Genetic diversity among different species of the genus *Leiurus* (Scorpiones: Buthidae) in Saudi Arabia and the Middle East

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Peer review under responsibility of King Saud University.

ARTICLE INFO

Article history:
Received 22 June 2020
Revised 11 July 2020
Accepted 30 August 2020
Available online 6 September 2020

Keywords:
Scorpiones
Buthidae
Leiurus
Phylogenetic
Saudi Arabia

Abstract

The molecular phylogenetic relationship among two species of genus *Leiurus*, from Saudi Arabia with additional comparative sequence data available from Egypt, Oman and Turkey is presented. The molecular phylogeny was performed using maximum parsimony, neighbor joining and bayesian inference. Our results indicate a clear deep splitting between the Western clade, which represented by *L. quinquestriatus* sequences from Egypt and those from the Eastern clade which encompassing different *Leiurus* species from Saudi Arabia, Oman and Turkey was shown. Also, the phylogenetic relationship represents additional support for the taxonomic status of Arabian *Leiurus* species.

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1. Introduction

The genus *Leiurus* was originally introduced as a subgenus of *Androctonus* by Ehrenberg in Hempich and Ehrenberg (1829). In 1949, Vachon elevated *Leiurus* to full generic rank with a single species with two subspecies represented for many years; *L. quinquestriatus* quinquestriatus (Ehrenberg, 1828) and *L. quinquestriatus* hebraeus (Birula, 1908). As of 2009, four scorpion species of genus *Leiurus* were added (Lourenço et al., 2002, 2006; Kovarík, 2007; Yagmur et al., 2009). Recently, Lowe et al. (2014) made a revision of the genus *Leiurus*, mainly with the Arabian Peninsula populations based on morphological characteristics. Subsequently, four new species have been witnessed from Africa in the past few years (Lourenço and Rossi, 2016; Lourenço et al., 2018; Lourenço, 2019; Kovarík and Lowe, 2020). Finally, two new species were described of *Leiurus* from Kuwait and Mauritania (Lourenço, 2020a,b). With these two new species, the total number of known species of this genus is elevated to sixteen, spread across Africa and the Middle East. Of these, *L. arabis* from the central Najd plateau to the east of Saudi Arabia; *L. haenggi* from the coastal mountains of the Red Sea in Saudi Arabia, Yemen and Oman; and *L. macroctenus* from coastal plains and steppes in Oman (Lowe et al., 2014; Alqahtani et al., 2019).

Recently, several studies used the molecular phylogeny as a tool for interpreting phylogenetic relationship of many scorpions within and among populations (Ben Ali et al., 2000; Fet et al., 2003; Ben Othmen et al., 2009; Alqahtani and Badry, 2020). Moreover, the molecular analysis within scorpion species *L. abdullah-bayrami* and *L. quinquestriatus* populations were constructed based on 16S rRNA sequence data (Ayhan et al., 2018; Sarhan et al., 2020). In order to the recent taxonomic changes in the genus, it is necessary to assess the taxonomic status of two *Leiurus* species in Saudi Arabia especially *L. arabis* and *L. haenggi* with additional comparative sequence data available from Egypt, Oman and Turkey.

2. Material and methods

2.1. Scorpion collection

Four adult specimens of two different species belong genus *Leiurus* were collected from two different localities in Saudi Arabia during the period from September 2017 to July 2019 (Fig. 1, Table 1). Scorpions were collected during daytime by randomly searching the scorpions under rocks and any other shelters (rock rolling).
In the night period, the UV lights were used (Stahnke, 1972). The collected scorpions were preserved in 95% ethanol for DNA isolation as described by Prendini et al. (2003).

### 2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from leg or pedipalp muscle tissue dissected from fresh or preserved (95% ethanol) scorpion specimens using DNeasy extraction kit (Qiagen) and following manufacturer’s instructions. Fragments of the mitochondrial 16S rRNA gene were amplified by polymerase chain reaction (PCR) using the 16S rRNA scorpion specific primers as described by Gantenbein et al. (1999). Amplified products were purified and sequenced on an ABI 3500 automated sequencer (Applied Biosystems Inc., USA).

### 2.3. Phylogenetic analysis

Sequence were checked and analyzed using BioEdit program\(^5\), version 7.2.5 (Hall, 1999). Sequence data were downloaded as ingroup from GenBank for *L. quinquestriatus* (Ehrenberg, 1880) from Egypt (MT111845, MT111856, MT111862, MT111864, MT111865, MT111866), *L. macroctenus* (AY226174.2, DNA record of Fet et al., 2003”) *L. quinquestriatus* used in GenBank was corrected in the synonymy listing of *L. macroctenus* in Lowe et al., 2014) and *L. abdullahbayrami* Turkey (KU318423.1) (Fig. 1, Table 1). Also, *Androctonus crassicauda* downloaded as out group (AJ277598.1). The sequence data was edited using Mega 6 (Tamura and Nei, 1993) and subsequently aligned using the default settings of ClustalW. Nucleotide composition from the ingroup sequences only was calculated. The genetic distances (p-distance)
were performed to estimate the sequence divergence for the entire data set, using Mega 6 (Tamura and Nei, 1993). Also, The phylogenetic analyses; maximum-parsimony (MP), neighbor-joining (NJ) and Bayesian inference analyses (BI) of 16S data set (n = 13) were performed as proposed by Alqahtani and Badry (2020).

3. Results

3.1. Genetic data

The 16S rRNA data set consisted of 307 aligned nucleotides were analyzed. In total 110 (35.83%) bases were constant, 120 (39.08%) bases were variable and 77 (25.08%) were parsimony-informative. Nucleotide composition was highly biased towards A–T. The mean values of T, C, A and G within the sequence data were 35.4, 10.8, 38.2 and 15.5%, respectively. Within the 307 bp, 190 polymorphic segregating sites were detected. The sequence divergences among Leiurus lineages ranged from 0.00 to 0.17, with an average of 0.13.

3.2. Phylogenetic analyses

The topology resulting from MP is presented in Fig. 2, was identical to that of the NJ tree (Fig. 3). Also, the general topology of the maximum-parsimony tree was a little different from those obtained by BI analyses (Fig. 4). In all phylogenetic analyses (Figs. 2, 3 and 4), two major highly supported clades are represented by all Leiurus taxa. The first clade (the Eastern clade) includes all Arabian and Middle East forms. This clade is further split into two subclades, includes L. abdullahbayrami from Turkey and those of the Arabian part (Saudi Arabia and Oman), the later further divided into two subclades includes; L. arabicus from Riyadh as a basal clade to L. haenggii from southwestern Saudi Arabia and L. macroctenus from Oman which grouped together as a sister group. While the second clade includes all Egyptian forms. The population of the Sinai Peninsula (Asian part of Egypt) and those of the African part of the country.

3.3. Genetic distance

The genetic distances from the entire data set was calculated and given in Table 2. The divergence between the Arabian species L. arabicus and L. haenggii had the lowest divergence (11%). Similarly, the distance between the Egyptian Leiurus was 2.0%. The highest divergence was observed between L. haenggii and other Leiurus species, ranged from 12%, 19%.

Fig. 2. MP phylogenetic tree of genus Leiurus mtDNA sequences fragment of the 16S rRNA gene from Saudi Arabia, Egypt including Sinai, Oman and Turkey. Number above branches indicate bootstrap values calculated with 1000 replicates.
Fig. 3. NJ phylogenetic tree of genus *Leiurus* mtDNA sequences fragment of the 16S rRNA gene from Saudi Arabia, Egypt including Sinai, Oman and Turkey. Number above branches indicate distance values.

Fig. 4. BI tree phylogenetic tree of genus *Leiurus* mtDNA sequences fragment of the 16S rRNA gene from Saudi Arabia, Egypt including Sinai, Oman and Turkey. (50% majority rule consensus tree). Numbers above nodes indicate the posterior probabilities.
4. Discussion

The phylogenetic analysis based on 16S rRNA data (Figs. 1, 2, and 3) reveals high diversity of species belonging to the genus Leiurus, of which cover some species belong Africa and Asia. Our phylogenetic analyses presented here indicate a clear deep splitting between the Western clade, which represented by L. quinquestriatus sequences from Egypt and those from those of the Eastern clade which encompassing different Leiurus species from Saudi Arabia, Oman and Turkey. Furthermore, the genetic divergence supported the distinction of the L. quinquestriatus from Egypt including Sinai from the Eastern clade species (12–19%) (Table 2). Levy and Amitai (2000) noted that among the Egyptian and Arabian Buthacus species and suggested that it might be due to historical speciation. In addition, many authors refer to the vicariance which associated with the Red Sea formation, drove simultaneous difference between Arabian and African lineages in several faunal group (Sanmartín, 2003; Amer and Kumazawa, 2005; Derricourt, 2005; Lourenço and Rossi, 2016; Saleh et al., 2018).

Within the Eastern clade, L. abdullahbayrami was basal to the L. arabicus and L. haenggii from Saudi Arabia. While L. macroctenus from Oman nested as a sister group to L. haenggii. L. abdullahbayrami, can be distinguished from the above species as proposed by Lowe et al. (2014) by several morphological and morphometric characters. This species is distributed belong semi-arid habitats in Turkey and northern Syria (Khail and Yağmur, 2010; Lowe et al., 2014). The divergence distance between L. abdullahbayrami and the others of genus Leiurus ranged from 12 to 14% (Table 2). Lourenço (2020a), described a new species of Leiurus from Kuwait, and noted that it might correspond to vicariant elements during the late Miocene, of which it might support the diversification among the Arabian species. Our results suggested that the same vicariant mechanisms, also acted to split off the Arabian clade from L. abdullahbayrami.

With regards to the relation between L. haenggii and L. arabicus, both have “medial intercarinal surfaces of tergites II–III smooth or sparsely shagreened”, without dense granulation. However, L. haenggii can distinguished from L. arabicus by having “more robust leg, pedipalp and metasomal segments; and smooth or weakly granulated median carinae on sternites III–V of females” (Lowe et al., 2014). Also, the L. macroctenus, can be distinguished by large pectine teeth from the most other species of genus Leiurus (Lowe et al., 2014, Kovářík and Lowe, 2020). The divergence between the Arabian species ranged from 11 to 12% (Table 2). Lowe et al. (2014) refer that L. arabicus is a more psammophilous ecomorphotype and it’s consistenced from the L. haenggii lineage. On contrary, our results showed that L. haenggii from southwestern Saudi Arabia and L. macroctenus from Oman grouped together in all phylogenetic analyses as a sister clade and it were predicted to have diverged from L. arabicus. It could be explained by the vicariant event caused by progressive aridification during the Late Pleistocene and the early Holocene, which served the diversification belong the Arabian species (Bray and Stokes, 2004; Lowe et al., 2014). Also, The effects of paleoclimatic conditions had a major impact on the distribution and differentiation of various species of Buthus, Mesobuthus and Tityus have been previously reported by several studies (Sousa et al., 2010, 2012; Fet et al., 2018; Lourenço, 2016, 2020c).

In conclusion, a clear deep splitting between L. quinquestriatus sequences from Egypt and those from Arabia and Turkey. Also, the phylogenetic relationship represents additional support for the taxonomic status of Arabian Leiurus species. Furthermore, additional detailed morphological revision utilizing type specimens of “L. jordanensis and L. brachycentrus” from the northern and southwestern parts of Saudi Arabia, is required to clarify their relationships with other species of this genus.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors express their gratitude to Prof. Dr. Sayed Amer, Department of Zoology, Faculty of Science, Cairo University, Egypt, for reviewing this article and for providing invaluable suggestions. Also, we would like to thank Dr. Moustafa Sarhan, Professor of Molecular Biology, Department of Zoology, Faculty of Science, Al-Azhar University (Asyut Branch) for his help in the molecular study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Table 2

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---------|---|---|---|---|---|---|---|---|---|----|----|----|----|
| 1. L. arabicus, KSA | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2. La2, L. arabicus, KSA | 0.00 | – | – | – | – | – | – | – | – | – | – | – | – |
| 3. Lh1, L. haenggii, KSA | 0.11 | 0.11 | – | – | – | – | – | – | – | – | – | – | – |
| 4. LH2, L. haenggii, KSA | 0.11 | 0.11 | 0.00 | – | – | – | – | – | – | – | – | – | – |
| 5. AQ220174.2 Leiurus macroctenus, Oman | 0.11 | 0.12 | 0.12 | 0.12 | – | – | – | – | – | – | – | – | – |
| 6. XU318423.1 L. abdullahbayrami, Turkey | 0.14 | 0.14 | 0.12 | 0.12 | 0.13 | – | – | – | – | – | – | – | – |
| 7. MIT11814.1 Abu Mingar Oasis, Egypt | 0.14 | 0.14 | 0.19 | 0.19 | –0.17 | 0.17 | – | – | – | – | – | – | – |
| 8. MIT11855.1 Fijiom, Egypt | 0.15 | 0.15 | 0.18 | 0.18 | 0.17 | 0.14 | 0.02 | – | – | – | – | – | – |
| 9. MIT11862.1 Wadi Khodae, Eastern desert, Egypt | 0.14 | 0.14 | 0.19 | 0.19 | 0.17 | 0.14 | 0.00 | 0.02 | – | – | – | – | – |
| 10. MIT11864.1 Siwa, Egypt | 0.15 | 0.15 | 0.18 | 0.18 | 0.17 | 0.14 | 0.02 | 0.00 | 0.02 | – | – | – | – |
| 11. MIT11865.1 Wadi Shafallah, South Saini, Egypt | 0.14 | 0.14 | 0.19 | 0.19 | 0.17 | 0.13 | 0.10 | 0.09 | 0.10 | 0.14 | – | – | – |
| 12. MIT11866.1 Wadi Arada, South Saini, Egypt | 0.14 | 0.14 | 0.19 | 0.19 | 0.17 | 0.13 | 0.10 | 0.09 | 0.10 | 0.08 | 0.08 | – | – |
| 13. AJ277598.1 Androctonus crassicauda | 0.54 | 0.54 | 0.56 | 0.56 | 0.56 | 0.56 | 0.53 | 0.53 | 0.52 | 0.53 | 0.53 | 0.53 | 0.53 |
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