for geographic origin assignment of the pig-nosed turtle *Carettochelys*



Matthew J. Young¹, Andrzej Kilian², Carla Eisemberg¹, Dianne M. Gleeson¹ and Arthur Georges¹

¹ Institute for Applied Ecology, University of Canberra, Australia
² Diversity Arrays Technology, University of Canberra, Australia



Introduction

The pig-nosed turtle (*Carettochelys insculpta*) is a large freshwater turtle (up to 22 kg, 57 cm carapace length). Its distribution is restricted to the Daly, Victoria, South Alligator and East Alligator river drainages of northern Australia and the rivers/coast of southern New Guinea (Papua New Guinea and Western Papua) (Figure 1).

Carettochelys is the sole surviving species of the family Carettochelyidae, is listed as Vulnerable by the IUCN, and is highly sought-after by animal poachers for the international pet trade, as bush-meat in food markets and as a constituent of traditional Asian medicine.

Intensified exploitation in recent decades is unsustainable, and has led to severe declines (~57%) in Kikori River populations since the early 1980's (Eisemberg *et al.*, 2011). A study of the *Carettochelys* in the Vrienschap River, Papua Province, found that 100% of nests were harvested (Triantoro, 2012), while on the Kikori River, Papua New Guinea, more than 95% of nests (up to 100% in some locations) were harvested by humans or a combination of humans and native predators (Eisemberg *et al.*, 2011).

Despite blanket legal protection in source countries prohibiting and restricting all capture and trade, and listing in CITES Appendix II, poaching and trade does occur with regular seizures by officials occurring at points of export in Indonesia (~81,000 *Carettochelys* seized between 2003-2012) (Burgess and Lilley, 2014).

Project Aims

Repatriation of seized *Carettochelys* is hindered by the inability to accurately determine the population of provenance. This study aims to determine whether SNP loci generated using DArTseq can be used to develop genetic profiles for geographic origin assignment of *Carettochelys* populations.

Pilot Study: Methods & Results

Available *Carettochelys* samples (Figure 1) were sequenced using DArTseq, available from Diversity Arrays Technology (DArT) {see QR code} generating single nucleotide polymorphisms (SNPs) via double digest Restriction-Site Associated DNA sequencing (ddRADseq). DArTseq uses a genome complexity reduction approach where high quality genomic DNA is digested with two restriction enzymes, the resulting fragments are then size-selected and sequenced on Next Generation Sequencing (NGS) platforms. A total of 20,199 SNPs were scored for 89 individuals spanning the Daly and South Alligator rivers of Australia and the Kikori, Fly, Merauke, Eilanden and Bamu rivers of New Guinea.

The SNP data was read into an adegenet genlight object for processing and subsequent analysis using the R package dartR {see QR code}. Stringent filtering on reproducibility and callrate reduced the number of SNP loci to 14,074. Principle Coordinate Analysis (PCoA) was used to represent genetic distances between individual *Carettochelys*. Fixed difference analysis determined preliminary Operational Taxonomic Units (OTUs) by identifying alleles that have become fixed to alternative states in pairwise population comparisons.

Carettochelys show strong structure between Australia and New Guinea, with three major PCoA clusters (Figure 2) representing (1) the Daly River of Australia, (2) the Eilanden and Merauke rivers of Papua Province and (3) rivers of Papua New Guinea. Fixed difference analysis determined 5 preliminary OTUs (Table 1), which correspond to the PCoA clusters where sample sizes are adequate.

At a finer scale, within New Guinea there is evident admixture between the Kikori, Bamu and Fly rivers, Papua New Guinea (Figure 3), which have been amalgamated into an OTU due to the lack of fixed allelic differences (Table 1).

Discussion

Our results indicate that there is enough resolution provided by the DArTseq SNP data to distinguish populations. Future work will involve obtaining *Carettochelys* genetic samples from multiple populations across the native range in Australia, Papua New Guinea and Papua Province. These samples will then be used to develop SNP profiles for each functional interbreeding population. SNP profiles will form the basis for a geographic assignment test to identify population provenance of seized *Carettochelys*. DArTseq sequences will also be mined for entire mitochondrial genomes for investigation of maternal lineage structuring.

Acknowledgments

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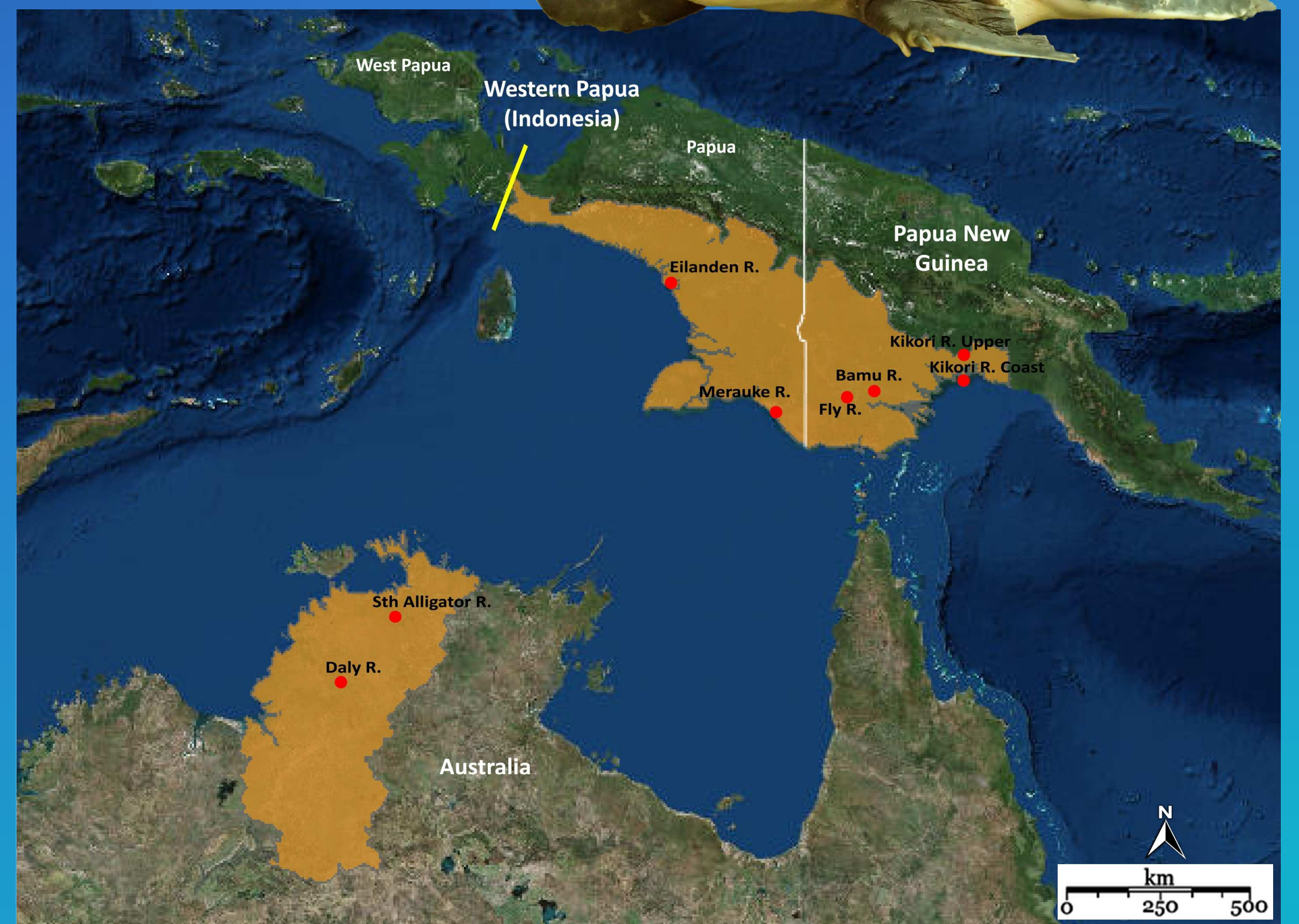


Figure 1. Native distribution of *Carettochelys insculpta* across Australia and New Guinea (orange shading). The border between the provinces of West Papua and Papua is approximated by the yellow line. Red dots indicate sample locations.

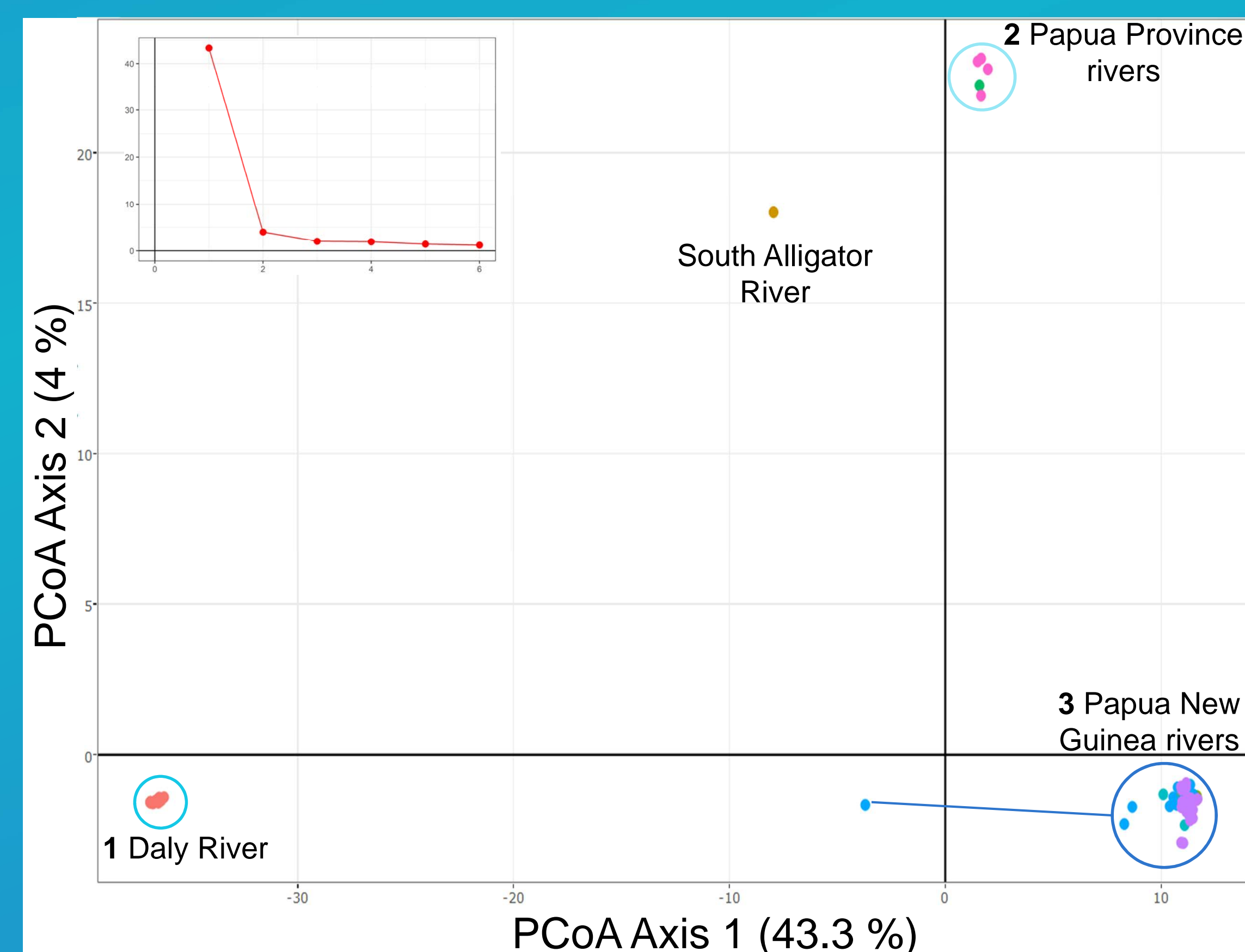


Figure 2. PCoA plot for *Carettochelys* from Australia and New Guinea showing genetic relationships between individuals based on SNP loci. A total of 49.3% of the variation was explained by the first three axes. Inset Scree Plot shows percentage contribution to observed variation in the PCoA of the first six axes.

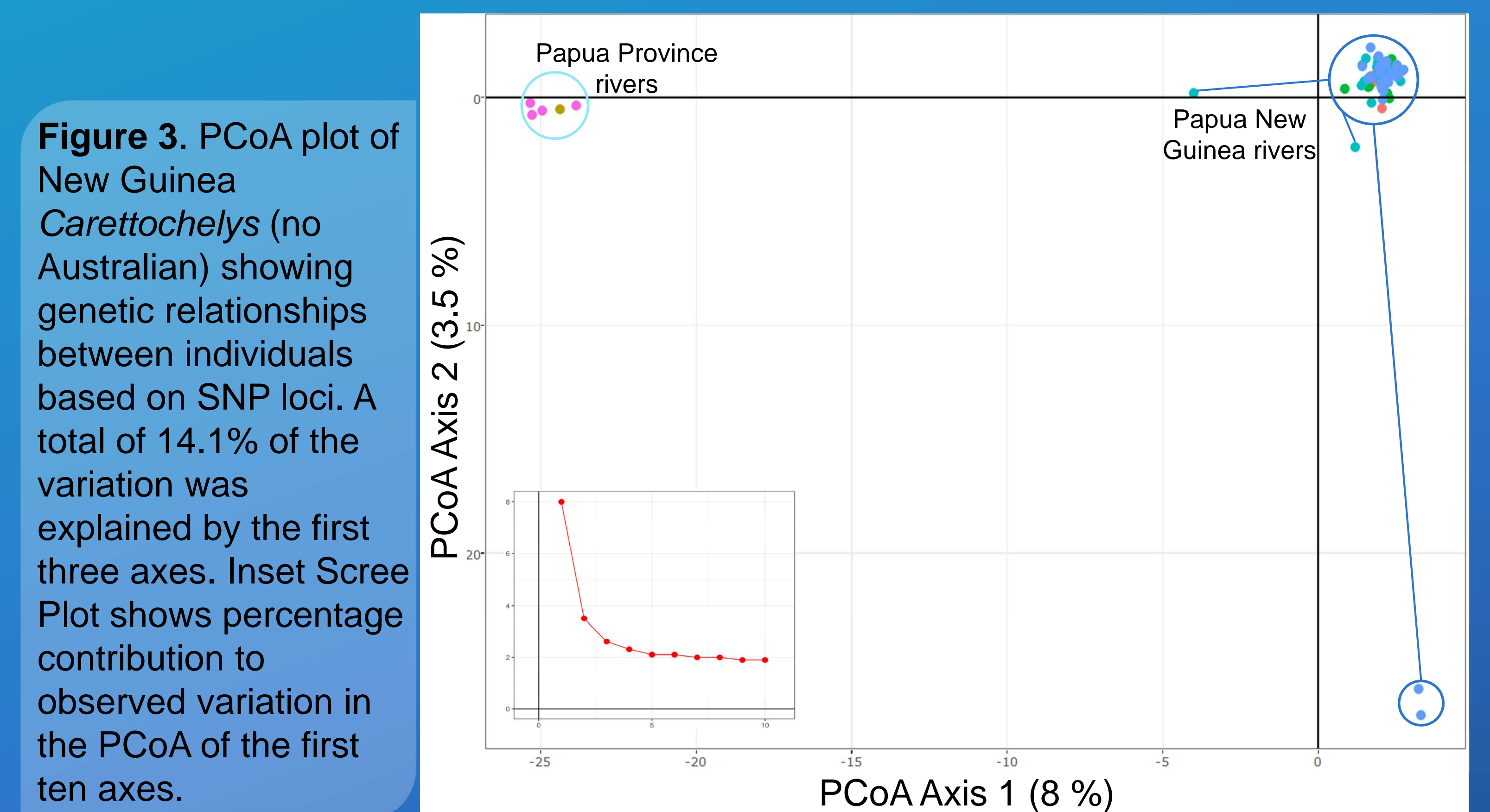


Figure 3. PCoA plot of New Guinea *Carettochelys* (no Australian) showing genetic relationships between individuals based on SNP loci. A total of 14.1% of the variation was explained by the first three axes. Inset Scree Plot shows percentage contribution to observed variation in the PCoA of the first ten axes.

Table 1. OTUs based on fixed allelic differences between populations.

Population	Location	n	O.T.U
Daly River	Australia	19	1
South Alligator River	Australia	1	2
Eilanden River	Papua Province	1	3
Merauke River	Papua Province	4	4
Kikori River headwaters	Papua New Guinea	27	5
Kikori River coastal	Papua New Guinea	25	5
Bamu River	Papua New Guinea	3	5
Fly River	Papua New Guinea	9	5



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