

Baraminic Classification of the Tuatara (*Sphenodon punctatus*) among Reptiles Based on Whole-Genome K-mer Signature Comparison

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Abstract

The baraminic status of the tuatara (*Sphenodon punctatus*), the only extant species of the reptilian order Rhynchocephalia, is an intriguing question for creation science. This animal superficially resembles lizards in its anatomy, yet has special physical and genomic characteristics which make it discontinuous with all other reptiles. A previous morphological baraminology study strongly suggested that the tuatara belongs to its own distinct group. In this study, the genomic analysis of 61 reptile genomes using the whole-genome k-mer signature reinforces this conclusion.

Introduction

The tuatara (Figure 1) is a unique reptile found on approximately 30 small islands around New Zealand (Evans, 2003). Its name comes from the Māori language and refers to the spiny crest on its back. It has a host of unique morphological and genomic features. For example, its brain fills only half of its endocranium. A row of pyramid-shaped acrodont teeth are on its lower jaw; two rows of blade-like teeth are on the upper jaw, which fit over the lower teeth when chewing. A parietal eye with retina and lens-like structures detects light, and is nested between the scales on the back of its head. It has no penis, rather sperm enters the female's body when the cloaca of

the male and the female meet together. The tuatara also has a cold-adapted physiology, temperature-dependent sex determination, and can also live up to 80 years (Young, 1950, p. 384; Jones and Cree, 2012).

The tuatara is the sole living member of the reptile order Rhynchocephalia (“beak-head” in Latin), although some researchers recognize a second subspecies as a full species, *Sphenodon guentheri* (Hay et al., 2010). This order is thought to have had a worldwide distribution at one time in Earth's history (Evans, 2003). The tuatara allegedly shared a common ancestor with squamates (scaled reptiles, such as lizards, worm lizards, and snakes) some 250 million

years ago (Gemmell et al., 2020). Many of its features were previously thought to be primitive but are now thought to have been acquired over time (Jones et al., 2009). For example, the *Sphenodon* skull is held to be fully diapsid, and the lower bar of the temporal bone is thought to have been lost in its descendants, only to reappear later in recent species (Evans, 2003).

Evolutionists consider the tuatara to be an important link to those alleged stem reptiles which they claim gave rise to dinosaurs, modern reptiles, birds, and mammals. Interestingly, the tuatara resembles several sphenodontine fossils, allegedly from the Cretaceous, classifying it as yet another living fossil (Hay, 2008). Over forty rhynchocephalians exist in the fossil record, and it is possible that *S. punctatus* is the sole survivor of its kind from the Genesis Flood. Considering that dinosaurs have all gone

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Figure 1. The tuatara (*Sphenodon punctatus*). Source: animals.sandiegozoo.org/animals/tuatara

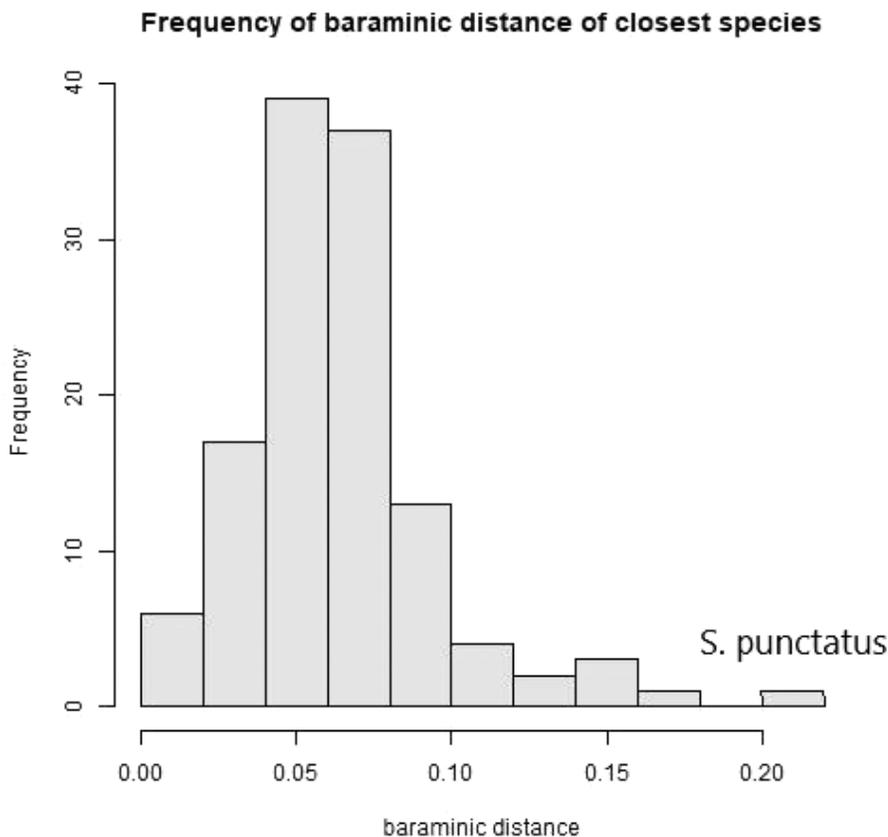


Figure 2. Histogram of closest baraminic distance values for each of the species in the morphological analysis of Cserhati, 2020a. The baraminic distance was noted for all species in that study and the species closest to it. These closest baraminic distances are then depicted as a histogram.

extinct, it is reasonable that something similar could have happened in Rhynchocephalia.

A previous baraminology study by Hennigan (2014b) places the tuatara into its own baramin. He bases his classification based on a number of skull characteristics which separate the tuatara from all other squamates. He also refers to the delay of hatching from eggs as much as 16 months after laying, and the lowest incubation temperature of all extant reptiles. In Figure 3 of Cserhati, 2020a, *S. punctatus* is fairly separate from all other species.

Upon examination of the baraminic distance matrix, the distance between *S. punctatus* and the species closest to it is 0.206. For each of the species in the study, the baraminic distance to the closest species to it was noted, and is visualized in the histogram in Figure 2. As we can see, the largest (closest) baraminic distance is that of *S. punctatus* (0.206, with *Physignathus cocincinus*, the Chinese water dragon). The next highest (closest) baraminic distance is 0.168, which belongs to *Anniella pulchra* (the California legless lizard, with *Sphenomorphus solomonis*, a skink species). This means that morphologically, *S. punctatus* is discontinuous with (or distinct from) all other reptiles, despite some superficial resemblance to lizards. But what about its genetics? Does a difference in phenotype also mean a difference in genotype as well?

Tuatara Genetics

Genetically, the tuatara is also a rather interesting animal. Its genome appears to be an amalgam, containing transposons from reptiles, monotremes, and placental animals. Its genome is 4.3 Gb in size with an estimated 17,448 genes, 75% of which are homologous to genes in birds, turtles, and crocodiles. Gene order is also similar between the tuatara, turtle, chicken and human. Furthermore, 64% of the tuatara genome is



Figure 3. Heat map showing Pearson Correlation Coefficient (PCC) values between species pairs among the 61 reptile species in this study. Darker colors denote higher PCC values, representing related species from the same cluster (baramin). Lighter colors denote lower PCC values, suggesting unrelated species from different baramins.

made up of repetitive elements, which are actually more similar to mammals than reptiles (Gemmell, 2020). If the tuatara genetically resembles reptiles and mammals, it cannot be an ancestor to either one of them. Rather, the evidence shows that the tuatara belongs to its own special group.

Remarkably, Hay and colleagues (2008) isolated mitochondrial DNA from tuatara fossils, suggesting from maximum likelihood analysis that the ratio and the sites of C to T and T to C substitutions are almost the same between modern and ancient tuatara populations (2.71 and 2.66 respectively). Furthermore, the positions where substitutions occur in the genome are also the same, another indication that the tuatara did not change much after its creation.

Object of this Study

The tuatara is interesting for baraminology studies because of its unique features. Some researchers believe that the tuatara most closely resembles squamates. A previous baraminology study on squamates showed that snakes and lizards are separate apobaramins (Cserhati, 2020a). It would be interesting to see how the tuatara can be classified, either as a member of its own or of another existing baramin. As we have seen, the tuatara is morphologically different from all other reptiles. Is the tuatara different genetically as well? Does the tuatara belong to an already existing reptile holobaramin, or does it form its own group? In this paper I will compare the tuatara with other reptile species by analyzing genomic data with a molecular baraminology algorithm to clarify its baraminic status.

Materials and Methods

Data availability

The whole-genome sequences (WGS) of 61 reptiles were downloaded from

NCBI, which were then analyzed with the whole-genome k-mer signature (WGKS) algorithm. This algorithm has been used in previous molecular baraminology studies to measure the global genomic similarity between species to determine baraminic relationships. Octamers were analyzed, according to protocol in Cserhati, 2020b. A list of these species and a link to their whole-genome sequences are available in Supplementary File 1. Molecular and morphological data from a previous study on squamates was also included in this study on the tuatara (Cserhati, 2020a). This data is available at github.com/csmaty/squamates. All supplementary files for the current study are available online at this web address.

All analyses were done using R version 4.0.1. Heatmaps were created using the heatmap command in R, using the 'ward.D2' clustering algorithm. Clustering was done using the Partition Around Medoids (PAM) algorithm, using the pam command in the cluster R library, with the algorithm parameter set to 'Forgy.' The PAM algorithm was designed to find a set of objects called 'medoids,' which are found in the middle of their own cluster. The algorithm classifies each object in such a way so as to minimize the dissimilarity between the object and their corresponding medoid. The Forgy algorithm is a widely used k-means clustering algorithm, which classifies entities based on their minimal distance to k previously determined centroids, each of which represents one of the k clusters, as selected by the user. The centroids can be determined by selecting those k entities which are the farthest from one another. In each iterative step, entities are assigned to one of the k centroids, after which the centroids are recalculated (Morissette and Chartier, 2013).

Results and discussion

Figure 3 is a heat map depicting the Pearson Correlation Coefficient (PCC)

values. Darker, redder colors depict PCC values closer to 1, indicating two species from the same cluster. Lighter colors depict PCC values closer to 0, indicating two species from two different clusters. The PCC matrix has a Hopkins clustering statistic value of 0.863, which is very good for clustering. Several large and small clusters, and individual species can be seen in the figure. Using the PAM clustering method, 30 clusters were chosen; those with two or more species are listed in Table 1. In the following paragraphs, only clusters with three or more species will be viewed as putative baramins.

Cluster 1 consists of six turtle species (*Actinemys marmorata*, *Chrysemys picta*, *Malaclemys terrapin*, *Platysternon megacephalum*, *Terrapene carolina*, *Trachemys scripta*). These species belong to a group including the family Emydidae (terrapins), plus Platysternidae (big-headed turtles).

Cluster 2 is made up of four species from the order Crocodylia: *Alligator mississippiensis*, *Alligator sinensis*, *Crocodylus porosus*, *Gavialis gangeticus*. These species belong to a baramin comprised of reptiles with long bodies and long snouts. Hennigan (2014) puts crocodiles, alligators (caimans) and gharials into their own separate kinds because of strong cognita and reported interspecific hybridization within (but not across) some families. However, he suspects that Crocodylia possibly forms a holobaramin, despite the lack of hybridization data between the different families. The results in this paper support the latter.

Cluster 6 is made up of three species, *Chelonia mydas* (green sea turtle) from the family Cheloniidae, *Chelydra serpentina* (common snapping turtle), from the family Chelydridae, and *Dermatemys mawii* (Central American river turtle, or hickatee), from the family Dermatemydidae. The green sea turtle is a marine turtle, albeit the female comes to shore to lay eggs. The other two species are aquatic and sometimes submerge

Table 1. Statistics for 11 putative baramins with two or more species based on PCC values calculated for the 61 species.

Cluster	Species	Min	Mean	Max	Stdev	P-Value
1	6	0.792	0.899	0.977	0.062	4.36E-23
2	4	0.872	0.913	0.962	0.035	3.48E-09
4	2	0.831	0.831	0.831	NA	NA
6	3	0.642	0.652	0.662	0.01	3.00E-30
7	3	0.819	0.885	0.937	0.06	9.22E-4
8	4	0.843	0.902	0.978	0.055	3.14E-08
10	2	0.979	0.979	0.979	NA	NA
13	2	0.831	0.831	0.831	NA	NA
15	6	0.732	0.84	0.977	0.067	4.43E-18
16	5	0.727	0.831	0.977	0.101	9.16E-09
20	3	0.938	0.958	0.996	0.033	2.15E-06
28	3	0.6	0.739	0.899	0.151	0.039

into rivers. These three species may belong to different families, and therefore it is very tentative that they belong to the same baramin. However, both *D. mawii* and *Ch. mydas* both have a diploid number of 56, and their chromosomes can be arranged into groups A, B, and C following the 7:5:16 pattern (Carr et al., 1981).

Cluster 7 is made of three species, *Chelonoidis abingdonii*, *Gopherus agassizii*, and *Gopherus evgoodei*, the last two which are fossorial turtle species. All three species belong to the tortoise family (Testudinidae).

Cluster 8 is made of four species from the family Viperidae (vipers): *Crotalus horridus*, *Crotalus viridis*, *Protobothrops flavoviridis*, and *Protobothrops mucrosquamatus*.

Cluster 15 is made of six snake species from the superfamily Colubroidea, which includes colubrid and elapid snakes. These six species are: *Hydrophis cyanocinctus*, *Hydrophis hardwickii*, *Hydrophis melanocephalus*, *Notechis scutatus*, *Ophiophagus hannah*, and *Pseudonaja textilis*.

Cluster 16 is made of five lizard species, *Lacerta agilis*, *Lacerta bilineata*, *Lacerta viridis*, *Podarcis muralis*, and *Zootoca vivipara*. These species all belong to the family Lacertidae (true lizards), which may comprise a holobaramin.

Cluster 20 is made of three snake species, *Pantherophis guttatus*, *Pantherophis obsoletus*, and *Ptyas mucosa*. All three belong to the family Colubridae (colubrid snakes, which have grooved fangs in the rear of the upper jaw).

Cluster 28 is made of three more snake species, *Thamnophis elegans*, *Thamnophis sirtalis*, and *Thermophis baileyi*. All three species belong to the family Colubridae.

There are several species which are grouped by themselves. Some of these species also belong to families which contain species from other clusters listed here. It may be that these families are polybaraminic. Such species include *Crotalus pyrrhus* and *Vipera berus* (Viperidae), *Dermochelys coriacea* and *Dermatemys mawii* (Dermochelyidae), *Gekko japonicus* and *Paroedura picta*

(Gekkonidae), *Emydocephalus ijimae*, *Laticauda colubrina*, *Laticauda laticaudata*, and *Naja naja* (Elapidae).

Figure 3 clearly shows that *Sphenodon punctatus* belongs to its own cluster. *S. punctatus* has a mean PCC of 0.206 with all 60 other reptile species in this study. The mean PCC value in the PCC matrix is 0.321. The species with the top five highest PCC values compared to the tuatara belong to *Emydura subglobosa* (0.309), *Alligator mississippiensis* (0.291), *Mesoclemys tuberculata* (0.283), *Crocodylus porosus* (0.275), and *Varanus komodoensis* (0.268). These are turtle, crocodile, and lizard species, indicating that *S. punctatus* is discontinuous with all other reptile groups.

Figure 4 is a histogram showing the number of species whose closest species (measured by the PCC value) is depicted on the x-axis. For species within the same holobaramin, the closest species would come from the same holobaramin and the PCC value would be very high. This is why there is a maximum at around 0.9. The species closest to *S. punctatus* is the turtle species, *Emydura subglobosa*, with a PCC value of only 0.309 between it and *S. punctatus*. In comparison, only one other species, *Anolis carolinensis*, has a PCC value lower than this when compared to any other species (namely *Zootoca vivipara*, with a PCC value of 0.248). This also illustrates the fact that *S. punctatus* is genomically discontinuous with all other reptiles.

Summary and Conclusion

According to this study, the tuatara is clearly genetically distinct from other reptiles. Therefore, I suggest putting it into its own holobaramin. The tuatara seems to be the sole survivor of its kind after the Flood. This is in agreement with a previous study based on morphological characteristics, as well as a previous creationist analysis. The current study illustrates how morphologi-

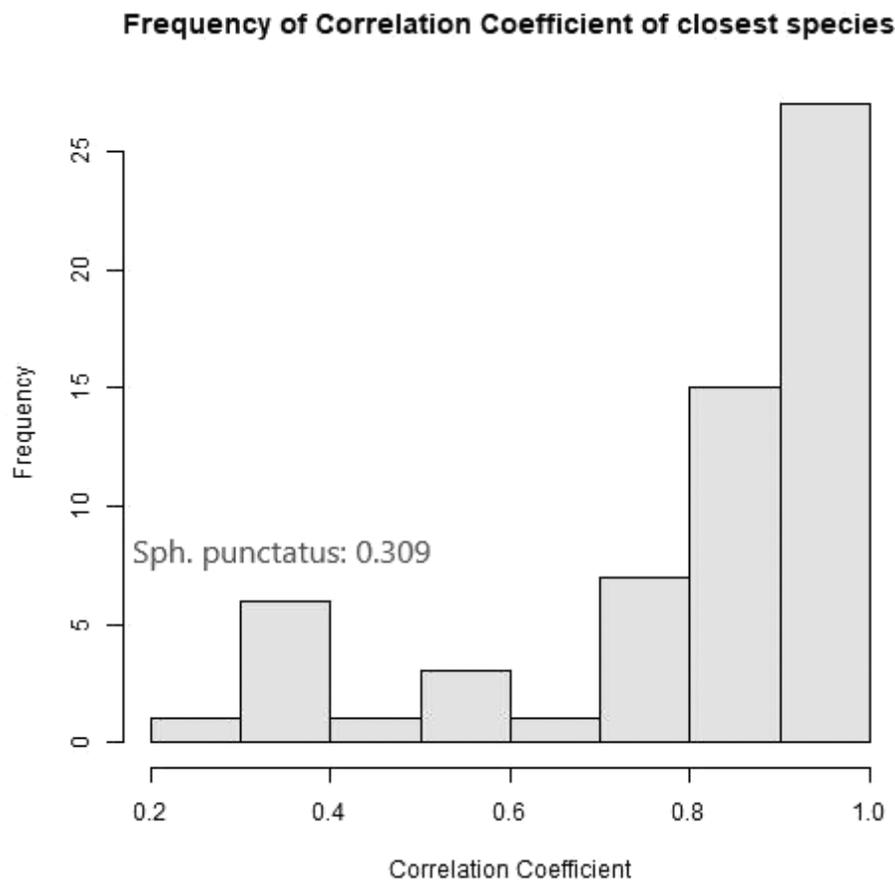


Figure 4. Histogram of PCC values of closest species to each of the 61 species in this study. In other words, for each species, the PCC of the species closest to it is noted, and all of these 61 ‘closest species PCC values’ are depicted on the histogram.

cal and genomic baraminology studies can nicely complement one another. Besides morphology and genetics, fossil data also appears to support the discontinuity between the tuatara and all other reptile groups, consistent with the creationist narrative that distinct kinds of animals were created at the beginning of history.

Acknowledgements

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