

An Integrative Study on *Mesobuthus rakhshanii* (Scorpiones: Buthidae)

Hossein Barahoei

h.barahoei@uoaz.ac.ir

Research Institute of Zabol <https://orcid.org/0000-0001-5195-7679>

Seyed Massoud Madjdzadeh

Shahid Bahonar University of Kerman

Asma Moeinadini

Shahid Bahonar University of Kerman

Research Article

Keywords: Biology, COI, litter size, *Mesobuthus*, phylogeny, species distribution modeling, Taxonomy

Posted Date: October 14th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7697350/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at *Biologia* on April 13th, 2026. See the published version at <https://doi.org/10.1007/s11756-026-02170-z>.

Abstract

Mesobuthus Vachon, 1950, with 30 described species is widely distributed from the Eastern Mediterranean area to the Eastern Palaearctic. *Mesobuthus rakhshanii* Barahoei, 2022 is described from the north of Sistan & Baluchestan and southern parts of South Khorasan Provinces. Totally, 213 specimens of *M. rakhshanii* were collected from the various habitats of these regions during 2021 to 2023. Specimens were studied from morphological, molecular, biological, and ecological point of view. Bayesian inference (BI) analysis based on the COI gene was conducted. Thirty pairs of alive specimens were used for biological study. The maximum entropy model (MaxEnt) was utilized to evaluate the contribution rates of bio-climatic factors and to project suitable habitats for of *M. rakhshanii*. Unlike other *Mesobuthus* species, the central median and posterior median carinae of the carapace of *M. rakhshanii* are not connected. According to the BI, *M. rakhshanii* forms a sister group with *M. navidpouri*, indicating that they are the two youngest species within the genus. *Mesobuthus mirshamsii* was sequenced for the first time and the validity of this species was confirmed. The observed litter size for *M. rakhshanii* ranged from 22 to 36. Our findings indicate that annual precipitation and the mean temperature of the coldest quarter are the most influential variables affecting the potential distribution of this species. The estimated optimal distribution area for *M. rakhshanii* is approximately 943,578 km², representing about 57.25% of Iran's land area. Among the suitable areas, 10.23%, 41.5%, and 5.52% were classified as low, moderate, and high suitability zones, respectively.

Introduction

Members of *Mesobuthus* are non-burrowing scorpions that rest for days under stones and bark of tree and in other sheltered places (Firoozfar et al. 2019). Due to the high adaptability, they inhabit a wide range of environments with various climatic conditions. They frequently enter human dwellings and can be found under carpets and household items where moisture levels are higher.

Mesobuthus Vachon, 1950 belongs to the family Buthidae and currently includes 30 recognized species (Rein, 2024). These species are distributed across a vast range extending from Turkey to China (Kovařík et al., 2022).

Recently, seven Iranian *Mesobuthus* subspecies were elevated to species rank, and seven new species were described by Kovařík et al. (2022) for the Iranian scorpion fauna. A survey in the Sistan region (northern Sistan and Baluchestan Province) resulted in the description of *Mesobuthus rakhshanii* Barahoei, 2022, along with the documentation of seven species from this region (Barahoei, 2022). The original description was published in

Persian; thus, a re-description is provided here for comparison with closely related species.

Yagmur et al. (2024) not only provided new distribution records for *M. rakhshanii* but also illustrated its diagnostic features and compared it with two other species, *Mesobuthus kirmanensis* (Birula, 1990) and *M. navidpourii* Kovařík et al. (2022), both reported from central part of Sistan and Baluchestan Province.

The litter size in *Mesobuthus* species ranges approximately from 12 to 40 offspring (Lourenço, 2007; Dehghani et al., 2018). Due to their medium size, members of this genus primarily prey on insects, thereby playing a significant role in natural pest control. They are also of interest for biological control programs. Additionally, their venom contains bioactive compounds that may be useful in developing pesticides against major agricultural pests.

The main objective of the present study is to explore the less-known aspects of *M. rakhshanii* by applying an integrative approach combining taxonomy, molecular phylogeny, ecological niche modeling, and biological observations.

Material and Methods

Sampling

A total of 213 specimens of *M. rakhshanii* (125♂, 73♀, 15 subadults) were collected from the southern parts of south Khorasan and the northern parts of Sistan & Baluchestan provinces between 2021 to 2023. Specimens were collected at night using ultraviolet light and preserved in 80% ethanol for morphological, molecular and ecological studies.

Morphology

Specimens from the northern Sistan & Baluchestan and southern South Khorasan regions were morphologically examined based on diagnostic characters used in species identification (Barahoei 2022). Nomenclature and measurements followed Stahnke (1971) and Kovařík (2009). Morphological traits were studied using an Optika stereomicroscope (Italy) and identification was carried out using valid taxonomic identification keys (Barahoei et al. 2020; Barahoei 2022; Kovařík et al. 2022). Measurements were taken with a calibrated ocular micrometer applied to stereomicroscope.

All specimens were compared with descriptions of previously identified species from neighboring regions. Intraspecific variation, sexual dimorphism, and differences between juveniles and adults were considered in the differentiation and re-description of *M. rakhshanii*.

Specimens were photographed using a Canon® EOS 800D digital camera (Japan), and the figures were edited using Adobe Lightroom v6.0 and Adobe Photoshop 2025.

Molecular study

The manus of the pedipalp was removed and placed in tubes containing 99.8% ethanol. DNA was extracted from fixed tissue using the FavorPrep™ Tissue Genomic DNA Extraction Mini Kit (Pingtung, Taiwan). Amplification of the mitochondrial cytochrome c oxidase I (COI) gene was performed using the LCO1490 and HCO2198 primers (Folmer et al., 1994). The PCR thermal cycle was as follows: initial denaturation at 94°C for 90 seconds, followed by 35 cycles of 94°C for 45 seconds, 52°C for 60 seconds, and 72°C for 60 seconds, with a final extension at 72°C for 5 minutes.

PCR products were purified and sequenced by Niagene Noor Company (Tehran, Iran). Sequences were edited using BioEdit v7.1.9 (Hall, 1999), and genetic distances within and between species were calculated using MEGA v7.0 (Kumar et al., 2016). A Bayesian inference (BI) was constructed using MrBayes v3.2.7a (Ronquist & Huelsenbeck, 2003) via the CIPRES Science Gateway (<http://www.phylo.org>; Miller et al., 2010), using 50 million generations. The resulting tree was visualized and edited using FigTree v1.4.4 and Adobe Photoshop 2025.

Six newly generated COI sequences were deposited in GenBank under accession numbers PP392847–PP392850 and PP392795–PP392796 (Table 1).

Biological observations

Sixty live specimens of *Mesobuthus rakhshanii* (30 females and 30 males) collected from the Sistan region were used to study the species' reproductive biology and litter size. The specimens were housed in 30×20×20 cm plastic containers filled with soil from the collection site and included rocks and clods to mimic their natural environment. Each male-female pair was placed in a container for mating. Gravid females were then isolated to monitor pregnancy.

The number of juveniles per female was recorded, along with the timing of initial molting and juvenile dispersal. The timing of subsequent molts was also documented. Some females were separated from their juveniles at different stages to assess maternal behaviors. In addition, a few females were kept isolated for a second year to assess the possibility of parthenogenetic reproduction.

Specimens that died during the observation period were preserved in 96% ethanol for further morphological and molecular analyses.

Species Distribution Modelling

To illustrate the suitable habitats and distribution modelling of *Mesobuthus rakhshanii*, 23 localities were used (Figure 1). We retrieved localities' information from our field observations. The 19 bioclimatic variables (1970-2000) and altitude layer used in this work were downloaded from Worldclim (www.worldclim.org) with a spatial resolution of 30 arc second (~1 km) (Fick and Hijmans 2017). The slope layer was created using ArcGIS 10.3.1 from the original altitude layer. The correlation analysis was assessed by OpenModeller version 1.0.7 (Muñoz et al. 2011) to avoid highly correlated and redundant variables. A Pearson correlation coefficient higher than 0.75 shows highly correlated variables and these were eliminated from the main analysis. Finally, five bioclimatic variables including Annual precipitation (bio12), mean temperature of coldest quarter (bio11), mean diurnal range (bio2), precipitation of driest quarter (bio17), precipitation seasonality (bio15) and slope layer as a topology predictor, were applied to construct the current potential distribution of the species. MaxEnt is an ecological niche model based on the theory of maximum entropy and constructed on the Java platform (Phillips et al. 2006). MaxEnt is known to simulate species distribution using the minimum number of records and generate accurate species distribution models (van Proosdij et al. 2016). We utilized the MaxEnt model version 3.4.4 to estimate the likelihood of species presence by maximizing entropy based on presence records and randomly generated background points. A final distribution model was derived from the average logistic outputs of 15 replicated runs with cross validation, a maximum of 10,000 background points, and a maximum of 1000 iterations. These outputs yield the probability of presence, ranging from 0 (unlikely) to 1 (highly likely). Receiver Operating Characteristic (ROC) curves and Area under the ROC Curve (AUC) values were used to test the accuracy of the model output. The ROC curve is an acceptance curve with the horizontal coordinate indicating the false positive rate (1-specificity) and the vertical coordinate indicating the true positive rate (1-omission rate) (Fan et al. 2006). The AUC values not affected by thresholds are more objective than others for model assessment. An AUC value closer to 1 indicates that the model result is better. The evaluation criteria of model simulation accuracy were as follows: poor ($AUC \leq 0.50$), available ($0.5 < AUC \leq 0.80$) and excellent ($0.80 < AUC \leq 1.00$) (Swets 1988).

Specimen deposition

The holotype is deposited at the Research Institute of Zabol, Iran (RIZ). Additional specimens are deposited at the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM), the Zoological Museum, Department of Biology, Shiraz University (ZM-CBSU), and the Zoological Museum of Shahid Bahonar University of Kerman (ZMBK).

Holotype is deposited at Research Institute of Zabol, Iran (RIZ). Some specimens are deposited in the Zoological Museum of Ferdowsi University of Mashhad, Iran (ZMFUM), Zoological Museum, Collection of Biology Department, Shiraz University (ZM-CBSU) and Zoological Museum of Shahid Bahonar University of Kerman, Iran (ZMBK).

Results

Systematics

Family Buthidae C. L. Koch, 1837

Genus *Mesobuthus* Vachon, 1950

Mesobuthus rakhshanii Barahoei, 2022

Synonyms and references

Buthus (Buthus) macmahoni: Birula, 1917a: 214.

Buthus (Buthus) zarudnyi macmahoni: Birula, 1917a: 240.

Mesobuthus macmahoni: Vachon, 1950: 153; Vachon, 1952: 325; Vachon, 1958: 146–148, figs. 30–31; Fet and Lowe, 2000: 177 (complete references list until 1998); Mirshamsi *et al.*, 2011b: 20 (in part); Navidpour *et al.*, 2011: 13 (in part); Kovařík, 2019: 17; Barahoei *et al.*, 2020: 397 (in part).

Material examined

(125♂, 73♀, 15 subadults)

HOLOTYPE, ♂, Iran, Sistan & Baluchestan Province, Hamun County, Lootak district, Rahmatabad village (30°45'N, 61°21'E, 481 m a.s.l), 10 May 2021, H. Barahoei leg. (RIZ-Mes-180).

Other material examined

Iran, Sistan & Baluchestan Province: 1♂, Hamun county, Akhund-e Gholami, 30°50'35.89"N, 61°20'19.82"E, 09 July 2022, M.S. Barahoei leg. – 1♂, Cheleng village, 30°53'53.01"N, 61°23'55.07"E, 23 August 2023, Hossini Tabatabaei leg. – 2♀, Dolat Abad, 30°50'17.83"N, 61°23'02.60"E, 15 May 2022, H. Alizaei leg.– 4♂, 1♂, Kusheh Olya, 30°57'34.51"N, 61°29'28.00"E, 29 March 2022; 1♂, 01 July 2022, K. Barahoei leg.– 4♂, 5♀, 3 subadults, Lootak, Rahmatabad village, 30°45'33.16"N, 61°21'09.95"E, 10 May 2021; 5♂, 1♂, 2 subadults, 30 March 2022; 2♀, 08 April 2022, M. Barahoei leg.– 2♂, 1♂, 1 subadult, Peere Sabz village, 30°51'21.35"N, 61°20'06.20"E, 10 May 2021; 4♂, 1♂, 23 July 2021; 2♀, 2♂, 16 August 2021; 1♂, 17 March 2022, H. Barahoei leg.; 8♂, 2 subadults, 31 March 2022; 2♂, 1♂, 20 May 2022, Z. Barahoei leg.; 5♂, 15♀, 25 June 2022; 1♂, 07 October 2022; 2♂, 1♂, 14 June 2023, H. Barahoei leg.– 3♂, 3♀, Nimruz county, Sefidabeh, 30°58'09.71"N, 60°31'47.11"E, 17 June 2022, H. Barahoei leg. – 2♂, 3♀, 1 subadult, Mirjaveh county, Mirjaveh, 29°00'30.46"N, 61°27'03.25"E, 11 July 2022, F. Vahidinia leg.– 1♂, Zabol

county, Hasan Abad, 30°00'13.28"N, 61°30'05.42"E, 13 November 2021, Z. Barahoei leg.; 1♂, 17 February 2022; 2 subadults, 30 March 2022, M.M. Barahoei leg. ; 1♂, 30 October 2022, H. Barahoei leg.– 11♂, 6♀, 3 subadults, (Figure 2) Hirmand county, Jahanabad-e sofla, 30°01'58.21"N, 61°46'38.53"E, 30 October 2023, F. Vahidinia leg.– 4♂, 1♀, Tappeh Daz village, 31°00'02.49"N, 61°35'46.09"E, 06 June 2022 – 1♂, Khomeini St., 31°02'09.23"N, 61°29'26.23"E, 10 April 2023, H. Barahoei leg.; 1♂, 11 November 2023, Lotfi leg.– 2♂, 3♀, Tappeh Daz village, 31°00'02.49"N, 61°35'46.09"E, 07 July 2022, P. Hormozi leg.– 1♂, Zahak county, Chah Nimeh, 30°50'22.34"N, 61°43'01.45"E, 29 September 2022, H. Barahoei leg., 2♂, 16 December 2023, M. Poudineh leg.– 1♂, Zahedan county, Manzel Aab village, 29°21'52.93"N, 60°45'25.98"E, 24 July 2022; 1♂, 12 June 2023; 10♂, 3♀, 2 subadults, 28 October 2023, F. Vahidinia leg.– 1♂, Hormak, 29°58'57.07"N, 60°50'39.97"E, 16 November 2023, F. Vahidinia leg.; **South Khorasan Province:** 3♂, Darmian county, Khonik, 31°28'01.60"N, 60°05'51.26"E, 30 June 2022– 4♂, 3♀, Nehbandan, 31°31'16.79"N, 60°02'03.22"E, 25 June 2022; 15♂, 3♀, 3 July 2022; 6♂, 5♀, 25 July 2022, Monday; 3♂, 3♀, 23 June 2022; 4♂, 2♀, Khansharaf, 31°33'30.81"N, 60°06'03.97"E, 17 June 2022 – 3♂, 1♀, Shosf, 31°48'14.93"N, 60°01'09.18"E, 10 June 2022– 5♂, 3♀, Tabaseyn-e Olya, 31°26'57.87"N, 60°40'00.97"E, 15 July 2022, N. Hashemzahi leg.

Geographical distribution

This species is endemic to Iran, collected from the north of Sistan & Baluchestan and south of South Khorasan provinces.

Etymology

The name of this species is in honor of the author's friend and colleague, Prof. Ehsan Rakhshani (Professor of the Department of Plant Protection, Faculty of Agriculture, University of Zabol, Zabol, Iran).

Re-description

Female HOLOTYPE (Figures 3-5)

Size: The total length of the body is 61 mm.

Coloration: overall color yellow, middle and lateral eyes, chelicer teeth and tip of telson are black, carinae of first to sixth tergites and ventral carinae of fourth and fifth segment of metasoma slightly darker than body color (Figures 3).

Chelicer: has two lateral teeth on the ventral surface of the fixed finger, the movable finger has inner and outer teeth, approximately same size.

Carapace: trapezoidal, tip width greater than length, surface with large granules with low density and some areas without granules, carinae fully developed but central median and posterior median carinae not connected. All carinae granulated, anterior median and dorsal median furrows shallow, dorsal lateral groove wide, deeper, and curved. In the lateral view, the front margin of the carapace is straight and has eight very long hairs. Median eyes are located in the front part of prosoma and have four pairs of lateral eyes (Figure 4A).

Legs: Tarsomeres with long, dense hairs, hair comb with seven to nine long, almost dense hairs on base of tarsus of legs.

Pedipalp: Segments are almost short. Femur (Figure 4C) 2.9 times as long as wide, intercarinal surface with granules on dorsal surface, with five distinct carinae, prodorsal and retrodorsal carinae with dense granules, retroventral carina faded, proventral carina with dense granules, promedian carinae with large separate conical granules. Patella (Figure 4C) 2.5 as long as wide, the intercarinal surface has very scattered granules on the dorsal surface, with eight carinae, the prodorsal carina with short granules, proventral carina almost with long separate conical granules, the dorsal median carinae, retrodorsal, ventral median, retroventral, and retromedian obliterated (with smooth ridge). Chela (Figure 4C) smooth, carina obliterated or smooth, manus wider than patella, length of movable finger about 1.5 times as length of manus, movable finger with 12 rows of oblique teeth, presence of internal and external teeth, with five terminal granules, fixed finger with 10 rows of oblique teeth, with internal and external teeth.

Trichobothriotaxy: Type *Aβ*, with 39 trichobothria on each pedipalp. Femur with 11 trichobothria (5 dorsal, *d2* reduced, 4 internal lateral, 2 external lateral). Patella with 13 trichobothria (5 dorsal, 1 median lateral, 7 external lateral). Chela with 15 trichobothria (8 on manus, 7 on fixed finger). Trichobothria *esb*, *Esb* and *Eb3* slightly reduced. Trichobothria *et* located close to the middle part of tooth row 5 and Trichobothria *est* close to the middle part of tooth row 7.

Mesosoma: All tergites granulated, tergites I-VI with three carinae, seventh tergite with five carinae, the median carina is present only at the beginning of the segment and granulated. Sternites III-VI without carina, sternite VII with four nearly developed carinae, sublateral carina present only in middle half of segment, median carina absent in first two thirds of segment. Pectinal teeth number 20 on the right side and 21 on the left side (Figure 4B), tip of pecten reaches to beginning of the fourth sternite and junction of the trochanter with the coxa in the fourth leg. Pecten with three marginal lamellae and eight middle lamellae, lamellae with many black hairs, each fulcrum with two to five black hairs. Sternum of semi-pentagonal type *I*, longer than wide, with a deep median depression. Genital operculum completely divided longitudinally, with short and smooth spines.

Metasoma (Figure 5): Segment I with 10 carinae, dorsal lateral and lateral dorsal carinae with longer granules at the end, median lateral and ventral lateral carinae with distinct granules, median ventral carinae with longer serrations at the end. Segments II-III with eight carinae, dorsal lateral and lateral dorsal carinae with longer granules in the distal part, median lateral carina obliterated, with eight and four large granules at the end of the segment II and III respectively, ventral lateral carina granulated, median ventral carina with longer tooth at the end. Segment IV with eight carinae, dorsal lateral carina with longer granules in terminal part, median lateral carina absent, dorsal and ventral lateral carina granulated, median ventral carina with smooth edges. Segment V with five carinae, the dorsal lateral carina with granule at the beginning and a smooth edge in the terminal part, absence of median and dorsal lateral carinae, ventral lateral carina is toothed, with long conical granule in the terminal part, median ventral carina with serrated granules, presence of eight long hairs on the lateral surface of segment (Figure 5).

All segments sparsely hirsute, segment I wider than or as wide as long (Figure 5A), other segments longer than wide. Anal arch with three toothed lobes in lateral part, in females often first lobe with a groove (Figure 5C). Ventral intercarinal surface of segment V with large granules sparsely (Figure 5B).

Telson: Elongate (Figure 5C), with 12 almost long and erect hairs, without subcaval teeth, ventral surface with ridges, dorsal surface smooth. Height/length ratio = 0.35 and width/length ratio = 0.38.

Male description (Figure 6)

The male is similar to the female with the following differences: Total length of body 52 mm, femur 3 times as long as wide; Patella 2.5 times as long as wide, movable finger 1.4 times as long as manus, movable finger with 11 rows of oblique teeth, fixed finger with 10 rows of oblique teeth; Pectinal teeth number 27 on the right and 29 on the left pectin, the tip of the pectin reaches the first half of sternite VII and after the junction of the trochanter with the coxa in fourth leg (Figure 6B); Pectin has eight middle lamellae, each fulcrum with two to five black hairs; Median lateral carina of metasomal segments II and III faded and with eight and four large granules at the end of the segment, respectively, the anal arch has three toothed lobes in the lateral part; Telson has 10 long and straight hairs; Telson height/length ratio = 0.33 and width/length ratio = 0.35.

Affinities

Members of *Mesobuthus rakhshanii* Barahoei, 2022 are medium-sized scorpions. The body length of adults is 38 to 52 mm in males and 51 to 61 mm in females. The number of pectin teeth is 25 to 29 in males and 18 to 21 in females. Pedipalp movable finger has 11-12 rows

of oblique teeth and five terminal teeth. The lateral anal arch is divided into three parts. In females, the first lobe has a short groove.

The members of this species are separated from many species of this genus by having a ratio of length to height of the third metasoma between 1.50 and 1.90. In this species, the central median and posterior median carinae of the carapace are not connected and do not form a lyre. With this trait, it is distinguished from other species except for *M. macmahoni* (Pocock, 1900).

Members of the *M. macmahoni* with a total size of 38 (male) to 55 (female) mm are distributed in the low altitude areas of southern Pakistan. The number of pectin teeth is 23 to 26 in males and 19 to 23 in females. In addition, the general color of the body is darker than *M. rakhshanii*.

Phylogenetic Study

Eight sequences of *Mesobuthus rakhshanii* Barahoei, 2022, collected from Sistan & Baluchestan and South Khorasan provinces, two sequences of *Mesobuthus mirshamsii* Kovařík et al., 2022, collected from Hormozgan province, and 13 sequences obtained from NCBI (National Center for Biotechnology Information) were analyzed (Table 1). The aligned COI sequences were 648 base pairs in length, comprising 494 (76.23%) conserved sites, 154 (23.77%) variable sites, and 111 (17.13%) parsimony-informative sites.

Based on Bayesian inference, *M. rakhshanii* was placed as the sister group to *M. navidpourii*, representing two of the newest species at the terminal branches of the phylogenetic tree (Figure 7). *M. mirshamsii* was positioned as the sister group to *M. kirmanensis*. Genetic distance analyses further supported the validity of *M. rakhshanii* and *M. mirshamsii* as distinct species (Table 2).

Biological Study

Sixty pairs of *M. rakhshanii* specimens collected from the Sistan region were used to investigate the species' biology and litter size. Each adult male and female was placed together in a container for mating. Gravid females, except for three individuals, were transferred to separate containers to facilitate pregnancy.

Each female gave birth to between 22 and 36 juveniles (Figure 8), with older females generally producing larger litters. The offspring remained mounted on their mother's back and separated after their first molt, occurring within 7–9 days (Appendix 1). Three mothers did not separate from their nymphs. During the initial days, mothers occasionally consumed some of their young. Even after the nymphs became independent and were fed mealworms, instances of cannibalism were still observed.

Molting times of the juveniles were recorded (Appendix 1). Molting occurred at shorter intervals during early development and lengthened as the scorpions aged (Figure 9). Adequate and timely feeding appeared to influence the molting schedule positively. Occasionally, exuviae were crushed by other specimens, making precise molting times and counts difficult to determine. Specimens that died during the study were preserved in 80% ethanol for subsequent morphological and molecular analyses

Species distribution modelling

The generated models based on 23 presence records of the East of Iran performed well and presented good models with a MaxEnt-generated AUC evaluation. The potential distribution models of *M. rakhshanii* showed good AUC test value, with 0.94 ± 0.01 and 0.92 ± 0.01 for training and test data, respectively (Figure 10A). Furthermore, the binomial omission test with the lowest presence threshold was statistically significant and the test omission rates did not exceed 5%.

According to the Jackknife analysis of regularized training gain, when used in isolation, Annual precipitation (bio12) was the strongest predictor with an average contribution of 72.8%. The next important variables were mean temperature of coldest quarter (bio11) with an average contribution of 13.5% and mean diurnal range (bio2) with an average contribution of 7.5%. Together, these three environmental factors contribute to a total of 93.8%, highlighting their significance in the distribution of *M. rakhshanii*. Following these three variables, the species was also influenced by precipitation of driest quarter (bio17) with an average contribution of 3.1%; precipitation seasonality (bio15) with an average contribution of 2.9% and slope with an average contribution of 0.3% (Figure 10B).

The MaxEnt model was employed to forecast the suitable regions for *M. rakhshanii* in the current climate, as depicted in Figure 11, the high suitable area spans 90,841 km², constituting 5.52% of Iran's total land area. The moderate suitable area includes 684,087 km², equivalent to 41.51% of Iran's land area. The low suitable area covers 168,650 km², accounting for 10.23% of Iran's land area. These regions include Yazd province, east of Isfahan province, South Khorasan province, north to center of Sistan & Baluchestan province and north of Kerman province. The potential distribution areas of *M. rakhshanii* included most of the lowland to low-altitude mountainous areas, but not in high-altitude ones. The model predicts highly suitable areas along Bafq, Mehriz, Meybod, Zabol counties and a small part of the border between Iran and Pakistan.

Discussion

The studied population of *Mesobuthus*, collected from Sistan & Baluchestan province in eastern Iran, exhibited morphological differences compared to other previously described

species from neighboring regions (as detailed in the Affinities section). *Mesobuthus macmahoni*, distributed in southern Pakistan, is considered the most closely related species to *M. rakhshanii*. This study expanded the known distribution range of *M. rakhshanii*. With the description of *M. rakhshanii*, the number of species of this genus reported from Iran reached 16. However, further investigations are still required in some eastern regions of Iran.

Mesobuthus scorpions are generally regarded as non-burrowing species. Although they may take shelter in soil crevices, cracks, or beneath rocks, they do not typically construct deep burrows like some other scorpion species. These scorpions are commonly found in both urban and rural environments, including deserts and mountainous areas.

Populations of *M. rakhshanii* inhabiting clay soils in low-altitude areas (e.g., the Sistan Plain) display a light brown coloration, whereas populations dwelling in sandy soils on mountain slopes (e.g., Nehbandan and Sefidabeh) tend to have a darker coloration. In these specimens, the carinae of the tergites exhibit a dark hue, which is also observable in immature individuals. Occasionally, the ventral side of the metasomal segment V in juveniles also appears dark.

Juvenile males and females are morphologically similar at early developmental stages, but sexual dimorphism usually becomes evident after three months. Male body coloration is typically darker than that of females. The movable fingers of immature specimens (less than one year old) are elongated, with narrow chelae. Sometimes, the median carina projects beyond the ends of the mesosoma tergites, causing these juveniles to be mistaken for members of the genus *Sassanidotus*. In males, metasomal segments are generally longer than wide, whereas in females, the first metasomal segment is distinctly wider than long. Females have wider tergites and consequently a larger abdomen, likely due to egg storage. Behaviorally, males tend to be more agile and active, while females are typically more sedentary.

The phylogenetic tree based on the COI gene shows that *M. rakhshanii* and *M. mirshamsii* cluster close to geographically neighboring species, namely *M. navidpouri* and *M. kirmanensis*, respectively (Figure 7). Genetic analysis comparing samples from the Sistan region with previously described species (Table 2) confirmed that the population in this area represents a new valid species. The intraspecific genetic divergence within this population was very low (0.002).

Although *M. rakhshanii* is morphologically similar to *M. macmahoni*, phylogenetic analysis places it as a sister group to *M. navidpouri* (Figure 7), with the smallest genetic distance observed between these two species (Table 2). This morphological similarity is likely due to adaptation to similar climatic conditions in their habitats.

Additionally, this study sequenced two samples of *M. mirshamsii* for the first time, confirming the validity of this species.

Scorpions were fed every 7 to 10 days, primarily with dark beetle larvae and occasionally with grasshoppers or crickets. If juveniles refused to eat larvae, no alternative food was offered. The size of the prey was selected according to the scorpion's size.

Litter sizes recorded for *M. iranus* (from Isfahan) and *M. crucittii* (from Khuzestan) ranged from 14 to 29 offspring (Dehghani et al., 2018), whereas in this study, litter size for *M. rakhshanii* ranged from 22 to 36 (Appendix 1).

A total of 626 juveniles were born from 22 mothers between April and October, with peak births in May and August (7 cases each). The average number of offspring per mother was 28.45. Survival data from April 2022 to March 2024 showed that 54 juveniles survived, with an average survival rate of 6.74 per mother (among 16 mothers with live offspring until March 2024). Major causes of juvenile mortality included cannibalism by mothers and siblings, mortality during molting caused by mealworms, predation by ants, and drowning in water containers (Appendix 1).

Early developmental stages exhibited frequent molting (approximately twice per month) due to rapid growth (Appendix 1). Despite being fed larvae of varying sizes appropriate to the juveniles, cannibalism was observed, likely due to limited space. Such behavior probably occurs in nature and may contribute to the survival of stronger individuals. During molting (Figure 12A, B), scorpions become immobile and were sometimes attacked and killed by mealworms (used as food) that consumed their pectines, entered their bodies, and fed on internal tissues.

After separation, mothers were housed individually to prevent re-mating. None produced offspring in the subsequent year. Some specimens laid eggs (Figure 12C, D), indicating that only one reproductive event occurs per mating.

Causes of death in scorpions during various life stages included natural mortality, intraspecific aggression, and predation by larvae during molting.

Members of the genus *Mesobuthus* possess venom that is not lethal to humans. Their venom affects nerves and causes moderate to severe localized pain lasting from one to several hours (Dehghani et al., 2023). Correspondence with patients and health centers in the Sistan region confirmed that stings of *M. rakhshanii* result in severe localized pain and mild edema during the initial hours post-sting.

Determining the potential distribution of *M. rakhshanii* as a newly described species is essential for further ecological and biological studies. Accurate distribution data will facilitate prevention of scorpion stings, improvement of treatment protocols, and

development of conservation and socioeconomic management plans (Kafash et al., 2023; Almeida et al., 2016).

The MaxEnt model, based on the maximum entropy principle, analyzes species distribution patterns by predicting the most uniform distribution under environmental constraints (Kong et al., 2019).

In this study, the potential geographic distribution of *M. rakhshanii* in Iran was modeled using MaxEnt. The results confirm the known distribution and highlight additional potential habitats where the species has not yet been recorded. High AUC scores (>0.9) indicate strong predictive performance based on presence-only data (Renner and Warton, 2013).

Mesobuthus rakhshanii is confined to eastern Sistan & Baluchestan and southern Khorasan provinces. The model predicts suitable habitats extending into southeastern Iran and the central plateau (Figure 11).

Species distribution modeling suggests that lowland to low-altitude mountainous regions in eastern Iran provide suitable climatic conditions for *M. rakhshanii*. In the western part of this belt (Bafq, Mehriz, Meybod counties), *M. vignolii* occupies similar climatic niches. Additionally, on the border of Saravan County with Pakistan, where conditions are alike, *M. macmahoni*, the species most morphologically similar to *M. rakhshanii*, is found. This shared climatic context likely explains their morphological resemblance.

Environmental variables are the primary drivers of scorpion community distribution patterns (El Hidan et al., 2017). This study identified the key factors influencing *M. rakhshanii* distribution (Figure 10B). Annual precipitation (bio12) emerged as the most significant variable, as it correlates with water availability in the arid regions of southern Iran and strongly influences species presence. The model shows that increased precipitation positively affects habitat suitability by supporting vegetation growth, shelter formation, and insect prey availability. The mean temperature during the coldest quarter (bio11) was the next most influential factor affecting the species' climatic niche. Other variables had minor effects.

Applying the selected binary threshold (10th percentile training presence), approximately 57.25% of Iran (~943,578 km²) is climatically suitable for *M. rakhshanii*, with about 5.52% (~90,841 km²) classified as highly suitable habitat. The species' distribution likely extends into neighboring countries on Iran's eastern border, such as Afghanistan and Pakistan. Further sampling in these regions is needed to clarify the species' true range and endemism status.

Declarations

Acknowledgements

This research was supported by project number PR-RIOZ-1403-8576-1 from the Research Institute of Zabol, Zabol, Iran.

ETHICAL STATEMENT

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethical approval: Not applicable. No experiments were performed on humans or live vertebrates.

Informed consent: Not applicable. This study did not involve human participants.

Author contribution: H. Barahoei: Conceptualization, fieldwork, morphological study, molecular analysis, figures preparation, manuscript writing; S.M. Madjdzadeh: Fieldwork, morphological study, manuscript editing. A. Moeinadini: Data curation, literature review, figures preparation, niche modeling analyses, manuscript writing. All authors read and approved the final manuscript.

Data Availability Statement: All data generated or analyzed during this study are included in this published article and its supplementary files. Additional datasets are available from the corresponding author on reasonable request.

References

1. Almeida TS, Fook SM, França FO, Monteiro TM, Silva EL, Gomes LC, Farias AM, 2016. Spatial distribution of scorpions according to the socioeconomic conditions in Campina Grande, State of Paraíba, Brazil. *Rev Soc Bras Med Trop* 49:477-85.
2. Barahoei H, 2022. Fauna of Sistan Scorpions (Arachnida: Scorpiones), Southeast Iran. *Taxonomy and Biosystematics* 14:27-70.
3. Dehghani R, Kamiabi F, Kassiri H, Hashemi A, Mohammadzadeh N, Gharagazloo F, 2018. A Study on Litter Size in Several Important Medical Scorpions Species (Arachnida: Scorpionida), I.R. Iran. *J Entomol* 15:155-160.
4. Dehghani R, Ghorbani A, Varzandeh M, Karami-Robati F, 2023. Toxicity mechanism of dangerous scorpion stings in Iran. *J Arthropod Borne Dis* 17:105-119.
5. El Hidan MA, Touloun O, Boumezzough A, 2017. Spatial relationship between environmental factors and scorpion distribution in Morocco. *J Entomol Zool Stud* 5:674-678.

6. Fan J, Upadhye S, Worster, A. 2006. Understanding Receiver Operating Characteristic (ROC) Curves. *Can J Emerg Med* 8:19–20.
7. Fick SE, Hijmans, RJ, 2017. WorldClim 2, New 1 km spatial resolution climate surfaces for global land areas. *Int J Climatol* 37:4302–4315.
8. Firoozfar F, Saghaipour A, Vatandoost H, Bavani MM, Taherpour M, Jesri N, Yazdani M, Arzamani K, 2019. Faunistic composition and spatial distribution of scorpions in North Khorasan Province northeast of Iran. *J Arthropod Borne Dis* 13:369–377.
9. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3:294–299.
10. Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 4:95–98.
11. Kafash A, Hanafi-Bojd AA, Mohammadi Bavani M, Shahi M, Akbari M, Rafinejad J, Bozorg Omid F, Hassanpour Gh, 2023. Mapping current and future risk of scorpion sting from a species with low medical concern, *Mesobuthus phillipsii* (Scorpiones: Buthidae) in Iran. *J Med Entomol* 60:1314–1320.
12. Kong WY, Li XH, Zou HF, 2019. Optimizing MaxEnt model in the prediction of species distribution. *Chin J Appl Ecol* 30:2116–2128.
13. Kovařík F, Fet V, Gantenbein B, Graham MR, Yağmur EA, Štáhlavský F, Poverennyi NM, Novruzov NE, 2022. A revision of the genus *Mesobuthus* Vachon, 1950, with a description of 14 new species (Scorpiones: Buthidae). *Euscorpius* 348:1–189.
14. Kumar S, Stecher G, Tamura K, 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874.
15. Lourenço WR, 2007. Litter size in micro-buthoid scorpions (Chelicerata, Scorpiones). *Boletín de la SEA* 40:473–477.
16. Muñoz MES, Giovanni R, Siqueira MF, Sutton T, Brewer P, Pereira RS, Canhos DAL, Canhos VP, 2011. OpenModeller: a generic approach to species' potential distribution modeling. *Geoinformatica* 15:111–135.
17. Phillips SJ, Anderson RP, Schapire RE, 2006. Maximum Entropy Modeling of Species Geographic Distributions. *Ecol Modell* 190:231–259.
18. Renner IW, Warton DI, 2013. Equivalence of MAXENT and Poisson point process models for species distribution modeling in ecology. *Biometrics* 69:274–281.
19. Swets J, 1988. Measuring the Accuracy of Diagnostic Systems. *Science* 240:1285–1293.
20. van Proosdij AS, Sosef MS, Wieringa JJ, Raes N, 2016. Minimum required number of specimen records to develop accurate species distribution models. *Echography* 39:542–552.

21. Yağmur E.A, Baghernavesi Z, Taherkhani Z, Akbari, P, Moradi M, 2024. New records of *Mesobuthus rakhshanii* Barahoei, 2022 in Iran (Scorpiones: Buthidae). *Euscorpius* 389:1-9.

Tables

Table 1 Data of scorpion specimens were used for sequencing of *COI* gene of *Mesobuthus* spp. and *Androctonus rostami* as outgroup.

Species	Collection	Locality	GenBank
<i>M. rakhshanii</i> Barahoei, 2022	RIZ-Mes-151	Iran, Sistan & Baluchestan Province, Hamun county, Peer-e Sabz village	PP392845
	RIZ-Mes-152		PP392846
	RIZ-Mes-152		PP392847
	RIZ-Mes-166		PP392848
	RIZ-Mes-168A		PP392849
	RIZ-Mes-168B		PP392850
	RIZ-Mes-170		
	RIZ-Mes-201	Iran, Sistan & Baluchestan Province, Zabol county, Hasan Abad village	PP392851
	RIZ-Mes-224	Iran, Sistan & Baluchestan Province, Zabol county, Zabol	PP392852
<i>M. mirshamsii</i> Kovařík et al., 2022	RIZ-Mes-136A	Hormozgan Province, Bandar Abbas, Dehnow Bala village	PP392795
	RIZ-Mes-136B		PP392796
<i>M. haarlovi</i> (Vachon, 1958)	-	Afghanistan, Uruzgan Province	OM905089
<i>M. kaftani</i> Kovařík et al., 2022	-	Iran, Razavi Khorasan Province	HM567371 HM567390 HM567391 HM567392 HM567393
<i>M. kirmanensis</i> Kovařík et al., 2022	-	Iran, Sistan & Baluchestan Province	HM567368 HM567381 HM567382 HM567383
<i>M. macmahoni</i> (Pocock, 1900)	-	Pakistan	OM905090
<i>M. navidpouri</i> Kovařík et al., 2022	-	Iran, Sistan & Baluchestan Province	HM567369
<i>Androctonus rostami</i> Barahoei et al., 2025	-	Iran, South Khorasan Province	HM567333

Table 2 Average Kimura 2-parameter (K2P) genetic distances among and within (bold) species of *Mesobuthus* and *Androctonus rostami* sequencing of *COI* gene.

	1	2	3	4	5	6	7
Species							
1. <i>M. haarlovi</i>	N/A						
2. <i>M. kaftani</i>	0.133	0.011					
3. <i>M. kirmanensis</i>	0.144	0.124	0.019				
4. <i>M. macmahoni</i>	0.119	0.126	0.112	N/A			
5. <i>M. mirshamsii</i>	0.157	0.125	0.093	0.130	0.012		
6. <i>M. navidpouri</i>	0.136	0.093	0.117	0.106	0.136	N/A	
7. <i>M. rakhshanii</i>	0.151	0.078	0.095	0.115	0.124	0.074	0.002
8. <i>A. rostami</i>	0.155	0.150	0.153	0.152	0.181	0.146	0.148

Figures

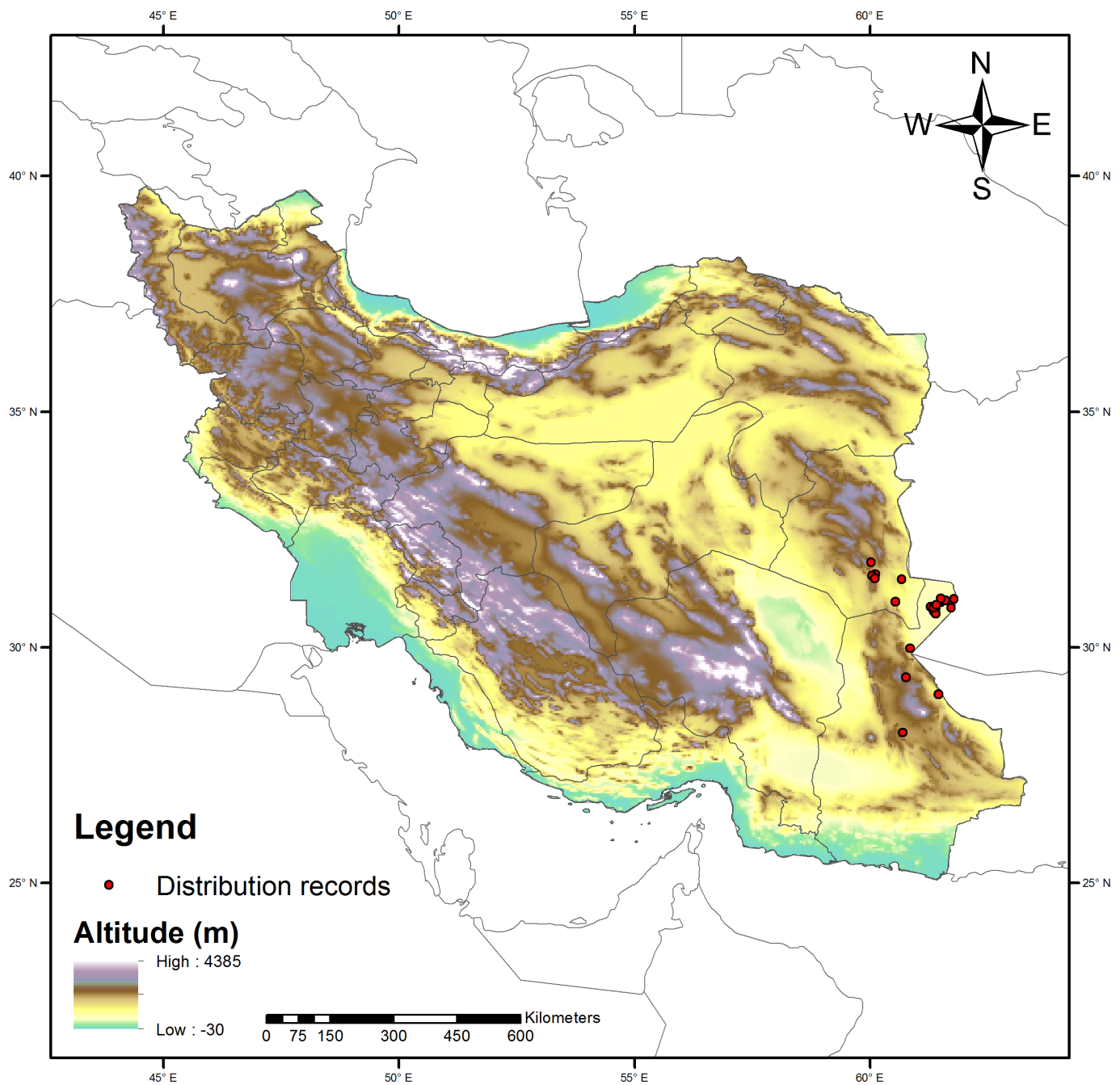


Figure 1

Occurrence records of *Mesobuthus rakhshanii* Barahoei, 2022 in east of Iran (Author's Work).



Figure 2

Alive specimens of *Mesobuthus rakhshanii* Barahoei, 2022 collected from Iran, Sistan & Baluchestan province, Hirmand county (Author's Work).



Figure 3

Female holotype of *Mesobuthus rakhshanii* Barahoei, 2022, dorsal (A) and ventral (B) views (Author's Work).

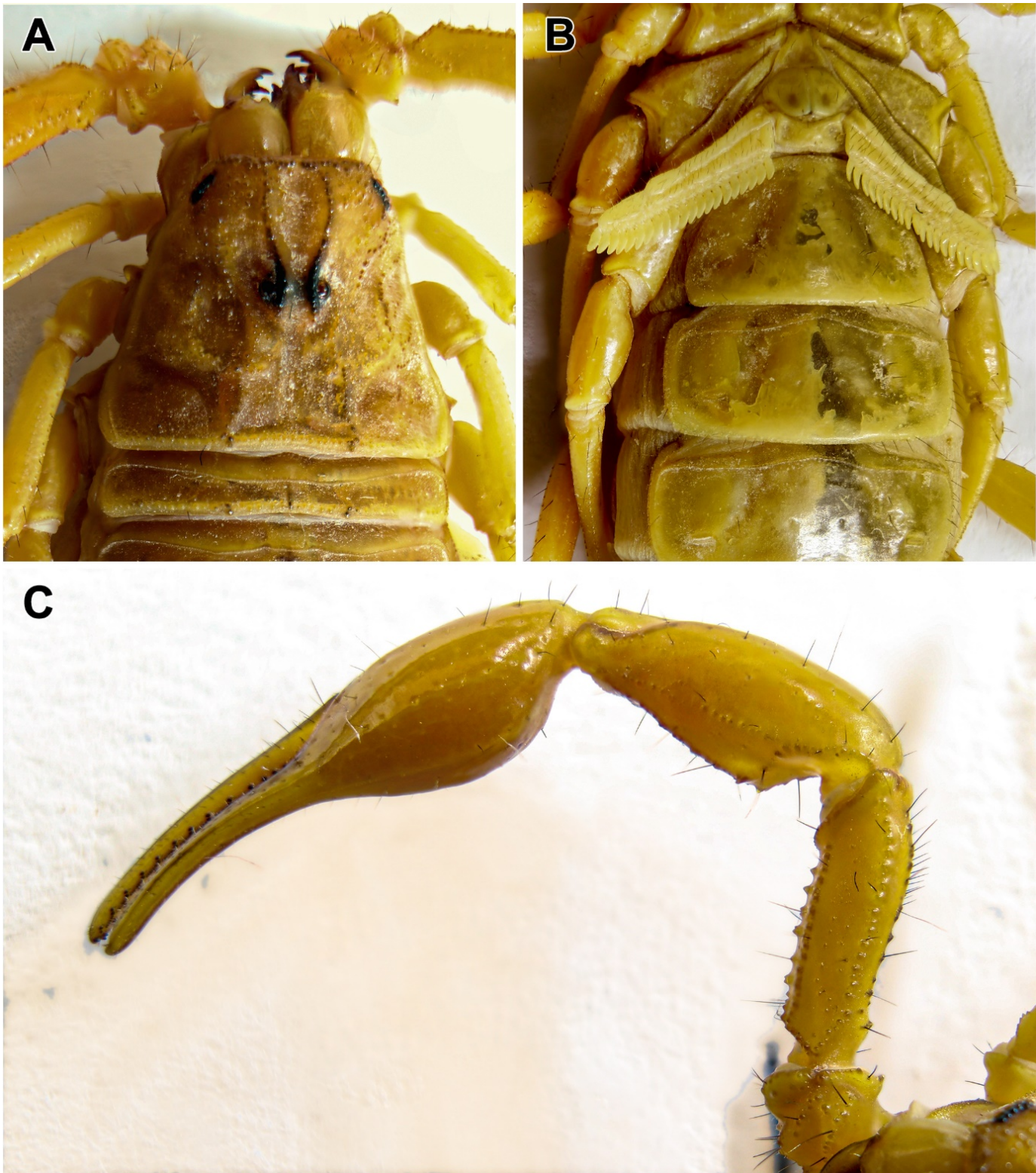


Figure 4

Female holotype of *Mesobuthus rakhshanii* Barahoei, 2022, A) chelicerae, carapace and tergites I; B) sternopectinal region and sternites; C) dorsal view of pedipalp (Author's Work).



Figure 5

Dorsal (A), ventral (B) and lateral (C) view of metasoma and telson of Female Holotype of *Mesobuthus rakhshanii* Barahoei, 2022 (Author's Work).

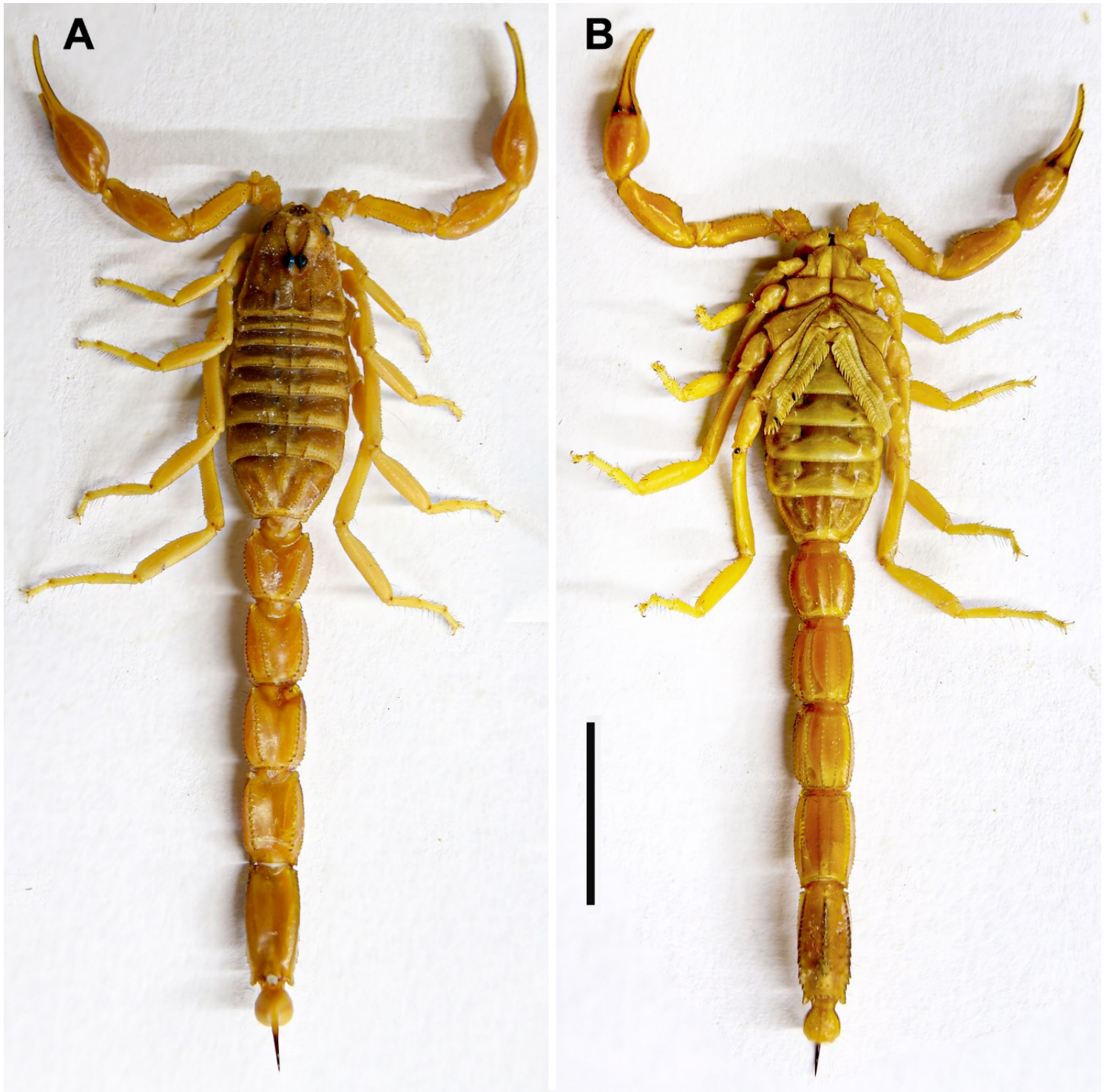


Figure 6

Male paratype of *Mesobuthus rakhshanii* Barahoei, 2022, dorsal (A) and ventral (B) views (Author's Work).

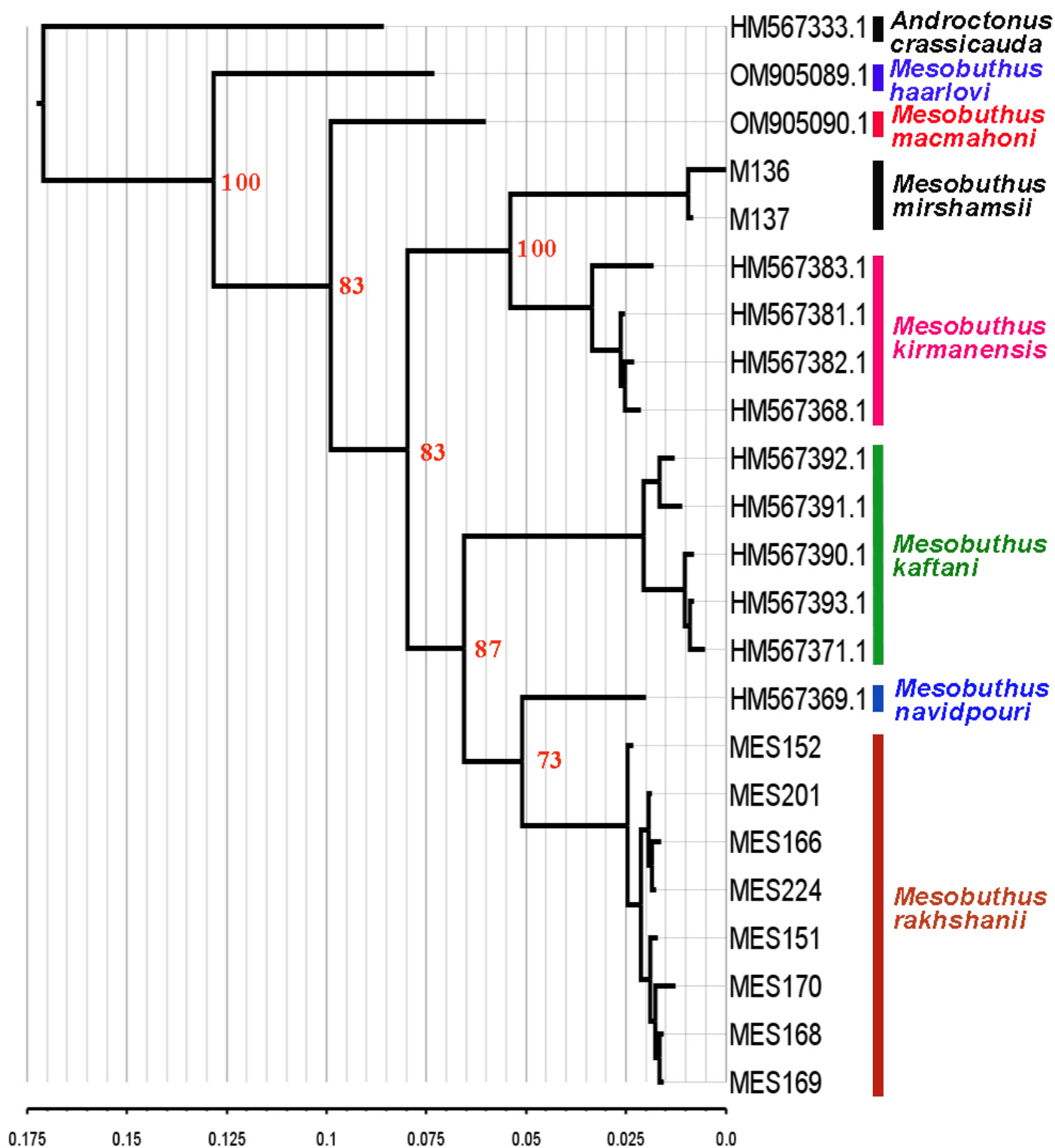


Figure 7

Bayesian inference (BI) was obtained by analysis of *COI* gene of *Mesobuthus* spp. and *Androctonus rostami* as an outgroup. Posterior probability values were added for each node (Author's Work).

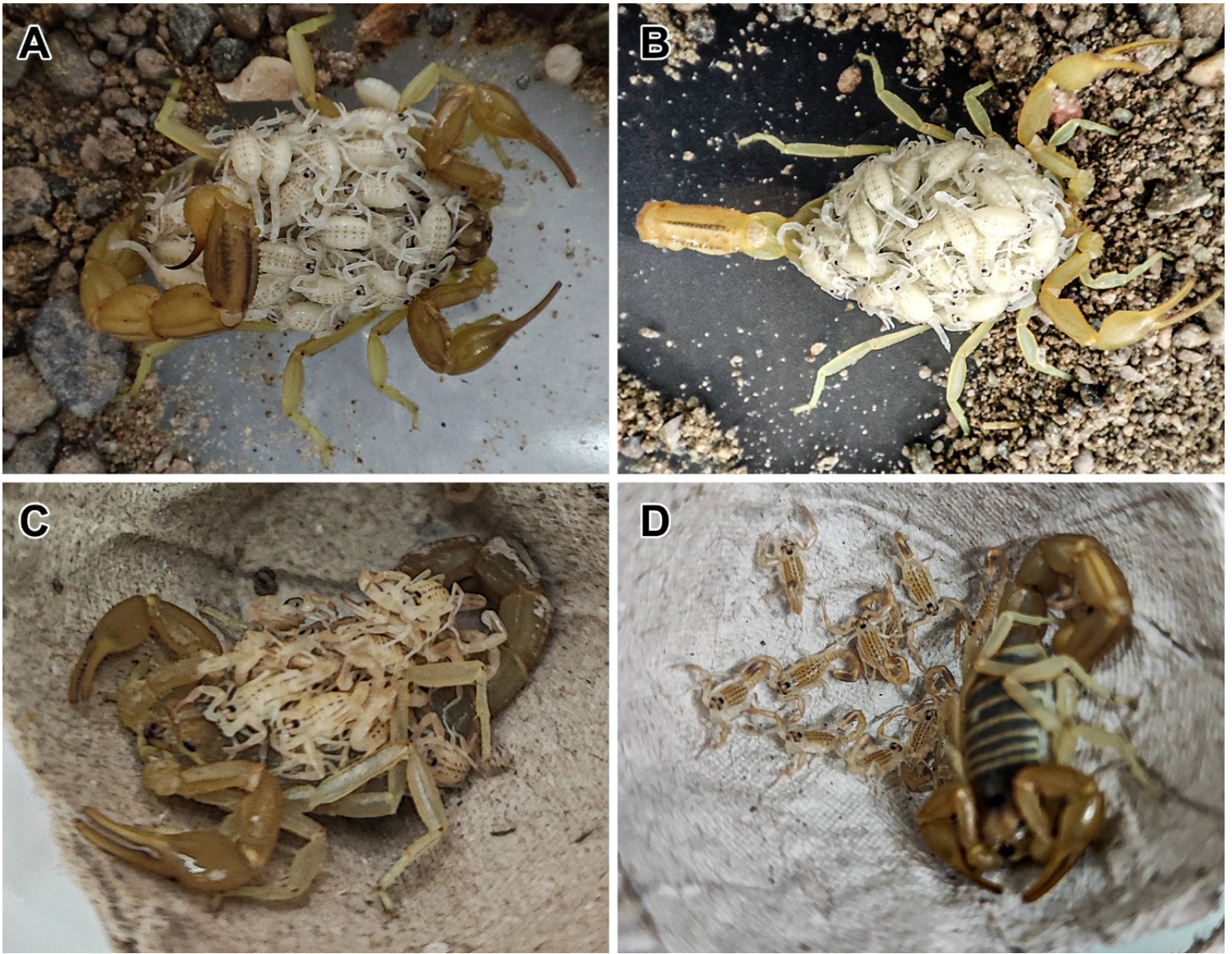


Figure 8

Female *Mesobuthus rakhshani* specimens carrying first instar larva after giving birth, A) M-01: fourth day; B) M-03: second day; C) M-14: sixth day; D) M-15: second week (Author's Work).



Figure 9

Molting of Nymphs of *Mesobuthus rakhshanii*, A) M-08: second month; B) M-15: third month; C) M-17: third month; D) M-11: fifth month (Author's Work).

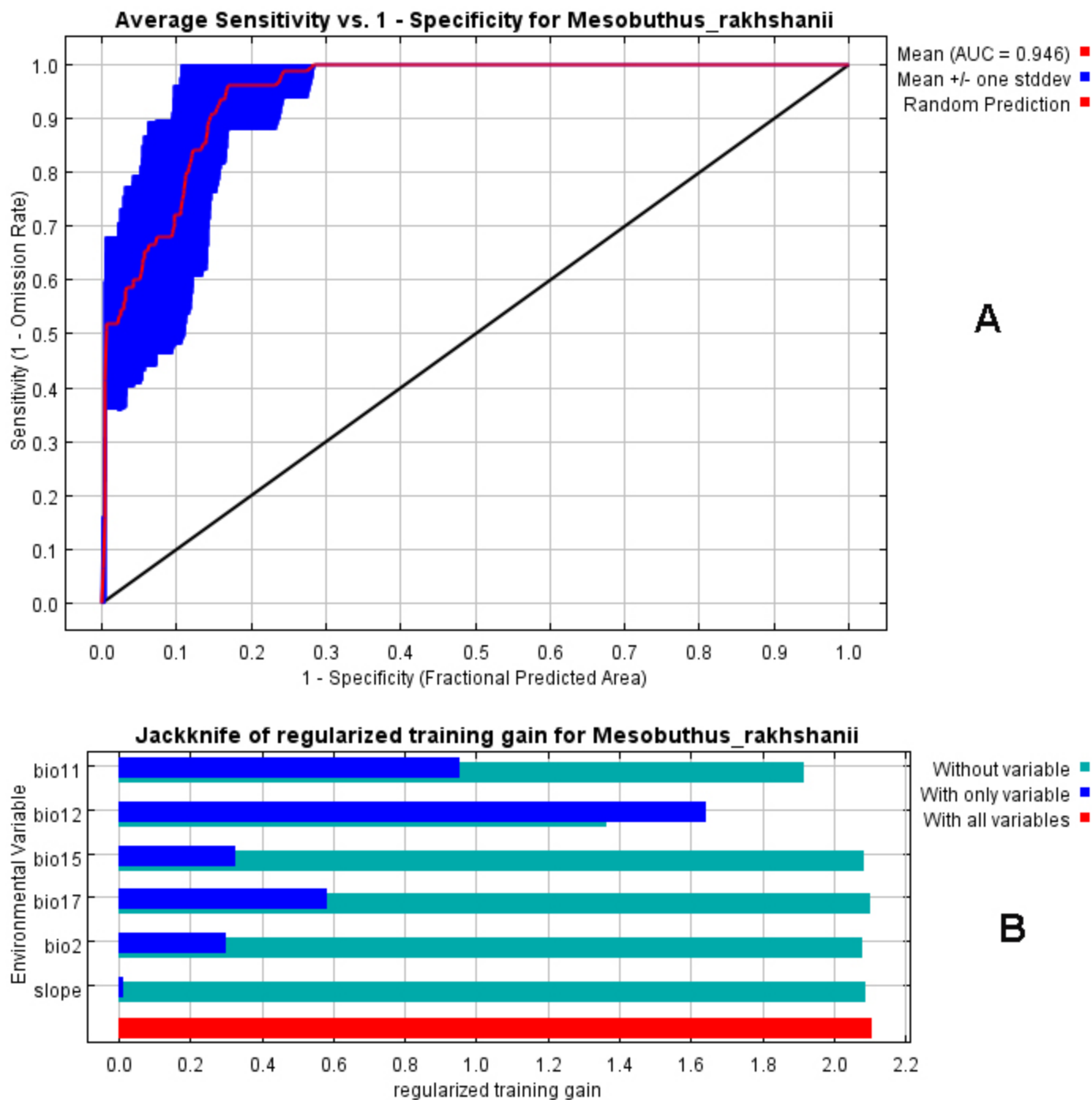


Figure 10

MaxEnt output for *Mesobuthus rakhshanii* Barahoei, 2022: A) model accuracy for current conditions; B) results of jackknife test of the relative importance of predictor variables in Iran (Author's Work).

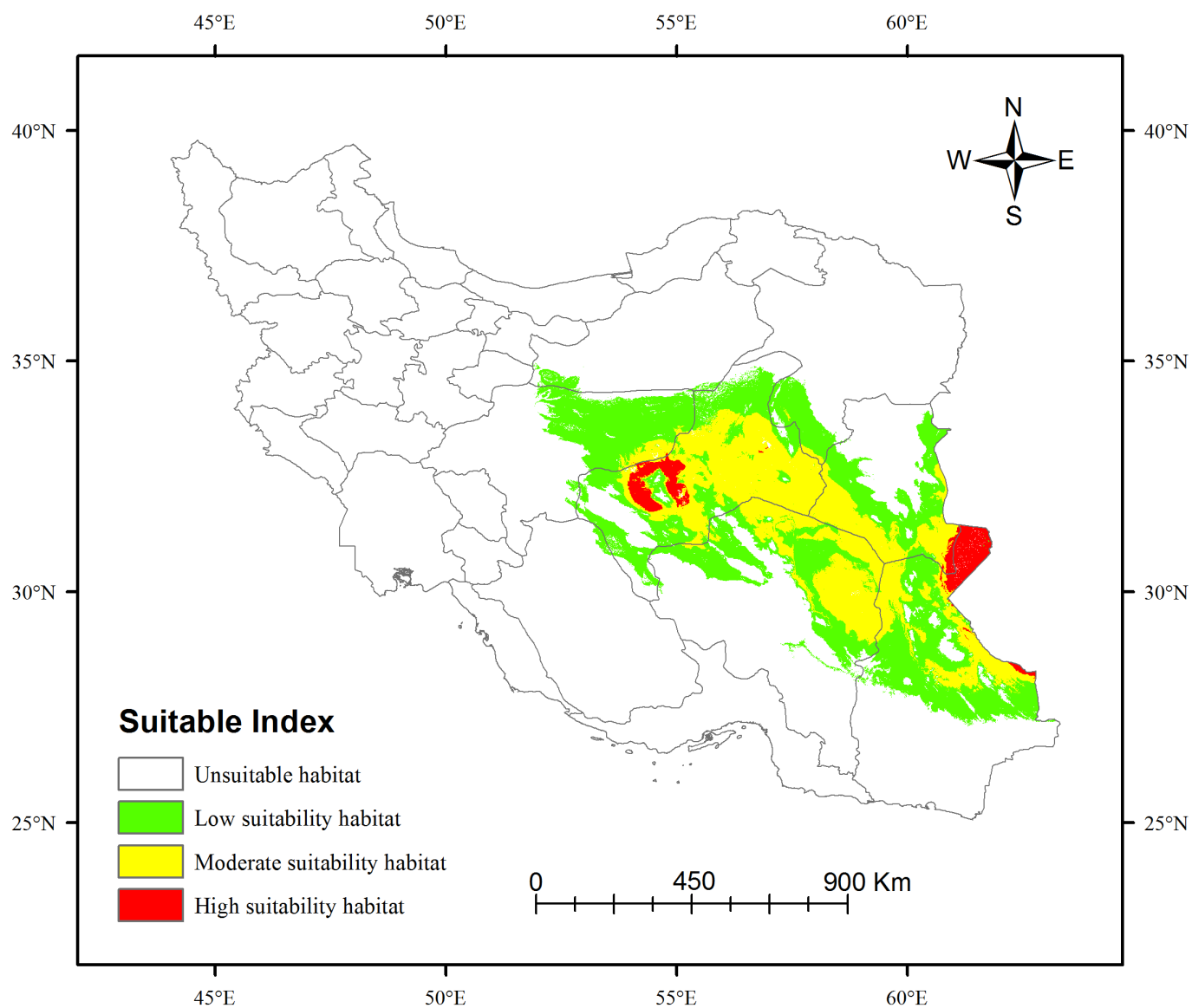


Figure 11

Potential suitable habitats for *Mesobuthus rakhshanii* Barahoei, 2022 in Iran under current climate (Author's Work).

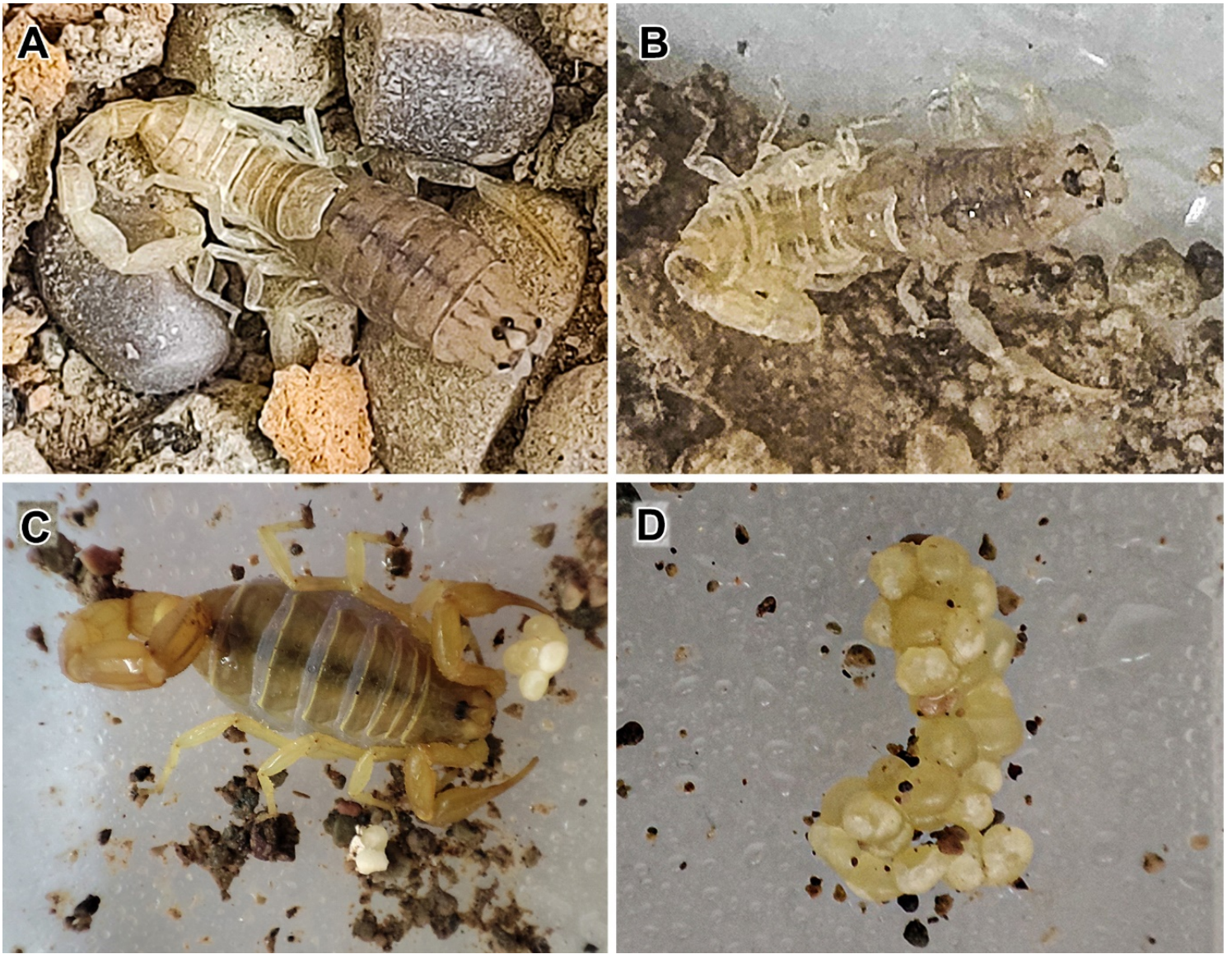


Figure 12

Nymphs of *Mesobuthus rakhshanii* were attacked by mealworms during molting, A) M-01: sixth month; B) M-18: third month; C, D) Eggs were laid by *Mesobuthus rakhshanii*(M-07) one year after giving birth (Author's Work).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Appendix1DataofMesobuthusrakhshaniispecimenswereusedforbiologicalstudy.docx](#)