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Eublemma scitula (Rambur) (Lepidoptera: Erebidae): first evidence of a predator of the invasive barnacle scale, *Ceroplastes cirripediformis* comstock (Hemiptera: Coccidae)

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Abstract

Background: The invasive barnacle scale, *Ceroplastes cirripediformis* Comstock (Hemiptera: Coccidae), is a pest native to South America and the Caribbean islands that has recently emerged as a serious threat to agricultural, horticultural, ornamental, medicinal, and aromatic plants. Finding indigenous natural enemies to control the invasive pests is the first step for developing a biological control program.

Results: The present study reports *Eublemma scitula* (Rambur) (Lepidoptera: Erebidae) for the first time as a predator of *C. cirripediformis* in India. The identity of *E. scitula* was confirmed morphologically based on male genitalia and mitochondrial cytochrome oxidase I (mt COI) gene sequences. *E. scitula* larvae were found as dominant predators of *C. cirripediformis*. The predatory activity of *E. scitula* was observed from June to September, with its peak population recorded during mid-July (2.1 larvae per 25 cm of infested shoot).

Conclusion: This is the first global record of *E. scitula* predating on an invasive barnacle scale insects from India. Furthermore, studies on the feeding potential of *E. scitula* under controlled and field conditions need to be evaluated for utilizing them as a biocontrol agent against *C. cirripediformis*.

Keywords: *Eublemma scitula*, Predator, *Ceroplastes cirripediformis*, Invasive pest, Biological control, India

Background

The barnacle scale, *Ceroplastes cirripediformis* Comstock (Hemiptera: Coccidae), is an invasive and most destructive pest of agricultural, horticultural, ornamental, medicinal, and aromatic crops. The pest is native to South America and the Caribbean islands, and is widely distributed in Nearctic, Neotropical, Palearctic, Indo-Australian, and Oriental regions (García et al. 2022). Recently, *C. cirripediformis* was designated as an invasive scale insect pest by Center for Agriculture and Bioscience International (Wang et al. 2020). This scale insect is highly polyphagous with distribution across 33 countries and found feeding on 119 genera of plants encompassing 63 families (García et al. 2022). This invasive scale insect causes direct damage to the plant by sucking plant sap and indirect damage by injecting salivary secretions and depositing sugary honeydew on the plant surface. The honeydew deposition promotes sooty mold growth, which impedes the photosynthetic activity of the plant and, in severe infestations, resulting in tree vigor reduction and shoot death (Wang et al. 2020). The crawlers and second nymphal instars of this pest infest host plants leaves and migrate to woody tissues during the third

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instar, whereas the adult female protects the eggs until hatching (Hamon and Williams 1984).

Potential spread of *C. cirripediformis*, based on climatic variables and the availability of highly suitable habitats for the pest, has been predicted for most zoogeographic regions of the world (Wang et al. 2020). Recently, occurrence and spread of this invasive scale insect has been noted in India, where it reported to feed on 46 host plants constituting 30 families (Joshi et al. 2021). The rapid spread of this scale insect in to the new geographic regions is due to a wide host range, suitable climatic conditions, global transport of fruits, live plants, and timber (Ouvrard et al. 2013). Due to the lack of natural enemies in invaded areas, the invasion of herbivorous insects, especially scale insects, is considered a critical pest problem (Miller et al. 2005). Nevertheless, scale insects are more vulnerable to natural enemies due to their sedentary habits and colonial distribution (Peeters et al. 2017). Therefore, it is imperative to find out potential natural enemies that can regulate invasive scale insects population in the invaded regions.

Numerous parasitoids on *C. cirripediformis* have been reported from different regions (García et al. 2022). However, there is limited information on the predators of *C. cirripediformis* from different parts of the world and no information from India, where this pest has become an invasive one. This forced for a consistent exploration and resulted in perceiving a lepidopterous predator, Grey Eublemma, *Eublemma scitula* (Rambur) (Lepidoptera: Erebidae) found devouring on the colonies of *C. cirripediformis* infesting the medicinal plant, *Cestrum diurnum* L. (Solanaceae: Solanales) at the research farm of CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India. Thus, in the present study, occurrence of the predatory *E. scitula* on invasive *C. cirripediformis* infesting *C. diurnum* was reported and the identity of the species was confirmed by morphology (male genitalia) and molecular analysis using COI gene of mitochondrial DNA.

Methods

Collection and identification of *E. scitula*

Predatory larvae of *E. scitula* feeding on colonies of *C. cirripediformis* infesting *C. diurnum* (Fig. 1) were collected from the CSIR-CIMAP Research Farm, Lucknow, Uttar Pradesh, India (26°53′38" N; 80°58′50" E, elevation:120 MSL) during June 2021. Five barnacle scale infested shoots (25 cm long) of *C. diurnum* were collected from 20 plants and placed in bags individually, brought to the laboratory and placed in the insect-rearing cages ($30 \times 30 \times 30$ cm^3) and kept under the laboratory under controlled conditions (25 ± 5 °C; 65 ± 5% RH and 12L:12D photoperiod) for further observations. After adult eclosion, the adult moths were dissected and the predator was identified based on male genitalia at the National Pusa Collection, Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi), and the identity was also confirmed using sequences of COI gene of mitochondrial DNA.

For molecular identification, *E. scitula* last larval instar was collected from the field and reared in insect-rearing cages (dimension: 90×40 mm, shape: circular, mesh pore size: 0.053 µm, Hi-Media®, India) under above-described

Fig. 1 **a**. *C. cirripediformis* infested shoots of *C. diurnum*; **b**. *E. scitula* on *C. cirripediformis* infested shoots of *C. diurnum*; marked with red circle in picture; **c**. shield-like structure formed by *E. scitula* by using remained scale cells of *C. cirripediformis*; **d**. larva of *E. scitula* inside the shield-like structure; **e**. adult moth of *E. scitula*

laboratory conditions until adult emergence which were utilized for DNA extraction and identification.

DNA extraction and amplification, sequencing and alignment

Insect DNA was extracted, following standard protocols of Dey et al. (2021). A fragment of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified by polymerase chain reaction (PCR), using 2 × PCR master mix (Thermo Fisher Scientific, USA), using primers, LCO1490 (5'-GGTCAACAAATCATAAAGATATTG G-3') and HCO2198 (5' TAAACTTCAGGGTGACCA AAAAATCA-3') (Folmer et al. 1994). The following thermal cycle parameters for 25μL amplification reaction: initial denaturation step of 94 °C for 1 min, followed by 4 cycles of 94 °C, 30 s; annealing at 45 °C for 90 s, 72 °C for 1 min; followed by 35 cycles of 94 °C, 30 s; 51 °C for 90 s, 72 °C for 1 min and the final extension at 72 °C for 5 min were used. PCR product was tested by electrophoresis on an agar gel, and if a single band was observed, it was purified, using a SureExtract PCR purification kit (Genetix Biotech Asia Pvt. Ltd.) and subjected for sequencing.

Sequence analysis and phylogenetic tree construction

PCR product was sequenced by the Sanger method; sequencing was performed bidirectionally by the automated sequencer (ABI Prism 3130 XL DNA Analyzer, USA) at the specific commercial facilities (Biokart Pvt. Ltd., Bangalore, India). Sequence was assembled and edited in Codon Code Aligner 10.0.1. Partial sequences for cytochrome oxidase I (COI) were compared in the GenBank database with the already available reference sequences. Multiple sequence alignment was performed employing present study sequence and database available sequences by using ClustalW and constructed phylogenetic tree by neighbor-joining (NJ) method with 1000 bootstrap iterations using MEGA X software (Kumar et al. 2018).

Occurrence of *E. scitula*

Experiment was conducted to observe the occurrence of predatory larvae of *E. scitula* on *C. cirripediformis* from June to September 2021. Field abundance of *E. scitula* was enumerated by investigating 15 plants of *C. diurnum* naturally infested with *C. cirripediformis*. Two infested shoots (25 cm in length) from each plant were observed for the occurrence of predatory larvae feeding in colonies of *C. cirripediformis* (Dean and Meyerdirk 1982) and recorded the number of larvae at 10-day intervals. The count of shield-like scale structures on the infested shoots was used to calculate the total larval population of *E. scitula*. No insecticides were applied to the plants during the study period.

Results

Identification of *E. scitula*

This is the first record of *E. scitula* predating on the invasive barnacle scale, *C. cirripediformis*.

The identity of *E. scitula* was confirmed based on its morphological characters and molecular analysis (Fig. 1e). The observed *E. scitula* larvae were bright pink to reddish in color with enlarged posterior body. On the *C. cirripediformis* infested shoots (Fig. 1a), the larvae of *E. scitula* (Fig. 1b) seen covering with light silk web along with debris of the scale insects, which provides a shield or enlarged scale insect like appearance during larval movement on the host plant (Fig. 1c,d).

To identify and determine the correct phylogenetic position of the *E. scitula* voucher CIMAP SK3 performed molecular genetic analysis, as shown in (Fig. 2), the size of the amplified COI region of genomic DNA was approximately 666 bp, which is the expected size of COI region in Lepidoptera. The alignment and comparisons of the mtCOI sequences of the *E. scitula* voucher CIMAP SK3, to the published mtCOI sequences in the GenBank database by BLAST search, yielded that the sequence results of the mtCOI region of the *E. scitula* voucher CIMAP SK3 seem to be highly homologous to *E. scitula* voucher BTS131 with 94.89% sequence identity. To confirm the position of *E. scitula* voucher CIMAP SK3 in the phylogeny, sequences representing *Eublemma* spp. and

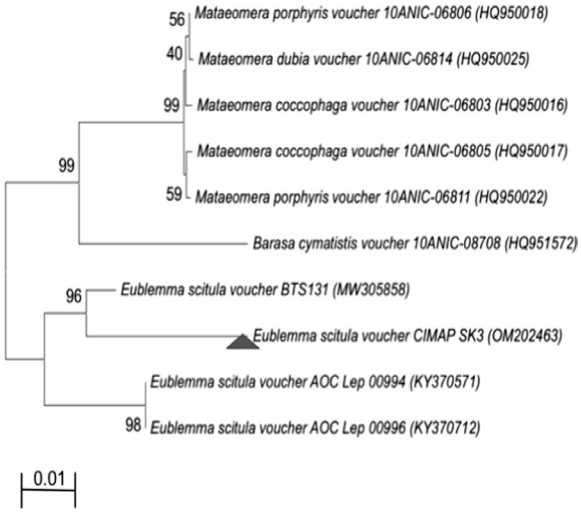

Fig. 2 The evolutionary history was inferred using the neighbor-joining method, and the sequences have been retrieved from NCBI database. The numbers at nodes show the level of bootstrap support based on data for 1000 replication. Bar, 0.01 substitutions per nucleotide position and numbers in parenthesis represent GenBank accession numbers. Evolutionary analyses were conducted in MEGA X. *E. scitula* voucher CIMAP SK3 is indicated by black triangle sign

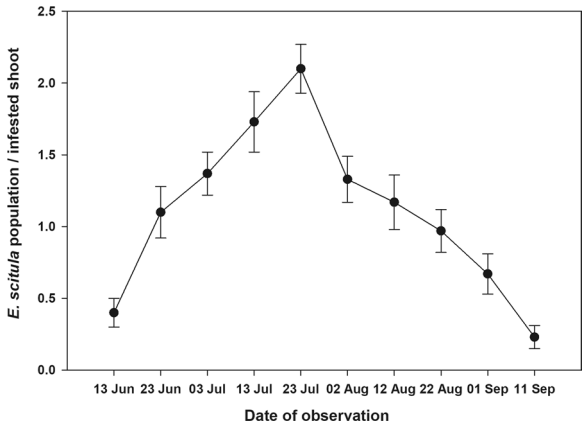

Fig. 3 Field abundance of *Eublemma scitula* on *C. cirripediformis* infesting *C. diurnum* plants (mean ± SE) during June to September 2021

other lepidopteran species totaling 9 reference sequences with more than 90% sequence identity selected from the GenBank database and constructed a phylogenetic tree, which indicated that *E. scitula* voucher CIMAP SK3 and *E. scitula* voucher BTS131 shared one clade. Therefore, the voucher CIMAP SK3 was identified as *E. scitula*.

Field abundance of *E. scitula*

Field occurrence study revealed that *E. scitula* larvae were found to be presented in the colonies of *C. cirripediformis* infested shoots, stems, and reproductive parts such as flowers and berries of *C. diurnum* during June 2021 and remained active until September 2021. The larval density of *E. scitula* during second week of June 2021 was 0.40 ± 0.10 larva per shoot, which gradually increased to 2.10 ± 0.17 larvae per shoot by third week of July 2021 (Fig. 3). Thereafter, the population gradually decreased

Table 1 Occurrence of *Eublemma scitula* on different species of scale insects

Scale insect	Host plant	Countries	References
Saissetia coffeae	Point gourd	India	Pathak and Yadav (2000)
Saissetia coffeae	Olive	Egypt	Morsi (2010b)
Saissetia oleae	Citrus	France	Panis (1974)
Coccus hesperidum	Citrus	France	Panis (1974)
Ceroplastes sinensis	Citrus	France	Panis (1974)
Euphilippia olivina	Olive	France	Panis (1974)
Pollinia pollini	Olive	France	Panis (1974)
Filippia oleae	Arbutus	France	Panis (1974)
Ceroplastes rusci	Fig, arbutus, myrtle	France	Panis (1974)
Eulecanium prunastri	Plum, apricot	France	Panis (1974)
Parthenolecanium carni	Grapevines	France	Panis (1974)
Kermes vermilio	Oak	France	Panis (1974)
Lecanodiaspis sardoa	Cistus	France	Panis (1974)
Coccids	Oleander, Yucca	Israel	Kravchenko et al. (2007)
Acacia nilotica, Tamarix articulata, Cajanum indicus, Saissetia oleae, Disdemococcus unifasciatus, Planoccoccus lilacinus, Udinia catori, Gascardis mimosae, Ceroplastes sinensis, Coccus hesperidum	-	Egypt	Salem (2021)
Saissetia oleae	Olive		Rouzaud (1893); Balduf (1931)
Saissetia oleae	Olive	Tunisia	Mansour et al. (2011)
Saissetia oleae	Citrus	Spain	Tena-Barreda and Garcia-Marí (2006)
Stictococcus dimorphus	Cacao, Pigeon pea	Nigeria	Strickland (1947)
Inglisia conchiformis	Cacao	Uganda	Strickland (1947)
Lecanodiaspis africana	Guava	Egypt	Morsi (2010a)
Tachardia lacca	Zizyphus, Rain tree, Butea	India	Ayyar (1929)
Lecanium hemisphcericum	Ferns, Sandal Wood, Guava	India	Ayyar (1929)
Pseudococcus lilacinus	*Ailanthus excelsa*	India	Ayyar (1929)
Pulvinaria maxima	Nim (Melia)	India	Ayyar (1929)
Anomalococcus indicus	*Acacia arabica*	India	Ayyar (1929)
Ceroplastes cajani	*Ocimum sanctum*, Red gram, Lablab	India	Ayyar (1929)
Ceroplastes ceriferus	*Lawsonia*, Mango	India	Ayyar (1929)

at the August first week and remained active until the second week of September (0.23 ± 0.08 larva per shoot). In addition to *E. scitula*, 4 other predators, 3 (Coleoptera: Coccinellidae), namely *Coccinella septempunctata* Linnaeus, *Anegleis cardoni* (Weise), *Cheilomenes sexmaculata* (Fabricius), and one (Hemiptera: Geocoridae), *Geocoris ochropterus* (Fieber) were also found predating on *C. cirripediformis*. However, the activity and numbers of these predators were negligible.

Discussion

Eublemma scitula was found predating on the scale insect species, *C. cirripediformis* (Table 1). This is a new record of *E. scitula* as a predator of *C. cirripediformis* infesting *C. diurnum* in Lucknow, India. Its population was observed from June to September under field conditions, with a peak activity (2.10 ± 0.17 larvae/shoot) by mid-July. Monobrullah et al. (2015) reported a similar peak activity of *Eublemma amabilis* (Moore) predating on *Kerria lacca* (Kerr). In the present study, *E. scitula* was the dominant predator of *C. cirripediformis* as it occurred abundantly by (2.10 larvae per shoot). Pathak and Yadav (2000) studied the predatory potential of *E. scitula* and found that its individual larva consumed a maximum of 22 mature females of the brown scale insect, *Saissetia coffeae* (Walker). Identification of indigenous natural enemies is an important prerequisite to suppress and control of invasive pest populations in invaded regions (Mani 2016). Therefore, identification, mass rearing and augmentative releases of the native predator against the invasive scale insect, *C. cirripediformis*, are required. Pathak and Yadav (2003) mass-reared *E. scitula* predatory larvae on the host, *S. coffeae*, and recommended its release in the field to control the pest during the early stages of infestation in pointed gourd. However, little information is available on the predatory feeding habits of *E. scitula*. The worldwide occurrence of *E. scitula* on various preys suggests that it is a generalist predator of scale insects. *E. scitula* has expanded its host range and found feeding on the invasive barnacle scale insect *C. cirripediformis* in the native environment. Therefore, the augmentation and conservation of this indigenous predator is required to suppress the population of the invasive *C. cirripediformis*.

Conclusions

The present study is the first detection of *E. scitula* larva found feeding on the invasive barnacle scale insect *C. cirripediformis*. The preliminary study on the occurrence of *E. scitula* suggests that the larvae are potential predators of *C. cirripediformis* infesting the medicinal plant *C. diurnum*. Conservation and augmentation of the predator are required to suppress the invasive pest population. Further studies on the feeding potential of *E. scitula* against *C. cirripediformis* need to be conducted under controlled and field conditions.

Abbreviations

N: North; E: East; MSL: Mean sea level; COI: Cytochrome oxidase I; NCBI: National Center for Biotechnology Information; DNA: Deoxyribonucleic acid; cm: Centimeter; mm: Millimeter; µL: Microliter; min: Minute; s: Seconds; ng: Nanogram; w:v: Weight: volume; %: Per cent; ±: Plus-minus; /: Per; °C: Degree centigrade; RH: Relative humidity; L:D: Light and dark period; PCR: Polymerase chain reaction; PvtLtd: Private limited; ICAR: Indian Council of Agricultural Research.

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Author contributions

SCK and MBG collected the predator and recorded observations. DCK performed the molecular analysis and PRS carried out the morphological identification of the predator. SCK, MBG, and DCK wrote and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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