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Some biological aspects of the central Indian endemic scorpion *Hottentotta jabalpurensis* Kovařík, 2007 (Scorpiones: Buthidae)

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Abstract: The present study reports the distribution and bionomics of *Hottentotta jabalpurensis* Kovarik, 2007, a central India endemic species. Information on courtship, maternal care, feeding habits, and kin recognition has been provided based on observations made in captivity. Morphological and morphometric details of the pre-insemination spermatophore of the species have been detailed for the first time for the species.

Keywords: Behaviour, breeding, distribution, kin recognition, Madhya Pradesh, spermatophore.

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Author contribution: PP carried out field surveys, reared the animals, conducted the research work and wrote the draft manuscript. PPM supervised the research work, validated the information and helped in manuscript writing. DBB validated the observations, guided the research work and helped in editing the manuscript.

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INTRODUCTION

The genus *Hottentotta* Birula, 1908 consisting of 55 species (Rein 2020) is one of the most widely distributed genera of the family Buthidae, distributed across Africa, the Arabian Peninsula, and in Asia (Kovařík 2007). In India *Hottentotta* is represented by eight species, namely: *H. tamulus* (Fabricius, 1798), *H. pachyurus* (Pocock, 1897), *H. rugiscutis* (Pocock, 1897), *H. jabalpurensis* Kovařík, 2007, *H. stockwelli* Kovařík, 2007, *H. keralaensis* Ashwathi, Sureshan & Lourenço, 2016, *H. reddyi* Lourenço, 2015, and *H. vinchu* Mirza, Ambekar & Kulkarni, 2019 (Kovařík 2007; Bastawade et al. 2012; Lourenço 2015, Ashwathi et al. 2016; Mirza et al. 2009). The genus is distributed in six out of 10 biogeographic zones of India (Rodgers et al. 2000), namely Gangetic plains, desert, semi-arid, Deccan Peninsula, Western Ghats, and coasts (Bastawade et al. 2012). *Hottentotta* is a medically important genus (Ward et al. 2018) and has evolved to inhabit closely to human dwellings, agricultural fields, and open areas (Tikader & Bastawade 1983; Ranawana et al. 2013; Mirza et al. 2019) which increases its frequent interaction with humans. *Hottentotta jabalpurensis* (Type locality: Jabalpur, Madhya Pradesh) was described based on some morphological characters in which it differs from its sister species *H. tamulus* in having metasoma densely hirsute, and the patella of pedipalp with long hair (Kovařík 2007).

Scorpions are predatory animals and are known for their cannibalistic behaviour, however, apart from an obligatory mother-young association, a few species are known to live in social groups. Sub-social behaviour has been observed in species like *Heterometrus fulvipes* and *H. swammerdemi* in the family Scorpionidae and on the other hand cohabitation and gregarious interaction have been seen in *Compsobuthus werneri judaicus* (Shivashankar 1994; Mohapatra & Pandey 2020; Warburg 2002). Similarly, studies on various behavioural aspects of courtship and breeding have been undertaken on some Indian scorpions of the family Scorpionidae and Buthidae (Bastawade 1992; Mirza & Sanap 2009; Mirza et al. 2009; Mohapatra & Pandey 2020).

Scorpions like many other arachnid groups have an indirect way of sperm transfer through a sperm packet called spermatophore (Polis 1990). The courtship and breeding process can be divided into four stages, i.e., initiation, Promenade á Deux, sperm transfer and separation (Ross 2009). Furthermore, spermatophore and hemispermatophores are considered to have characters having taxonomic significance and phylogenetic values (Francke 1979; Monod et al. 2017). Among the Indian scorpions, studies on the morphology of spermatophore and hemispermatophore are relatively rare and are restricted to a few species (see Mathew 1956; Bastawade 1992, 1994; Mirza & Sanap 2009; Mohapatra & Pandey 2020).

Scorpions are viviparous arthropods with a long gestation period (Polis 1990). The females during parturition form a birth basket by using the first pair of legs, crossing each other medially (Francke 1982). After birth, the babies climb up to the mother's back and settle in a particular orientation. The larval orientation can be random, transverse, or longitudinal depending on different families or species (Savary 1996). They continue to do so for a varying number of days after the young scorpions undergo their first ecdysis to second-instar, after which the vagile second-instar young ones disperse from the mothers' dorsum and become free-living (Williams 1971; Polis & Sissom 1990; Lourenço 2018). Studies on such mother and young associations in Indian scorpion species is very rare and has been mentioned by Mathew (1962) for *Lychas tricarinatus*, Mirza et al. (2009) for *Hottentotta pachyurus* (Pocock, 1897), and Mirza & Sanap (2009) for *Heterometrus phipsoni* (Pocock, 1893).

The present study emphasizes the breeding behavior of *H. jabalpurensis* along with distribution and natural history observations. Information on the Morphological description of the spermatophore in the pre-insemination state, parturition, maternal care, kinship behaviour and cannibalism has been provided based on observations in captivity.

MATERIALS AND METHODS

The study was undertaken during a seven months dissertation work carried out by the first author from January to July 2018 at the Zoological Survey of India (ZSI), Central Zone Regional Centre (CZRC), Jabalpur, Madhya Pradesh. Scorpions were sampled randomly by lifting rocks, finding them in leaf litter, peeling off bark and digging the burrows in various sampling localities outside protected areas. During the night, scorpions were searched with the aid of an ultraviolet torch. Animals were handpicked with the help of a forceps when located and were kept separately in plastic boxes to avoid being eaten by larger ones. Photographs were taken in their natural habitat and the captive individuals were photographed regularly to record their behavior with a Nikon D5100 camera fitted with Nikon-100 macro or Tamron 90mm lens. A total of 18 adult individuals of

Hottentotta jabalpurensis comprising four adult males and 14 adult females were collected from Sidh Baba Mandir area, Katangi, and Paatbaba area, Jabalpur, during the month of March–June 2018. All the animals except two males, from which the spermatophores were obtained, were released back in their respective habitats after the completion of the experiment during September 2019. To record various behavioral aspects, live scorpions were maintained in the laboratory in terrariums or plastic boxes of 6´´X 3´´X 3´´ sizes, with a layer of 1´´ soil substratum. The room temperature was maintained in an air-conditioned room (24–30°C) and water was provided in each terrarium in small bowls to maintain humidity and avoid desiccation. The animals were fed with live mealworms maintained in the laboratory. Other prey species such as gryllids, small geckos (*Hemidactylus* spp.), skinks (*Eutropis* spp.), and termites were also fed from time to time. Behavioral aspects such as feeding, courtship, and kin recognition were recorded during the study period. Spermatophores of the scorpions were obtained from the captive breeding groups and were preserved in 70% ethyl alcohol. Photographs were taken to see the natural coloration. Morphological data were collected under a stereo-zoom microscope (Leica M-2054) and measurements were taken using Mitutoyo™ digital calipers to the nearest 0.1mm. Morphometric details of the spermatophore were taken to the nearest 0.01mm under the microscope. Distribution localities of the species were recorded based on the meta-data available for the specimens housed in the national zoological collections of ZSI, CZRC. Each specimen was identified by evaluating standard taxonomic characters in a datasheet to record quantitative (i.e., mensural and meristic) and qualitative taxonomic characters following standard taxonomic keys (Bastawade 1992; Kovařík 2007; Lowe 2010; Monod et al. 2017).

RESULTS

Distribution (Figure 1): *Hottentotta jabalpurensis* was found to be distributed in Damoh, Sagar, Narsinghapur, Jabalpur, Chhindwara, Raisen, Dewas, Dheona, Panna,

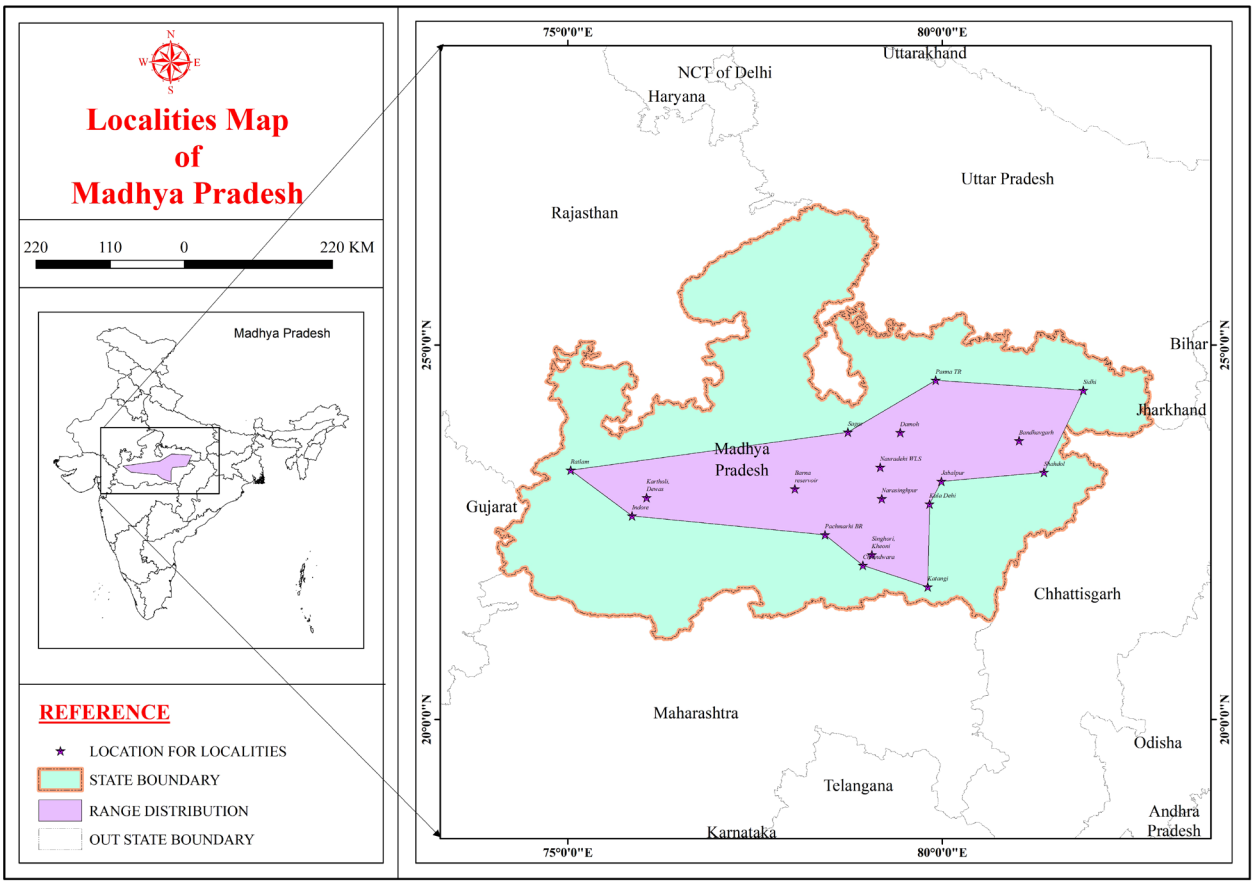

Figure 1. Distribution localities of *Hottentotta jabalpurensis* in Madhya Pradesh.

Shahdol, Shivpuri, Sidhi, Indore, Hoshagabad, Ratlam, and Umaria districts of Madhya Pradesh (Table-1). This species was found mostly below rocks and large boulders, sometimes clinging on to the inner surface of rocks and sitting on wood logs in wet regions. Although, this species was generally found solitary, males and females were found to be under the same rock during January–March, probably due to breeding activities.

Feeding habits (Image 1,2): In captivity, scorpions were fed with small crickets, mealworms, centipedes, termites and grasshoppers. Prey larger than the body size was stung to overpower and feed on it. Juveniles after a few days of second molting started using the stinger and in comparison to adults the use of stinger was more frequent in the juveniles. The juveniles were also found to hunt in a group to overpower larger prey. Adults as well as juveniles were found to manipulate the orientation of the prey to feed on the head at first, no matter from which side it was captured. The prey was eaten as a whole or it was torn into pieces and the undigested parts were discarded after the full meal. Smaller preys such as termites, mealworms, etc., were devoured fully.

Sexual dimorphism: Marked sexual dimorphism was found in *H. jabalpurensis*. Females were larger than males with a total length of 50–80 mm in females and 48–65 mm in males. The males also had a higher number of pectinal teeth (30–36) than females (25–30). In males, the body was slender with chela of pedipalp more robust than females and the males had a prominent protuberance at the proximal end of the moveable chela and scalloped on the immovable finger. Furthermore, adult males were found to have yellowish legs, metasoma and pedipalps whereas the females, were mostly reddish-brown overall.

Table 1. Geocoordinates of the localities of distribution of *Hottentotta jabalpurensis* in Madhya Pradesh (WGS-84).

Location	GPS coordinates
Damoh	N23.8210°, E79.4514°
Narsinghpur	N22.9473°, E79.1923°
Sagar	N23.8388°, E78.7378°
Ranjhi, Jabalpur	N23.1815°, E79.9864°
Patbaba area, Jabalpur	N23.1702°, E79.9747°
Sidh Baba Mandir area, Katangi	N23.4645°, E79.7980°
Nauradehi WLS	N23.3683°, E79.1718°
Chhindwara	N22.0574°, E78.9382°
Barna reservoir, Raisen	N23.0795°, E78.0310°
Kartholi, Dewas	N32.6307°, E74.9490°
Veerangana Durgavati WLS, Damoh	N21.6970°, E77.7954°
Kesli, Sagar	N23.4226°, E78.8184°
Madhav NP, Shivpuri	N25.4317°, E77.7391°
Lameta ghat, Jabalpur	N23.1092°, E79.8282°
Kala Dehi, Jabalpur	N22.8787°, E79.8293°
Panna TR, Panna District	N24.7166, E80.2000
Shahdol	N23.3022, E81.3267°
Sidhi	N24.3956°, E81.8825°
Indore	N22.6791°, E75.8580°
Singhori WLS, Kheoni	N22.1983°, E79.0579°
Pachmarhi BR	N22.4674°, E78.4346°
Ratlam	N23.3315°, E75.0367°
Bandhavgarh N. Park, Umaria	N23.7224°, E81.0242°

Mating behavior (Image 3): The mating behaviour was observed in three pairs and the findings are as follows. The process took place in captivity on three occasions on 06.iv.2018, 23.iv.2018, and 24.iv.2018

Image 1. female *Hottentotta jabalpurensis* feeding on a praying mantis while carrying second molt scorplings.

Image 2. *Hottentotta jabalpurensis* feeding on a scolopendra (centipede).

Image 3. Courtship in *Hottentotta jabalpurensis*. The male (on right) holding the pedipalps of the female (on left).

when the male and female were introduced into the same box. The courtship was always initiated by the male and he started with patting the female and trying to grasp her from pedipalps. Of the breeding pair on 06.iv.2018, the female remained immobile and withdrew the pedipalps after the first attempt made by the male. The male juddered several times while vigorously pulling the female towards him which led to holding each other through pedipalps to perform the ritual dance 'promenade á deux'. Also, occasional cheliceral massage and tail raising were observed. After a halt for 10–12 minutes, the male again pulled the female using just one pedipalp and keeping away the unused one while constantly searching for a suitable substratum with raised pectines. Occasionally the female resisted the movements, but the male moved closer to her and tried pulling her with elevated body and metasoma while the female kept her body close to the substratum with raised pectines. The male deposited the spermatophore on a small piece of wood (Image 6) following vigorous juddering and waited for the spermatophore to get dried up. The breeding process from initiation till deposition of spermatophore lasted for 30 minutes. The second pair (on 23.iv.2018) bred in the same way and the process lasted for 40 minutes. The male deposited the spermatophore on a paper. Of the third pair, which was observed on 24.iv.2018, the courtship was initiated naturally when a male entered a female's compartment. The female in this case did not resist the male, rather she was found juddering and trying to take a hold of the male's pedipalp. Although juddering in this female was not as vigorous as it was observed in the males, but the male did not produce spermatophore even after 45 minutes of courtship. Also, it was noted that in the above two cases, the females did not show any interest in sperm transfer and the males were always observed to escape the site immediately after mating. In another case where a male was kept with a female, the pair did not show any affinity for courtship until 20 hours after staying together. Another interesting behaviour observed during the study period was when a male was found to be clasping the female sitting very close to her and later fed upon the female after two days.

Spermatophore (Image 6–8): Two pre-insemination spermatophores of *H. jabalpurensis* bearing registration numbers ZSI-CZRC-7264 and 7265 were studied. The spermatophore when extruded was semi-solid at the pedicle, which came out first and got firmly stuck on the substratum. It was translucent with a pinkish brown tinge, capsule dark brown, stem dark brown on the sides and paler at the middle portion, flagellum whitish. Pedicel flat, little broader at the base and creamy white. After extrusion, it turned solid and brownish within five minutes. The capsular region was reddish-brown comprising base, capsular distal carina, capsular basal carina and basal hook. When the spermatophore was extruded by the male it got glued to the substratum by the pedicel at one end while the flagellum got attached to the other end of the substratum. The stretching of spermatophore probably helps in maintaining a particular direction after it dries up.

The spermatophores (ZSI-CZRC-A-21455 and 21456) show slight variation in stem length (6.4mm in the former and 6.7mm in the latter) and length of the flagellum (4.9mm in the former and 3.7mm in the latter). The spermatophore of ZSI-CZRC-7264 is described as follows. Most part of the flagelliform spermatophore comprises a tubular stem, which is slender, elongated, translucent and hollow, 6.4mm in length, 0.9mm width and 0.7mm in depth. The capsule is the complex part

Image 4. Female *Hottentotta jabalpurensis* with newborn babies on her back.

Image 5. Female with second instar babies.

of the spermatophore and functions as a storage for spermatozoa and responsible for sperm transfer. It is broader at the base, 1.0mm in length and tapered towards the apex, comprising a pair of tapered capsular distal carina (0.14mm in length), pair of capsular basal carina, pair of stout and apically pointed basal hook (0.07mm length). The capsule width at the region of basal hook was 0.25mm. The flagellum was 4.9mm long, extending from the ventral side of the capsular region and is divided into a thicker part, pars recta, and a distal thinner part called pars reflecta. There is a raised portion on the dorsal side of the flagellum just below the distal end of the ventral process (termed as hook).

Parturition and maternal care (Image 4,5)**:** *Hottentotta jabalpurensis* females (n=9) gave birth to 23–45 juveniles in captivity. The parturition mostly

Image 6. Pre-insemination spermatophore of *Hottentotta jabalpurensis.*

Image 7. Dorsal and ventral aspects of pre-insemination spermatophore of *Hottentotta jabalpurensis.* © Pratyush P. Mohapatra.

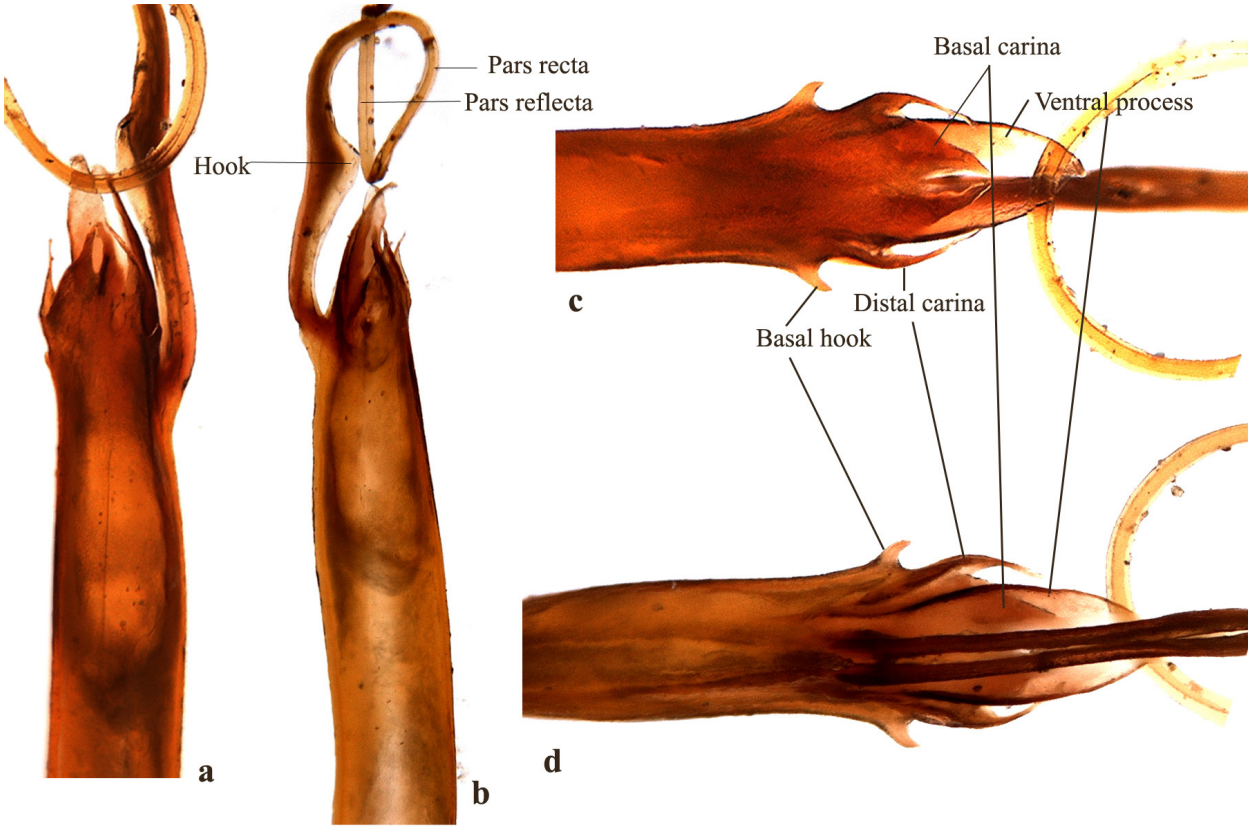

Image 8. Various structures of spermatophore of *Hottentotta jabalpurensis*: a—dorso-lateral side | b—lateral aspects | c—dorsal aspect of capsular region | d—ventral aspect of capsular region. © Pratyush P. Mohapatra.

took place during early evening hours of 17.00–20.00 h. (n= 6), or during late night 11.00–01.00 h. (n= 2) and on one occasion it took place at 14.00h. Prior to parturition the females dug a pit using their first two pairs of her legs and was found resting in a peculiar posture by bending its first pair of legs at the patellar-tibial joint making a 'birth basket', thus fencing the area in front of the genital operculum. During the process of parturition, babies came out of the genital operculum at an interval of 3–5 minutes. After emergence, the babies climbed onto the dorsum of the mother. Babies were oriented randomly on the dorsum and were found stacked in two or three layers on the dorsum, sometimes extended to the ventral part also. It was observed that juveniles remained on the dorsum till the completion of second molting after which their exoskeleton becomes hardened and they start becoming independent. It was observed that till the second molt, the babies were not feeding, although the mother was accepting food just after parturition. One among the female retained the

Image 9. Kin recognition behavior in two female *Hottentotta jabalpurensis* while still carrying second molt scorplings.

parturition after giving birth to five babies till the second ecdysis of the young ones already on her body and later when it was shifted to another box after 10 days, it again gave birth to 18 more babies.

Maternal care was found to be obligatory and while carrying babies, the mother was very attentive, cautious and mostly aggressive. In one incident one male was introduced into the box having a gravid female and when it tried to interact with the female, the female showed no interest at first but later got very aggressive and attacked the male following which the male was separated immediately. Juveniles became independent and aggressive after the second molt, i.e., after 8–10 days of birth but preferred to stay near the mother. While the babies were found moving away from the mother, in the case of the slightest disturbance they immediately ran towards the mother.

The difference between birth and first molt was 2–3 days and between the first and second molt this difference was 4–5 days (n=9). Scorplings were pale yellow at birth with translucent and slight orange in color pedipalps and metasoma, possessing clearly visible median and lateral carinae on the mesosoma as well as dorsal, lateral and ventral carinae on the metasoma. Chelicerae and legs are translucent with black patches. They have a soft exoskeleton and under-developed stinger. Hence, they cannot sting or feed and utilize the stored nutrients. After the first molt they start to change color from pale yellow to brownish-orange, carinae becomes obtrusive, the exoskeleton gets thicker and the stinger becomes hard and sharp. Colour turns brownish-black on the mesosoma and legs and orange on the pedipalp and metasoma with well-marked carinae after the second molt and characters become more prominent as they grow. After the second molt, the babies started feeding on supplied prey and sometimes, they consumed the leftover of the mother's or other juvenile's prey. Cannibalism was observed to be common among juveniles.

Kin recognition (Image 9): Juveniles interacted more than adults under captivity where they either used to avoid or feed on each other. Also, sometimes mothers were found to feed on babies soon after they were born. The most interesting observation includes two juvenile bearing females coming in contact and communicating with each other, being very close, patting one another using pedipalps. The females after being recognized stayed together for two days without harming each other or any juveniles. No cannibalism was found in this case and the juveniles of both the broods readily accepted supplied prey. Later, after a few days of second molting, when one of the females was kept in another box, she gave birth to 18 more babies.

DISCUSSION

Studies on Indian scorpions are mostly confined to taxonomy and regional checklists; however, information on bionomics is still understated. The present study is an attempt to expand the information on biological aspects of a species based on observations in captivity

as well as their natural microhabitat. This central Indian endemic species, *H. jabalpurensis* like other congeners such as *H. tamulus*, *H. pachyurus*, and *H. vinchu*, was found generally below the rocks and large boulders, and sometimes on fallen wood logs in wet regions. The coloration among males and females and the juveniles could be added as intraspecific taxonomic characters as the original description is based on old collections (Kovařík 2007). The present study extends the distribution range of the species beyond its type locality in other parts of Madhya Pradesh. As per the information available on maternal care in *H. pachyurus* by Mirza et al. (2009), the juveniles did not show much morphometric variation from that of *H. jabalpurensis*; however, the molting intervals differ as the former species showed just 3–4 days' time span between birth to the second molt which is quite fast whereas it was found to be 8–10 days in the latter species. Also, unlike *H. pachyurus,* juveniles in *H. jabalpurensis* were not found consuming their molt.

The spermatophore study on the Indian scorpion is limited to a very few species, hence an attempt has been made to compare the available data on spermatophore/ hemispermatophore of genus *Hottentotta*. The spermatophore of *H. tamulus* as reported by Bastawade (1992) varies by being placed at an angle of 20° and inverted backward whereas the spermatophore of *H. jabalpurensis* was placed at an angle of 10°. This variation might be due to differences in the state of the spermatophore and the inversion of *H. tamulus* spermatophore could be due to the female exerting pressure on the capsule while obtaining the spermatids as Bastawade (1992) described the post-insemination spermatophore. As mentioned by Bastawade (1992), the total length of the spermatophore of *H. tamulus* is 1.3–1.5 times longer and the stem is twice the length than that of *H. jabalpurensis* (present study). Variations on the capsular region between these two species could not be assessed because of a lack of mensural or meristic data on the capsular structure for *H. tamulus*. Furthermore, in comparison with the information on hemispermatophore of two chinese species, *H. pellucidus* and *H. saxinatans* provided by Lowe (2010) no comparative information could be inferred.

Iteroparity is a common phenomenon in scorpions as such reproductive strategy is observed in various species (de Albuquerquea & de Araujo Lira 2016) and there is some information available on intervals between parturitions (Lees 1955; Mathew 1962; Polis & Farley 1979; Warburg 2012). Among the Indian scorpions, Mathew (1962) discussed embryonic diapause in *Lychas tricarinatus* which was of 41–42 days. Our observation in one of the *Hottentotta jabalpurensis* giving birth to babies within an interval of 10 days is possibly a case related to unfavourable environmental conditions. Another interesting behavior observed in this study is female juddering in response to a male approaching for mating. Juddering is a common phenomenon in males during mating as a direct response to stimulate unreceptive females (Ross 2009). Even during the present study, we observed juddering as a mode of communication to suppress unreceptive counterparts. Furthermore, *Hottentotta jabalpurensis* males holding the females by engaging one of the pedipalps and keeping another free is an additional behavior recorded during the study. Hence, this study reports some additional behavioural observations in *Hottentotta jabalpurensis*, which can also be studied in other Indian species.

As envisaged from the study, there is no specific threat to the species. This species is highly adaptable and observed in various micro-habitat types ranging from human-modified habitats to undisturbed forests. *H. jabalpurensis* was found near human habitation, agricultural fields, scrub forest, deciduous forest and semi-evergreen forests and was found below small to large boulders, logs and crevices. This species also occurs in a large number of protected areas such as Veerangana Durgavati WS, Singhori WS, Kanha TR, Bandhavgarh TR, Satpura TR, Bori WS, and Sanjay NP. As this species is considered potentially dangerous for humans and its envenomation has the possibility of confusion with snake-bite, study on human-scorpion interaction can be undertaken to understand the prevalence of such conflicts. A case of envenomation as observed by one of the authors (PPM), following the sting on the right-hand thumb is described as follows. There was swelling and systemic pain around the affected area with a feeling of dizziness, followed by uncoordinated movements for about 6hr. The swelling became normal after 56–60 h following the envenomation.

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