Report on *Crioceris* sp. infestation and damage to medicinal crop *Asparagus racemosus* in New Delhi, India

ABSTRACT

The current study presents the identification and characterization of a newly reported insect species, *Crioceris paracenthesis*, discovered infesting *Asparagus racemosus* in New Delhi, India. Through comprehensive morphological and behavioural analyses, distinct sexual dimorphism was observed, with black males and yellowish-brown females displaying a characteristic black spot on the inner edge of the posterior part of the elytra. Detailed descriptions of morphological traits, including colouration, marking patterns, and antennae structure, were provided. Molecular identification confirmed its close relationship (88.15% identity) with *Crioceris paracenthesis*. Insights into the life cycle revealed hibernation during winter, emergence in spring for mating and egg-laying, and subsequent larval development through four instars before pupation in the soil. Observations on mating behaviour indicated polygynandrous mating, alongside defensive mechanisms such as feigning death in response to threats. The study underscored the significant damage inflicted by *Crioceris sp*. larvae and adults on *Asparagus racemosus*, leading to broken cladophylls, notches along leaf edges, and extensive defoliation, thereby impeding plant growth. This research enhances our understanding of Chrysomelidae beetle diversity associated with asparagus crops, calling for further taxonomic investigations to formally classify this newly discovered *Crioceris* species.

Key words: Medicinal plant**;** pest damage**;** coleopteran**;** molecular identification**;** chrysomelid.

1. INTRODUCTION

Asparagus racemosus Willd, commonly known as Shatavari, is a woody climber belonging to the Asparagaceae family. It is a perennial plant characterised by its highly branched structure, reaching a height of 1 to 2 metres. Shatavari bears small, needle-like leaves and white flowers with small spikes [1]. Thriving in gravelly, rocky soils, this species is typically found at elevations of 1300 to 1400 metres in the piedmont plains [2]. Its botanical description was initially recorded in 1799 in the United States. *Asparagus racemosus* is part of the diverse *Asparagus* genus, which encompasses over 250 species worldwide, with 22 species present in India. Its distribution spans across India, Sri Lanka, and the Himalayan regions, as well as certain areas of Australia and Africa. The cultivation of *A. racemosus* is practised in various Indian states, including Arunachal Pradesh, Himachal Pradesh, Delhi, Assam, Gujarat, Karnataka, Chhattisgarh, Kerala, Haryana, Punjab, and Jharkhand. The high demand *for A. racemosus* is attributable to its wide range of applications. Unfortunately, due to destructive harvesting, habitat degradation, and deforestation, the species is currently listed as endangered in its natural habitat.

Shatavari, a term rooted in Ayurvedic tradition, translates to "Who has a hundred husbands or is agreeable to many." Recognised as the "Queen of Herbs" in Ayurveda, this remarkable herb possesses an energy value of 22 kcal/100g [3] and finds utility in treating various conditions such as madhur rasam, madhur vipakam, seet-veeryam, som rogam, chronic fever, and internal heat [4]. Shatavari's efficacy is particularly notable in addressing issues related to the female reproductive system. References to Shatavari can be found in significant Ayurvedic texts like the Charak Samhita by Charak and the Ashtang Hridyam by Vagbhata, which provide insights into the treatment of women's health disorders [5-6]. The roots of *A. racemosus* contain steroidal saponins known as Shatavarins I–IV, which serve as the plant's

primary active components. These roots exhibit diverse pharmacological activities, including aphrodisiac, antioxidant, immunostimulant, antihepatotoxic, antibacterial, antidiabetic, anticarcinogenic, antidiarrheal, antiulcerogenic, and antioxytocic effects [7-8]

Asparagus racemosus (Shatavari) is susceptible to various pests that can cause harm to the plant. Aphids are a common pest that feeds on the plant's sap, resulting in stunted growth and distorted leaves. Red Spider Mites pose another threat, as they cause yellowing and the formation of webbing on the undersides of leaves. Whiteflies gather in clusters on the leaf undersides and extract the plant's juices, leading to yellowing, stunted growth, and the development of sooty mold. Thrips, slender insects, feed on the plant cells, causing a silvering or bronzing effect on the leaves. Mealybugs, covered in a waxy substance, extract sap from the plant and produce honeydew, which attracts sooty mold [9].

Apart from these pests, the Asparagus Beetle (*Crioceris asparagi*) is a particularly significant threat to *Asparagus racemosus* plants. These beetles are characterised by their distinctive red or orange colour with black spots. Both the adult beetles and their larvae feed on the foliage of asparagus plants, resulting in considerable damage. The adult beetles emerge in the spring and primarily feed on the tender shoots and foliage. They chew on the leaves, leaving behind characteristic feeding notches and causing skeletonization. The larvae, resembling small grubs and having a dark coloration, also feed on the foliage and can cause extensive harm if not controlled. The presence of Asparagus beetles weakens the plants, reducing their vitality. Severe infestations can lead to defoliation, decreased productivity, and even plant death. Moreover, the beetles lay eggs on the plants, contributing to the perpetuation of the infestation [10].

To examine the dynamics of insect pests, including their diversity, dispersal, and invasion within the *Asparagus racemosus* crop ecosystem, a periodic survey was conducted in various

locations across the National Capital Region (NCR) of New Delhi. This survey aimed to gain insights into the changing scenarios of these pests, providing valuable information for effective pest management strategies and the maintenance of healthy *Asparagus racemosus* crops.

2. MATERIALS AND METHOD

2.1 Collection, transportation, and preservation of the specimen:

In a 100-m^2 *Crioceris*-infested plot, ten randomly selected *Asparagus racemosus* plants were sampled for insect collection. The adult beetles were challenging to collect due to their active behaviour. However, the larvae were easily collected as they remained attached to the shoots. To collect the adult beetles, a sweep net was used, and they were later transferred to a killing jar. Subsequently, the adults were placed in 30 ml screw-capped vials filled with 70% alcohol and transported to the laboratory. In the lab, the specimens were taken out, and the alcohol was dried using blotting papers. The adult beetles were then pinned at the left elytra using standard insect pins and left to dry completely for three days. After drying, the dried and pinned specimens were stored in standard insect boxes. The holotype of this specimen is deposited at National Pusa Collection (NPC) laboratory. And for species identification, we compared reference specimens and pertinent literature.

For molecular identification, beetle samples were collected and stored in 70% ethanol at -20 degrees Celsius until DNA extraction. DNA extraction was performed using the DNA Sure Tissue Mini Kit (Qiagen #NP-61305) following the manufacturer's instructions. The extracted DNA was examined on a 0.8% agarose gel containing 0.5 g/ml of ethidium bromide, and the quantified DNA was used for further PCR analysis. A partial mtCOI gene

fragment was amplified using the universal primers LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAACTTCAGGGTGACCAAAAAATCA-3'). PCR amplification was carried out in a 25-µl reaction mixture containing 12.5 µl of PCR master mix (Promega M750A), 7.5 µl of nuclease-free water, 1 µl of forward and reverse primers, and 3 µl of DNA template [11]. The PCR-amplified product (3 µl) was electrophoresed at 100 V for 45 minutes on a 1.2% agarose gel in 1X TAE buffer. The purification and sequencing of the PCR-amplified products were outsourced. BLAST analysis was performed to search for homologous sequences using the National Centre for Biotechnology Information (NCBI) database (http://ncbi.nlm.nih.gov/BLAST). The obtained sequence was submitted to NCBI GenBank to obtain accession numbers. Multiple alignments for homology search were conducted using the Clustal W algorithm software, with the default gap penalty values being gap opening 10 and extension 0.2 with a p-distance model and pairwise gap deletion selected. Dendrograms by the neighbour joining method were generated using MEGA11 software, incorporating the isolate from the current study and reference strain sequences obtained from GenBank [12].

3. RESULT

During the survey, on February 22, 2023, at Rashtrapati Bhavan Amrit Udyan in New Delhi, India (latitude: 28.6156, longitude: 77.1984), the first report of *Crioceris* sp. on *A. racemosus* was documented. The adult insects displayed colour sexual dimorphism and measured approximately 6 to 7 mm long, with the male being black and the female being yellowish brown with a black spot on the inner edge of the posterior part of the elytra. Additionally, a faint black patch was observed on the posterior part of the elytra. Both adults had 11 segmented antennae, which were inserted in front of the eyes and widely separated. The front coxa was conical and contiguous, providing a distinctive feature. The prothorax was narrower

than the elytra at its base, and a strong middle constriction was typically present. The elytra exhibited repetitive punctures in several rows, and the tarsal claws were simple and broadly spread out from the base. On the ventral side, only five abdominal segments were visible, with the remaining segments concealed **(Fig. 1 and 2).**

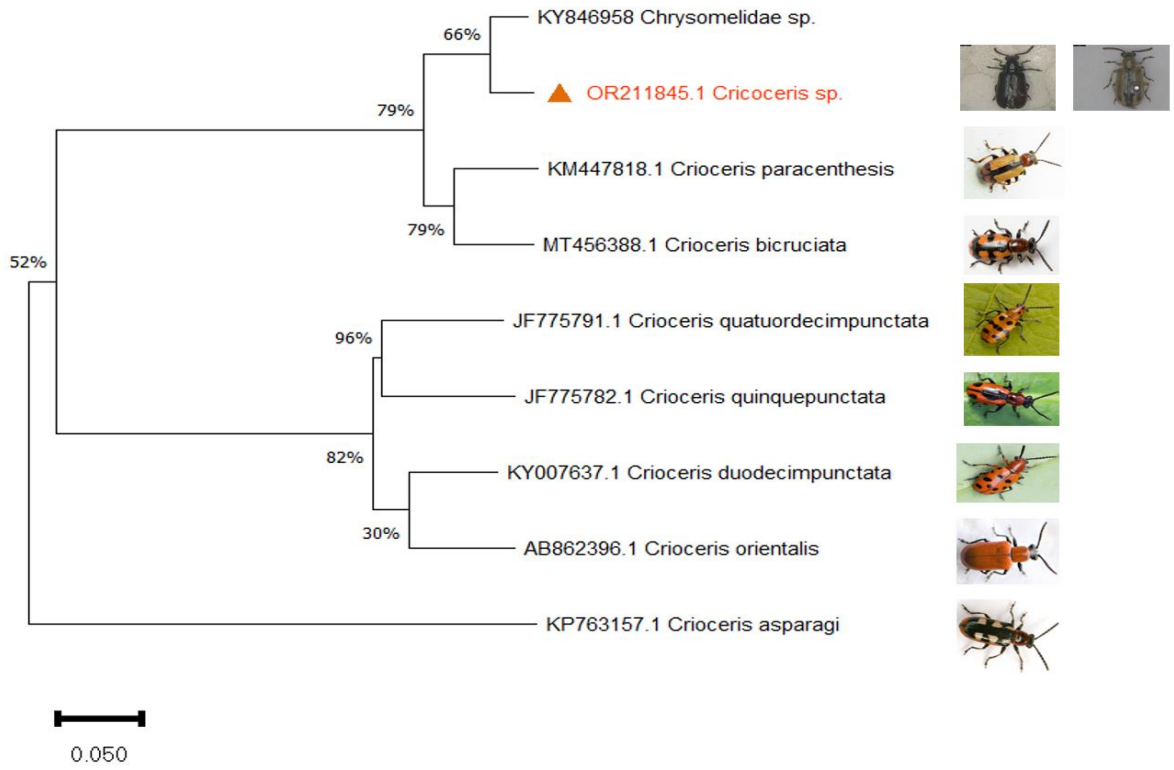
Fig. 1 Image of new black colour male of *Crioceris* sp. collected from Rashtrapati Bhavan, Herbal Garden: a: dorsal view adult; b: ventral view of adult; c: lateral view of adult

Fig. 2 Image of new brown colour female of *Crioceris* sp. collected from Rashtrapati Bhavan, Herbal Garden: a: dorsal view adult; b: ventral view of adult; c: lateral view of adult

The eggs of *Crioceris* sp. were elongate, conical, and relatively large compared to the adult body size. They displayed a greyish-brown colour. The larva of the species appeared dark greyish to creamish white, with a black head and three pairs of black legs **(Fig. 3).** Diagnostic colour characters are commonly used to distinguish between different insect species, and many leaf beetles, including the criocerine, exhibit such colour characters [13]. This new species differed from existing ones in terms of its morphological appearance, particularly in its varied coloration and marking patterns from that of available described species **(Fig. 4).** Furthermore, molecular identification using the NCBI BLAST algorithm indicated an 88.15% identity with *Crioceris paracenthesis*, and the obtained sequence was assigned an accession number (OR211845.1) (**Fig. 4).**

Fig. 3 Infestation of new *Crioceris* sp. larva on *Asparagus racemosus*

Fig. 4 Molecular identifications of *new Crioceris* sp.



The sequence highlighted in red colour indicates the sequences generated in this study.

During the winter survey, we observed that the adult *Crioceris* sp. went into hibernation and emerged in the spring. They laid their eggs on the new shoots of asparagus plants. The incubation period for the eggs ranged from 5 to 12 days, and upon hatching, the larvae immediately began feeding on the young asparagus. The larval period lasted for 2 to 3 weeks, during which they went through four instars. In the final instar, the larvae fell to the soil and formed a pupa in an earthen cell. Adults emerged after 5 to 8 days.

We noticed that this *Crioceris* sp. engaged in mating behaviour after the winter hibernation and a few days of feeding. This new species exhibited polygynandrous mating behaviour, where females paired with several males, and each male also paired with several different females. The males rode on the backs of females, while females exhibited behaviours such as kicking the males and moving their abdomens away to avoid mating. Shortly after mating, the

females oviposited singly or in groups on leaves or spears. Multiple eggs were laid in a row along the plant, and they were coated with a dark green or brown adhesive secreted by the female.

During our observations, we found that this insect species was highly active during the daytime, especially while running up and down the plant and evading predators. While they were capable of flight, they did not frequently engage in it. Additionally, when attacked by predators, they sometimes played dead as a defence mechanism.

In terms of plant damage, both the larvae and adults of this *Crioceris* sp. fed *on Asparagus racemosus* by chewing. The larvae fed within developing asparagus berries, consuming their way out, and also fed on young spears. The adults fed on young spears and consumed buds, causing damage early in the season. The damage was characterised by broken cladophylls and the removal of photosynthetic tissue **(Fig. 5a)**. The beetles chewed on the leaves, creating distinctive feeding notches along the leaf edges. These notches appeared as irregular, jagged edges. They also fed on the soft tissue between the leaf veins, leaving behind a network of veins and translucent leaf remnants. This gave the affected leaves a skeletal appearance. Severe infestations of these beetles could result in extensive defoliation of the plant **(Fig. 5b).** Their feeding activity weakened the plants, hindering their growth **(Fig. 5c)** compared to healthy plants **(Fig. 5d).** The damage caused by these pests included stunted growth, resulting in smaller and less productive plants. We noticed severe infestation accounting 60-80 grubs per spear and adults were 8 to 10 per spear.

Fig. 5 a) Skeletisation of spears by removal of photosynthetic tissue b) extensive defoliation of the plant c) extensive infestation led to plant death d) healthy plant

a) **b)**

c) **d)**

4. DISCUSSION

This new report presents information that is different from previous reports documented across the globe. In Europe and North America, two species, *Crioceris duodecimpunctata* (Linnaeus, 1758) and *C. asparagi*, are recognized as serious pests of *Asparagus officinalis* [14-15]. The first record of *C. asparagi* in Lithuania's Vilnius environs was published by Tenenbaum [16], and *Crioceris asparagi* and *Crioceris duodecimpunctata* were observed in western Massachusetts on *A. officinalis* by Capinera [17]. Major pests that contributed to the decline of Dutch asparagus production include the asparagus fly (*Platyparea poeciloptera* Schrank) and asparagus beetles (*Crioceris duodecimpunctata* L. and *C. asparagi* L.) [18].

Crioceris quatuordecimpunctata (Scopoli), commonly known as the asparagus beetle, has also been reported in *Asparagus acreage* in China [19]. The asparagus beetle observed in the wild asparagus field differs from the other two chrysomelidae beetles (*Crioceris asparagi* (L.) and *Crioceris duodecimpunctata* (L.)) reported on garden asparagus in California [20]. In 2015, a report of *Crioceris asparagi* (L.) and *Crioceris duodecimpunctata* on *A. racemosus* was documented in Nepal (PQPMC) [21].

In India, there have been previous reports on chrysomelidae beetles. *Crioceris hampsoni* from Niligiri hills India by Hampson [22]. And it differs from our specimen by having dark yellow colour with 12 pairs of dark spots on elytra**.** And another chrysomelidae beetle *Lema downesi* Baly, which was reported on wild asparagus [23]. Even Saravanan and Vipin [24] reported *Lema downesi* from *A. racemosus* in India. Additionally, *Crioceris aparagi* has been reported for the first time in India on *Asparagus filicinus* [25] and our specimen clearly differs from this in elytra colour pattern and also absence of three pairs of white spots **(Fig. 4).** Therefore, our study is the first to report the presence of the *Crioceris* genus on *Asparagus racemosus* in India. Moreover, our study identified a new species of *Crioceris* that differs significantly from previously described species worldwide based on morphological and molecular evidence. In order to name the new species further in-depth taxonomic study is needed.

Figure 6. Image of new black colour male of *Crioceris* sp. collected from Rashtrapati Bhavan, Herbal Garden: a: dorsal view adult; b: ventral view of adult; c: lateral view of adult

Figure 7. Image of new brown colour female of *Crioceris* sp. collected from Rashtrapati Bhavan, Herbal Garden: a: dorsal view adult; b: ventral view of adult; c: lateral view of adult

Figure 8. Infestation of new *Crioceris* sp. larva on *Asparagus racemosus*

Figure 9. Molecular identifications of *new Crioceris* sp.

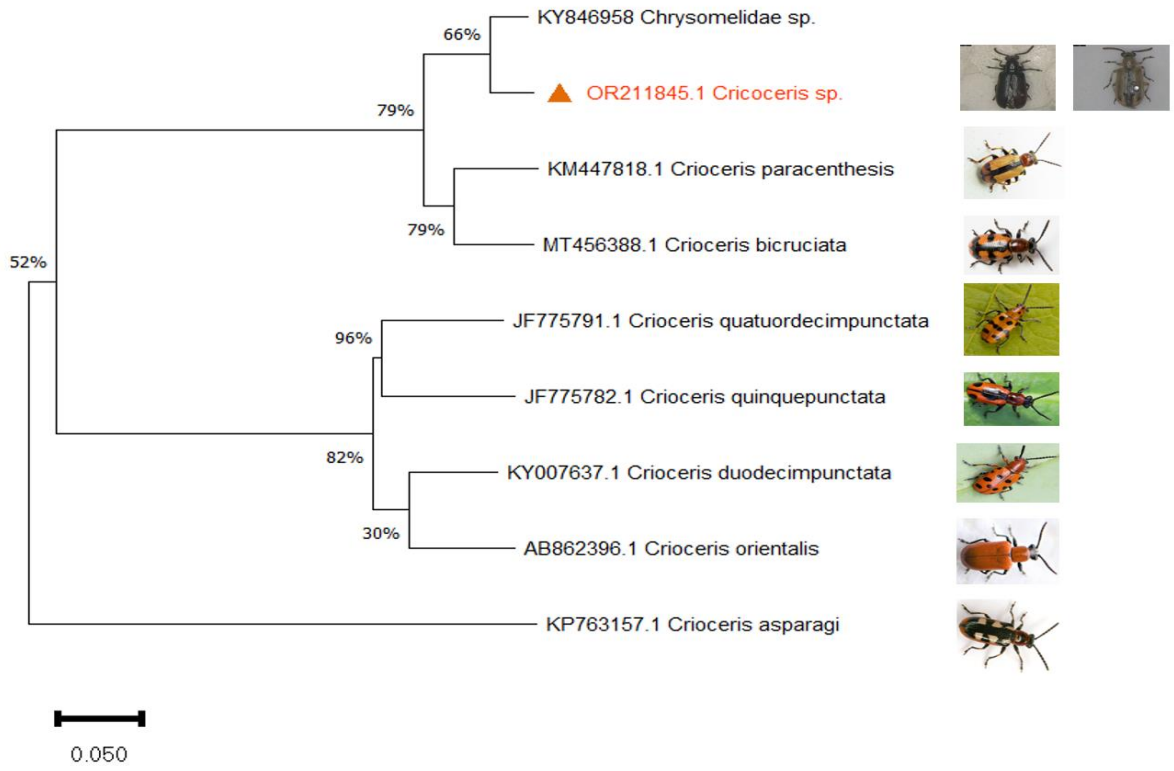
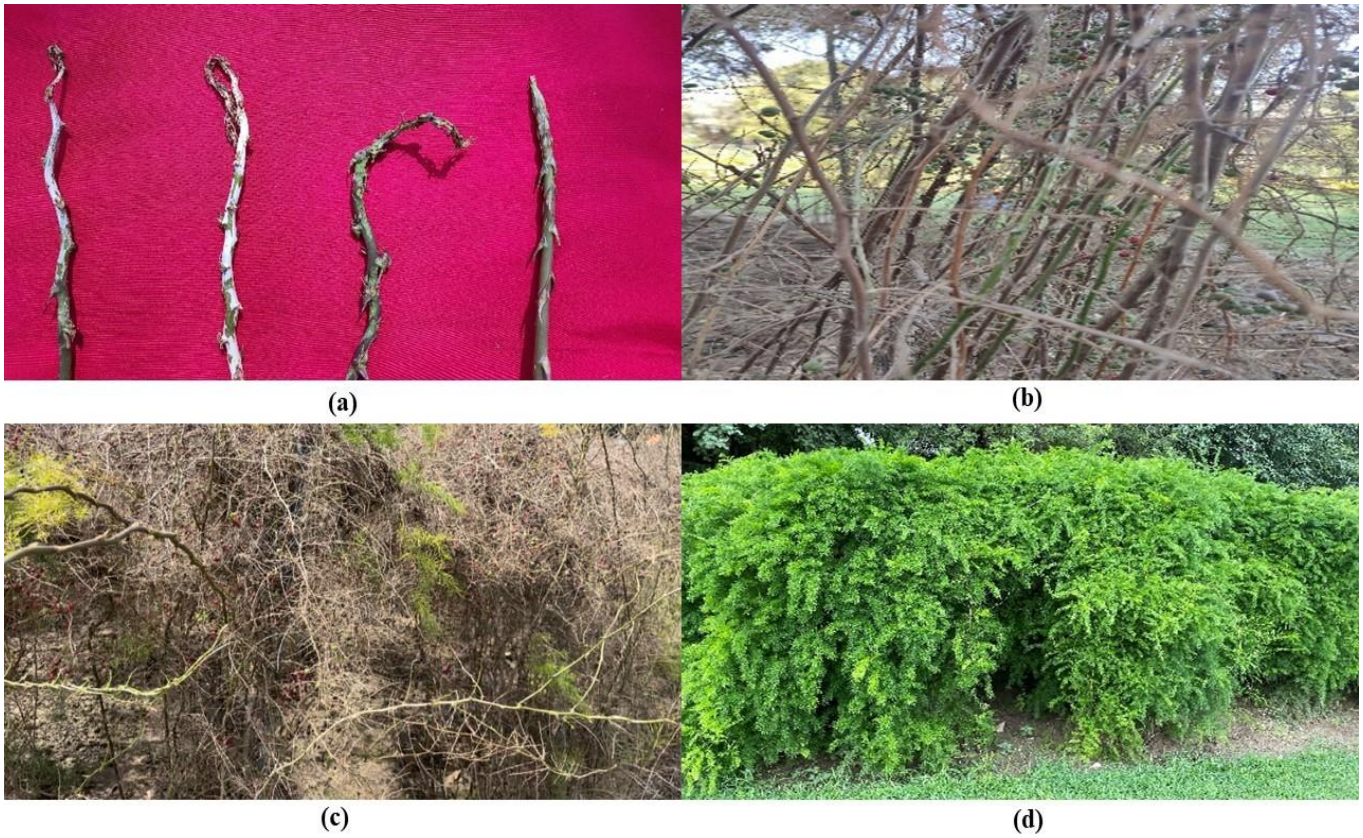


Fig. 10 a) Skeletisation of spears by removal of photosynthetic tissue b) extensive defoliation of the plant c) extensive infestation led to plant death d) healthy plant



(a) (b)

(c) (d)

5. CONCLUSION

Our survey conducted in New Delhi, India, documented the first report of *Crioceris* sp. on *Asparagus racemosus* in India. This new species displayed distinct sexual dimorphism and differed significantly from previously described *Crioceris* species worldwide. We observed its mating behaviour, egg-laying patterns, and feeding habits, which included damage to the asparagus plant at both the larval and adult stages. Our findings contribute to our understanding of the diversity and distribution of chrysomelidae beetles associated with asparagus crops. Further taxonomic investigations are necessary to officially name and classify this newly identified *Crioceris* species.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable, as no approval of research ethics committees was required to accomplish the

goals of this study because experimental work was conducted with an unregulated invertebrate species.

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