

MITOGENOME ANNOUNCEMENT

Complete mitochondrial genomes of Tuatara endemic to different islands of New ZealandElmira Mohandesan¹, Sankar Subramanian², Craig D. Millar¹, and David M. Lambert²¹Allan Wilson Centre for Molecular Ecology and Evolution, School of Biological Sciences, University of Auckland, Auckland, New Zealand and²Environmental Futures Centre, Griffith University, Nathan, Australia**Abstract**

Tuatara are the sister taxon to the Squamata (including lizards and snakes) and are regarded as the most distinctive surviving reptilian genus. They are currently inhabits on offshore islands around New Zealand and have been recognized as a species in need of active conservation management. In this study, we report a total number of five nearly complete mitochondrial genomes, which were sequenced by Sanger and Next Generation DNA sequencing methods. Our phylogenomic analysis revealed distinct clustering of tuatara populations from the north and south islands of New Zealand.

Keywords

Mitochondrial genome, mtDNA, sphenodon, tuatara

History

Received 30 August 2013

Accepted 30 August 2013

Published online 23 October 2013

Tuatara are significant in terms of global biodiversity and the evolutionary history of reptiles (Cree & Butler, 1993). Tuatara are the only living members of the order Sphenodontia and are exclusively inhabit on offshore islands around New Zealand that are free of introduced mammals (Duncan et al., 2002; Gaze, 2001; King, 2003). Although fossil records indicate little morphological change in modern tuatara compared to their Cretaceous relatives (Apesteguia & Novas, 2003; Benton, 1993; Carroll, 1988), molecular data suggest rapid molecular evolution in this iconic taxon (Hay et al., 2008; Subramanian et al., 2009).

In order to further examine the population genetics and phylogenetics of tuatara, we sequenced complete mitochondrial genomes of tuatara from five islands. The samples (blood and toe pad tissues) collected from individuals of various islands: Cuvier, Mercury, Stanley, Stephens and North Brother Islands (Figure 1). The complete genomes were obtained using long-range PCR of 4kb DNA fragments using a series of primers that were designed according to the mitogenome of *Sphenodon* (NC004815). The long-range PCRs were performed as described by Rest et al. (2003) with slight modifications. The PCR products were then sequenced using the Sanger/454-sequencing platform. Typically each tuatara mitogenome (15,181 bp) consists of 12 protein-coding, 21 tRNA, 2 rRNA genes and 2 control regions – identical to the reference genome reported previously (Rest et al., 2003). Although the structure and gene organization of mtDNA is highly conserved among various taxa, the tuatara mtDNA has

a distinctive gene order compared to other vertebrates (Rest et al., 2003). This genome lacks the origin of replication in light strand, which is usually found within the cluster of five tRNA genes (WANCY) and this is a common trait among all birds, crocodylians and *Sphenodons*. It has two tRNA Lys copies. The ND4 gene is followed by ND6, tRNA Glu, D-loop (823 bp), tRNA Leu, Cyt b, tRNA Pro, tRNA Ser and another D-loop region (926 bp). The sequences of these two D-loops are identical in the initial 750 bp in 5' direction, but can be different thereafter. The ND5, tRNA His, and tRNA Thr genes are absent. The average length of the mtDNA showed asymmetric base frequencies (π A = 33.3%, π T = 26.8%, π C = 25.9%, π AG = 14.0%). The maximum likelihood composite estimate of the pattern of nucleotide substitution showed a strong bias towards transition mutations (frequencies C ↔ T = 0.30, G ↔ A = 0.56) over transversion (0.14).

We constructed a maximum likelihood tree using the software MEGA (Tamura et al., 2011) by choosing the GTR (γ +I) model. The mitogenomic phylogeny showed that the individuals from Stephens and Brother islands – located near the south island of New Zealand, group together and separated from those from the northern islands (Figure 1). The individuals from Mercury and Stanley islands appear to be closely related compared to that from Cuvier Island. These results might be useful for future studies on the population genetics and phylogenetics of tuatara.

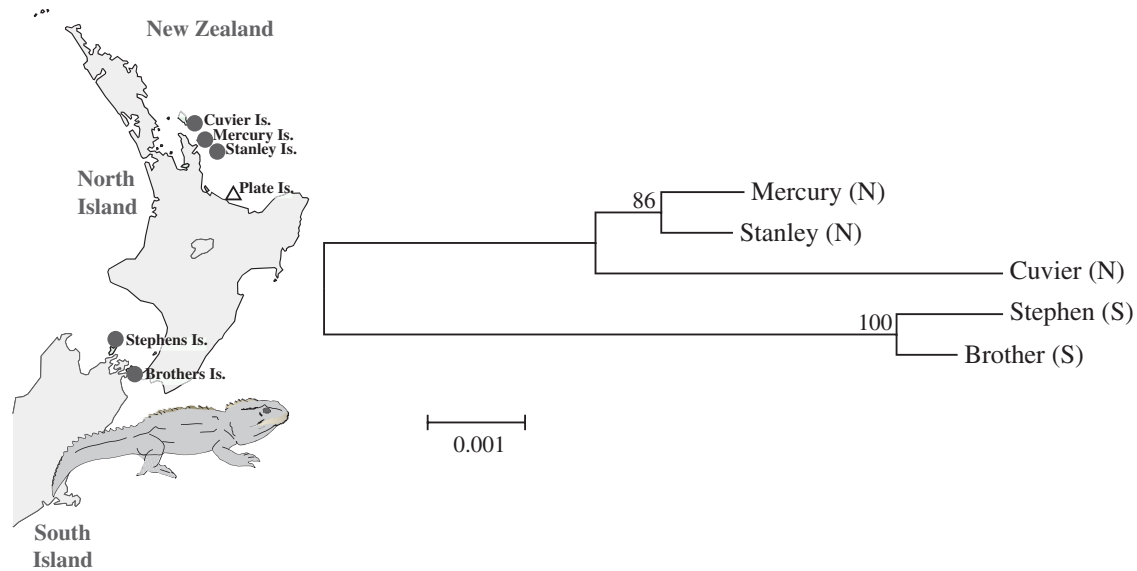


Figure 1. A maximum likelihood tree showing the phylogenetic relationship of tuatara belonging to five different islands of New Zealand. The numbers above the nodes are bootstrap (500 replications) confidence values. The letters N and S in parenthesis denotes northern and southern islands.

Acknowledgements

We wish to thank C. H. Daugherty and N. Nelson and J. M. Hay for providing us with samples. This research project was supported by a scholarship to E. M. from the Allan Wilson Centre for Molecular Ecology and Evolution. The project was also supported by Griffith University.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

- Apesteuguía S, Novas FE. (2003). Large Cretaceous Sphenodontian from Patagonia provides insight into Lepidosaur evolution in Gondwana. *Nature* 425:609–12.
- Benton MJ. (1993). Reptilia. In: Benton MJ, editor. *The fossil record 2*. London: Chapman and Hall. p 681–715.
- Carroll RL. (1988). *Vertebrate paleontology and evolution*. London: Freeman WH and Company.
- Cree A, Butler D. (1993). Tuatara recovery plan. Threatened species recovery plan 9. Wellington: Department of Conservation.
- Duncan RP, Blackburn TM, Worthy TH. (2002). Prehistoric bird extinctions and human hunting. *Proc Biol Sci* 269:517–21.
- Gaze P. (2001). Tuatara recovery plan. Threatened species recovery plan 47. Wellington: Department of Conservation.
- Hay JM, Subramanian S, Millar CD, Mohandesan E, Lambert DM. (2008). Rapid molecular evolution in a living fossil. *Trends Genet* 24: 106–9.
- King M. (2003). *The Penguin History of New Zealand*. Auckland: Penguin Books.
- Rest JS, Ast JC, Austin CC, Waddell PJ, Tibbetts EA, Hay JM, Mindell DP. (2003). Molecular systematics of primary reptilian lineages and the tuatara mitochondrial genome. *Mol Phylogenet Evol* 29:289–97.
- Subramanian S, Hay JM, Mohandesan E, Millar CD, Lambert DM. (2009). Molecular and morphological evolution in tuatara are decoupled. *Trends Genet* 25:16–18.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–9.