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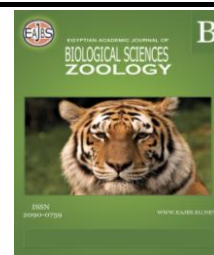


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Phylogeny of *Eryx Jayakari* Isolated from Ha'il Province

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ABSTRACT

Eryx jayakari (Arabian sand boa) is an important egg-laying boa that successfully reversed the oviparous ability about 60 million years after the transition of the group to viviparous. The current study aimed to infer the phylogeny of *Eryx jayakari* - isolated by our group from Hail province, Saudi Arabia- to other boas using mitochondrial 16S and 12S rRNA sequences and bioinformatics tools (MultAlin alignment, Mega program, and the Maximum Likelihood method). 16S rRNA phylogenetic tree inferred a strong relation between *E. jayakari* isolated from Ha'il (KSA) and that isolated from Khuzestan (Iran). 12S rRNA gene sequences showed a higher diversification than the 16S rRNA gene sequences in inferring the phylogenetic relationships for *E. jayakari*. A strong relation between *E. jayakari* and *E. miliaris* was also observed. It can be concluded that *E. jayakari* in Hail might be disseminated from Iran. More 12S rRNA sequences from Iran are required for further assessments.

INTRODUCTION

Eryx jayakari (also known as Arabian sand boa), is a small, non-venomous snake that lives mostly under the sand layer (Boulenger, 1988; Al-Sadoon and Al-Otaibi, 2014). *Eryx* eyes are located on the top of the head, rather than on the sides. This position allows the snake to scan around while the body remains buried in the sand. *E. jayakari* is disseminated from its origin in Western Asia or India and invaded extremely arid deserts in the Arabian Peninsula, Iran, and Africa, where low altitude and interior regions of the Arabian Peninsula are among the suitable habitats of the species (Eskandarzadeh *et al.*, 2018 a and b). *E. jayakari* covers the sandy areas of the Arabian Peninsula including the Ha'il region of Saudi Arabia (Gasperetti, 1988; Al-Sadoon, 1989; Dekinesh, 1991; Noonan and Chippindale, 2006;

Sindaco *et al.*, 2013; Al-Sadoon and Al-Otaibi, 2014; Alshammari *et al.*, 2017; Alshammari and Busais, 2020). Males and females showed a significant difference in “total length, snout-vent length, body diameter”, and growth rate (Fitch, 1960 and 1975; Wilbur, 1975; Vogt, 1980; Al-Sadoon and Al-Otaibi, 2014).

E. jayakari is a carnivorous, nocturnal forager feeding on reptiles (mainly lizards), small rodents, and arthropods where larger animals tend to feed on larger preys (Al-Johany and Al-Sadoon, 1996; Al-Sadoon and Al-Otaibi, 2014). Unlike most boas, which deliver live young (viviparous), the Arabian Sand Boa is one of the three boa species that lay eggs (oviparous) (Staub and Emberton, 2002; Bartlett and Bartlett, 2005; Lynch and Wagner, 2010). The clutch size is usually of seven eggs, which hatch after ~66 days (Hellyer and Aspinall, 2005). The egg lacks egg-tooth; that other oviparous snakes use to tear their way out of the egg; and the shell is very thin thus *E. jayakari* can force through the eggshell with their heads (Staub and Emberton, 2002).

Mitogenome or mitochondrial DNA (MtDNA) of vertebrates is distinguished by cellular abundance, rapid evolution, intronless sequence, and highly conserved content and order. Therefore, mitogenome represented a valuable tool for molecular taxonomy, phylogeny, and evolutionary studies (Qian *et al.*, 2018). Snake mitogenome is unusual for vertebrates; in having unique duplicated control regions (CRs), higher evolutionary rate than nuclear DNA, shorter tRNA genes, and other shortened genes; that make it an ideal model for exploring potential links between mitogenomic structure, function, and evolution (Douglas and Gower, 2010; Eberhard and Wright, 2016).

Regardless of the comprehensive review of the snakes in the Middle East, few studies were conducted to investigate molecular classification, phylogeny, and evolution of Saudi snakes (Pook *et al.*, 2009; Alshammari, 2011; Alshammari *et al.*, 2015). Thus, the present study was conducted to validate the use of 12S and 16S rRNA genes for the derivation of phylogenetic relationships among *Eryx* snakes (Boidae) that might reveal other species or subspecies. The isolated sequences will be used to establish a repository for the Saudi snakes as part of the ecological footprinting project that aims to maintain sustainable environmental development.

MATERIALS AND METHODS

Snakes were collected from Ha'il region in Saudi Arabia. Snakes were identified according to the morphological characteristics (Werner *et al.*, 1999; Egan, 2007; Amr and Disi, 2011), and blood samples were collected according to the guidelines stated by the New York Academy of Sciences (New York Academy of Sciences, 1988). DNA was extracted, 12 and 16S rRNA gene fragments (>300 bp; lengths of 420 and 526 bp for 12S and 16S rRNA; respectively) were amplified by PCR using sequence-specific primers, visualized, sequenced (Pook *et al.*, 2009; Alshammari *et al.*, 2015), and sequences were submitted to GenBank (Table 1).

Datasets and sequence alignment. - Two datasets were used to investigate the phylogenetic relationships and the genetic variability of 16 and 12S rRNA) *Eryx* snake (Boidae) sequences (Johnson *et al.*, 2008; Geer *et al.*, 2010). The fasta sequence of 12S rRNA and 16S rRNA were aligned in the MultAlin webserver (Mitchell, 1993; Pervez *et al.*, 2014). The gap penalties at the opening and gap penalties at extension were set as default. The high consensus value was set as 90% and the low consensus value was set as 50%. Phylogenetic trees were constructed in Mega (Kumar *et al.*, 2008 and 2018) with the Maximum Likelihood method (Dempster *et al.*, 1977; Yuan *et al.*, 2013; Glas, 2017) and a bootstrap value of 1000. The substitution model and ML heuristic method were set according to the Tamura-Nei model (Hall, 2013) and Nearest Neighbor Interchange (NNI).

Table1. Sequences of isolates from Ha'il.

| Accession number | | Family | Species | Locality | Isolate number |
|------------------|----------|--------|----------------------|-----------|----------------|
| 16S rRNA | 12S rRNA | | | | |
| HQ267803 | HQ658419 | Boidae | <i>Eryx jayakari</i> | Hail, KSA | 7 |
| HQ267804 | HQ658444 | | | | 35 |

RESULTS

16S rRNA Data Set:

The phylogenetic tree from *Eryx jayakari* 16S isolates revealed (Fig. 1A) that the *Eryx miliaris* family has a close relationship with their species. Besides, diversification was found in the *Eryx elegans* family as they are not closely related with the *Eryx jayakari* family. Interestingly, 16S isolates from another family, Boa constrictor has found relativeness with the *Eryx miliaris* family. Also, *Sanzinia madagascariensis* and *Epicrates alvarezi* family are related to *Eryx jayakari* 16S RNA sequence. Furthermore, multiple sub-species families of Boa family-like *Boa constrictor*, *Boa constrictor imperator*, *Boa constrictor ortonii*, *Boa constrictor sabogae*, *Boa constrictor orophias* evolutionarily related to *Eryx jayakari*. Furthermore, not only *Eryx jayakari* but also other subspecies of the *Eryx* family, *Eryx tataricus* has also closeness with the Boa family. The isolates from Ha'il Province (HQ267803 and HQ267804) showed close relation to the MN338724 from Iran (Khuzestan) Province.

12S rRNA Data Set:

The 16S rRNA sequence of the *Eryx jayakari* family did not show too much diversification like the 12S rRNA sequence (Fig. 1B). This sequence has an evolutionary relationship with a wide range of snake family members besides the Boa family. Boa constrictor family close relation with *Eryx conicus* and distantly related to *Charina trivigata* and *Gloyduis strauchi* family. This cluster of snake families did not have any familiarity with *Lampropeltis getula*, *Lapemis curtus* although later group possesses close evolutionary association. Another evolutionary cluster of the family was observed with *Enhydris punctate*, *Erpeton tentaculatm*, *Hydrophis curtus*, *Erepton tentaculatm*, *Gerarda prevostiana*, *Enhydris punctate*, *Fordonia leucobaila*, and interestingly they did not have a close relationship with above-mentioned snake family. On the other hand, Corallus, Cyllindrophis, Psammophis, Chironius family closely related to *Eryx* family. A large group of Boa families with Cantoria, Epicrates, Chrysopelea, Protobothrops, Python, Xenopeltis, Porthidium family also evolutionary related to *Eryx* family. The isolates from Ha'il Province (HQ658419 and HQ658444) clustered together with no relevance to other species as there were no similar isolates reported from Khuzestan Province.

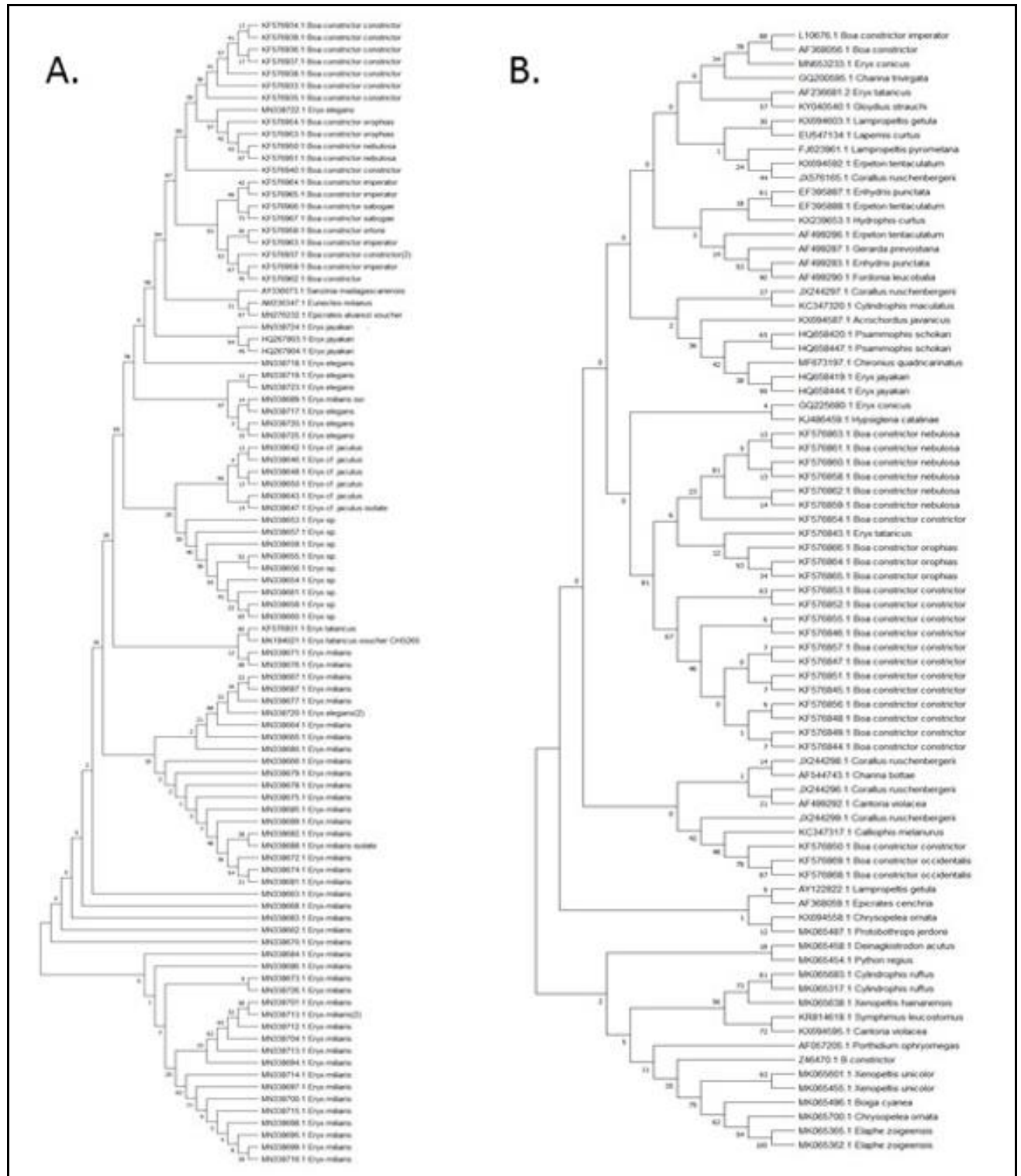


Fig. 1. Phylogenetic trees inferred from 16S (A) and 12S (B) rRNA.

DISCUSSION

The current study shows for the first time the higher diversification of 12S than the 16S rRNA gene in inferring the phylogenetic relationships for *E. jayakari*. It further supports the strong relation between *E. jayakari* and *E. miliaris* (Eskandarzadeh *et al.*, 2020). Also, it provides a correlation between *E. jayakari* isolated from Ha'il and that isolated from Iran

(Khuzestan) (based on 16S rRNA).

Eskandarzadeh *et al.*, (2020) reported that *E. jayakari* is the sister taxon to the other species-included in their study- and well distinguished from other species in the morphological cluster analysis. Also, they reported the highest niche segregation between *E. jayakari* and other species in Iran. In accordance with our findings, they reported a 0.9% overlap between *E. elegans* and *E. jayakari*.

In conclusion, *E. jayakari* in Hail might be disseminated from Iran and more 12S rRNA sequences from Iran are required for further assessments.

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