Research Article

Journal of Wildlife and Biodiversity

Volume 8(4): 184-192 (2024) (http://www.wildlife-biodiversity.com/)

On the identity and taxonomy of *Kraepelinia palpator* **(Birula, 1903) (Scorpiones: Buthidae) from southeast Iran**

Hossein Barahoei

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Department of Agronomy and Plant Breeding, Agricultural Research Institute, Research Institute of Zabol, Zabol, Iran, Orcid: <u>https://orcid.org/0000-0001-5195-7679</u>, Email: h.barahoei@uoz.ac.ir

Received: 27 May 2024 / Revised: 15 August 2024 / Accepted: 19 September 2024/ Published online: 26 September 2024. **How to cite:** Barahoei, H. (2024). On the identity and taxonomy of Kraepelinia palpator (Birula, 1903) (Scorpiones: Buthidae) from southeast Iran, Journal of Wildlife and Biodiversity, 8(4), 184-192. **DOI**: https://doi.org/10.5281/zenodo.13840937

Abstract

Kraepelinia Vachon, 1974 is a monotypic buthid scorpion genus, with its populations distributed in Iran and Turkmenistan. A recent series of specimens were collected from the Sistan region, southeast Iran, during 2019-2023 and used for morphological and molecular studies. The mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene was sequenced for molecular research. *Kraepelinia palpator* was re-described based on the new specimens. Based on phylogenetic analysis, *K. palpator* was placed sister to *Mesobuthus* Vachon, 1950. Phylogenetic trees and genetic distances confirmed the Monophyly of all studied genera of Buthidae in this study. This study found the highest genetic distance (0.155) between *K. palpator* and *Androctonus* sp., and the lowest (0.102) was with *Mesobuthus rakhshanii. Kraepelinia* runs as the most similar taxon to *Mesobuthus* based on morphological characteristics are the placement of *eb* trichobothria on the manus and having teeth on the ventral surface of the third metasomal segment. The members of this species are distributed in the lowlands areas.

Keywords: Kraepelinia, scorpion, morphology, COI, Sistan

Introduction

Kraepelinia Vachon, 1974 is a genus belonging to the Buthidae family with only one described species. Populations of this species are distributed in eastern Iran and Turkmenistan (Fet, 1984; Navidpour et al., 2011; Barahoei et al., 2020). The most essential characteristics of this genus are the small size (less than 36 mm in males and a maximum of 45 mm in females), overall yellow body, carapace granular with distinct carinae, length, and width of metasomal segment I–III almost equal, pectin with 15–18 teeth in females and 22–26 in males, pedipalp fingers thick and short, and with a chamfered and hairy end, length of movable finger equal to the length of the manus, trichobothria *eb* located on the manus (not on the fixed finger), Trichobothriotaxy type $A\beta$, cuneiform teeth on the ventral part of the third metasomal segment (Stahnke, 1972; Farzanpay, 1987; Barahoei et al., 2020).

Birula (1903) first placed this species in *Buthus* and then (1958) under *Buthacus*. Vachon (1948) accepted *Buthacus* as an independent genus close to *Mesobuthus*, but in 1974, due to morphological differences between this genus and *Buthacus*, he placed it in *Kraepelinia*. Farzanpay (1987) described this species in a Persian book without pictures. Lourenço & Leguin (2010) described one female specimen from Iran deposited in French in Muséum National d'Histoire Naturelle, Paris, France. Barahoei (2022) also presented the diagnostic characteristics of species by providing only complete pictures of male and female specimens. A molecular study of scorpions of the family Buthidae based on the mitochondrial ribosome *16S* gene showed that the only sequence of this genus was placed with *Liobuthus* as the sister group of *Mesobuthus* (Fet et al., 2003). No information is available on the

biology of this genus. Specimens of *Kraepelinia palpator* were collected from the areas adjacent to the location of the type specimen to re-describe the species and verify its position in the phylogenetic tree.

Material and methods

The specimens of *Kraepelinia* species were collected from the Sistan region in 2019 and 2023 and were deposited in the Research Institute of Zabol (RIZ). The morphological nomenclature follows Stahnke (1970) and Sissom (1990), the terminology of pedipalp carinae is based on Prendini (2016), metasomal carinae follows González-Santillán & Prendini (2013), and morphological measurements are based on Sissom et al. (1990). The morphological examination of the samples was done using an SZM-2 Optika stereomicroscope (OPTIKA, Italy) and based on valid references (Birula, 1903; Farzanpay, 1987; Barahoei et al., 2020). Photography was done with a Canon D800 camera (Japan), and editing of images was done using Adobe Lightroom and Adobe Photoshop software.

The mitochondrial *COI* gene was sequenced for molecular comparison of the samples. For this purpose, eight samples were selected for DNA extraction and molecular analysis. The COI gene was amplified with the help of appropriate primers (Folmer et al., 1994) and an already-defined temperature program (Barahoei et al., 2022). After checking on agarose gel, the quality cases were sent to Niagene Noor Company (Tehran) for sequencing. Editing and alignment of sequences was done using Bioedit 7.0.5.3 software (Hall, 1999). MEGA 7.0 software (Kumar et al., 2016) calculated genetic divergence between and within species. Maximum likelihood and Bayesian interference were drawn on http://www.atgcmontpellier.fr/phyml (Guindon et al., 2010) and the CIPRES website (http://www.phylo.org, Miller et al., 2010), respectively.

Results

Taxonomy

Buthidae C. L. Koch, 1837

Kraepelinia Vachon, 1974

Kraepelinia palpator (Birula, 1903)

Syntype locality and depository: *Buthus palpator* Birula, 1903: 72–74. Persia (now Iran), Sistan & Baluchistan province, between Sia-Kuhi (Godar-i-Siah-Kuhi) Pass and Bid (Kale-i-Bid) (125 km south of Zahedan City); ZISP.

Buthus palpator: Birula, 1903: 72-74.

Buthacus palpator: Vachon, 1966: 210; Habibi, 1971: 43.

Kraepelinia palpator: Vachon, 1974: 950–951, figs. 235–239; Fet, 1984: 37–42, fig. 1; Fet, 1989: 82–83; Farzanpay, 1988: 38; Fet, 1994: 531; Kovařík, 1997a: 49; Kovařík, 1998: 112; Fet & Lowe, 2000: 154; Vignoli et al., 2003: 3; Lourenço & Leguin, 2010: 307-308, figs. 1-18; Navidpour et al., 2011: 9; Barahoei et al., 2020: 392, fig. 13; Barahoei, 2022: 42-46, figs. 5–6.

Examined materials (9 Female, 8 Male, 1 Subadult): *IRAN, Sistan & Baluchistan province*: 1 Female, 1 Male (Fig. 1A) (RIZ-Kra-010), Zahak, Chah Nimeh, 30°50'22.34" N, 61°43'01.45"E, 29 September 2022; 2 Female - 1 Male (RIZ-Kra-004), Hamun, Lootak, Rahmatabad village, 30°45'33.16"N, 61°21'09.95"E, 30 March 2022; 1 Female, 1 Male (RIZ-Kra-006), 29 November 2020; 1 Female (RIZ-Kra-007), 10 May 2021; 1 Female (Fig. 1B) (RIZ-Kra-011), 29 December 2023; 1 Male (RIZ-Kra-012), 07 June 2023; 1 Female (RIZ-Kra-001) - Hamun, Peer-e Sabz village, 30°51'21.35"N, 61°20'06.20"E, 2019 April 04; 1 Female (RIZ-Kra-002), 01 September 2019; 1 Subadult (RIZ-Kra-003), 01 September 2019; 1 Female, 1 Male (RIZ-Kra-005), 25 June 2022; 1 Male (RIZ-Kra-008), 17 September 2021; 2 Male (RIZ-Kra-009), 25 June 2022, coll. H. Barahoei.

General distribution: Kerman (Birula, 1903; Farzanpay, 1987; Navidpour et al., 2011), Sistan & Baluchistan (Birula, 1903; Mir et al., 2014; Barahoei et al., 2020) and South Khorasan provinces (Barahoei et al., 2020).

Comment: Specimens collected from Esfahan (Vignoli et al., 2003) and Turkmenistan (Fet, 1984, 1994) seem to belong to new species.



Figure 1. Alive male (A, RIZ-Kra-010) and female (B, RIZ-Kra-011) specimens of *Kraepelinia palpator* (Birula, 1903) collected from north of Sistan & Baluchistan province, Iran.

Diagnosis

The small size, the total length of adults 28–45 mm; overall color yellow; ventral carinae of metasomal segment IV–V dark, central lateral carinae of carapace connected to posterior median carinae; femur 2.6–2.8 times longer than wide; patella 2.2 times longer than wide; movable finger length equal with manus length, with 10–11 rows of compressed oblique teeth, with five terminal granules; trichobothria *eb* located on manus; pectinal teeth 22–26 in males and 17–18 in females; metasomal segments short, all segments sparsely setose, segment I wider than long, segments II–V longer than wide, anal arch with two lateral rounded and six ventral lobes, telson broad and short.

Re-description (Figs. 1-4)

Size: Scorpions with small size, adult body length 28 to 36 mm in males and 35 to 45 mm in females (Figs. 1, 2).

Coloration: Overall color yellow, legs lighter, area around median eyes black, chelicer yellow with dark teeth and without reticulation, ventral carinae of metasomal segment IV-V dark, end of the telson black (Figs. 1-4).

Chelicer: Has two lateral teeth on the ventral surface of the fixed finger, the movable finger with the same size internal and external teeth.

Carapace: Trapezoidal, wider than long, developed carinae, intercarinal surface smooth, front margin of the carapace straight in lateral view with two to four long setae, large ocular tubercle in the anterior part of prosoma, and three pairs of lateral eyes (Fig. 3A), small granular central median and posterior median carinae, large granulated central lateral carina, connected to posterior median carinae (Fig. 3A). **Legs**: Tarsomeres are densely hairy, with a dense comb of hair on all legs (Fig. 2).

Pedipalp: Segments short (Fig. 3C); femur 2.6 to 2.8 times longer than wide, intercarinal surface smooth, with five distinct carinae and scarred, prodorsal and retrodorsal carinae with dense granules, retroventral carina obliterated and with only a few granules, proventral carina with dense granules, promedian carina with large and separate cuneiform granules; patella 2.2 times longer than wide, intercarinal surface smooth, with eight carinae, promedian and prodorsal carinae with large and

separate cuneiform granules, dorsomedian carina with dense granules, retrodorsal, retromedian and retroventral carinae obscured (with smooth ridge), proventral carina with sparse granules; chela smooth, carina faded or smooth (Fig. 3C), manus wider than patella, movable finger length equal with manus length, movable finger with 10-11 rows of compressed oblique teeth, with internal and external teeth, with five terminal granules, fixed finger with 9-10 rows of compressed oblique teeth, with internal and external teeth (Fig. 3C).



Figure 2. General view of the male specimen of *Kraepelinia palpator* (Birula, 1903): A) Dorsal view, B) Ventral view (RIZ-Kra-012).

Trichobothriotaxy: Type $A\beta$, with 39 trichobothria on each pedipalp; femur with 11 trichobothria (5 dorsal, four median lateral, 2 external lateral); patella with 13 trichobothria (5 dorsal, one median lateral, seven external lateral); *d2* greatly reduced; chela with 15 trichobothria (8 manus, seven fixed finger); *eb* located on manus (Fig. 3C), *et* located near middle of row 5 and *est* near beginning of row 7 (Fig. 3C).



Figure 3. Body parts of the male specimen of *Kraepelinia palpator* (Birula, 1903): A) Carapace and mesosoma, B) Sternopectinal region and sternites, C) Pedipalp (RIZ-Kra-012).

Mesosoma: Protergites smooth, with some large granules at the end, post-tergites I–VI with three carinae, post-tergite VII with five carinae, median carina present only at the first half of the segment and granulated; sternites III–VI without carinae, sternite VII with four carinae, not granulated, lateral carinae present to 2/3 of segment, number of pectin teeth 22 to 26 in males (Figs. 2B, 3B) and 17 to 18 in females; in males marginal tip of pectin reaches to beginning of sixth sternite, also to coxa-trochanter joint of fourth leg (Fig. 3B), but it does not get this place in females; pectin with three marginal lamellae and six to seven middle lamellae, lamellae with numerous black setae, each fulcrum with three to five black setae; sternum type *I*, sub-pentagonal, longer than wide, with a deep median depression; genital operculum completely divided longitudinally, with short and smooth bristles (Fig. 3B).

Metasoma: Segments short (Fig. 4), all segments sparsely setose; Segment I wider than long, with 10 carinae, dorsal lateral and lateral median carinae with long and sparse granules, lateral inframedian and ventral lateral carinae with distinct and sparse granules, ventral submedian carinae with smooth ridges; Segments II-III longer than wide, with eight carinae, dorsal lateral and lateral median carinae with long

and sparse granules, lateral inframedian carina extended to middle of segment with sparse granules, ventral lateral carina granulated, ventral submedian carinae with long granules towards end of segment II, with five to six separate teeth in segment III (Figs. 4B-C); Segment IV longer than wide, with eight carinae, dorsal lateral carinae with long granules, absence of lateral inframedian carinae, dorsal lateral and ventral lateral carinae with small granules, ventral submedian carinae, dorsal lateral carinae with small granules, ventral submedian carinae, dorsal lateral carinae with small granules, ventral submedian carinae, dorsal lateral carinae with granule anteriorly and a smooth ridge posteriorly, absence of lateral median and lateral inframedian carinae, ventral lateral carina serrated, with very long granules posteriorly (Figs. 4B-C), ventral median carina with a number of small granules anteriorly and a smooth ridge posteriorly and a smooth ridge posteriorly, presence of six to eight long setae on the lateral surface of segment; Anal arch with two lateral rounded (sometimes the first one bifurcate) and six ventral lobes (Fig. 4).

Telson: Broad and short in both sexes, with sparse long setae, without teeth, ventral surface without ridges, dorsal surface smooth; height /length ratio = 0.38 in males and 0.44 in females, telson wide/length ratio = 0.47 in males and 0.53 in females (Fig. 4).



Figure 4. Metasoma and telson of the male specimen of *Kraepelinia palpator* (Birula, 1903): A) Dorsal view, B) Ventral view, C) Lateral view (RIZ-Kra-012).

Molecular study

Five sequences were obtained from *Kraepelinia*, 12 sequences from different genera of the Buthidae family (from Sistan & Baluchistan province), and a sequence of *Androctonus* sp. (the oldest genus) as an outgroup, were used to construct phylogenetic trees (Table 1).

The sequences were 618 bp long, including 462 conserved (74.76%), 156 variables (25.24%), and 135 parsimony positions (21.84%).

Species	Collection	Locality	Genbank	
Kraepelinia palpator (Birula, 1903)	RIZ-Kra-005		PP529594	
	RIZ-Kra-09A	Iron Siston & Deluchiston	PP529595	
	RIZ-Kra-09B	Province Hemun and Zabak	PP529596	
	RIZ-Kra-010	FIOVINCE, Hamun and Zanak	PP529597	
	RIZ-Kra-011		PP529599	
	RIZ-Mes-151			
Mesobuthus rakhshanii	RIZ-Mes-152	Iran, Sistan & Baluchistan	unpublished	
Barahoei, 2022	RIZ-Mes-166	Province, Hamun and Nimruz		
	RIZ-Mes-168			
Odontobuthus tirgari		Iran, Sistan & Baluchistan	MW655771	
Mirshamsi et al., 2013	-	Province, Zahedan		
Orthochirus persa (Birula,	RIZ-Ort-041 RIZ-Ort-042	Iran, Sistan & Baluchistan	unpublished	
1900)	RIZ-Ort-099	Province, Hamun		
Sassanidothus gracilis	RIZ-Sas-010 RIZ-Sas-32A	Iran, Sistan & Baluchistan	unpublished	
(Birula, 1900)	RIZ-Sas-32B RIZ-Sas-32C	Province, Hamun		
Androctonus sp.	PIZ And 263	Iran, Sistan & Baluchistan	DD535682	
	NIZ-AIIu-203	Province, Nimruz	FF 555082	

Table 1. Data from scorpion specimens were used to construct phylogenetic trees based on sequences of the COI gene of Kraepelinia and other genera of Buthidae in eastern Iran.

The results of Maximum likelihood and Bayesian interference were similar. Members of *Kraepelinia palpator* were placed at the top of the tree as the newest genus and sister group of *Mesobuthus* (Fig. 5).

Phylogenetic trees (Fig. 5) and genetic distances confirmed the validity of all genera. The highest genetic distance (0.155) was between *Kraepelinia palpator* and *Androctonus* sp., and the lowest (0.102) was with *Mesobuthus rakhshanii* (Table 2).



Figure 5. Consensus tree of Bayesian Inference (BI) and Maximum Likelihood (ML) was constructed based on *COI* sequences of scorpions in Sistan & Baluchistan province. Posterior probability values of BI analyses and bootstrap values of ML are mentioned on each node, respectively. *Androctonus* sp. is used as an outgroup.

Species	1	2	3	4	5
1. Kraepelinia palpator	0.002				
2. Mesobuthus rakhshanii	0.102	0.002			
3. Odontobuthus tirgari	0.137	0.149	-		
4. Orthochirus persa	0.154	0.159	0.124	0.005	
5. Sassanidotus gracilis	0.137	0.119	0.125	0.152	0.003
6. Androctonus sp.	0.155	0.148	0.114	0.127	0.137

Table 2. Genetic distances (Average Kimura 2-parameter) within (bold) and among *Kraepelinia* members and other genera of Buthidae in eastern Iran based on *COI* gene sequences.

Discussion

Geographical barriers (such as the formation of the Lut Desert, the creation of the Zagros mountains and the mountainous belt of the east of Iran, etc.), resulting from the collision of different plates play a key role in the speciation of scorpions. Scorpions with allopatric or parapatric distribution patterns easily undergo speciation (Barahoei et al., 2022). The type locality of Kraepelinia palpator (Birula, 1903) is mentioned between Siah Kouh and Qale Bid (Birula, 1903), which is located in the center of Sistan and Baluchistan province (not Kerman province). This species was also reported from Isfahan province (an immature specimen) (Vignoli et al., 2003). Since the mountains of the eastern belt of Iran have caused geographical separation and thus speciation in scorpions (as sedentary animals) (Lowe, 2010; Barahoei et al., 2022), therefore it seems that either the identification was wrong or the specimen belongs to a new species. In addition, the samples reported from Turkmenistan (Fet, 1984) probably belong to the new species because they are geographically far apart and live in different weather conditions. The recent material of this species was found near the Hamoun wetland (Sistan - 480 m a.s.l.) with a distance of about 300 km from the type locality (1350 m a.s.l.). In addition, this scorpion was found in some houses in villages. The most important morphological difference between Kraepelinia and other scorpions is the placement of eb trichobothria on the manus. In other genera, this trichobothrium is located at the end of the movable finger of the pedipalp. Another distinguishing feature of *Kraepelinia* is having teeth on the ventral surface of the second and third metasomal segments (similar to Odontobuthus). Kraepelinia is placed as a sister group of Mesobuthus in the phylogenetic tree (Fig. 5). These two genera have the lowest genetic distance (Table 2).

Conclusion

Members of the small scorpion, *Kraepelinia* Vachon, 1974, which can be identified by the placement of trichobothrium *eb* on the manus, live in Iran and Turkmenistan. So far, only one species, *K. palpator* (Birula, 1903), has been described. Morphological and molecular investigation of this species in southeast Iran confirmed the validity of the genus and species. Due to the low movement of scorpions and rapid speciation despite geographical barriers, there is a possibility of other species in other areas, such as the center and northeast of Iran.

Acknowledgments

This research was supported by project PR-RIOZ-1401-8576-1 of the Research Institute of Zabol, Zabol, Iran.

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