

Research Article

GENETIC RELATIONSHIPS OF THE SUBORDO SERPENTES BASED ON GENE ND 1

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Abstract:

The suborder Serpentes comprises a highly diverse group of reptiles commonly known as snakes, characterized by significant morphological, ecological, and behavioral variation. Due to the frequent morphological convergence and environmental adaptation among species, genetic approaches are necessary to clarify their phylogenetic relationships. This study aims to analyze the genetic diversity and infer the evolutionary relationships among selected species within the suborder Serpentes using the mitochondrial gene ND1 (NADH dehydrogenase subunit 1) as a molecular marker. A total of 10 species were analyzed using secondary data obtained from the NCBI Nucleotide database. Sequence alignment was conducted using BioEdit, and phylogenetic analysis was performed with MEGA software employing the UPGMA method and Kimura two-parameter model for genetic distance calculation. The resulting phylogenetic tree showed that species with lower genetic distances tended to cluster together, in accordance with their known taxonomic classifications. Similarity values derived from the genetic distance matrix revealed that species within the same genus had similarity above 90%, species within the same family but different genus showed 80–89% similarity, while species from different families within Serpentes showed similarity ranging from 70–79%. These findings indicate that the ND1 gene serves as an effective molecular marker for assessing genetic relatedness and diversity within Serpentes, and that *in silico* analysis using secondary genetic data can reliably reflect taxonomic relationships.

Keywords: Order Serpentes, ND 1, Phylogenetic

INTRODUCTION

Suborder Serpentes is a group of reptiles that includes all types of snakes and is widely known for its diversity in morphology, habitat, and survival strategies. Snakes have successfully adapted to various ecosystems, from tropical forests, and grasslands, to aquatic environments, indicating high rates of evolution and speciation in this group (Grundler & Rabosky, 2021; Hudry & Herrel, 2025). This diversity makes Serpentes an interesting taxonomic group to study, especially in order to understand the differences and relationships between species. However, morphological approaches alone are often insufficient to explain phylogenetic relationships in depth, given the presence of convergent forms and similar environmental adaptations among different species (Almeida-Santos et al., 2007).

The molecular approach is an effective method for uncovering relationships between species because it is based on objective genetic data and can be analyzed quantitatively. One of the molecular markers that can be used in animal taxonomy



and phylogenetic studies is the ND1 gene (NADH dehydrogenase subunit 1). ND1 is a gene in the mitochondrial genome that plays a role in the mitochondrial respiratory chain, especially in complex I (Uddin et al., 2018). The ND1 gene is not highly conserved, its moderate level of variation allows it to be used effectively in phylogenetic studies focusing on medium-level taxonomic categories (Zardoya & Meyer, 1996). In a comparative study of various DNA markers for species monitoring, ND1 demonstrated a major contribution to understanding diversity, especially in species-rich taxa (Mayer et al., 2007; Rach et al., 2017).

Genetic sequence data are now widely available and easily accessible in data banks as public repositories (Kane et al., 2020). One of them is the National Center for Biotechnology Information (NCBI), an institution under the United States National Institutes of Health (NIH). NCBI provides nucleotide and protein data sets from a variety of organisms around the world, making it possible to use documented genetic information as a basis for studies of diversity and relationships between species (Sayers et al., 2024). With these data sources, taxa diversity studies can now be carried out in silico, namely through computer-based analysis using secondary data without the need for DNA extraction or wet laboratory observations (Jeyasingh & Glory, 2024).

Considering the high level of diversity in the Serpentes suborder, it is necessary to reveal the relationship patterns between species within it. The use of molecular markers such as the ND1 gene allows phylogenetic analysis to be carried out. The availability of genetic data from public repositories such as NCBI can also help make analyses more efficient. Based on this background, this research aims to analyze the kinship relationships of several species in the Serpentes suborder based on the ND1 gene by utilizing secondary data from NCBI.

Methods

Genetic sequence information of ten species in the suborder Serpentes was obtained from the NCBI-Nucleotide database, with the ND1 (NADH dehydrogenase subunit 1) gene selected as a molecular marker. The accession numbers of each species are presented in Table 1.

Table 1. Accession number of ND1 sequence from several species in the suborder Serpentes

No	Species	Accession Numbers
1	<i>Naja naja</i>	NC_010225.1
2	<i>Ophiophagus hannah</i>	NC_011394.1
3	<i>Bungarus fasciatus</i>	NC_011393.1
4	<i>Python molurus</i>	NC_015812.1
5	<i>Python regius</i>	NC_007399.1
6	<i>Malayopython reticulatus</i>	NC_042397.1
7	<i>Bothrops atrox</i>	MK_313563.1
8	<i>Crotalus adamanteus</i>	NC_041524.1
9	<i>Agkistrodon contortrix</i>	MK_313581.1
10	<i>Thamnophis sirtalis</i>	MT_801477.1

Sequence alignment was carried out using BioEdit version 7.2 software, then continued with phylogenetic analysis using MEGA version 12 software. Genetic distances between species were calculated using the Kimura two-parameter method, and phylogenetic trees were constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method. Branch validation was carried out through a bootstrap test of 1000 repetitions. The results of the analysis include



a genetic distance matrix visualized with a phylogenetic tree and taxonomic grouping based on similarity values as a reference for classification thresholds.

Results

The final alignment of the ND1 gene sequences, obtained from NCBI and processed through trimming, resulted in a dataset of 943 base pairs. The pairwise genetic distances were calculated using the Kimura 2-parameter (K2P) model and are presented in Table 2.

Table 2. Accession number of ND1 sequence from several species in the suborder Serpentes

Species	1	2	3	4	5	6	7	8	9	10
1 <i>Naja naja</i>										
2 <i>Ophiophagus hannah</i>	0,2157									
3 <i>Python molurus</i>	0,2666	0,2622								
4 <i>Python regius</i>	0,2520	0,2490	0,0943							
5 <i>Malayopython reticulatus</i>	0,2319	0,2363	0,1460	0,1294						
6 <i>Bungarus fasciatus</i>	0,2095	0,2144	0,2569	0,2525	0,2509					
7 <i>Bothrops atrox</i>	0,2729	0,2601	0,2855	0,2682	0,2715	0,2929				
8 <i>Crotalus adamanteus</i>	0,2825	0,2704	0,2709	0,2554	0,2570	0,2739	0,2016			
9 <i>Agkistrodon contortrix</i>	0,2693	0,2545	0,2505	0,2400	0,2371	0,2649	0,1595	0,1713		
10 <i>Thamnophis sirtalis</i>	0,2707	0,2556	0,2474	0,2402	0,2309	0,2551	0,2522	0,2473	0,2378	

The genetic distance values ranged from 0.0943 (between *Python molurus* and *Python regius*) to 0.2929 (between *Bothrops atrox* and *Bungarus fasciatus*), indicating a wide spectrum of divergence levels. These genetic distance estimates formed the quantitative basis for constructing the phylogenetic tree to elucidate the relationships among the species. These genetic distance estimates formed the quantitative basis for constructing the phylogenetic tree to elucidate the relationships among the species. Based on this distance matrix, a phylogenetic tree was reconstructed using the UPGMA method, as shown in Figure 1.

Discussion

Based on the phylogeny tree resulting from analysis using the UPGMA method and Kimura distance calculations for the two parameters, it can be seen that the species that have the smallest genetic distance values in the matrix tend to be in the same clade (Saud & Alshami, 2021). For example, *P. molurus* and *P. regius* are combined in one branch with a high bootstrap value, this is supported by the very low genetic distance between the two, namely 0.0943, which indicates the Genus *Python* clade (iNaturalist, 2025a). Apart from that, *M. reticulatus* also belongs to the same clade as species from the *Python* genus, this shows that there is a close relationship between species in the Pythonidae family (iNaturalist, 2025b). This is in line with the genetic distance value to *P. molurus* (0.1460) and *P. regius* (0.1294) which is also relatively low compared to the distance between *M. reticulatus* and other species.

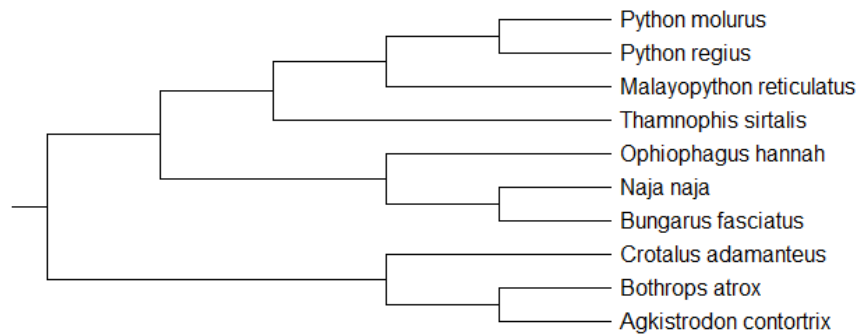


Figure 1. UPGMA Phylogeny Tree of Serpentes Suborder Based on the ND1 Gene

Interestingly, *T. sirtalis* (Colubridae) belongs to the same clade as the Pythonidae species, even though it comes from a different family (iNaturalist, 2025c). This is supported by the relatively low genetic distance to *M. reticulatus* (0.2309), *P. molurus* (0.2474), and *P. regius* (0.2402), as well as the low bootstrap value so that its existence in this clade is most likely due to limited data that only uses one species from the Colubridae family.

The next branch joins the Elapidae family clade, where in this clade *N. naja* and *O. hannah* are combined with high bootstrap values and supported by a low genetic distance value of 0.2157. Both of them were then grouped with *B. fasciatus* which also has relatively low genetic distance values between the two (0.2505 and 0.2509), which indeed proves the relationship between the three species (iNaturalist, 2025d).

The next branch was formed from the Elapidae family clade, namely the Viperidae family clade (iNaturalist, 2025e). In this clade, the *C. adamanteus* species is part of the *B. atrox* and *A. contortrix* branches, with high bootstrap values. The genetic distance of *C. adamanteus* to the two species is 0.2016 and 0.1713 respectively. Meanwhile, the genetic distance between *B. atrox* and *A. contortrix* is 0.1713, a small distance value means they are grouped in the same branch but with a bootstrap value that is not too high because the two species also come from different genus. Overall, a branching phylogenetic tree was formed according to the taxonomic classification between species (Figure 1).

In molecular identification, a threshold value of 98% for the ND1 gene is generally used as the minimum limit for sequence similarity between individuals in the same species (Morgan & Blair, 1998). Therefore, pairs of species that have similarities below this threshold are likely to come from different species, even though they may still be in the same genus or family (Wang et al., 2025). Based on the results of the similarity analysis, it is known that the similarity within one genus is above 90%, the similarity between species in the same family but different genera is around 80-89%, and the similarity between different families in the same suborder is around 70-79%.

Conclusions

The phylogenetic tree formed based on the ND1 gene shows a grouping pattern of species in the Serpentes suborder which is in accordance with the existing taxonomic classification. Species with low genetic distance tend to be in the same cluster, supported by high bootstrap values. Similarity analysis produces a range of ND1 values in the Serpentes suborder, namely above 90% for species in the same genus, 80-89% for species in the same family but different genera, and 70-79% for species from different families. Thus, the ND1 gene has been



proven to be able to be used as a molecular marker to reveal the diversity and relationships of species in the Serpentes suborder in silico.

Declarations

The authors declare that there is no conflict of interest

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