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Succession study on forensically important Coleoptera from India: a preliminary study and its forensic implications

Neha Singh[1*] and Madhu Bala[2]

Abstract

Background: Coleoptera is the second most important group of entomofauna associated with decomposition, yet little is known about its role in decomposition and postmortem colonization of carcasses in India and other parts of the world. Consequently, a preliminary study was conducted to study the succession of forensically important Coleoptera using goat carrion as an animal model.

Results: Five decomposition stages were observed during the experiment. A total of 1174 adult coleopterans belonging to 21 species and seven families were collected during this study. Throughout faunal succession, Histeridae and Silphidae were the dominant Coleopteran families, and they occurred mainly during the bloated and decay stages of decomposition; *Necrophila* (*Calosilpha*) *ioptera* was the dominant species present from the bloated to dry stage of putrescence. Two species, *Pachylister bellicus* and *Saprinus sternifossa* were reported for the first time from India.

Conclusion: This study provides baseline data regarding Coleopteran species associated with different decomposition stages, but more extensive studies need to be performed to develop a geographical database on arthropod succession from as many habitats as possible so that this kind of data can be used in forensic cases.

Keywords: Forensic entomology, Carrion, Decomposition, Insect succession, Coleoptera

Background

Forensic entomology applies evidence from insect to legal problems such as the estimation of the minimum postmortem interval (mPMI). It is, therefore, indispensable to know the insect fauna that is attracted to humans across varied geographic regions (Shin et al. 2015). Animal carcasses are widely used as experimental models to study faunal succession as they are a temporary and rapidly changing food source to many creatures. Insects, especially Diptera and Coleoptera, are generally the first to arrive because they are attracted by different odors and gases that are released by a carcass, and they use it as a source of food, reproduction, and larval development (Cornaby 1974). The environmental factors such as temperature, humidity, rainfall, season, and micro-climate of the surrounding postmortem habitat play a significant role in the determination of insect fauna on carrion, and they may combine differently at different crime scenes. If these factors are not considered, a falsifying effect on the accuracy of the mPMI estimate may emerge (Eberhardt & Elliot 2008).

Flies are used primarily as entomological evidence to estimate mPMI, and beetles have been found to be the primary entomological evidence for a corpse in the advanced decomposition stage but their usefulness to estimate the mPMI needs to be emphasized (Kulshrestha & Satpathy 2001). According to Villet (2011), the families of Coleoptera that are of forensic importance are Silphidae, Histeridae, Staphylinidae, Dermestidae, Cleridae, Trogidae, and Scarabaeidae. The scarcity of data on the ecology and behavior of beetles is the main hurdle in using them as forensic evidence. However, knowledge on the ecology of forensically important beetles could not only help in PMI/PAI estimation (Matuszewski 2011, 2012; Souza et al. 2014), but also be used to support mPMI estimates from Dipteran data

* Correspondence: nehasinghko@gmail.com
[1]Department of Zoology & Environmental Sciences, Punjabi University, Patiala, India
Full list of author information is available at the end of the article

(Goff & Flynn 1991). The fact that beetles populate at the corpse later than flies likely defines their specificity with a particular stage of decomposition (Varatharajan & Sen 2000) or as a form of adaptation to reduce competition with flies (Smith 1986). Beetles can be used as mPMI indicators, but knowledge of their biology and life cycle is essential to be used along with the expertise on systematic evidence. There are various studies where beetles have been observed on human corpses (Kulshrestha & Satpathy 2001; Barreto et al. 2002; Sukontason et al. 2007; Mariani et al. 2014; Sharma et al. 2018, Moemenbellah-Fard 2018). Moreover, some studies have shown the successful implication of beetle data collected from the human corpse to estimate the postmortem interval (Kumara et al. 2009; Ridgeway et al. 2013).

It is well established that arthropod appearance on carrion follows a predictable sequence (Payne 1965; Rodriguez & Bass 1983; Early & Goff 1986; Anderson & VanLaerhoven 1996; Grassberger & Frank 2004; Tabor et al. 2004; Gill, 2011) at the family level. The mPMI can be estimated by comparing the taxa found on human carrion with the known succession patterns in that particular habitat or geographical area using the animal models under controlled conditions (Tabor et al. 2005). Most of the families that are attracted to corpses are ubiquitous; however, their distribution can vary across regions which emphasize the need to perform local studies (Castro et al. 2013).

The purpose of this study was to observe the process of beetle succession using a goat carcass and identify different beetles that are associated with various stages of decomposition to provide baseline data. The current study was limited by a small sample size and was preliminary, but the data provides a summary of some of the forensically important beetles.

Material and methods

Study area

The experiment was performed during May 2014 (2nd–16th) in Kullu, Himachal Pradesh, India. This area is a broad open valley, with the highest temperatures ranging from 24 to 34 °C from May to August. During December and January, the lowest temperatures are observed, which range from −4 to 20 °C and is accompanied by some snowfall. The average annual rainfall is 800 mm.

The study was conducted in a private orchid farm (31° 57′ 35.9″ N, 77° 07′ 21.8″ E). The site is away from the urban area of the main city and was selected to avoid disturbances. The vegetation is characterized by native shrubs and apple, plum, almond, and permission trees, and the local vertebrate fauna comprises wild dogs, jackals, snakes, and lizards.

For the collection purposes, goat carrion weighing about 3 kg was used and covered with a cage (24 × 20 × 9 in) to prevent the scavengers. There were three replicates of the experiment (n = 3), separated each other by a distance of 600 m. Carcasses were partially in the shade depending upon the time of the day.

Insect collection and identification

Sampling was done by thoroughly inspecting the carrion during which adult specimens were collected with the help of forceps with minimum disturbance. The collection began 24 h after placing the carrion and was done two times a day, morning and evening, till the 11th day of decomposition, after which it took place once a day until skeletonization of the carrion. Temperature and relative humidity at the main and replicate experimental sites were recorded at the time of sampling using a portable thermo-hygrometer.

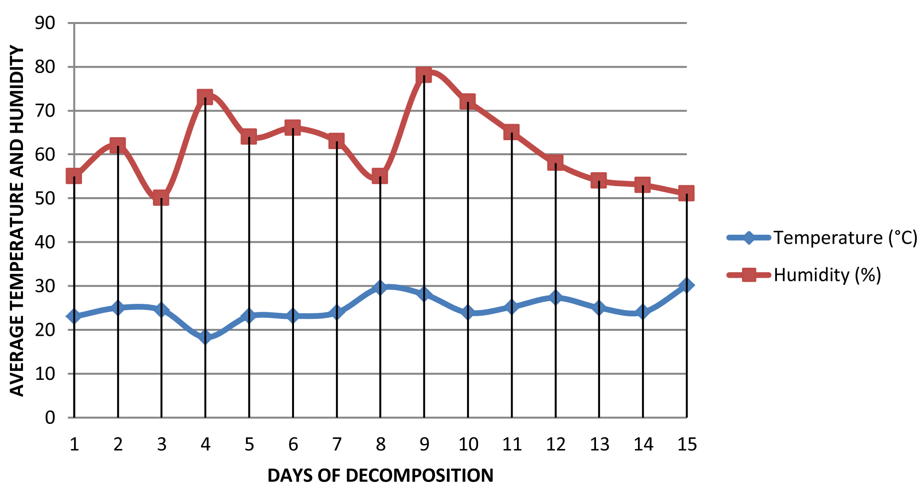

Fig. 1 Meteorological data showing average temperatures and humidity during the experiment. This figure describes the average temperature and humidity of each day during the whole decomposition process

All adult beetles were collected regardless of their behavior or feeding habits on the carrion. Collected specimens were categorized as necrophagous and non-necrophagous on the basis of their feeding behavior. Necrophagous beetles consisted of the ones which were observed feeding upon the decaying meat while the rest was considered as non-necrophagous. Collected fauna was stored in 70% alcohol in vials at the site until they were brought to the laboratory for the identification with the help of taxonomic keys (Peacock 1993; Ruzicka & Schneider 1996, 2002, 2011; Ruzicka et al. 2000, 2011, 2015). Beetle larvae belonging to silphids and dermestids were collected as an entity, but not identified to the species level; hence, they were not included in the study.

Data analysis

The variation of species with each decomposition stage and their feeding habits were analyzed with the Kruskal–Wallis test (Minitab 18). Chi-square was performed for comparison between necrophagous and non-necrophagous species in all replicates using a 5% confidence level.

Results

Meteorological data

The average ambient temperature and relative humidity were 24.9 °C and 61.2%, respectively, at all sites (Fig. 1). There was no significant change observed during replicates in terms of decomposition stages ($P > 0.05$, D.F. = 2).

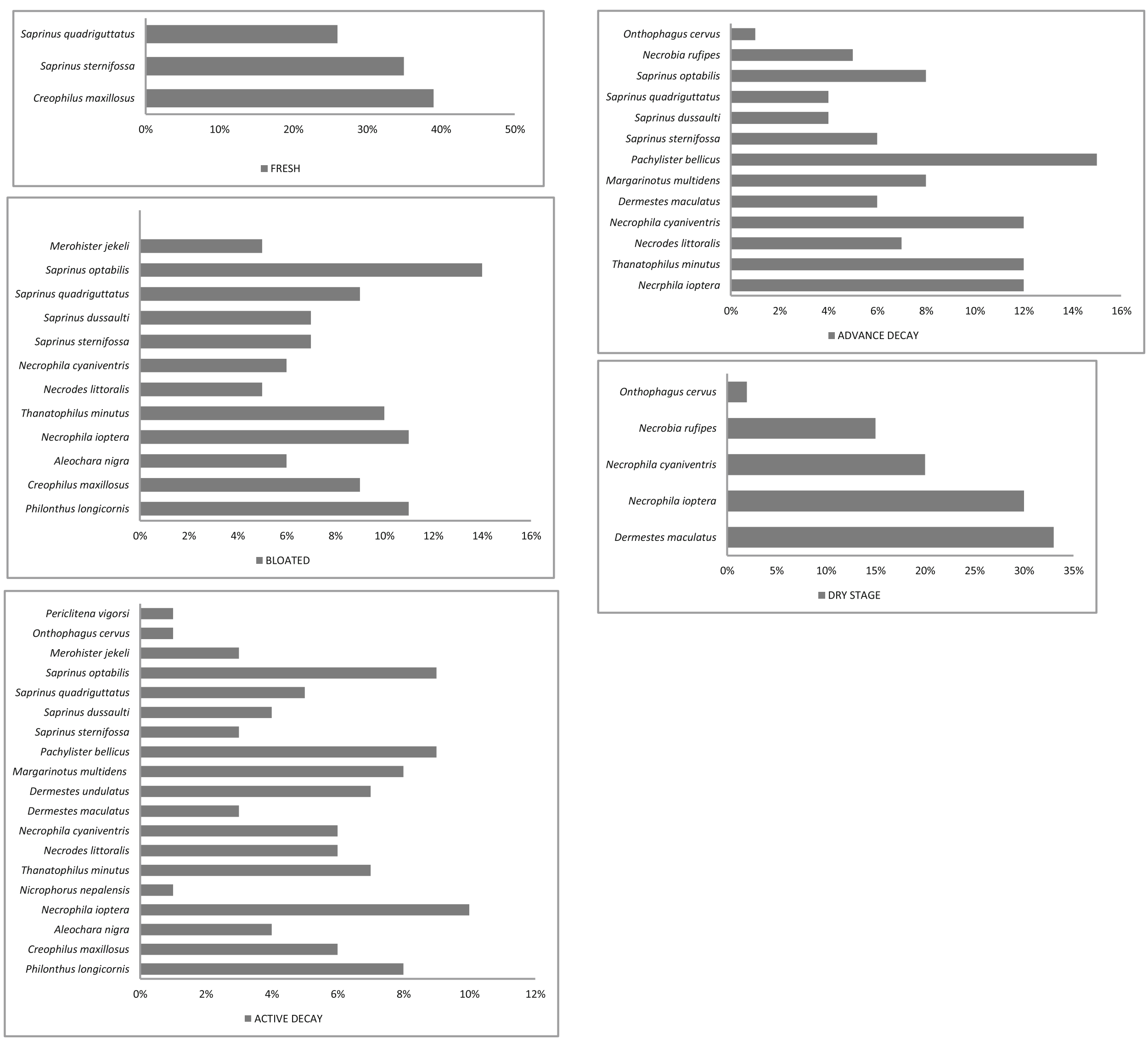

Fig. 2 Composition of Coleoptera (species) during five decomposition stages. These charts explain the percentage of each species found at every stage of decomposition during experiment

Decomposition process

Complete decomposition occurred in 15 days. Five decomposition stages were observed (fresh, bloated, active decay, advanced decay, and dry) in all the replicates which were in accordance with the stages mentioned by Anderson and VanLaerhoven (1996), and the composition of beetle species at each decomposition stage is shown in Fig. 2.

Beetle community and feeding habits

We collected 1174 adult coleopterans from 21 species and seven families during this study. Species richness and abundance reached a maximum (19 species) during the active decay stage, followed by the bloated and advanced decay stages having 13 species each, while the minimum number of species were observed during the fresh stage (Fig. 2). The chronological sequence of their arrival in relation to the decomposition days with all replicates is shown in Table 1. The Kruskal–Wallis test (Minitab 18) showed that there was no statistically significant difference between the number of individuals and families found at each decomposition stage among all replicates (P = 0.993 ≥ 0.05, D.F. = 2). Beetle species of different feeding habits (Table 2) were divided into two categories of necrophagous and non-necrophagous. In terms of abundance, no significant difference was found between necrophagous and non-necrophagous species in all three replicates (χ^2 = 1.037, P > 0.05, D.F. = 2).

Discussion

As previously discussed, insects play a significant role in the decomposition process, and they are intimate witnesses to the postmortem phase of carcasses (Krikken & Huibregts 2001); however, there are several factors that can affect the colonization of insects on carrion, and these factors can alter the rate of decomposition progression. Rainfall is a factor that can affect the rate of decomposition since rain can soak the carcass and expel fly larvae (Lyu et al. 2016). In this study, increased maggot activity was observed after a night of rainfall on the 9th day of decomposition.

A high proportion of beetles were collected during the early stages of decomposition, which is in contrast to previous studies that suggest the coleopterans do not occupy carrion until later stages of decomposition. However, there

Table 1 List of all beetle species and their number found during this study

Family	Species	No. of specimens (%)	Feeding habit[#]
Staphylinidae	*Philonthus longicornis* (Stephens, 1832)	72 (6.1%)	P
	Creophilus maxillosus (Linnaeus, 1758)	76 (6.5%)	P
	Aleochara nigra (Kraatz, 1859)	40 (3.4%)	P
Silphidae	*Necrophila* (*Calosilpha*)*ioptera* (Kollar & Redtenbacher,1848)	130 (11.1%)	N
	Nicrophorus nepalensis (Hope, 1831)	2 (0.2%)	N
	Thanatophilus minutus (Kraatz, 1876)	95(8.1%)	N
	Necrodes littoralis (Linnaeus, 1758)	63 (5.4%)	N
	Necrophila (*Calosilpha*) *cyaniventris* (Motschulsky, 1870)	90 (7.7%)	N
Dermestidae	*Dermestes maculatus* De Geer, 1774	49 (4.2%)	N
	Dermestes undulatus Brahm, 1790	31 (2.7%)	N
Histeridae	*Margarinotus multidens* (Schmidt, 1889)	57 (4.9%)	P
	*Pachylister bellicus** (Marseul,1884)	73 (6.2%)	P
	*Saprinus sternifossa** G.Muller, 1937	69 (5.9%)	P
	Saprinus dussaulti Marseul, 1870	44 (3.8%)	P
	Saprinus quadriguttatus (Fabricius, 1798)	74 (6.3%)	P
	Saprinus optabilis Marseul, 1855	107 (9.1%)	P
	Merohister jekeli (Marseul,1857)*Merohister jekeli* (Marseul,1857)	33(2.8%)	P
Cleridae	*Necrobia rufipes* (De Geer, 1775)	22(1.9%)	N
Scarabaeidae	*Onthophagus cervus* (Fabricius,1801)	10 (0.9%)	C
	Onthophagus sp.	7 (0.6%)	O
Chrysomelidae	*Periclitena vigorsi* (Hope, 1831)	3 (0.3%)	O
Total specimens		1174	
Total feeding categories			4

[#]N, necrophagous; P, predator; C, coprophagous; O, others
*First time reported from India according to Mazur (2011)

Table 2 Chronological sequence of beetle occurrence during different decomposition stages

Adult fauna			Days of decomposition														
			Fresh		Bloated			Active decay				Adv. decay			Dry		
S.No.	Family	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Staphylinidae	*Philonthus longicornis*															
		Creophilus maxillosus															
		Aleochara nigra															
2	Silphidae	*Necrophila* (*Calosilpha*) *ioptera*															
		Nicrophorus nepalensis															
		Thanatophilus minutus															
		Necrodes littoralis															
		Necrophila (*Calosilpha*) *cyaniventris*															
3	Dermestidae	*Dermestes maculatus*															
		Dermestes undulatus															
4	Histeridae	*Margarinotus multidens*															
		Pachylister bellicus															
		Saprinus sternifossa															
		Saprinus dussaulti															
		Saprinus quadriguttatus															
		Saprinus optabilis															
		Merohister jekeli															
5	Cleridae	*Necrobia rufipes*															
6	Scarabaeidae	*Onthophagus cervus*															
		Onthophagus sp.															
7	Chrysomelidae	*Periclitena vigorsi*															

are various reports in the literature supporting their early arrival on carcasses (Braack 1987; Midgley et al. 2010; Mayer & Vasconcelos 2013). Histeridae and Staphylinidae were the first to arrive after 24 h of carrion placement, which supports the claim of the early arrival of coleopterans on carcasses. The fresh stage was dominated by predators from the families Staphylinidae and Histeridae: *Creophilus maxillous* (39%), *Saprinus quadriguttatus* (35%), and *Saprinus sternifossa* (26%). During the bloated stage, out of the 13 species identified, the dominant

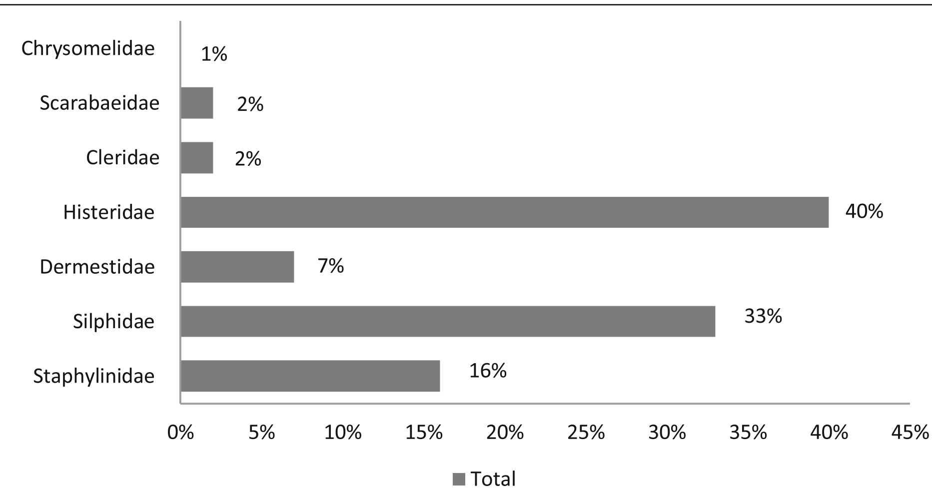

Fig. 3 Composition of Coleoptera (families) communities during decomposition. Bar chart explaining the number of specimens of seven families (%) found during the experiment

species were *Philonthus longicornis* (11%), *Necrophila* (*Calosilpha*) *ioptera* (11%), and *Saprinus optabilis* (14%). Active decay was dominated by *Necrophila* (*Calosilpha*) *ioptera* (10%). During advanced decay, when maggots and beetles had already devoured most of the flesh, the most abundant beetle species included *Pachylister bellicus* (15%), *Necrophila* (*Calosilpha*) *ioptera* (12%), and *Necrophila* (*Calosilpha*) *cyaniventris* (12%). At the dry stage when mostly the bones remained, *Dermestes maculatus* (33%) was dominant.

Histeridae (40%, Fig. 3) had the highest species richness of all families, among which *Saprinus optabilis* was the most abundant species. Seven species of histerid beetles were collected, with *Pachylister bellicus* (during active decay) and *Saprinus sternifossa* (during bloated and active decay) being first time reported from India as per Mazur (2011). This demonstrates that studies like the current one are not only beneficial for forensic but are also of taxonomic importance.

The invasion of Silphidae (33%) on the carrion began at the bloated stage, and they were maintained at a medium abundance during the active and advanced decay stages. *Necrophila* (*Calosilpha*) *ioptera* from this family was the dominant species in this study. Of the five species collected, *Necrophila* (*Calosilpha*) *ioptera* and *Nercophila* (*Calosilpha*) *cyaniventris* were present from bloated to dry stage whereas *Thanatophilus minutus* and *Necrodes littoralis* were present from the bloated to active decay stages (Fig. 2). *Nercophila* (*Calosilpha*) *cyaniventris* had been reported before similar studies conducted in India (Bharti & Singh 2003). *Nicrophorus nepalensis* is a carrion-feeding beetle and is known to be attracted to small carrions (Hwang & Shiao 2011), but there were only two specimens recorded during the active decay stage in this study. However, their appearance indicates its general presence in the study habitat and area.

Staphylinidae (16%) was the third most abundant family found in this study (Fig. 3), and it was observed during the bloated and active decay. And it appeared with *Creophilus maxillosus* after 24 h of bait placement. Three species of this family (*Philonthus longicornis*, *Creophilus maxillosus*, and *Aleochara nigra*) appeared from the bloated to the active decay stages and preyed on fly larvae, showing predatory behavior of these species on maggots as reported in the literature (Byrd & Castner 2010; Castro et al. 2010; Lyu et al. 2016). *Philonthus longicornis* was reported from the previous study conducted on pig carrion (Kaur & Bala 2018).

Both larvae and adults of Dermestids were observed. Two species of Dermestidae, *Dermestes maculatus* and *Dermestes undulatus*, represented the 7% of the total collected beetle fauna and were observed during the active decay stage but only *Dermestes maculatus* remained until the dry stage. Larvae of dermestids were the dominant insect fauna during the dry stages.

Necrobia rufipes is known to have a cosmopolitan distribution and widely reported in various forensic studies. Single species (2%) of the Cleridae family was collected during the advanced decay stage. However, only a few numbers of these were collected despite it being associated with forensic studies worldwide.

One species in the family Chrysomelidae, *Periclitena vigorsi*, was observed during one of the experiments. According to Anton et al. (2011), family Chrysomelidae is an accidental visitor to carrion. However, it has been reported in forensic literature (Miralbes 2002; Castro 2011; Lopes, 2012). Further studies need to be conducted to examine their forensic importance. Two species in the family Scarabaeidae, *Onthophagus cervus* and *Onthophagus* sp., showing coprophagous behavior were found during the experiments.

We agree that low replication limits the applicability of findings to some extent in legal cases, but this data could represent a valuable baseline for studies aiming to understand decomposition processes and to refine the identification for taxa of forensic importance in poorly studied regions of world such as India which is the seventh largest country in terms of area and it has a varied geographical and climatic conditions.

Conclusion

To the best of our knowledge, this is the first inventory conducted to study the beetle succession on carrion in India specifically. *Necrobia rufipes* and *Dermestes maculatus* are widely studied because of their forensic importance, and both were observed in this study. The presence of such species that has cosmopolitan distribution supports their forensic relevance, but lack of their developmental data is the primary hurdle in using these for the estimation of postmortem interval. There should be more developmental models of beetle species along with their succession information and ecology. More extensive studies need to be performed in order to develop a geographical database on arthropod succession from different habitats and their endemic species which can reinforce the practical use of insect species in forensic cases to estimate the mPMI.

Abbreviations

mPMI: Minimum postmortem interval; PAI: Pre-appearance interval; F: Fahrenheit; kg: Kilograms; m: Meters; h: Hours; D.F: Degree of freedom; Fig.: Figure; sp.: Species

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Authors' contributions

Both the authors have contributed in the study. All authors read and approved the final manuscript.

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Availability of data and materials
Not applicable

Ethics approval and consent to participate
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Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests

Author details
[1]Department of Zoology & Environmental Sciences, Punjabi University, Patiala, India. [2]Department of Zoology & Environmental Sciences, Punjabi University, Patiala, India.

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