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Original Research Paper

Molecular characterisation, biology, behaviour and feeding potential of a novel predator of the mango ecosystem: *Sycanus bifidus* (Fabricius, 1787)

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ABSTRACT

This study provides an overview of the comprehensive study on *Sycanus bifidus*, a new generalist predatory fauna collected from mango ecosystem, encompassing its molecular characterization, biology, behaviour and feeding potential. The research delves into the intricate details of *S. bifidus*, shedding light on its morphological features, and ecological significance. Molecular characterization techniques, including DNA sequencing and genetic analysis, play a pivotal role in elucidating the species genetic makeup, population dynamics, and potential adaptations. The study also explores the intricate biology and behaviour of *S. bifidus.* Furthermore, the research investigates the feeding potential of *S. bifidus*, emphasizing its role as a predator in controlling pest populations. An in-depth analysis of its feeding habits, and efficiency as a biological control agent provides valuable information for the development of sustainable pest management strategies.

Keywords: Feeding potential, mango, molecular characterization, predatory efficiency, *Sycanus bifidus*

INTRODUCTION

Sycanus is a genus of assassin bug (Heteroptera: Reduviidae: Harpactorinae: Harpactorini) with many species that are found in the African and Asian regions. The assassin bug, family Reduviidae, is the second largest family in Heteroptera, including about 7000 described species placed under 1000 genera and 25 sub-families (Weirauch et al., 2014). In India, Ambrose (2006) listed 464 species under 144 genera and 14 sub-families (Ambrose, 2006). Reduviidae constitute an important group of predatory insects (Ambrose, 1980), it is the largest family of predaceous Hemiptera and many of its members are found to be potential predators of a wide array of insect pests across many taxonomic groups (Ambrose, 1999 & 2003). The species of the genus *Sycanus* is known to be a predators of vegetable ecosystem (Truonga et al., 2020). Since, they are genaralist predators they may not be useful as predators on specific pests but they are valuable predators in situations where diverse, group of insect pest species occur. Hence, they should be conserved and augmented to be effectively utilized in the integrated pest management (IPM) programmes (Ambrose, 1999 & 2003). Conservation and augmentation of any biological control agent rely upon its comprehensive knowledge on bioecology, ecophysiology and behaviour (Ambrose & Claver, 2001; Das & Ambros, 2008). Many harpactorinae reduviids inhabit India, and the genus *Sycanus* is one of the most common. Its individuals are effective predators with high potential in biological control (Sahayaraj, 2004). *Sycanus* is a predacious reduviid that has a wide prey diet including several orders of insects, such as both the larvae and pupae of Lepidoptera, Coleoptera and Diptera (Sahayaraj & Balasubramanian, 2016) and is considered to have the potential to be a biological control agent of mango ecosystem. Lack of knowledge on the natural history of reduviids, proved to be a limiting factor in pursuing their effective utilization as biocontrol agents in the integrated pest management programme, a novel predator of mango, *S. bifidus* is one such example. Thus, an effort was undertaken to characterise the species and to investigate its potential for predation.

MATERIAL AND METHODS

Collection of bugs from mango foliage

Species belongs to genus *Sycanus* is known to dewll on foliage depending on the availability of its prey. Whereas, the novel predator, *S. bifidus* adults and nymphs observed to feed on lepidopteran mango pests were hand collected carefully using foreceps during October 2019. The collected bugs were divided into two groups: one group was placed in alcohol vials for fundamental taxonomy studies, and the other group was placed in tiny wooden rearing cages with zip-lock lids and taken to the labs.

Identification and description

The many available literatures, in particular Distant's Rhynchota volumes in the Fauna of British India Series (Distant, 1904 & 1910), were used to identify genera and species. The classification was based on the Maldonado (1990) catalogue. For confirmation, molecular confirmation was also carried out. Leica Application Suite (LAS) software, which was installed in the Leica M205A stereomicroscope with DFC 420 camera attachment, was used to measure a variety of morphological structures. Redescriptions of Zhao et al. (2024) were also used for identification of the specimens.

Molecular characterization

Collected bug samples were stored as dried/fresh tissue in cryo vials till DNA extraction. Genomic DNA was isolated from samples using the HiPurA™ Insect DNA Purification Kit following the manufacturer's instructions. Extracted DNA templates were amplified by polymerase chain reaction (PCR) using the universal primer pair (LCO LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') targeting a fragment of mitochondrial cytochrome c oxidase subunit I (COI) (Folmer et al., 1994). The PCR was executed using 12.5 µl of 2× PCR master mix (EmeraldAmp® GT PCR Master Mix, DSS Takara), 1 µl of 50 ng template genomic DNA, 0.5 µl of each primer (10 µmol/ml) and nuclease free water to make the final volume of 25 µl under subsequent condition: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 15 s, primers annealing at 58°C for 40 s, extension at 72°C for 40 s with a final extension at 72°C for 5 min. Thereafter, 5 µl of PCR products were subjected to electrophoresis on 1.2% agarose gel and visualized over the transilluminator to detect the amplification. During the whole procedure, extraction and PCR blanks were incorporated into the analysis to check the contamination. The PCR product was then purified using QIA quick PCR Purification Kit (Qiagen). The purified PCR product was submitted for sequencing using custom services. The obtained forward and reverse sequences were assembled and subjected BLASTn analysis. After validating all obtained sequence data, multiple sequence alignment (MSA) was performed. The intraspecies and interspecies distances were calculated using Kimura 2 parameter (K2P) using MEGA 11 software (Tamura 2021). Evolutionary distances were calculated using neighbor-joining (NJ) method, and the phylogenetic tree was constructed with 1000 bootstrap replicates using MEGA 11.

Biology studies

Adult reduviids were collected from mango orchards of ICAR-Central Institute for Subtropical Horticulture, in Uttar Pradesh (26°54'16"N 80°45'52"E). The collected adults were shipped to ICAR-Indian Agricultural Research Institute, New Delhi for detailed biology studies. The adults were identified and reared separately in glass bottles (500 ml capacity) using larvae of *Spodoptera litura*. Virgin males and females that are emerged in the laboratory were allowed to mate in glass rearing bottles. Only adults reared in the laboratory were used in the experimental studies. Adults were allowed to mate, then the mated females were maintained individually in order to record the number of egg batches and eggs per batch. The containers were carefully examined twice daily to record the per cent hatching and number of eggs laid. Twenty-five newly hatched nymphs were isolated soon after eclosion and reared individually on first and second instar *S. litura*. When the reduviid nymphs grew larger, fourth and fifth instar *S. litura* larvae were supplied to them as prey. Observations on fecundity, egg hatchability, ecdysis, nymphal mortality, adult emergence, sex ratio, and adult longevity were recorded. All the data were expressed as mean ± SE. The data was subjected to analysis to estimate the life table parameters.

For feeding potential studies, Ist, IInd, IIIrd, IVth, Vth instar nymphs and adults were starved in a separate celled tray to avaoid cannibalism for 6 hours. Each first and second instar was given with 10 second instar larvae of *S. litura* and later instars with 10 IIIrd instar *S. litura* every day. The numbers of prey killed were recorded every day. Behavioural observation of predator was observed in mango ecosystem.

RESULTS AND DISCUSSION

Diagnosis of *Sycanus bifidus* (Fabricius, 1787)

Body elongated and ovate; head long and slender, as long as pronotum and scutellum combined together; postocular nearly twice longer than anteocular, cylindrical; anterior pronotal lobe twice shorter than posterior, medially sulcate, posterior lobe rugose and granulate; scutellum armed with erect bifid spine (Fig. 1); paramere apically swollen; pygophore ovate, median pygophore process longly spinously produced.

Fig. 1 : Habitus of *Sycanus bifidus*

***Measurements* (n=3):** All measurements are in mm, body length: 13.36-13.91; length of head: 5.17-5.60; interocular distance: 0.76-0.84; interocellar distance: 0.36-0.38; length of scape: 6.66-7.13; length of pedicel: 2.33-2.45; length of anteocular: 1.93-2.12; length of postocular: 2.63-3.60; length of labial segment II: 2.49-3.11, III: 3.96-4.59, IV: 0.55-0.60; length of anterior pronotal lobe: 1.14-1.16; length of posterior pronotal lobe: 2.76-3.11; width of anterior pronotal lobe: 4.27-4.28; width of posterior pronotal lobe: 6.61-6.99; length of scutellum: 1.23-1.26; width of abdomen: 5.46-7.74.

***Distribution*:** Andhra Pradesh, Karnataka, Kerala and Uttar Pradesh

Material examined: INDIA: Uttar Pradesh, 4♀, 1♂, 13.iv.2019, Gundappa, B., Host: Mango; Prey: lepidopteran caterpillars.

Remarks: *S. bifidus* was listed in checklist of Assassin bugs prepared by Zoological Survey of India (Biswas and Mitra, 2011) but neither its location nor its host has been available clearly. This species externally resembles *Sycanus galbanus* in general body coloration and structure, but differs with the body measurements and scutellar spine. Phenotypic plasticity makes it difficult to identify hence, molecular support was taken to judge the species delimitation. Zhao et al. (2024) noted that polymorphism of body coloration brings difficulties in the species identification of *S. bifidus*.

Molecular identification: The amplification of ~600 bp was obtained after PCR. The forward and reverse sequences obtained after sequencing were assembled and the consensus sequence was submitted to NCBI vide accession number MW450669.1. The present isolate was sharing 100 per cent similarity with S. bifidus isolate HDX11 (KJ630088) and more than 97 per cent similarity with other *S. bifidus* isolates. Thus, confirming the identity of the present isolate as S. bifidus. The phylogenetic tree constructed using MEGA 11 software through neighbour joining method have clustered the present isolate along with other S. bifidus isolates with 99 per cent bootstrap value. Sineadiadema was taken as outgroup member in phylogentic analysis (Fig. 2).

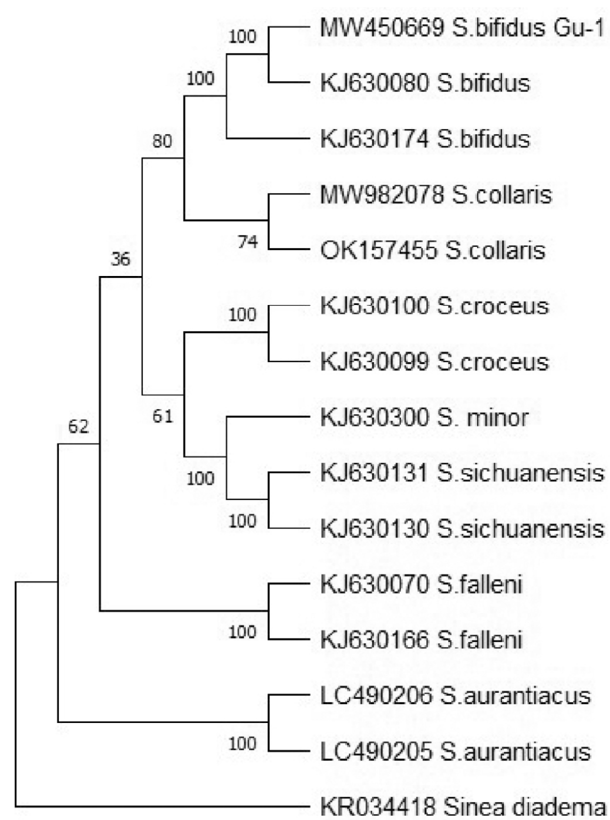

Fig. 2 : Phylogenetic tree of *S. bifidus*

Biology and feeding potential of *S. bifidus*

The details of biological parameters of the reduviid *S. bifidus* reared on *S. litura* are presented in Table 1. Biology of the *S. bifidus* was studied under the laboratory conditions. Egg incubation period was 13.4

Table 1 : Biology of *S. bifidus under laboratory conditions*

Stages	Days (Mean ± SE)
Egg incubation	13.4 ± 1.84
I instar	6.2 ± .58
II instar	6.6 ± 0.81
III instar	8.2 ± 0.37
IV instar	9.8 ± 0.86
V instar	11 ± 1.26
Longevity of adult male	17.6 ± 1.44
Longevity of adult female	20.2 ± 2.18
Preoviposition	5.2 ± 0.37
Oviposition period	3.8 ± 0.37
Post oviposition	7.6 ± 0.51
Fecundity (%)	83 ± 5.94
Hatching (%)	63.4 ± 4.52
Survival (%)	46.4 ± 3.78

± 1.84 days, five nymphal instars were observed I, II, III, IV and V instar was completed in 6.2 ± 0.58, 6.6 ± 0.81, 8.2 ± 0.37, 9.8 ± 0.86 and 11 ± 1.26 days respectively. The feeding potential of, Ist, IInd, IIIrd, IVth, Vth instar nymphs, adults male and female were 11.3 ± 2.49, 31.6 ± 4.94, 13.67 ± 0.62, 18.00 ± 0.82, 34.33 ± 5.17, 146.33 ± 3.97 and 34.33 ± 4.33 respectively (Table 2).

Table 2 : Feeding Potential of *S. bifidus under laboratory conditions*

Stages	No. of insects fed (x ± SE) Prey: *Spodopteralitura*
I instar	11.33 ± 2.49
II instar	31.67 ± 4.94
III instar	13.67 ± 0.62
IV instar	18.00 ± 0.82
V instar	34.33 ± 5.17
Adults female	146.33 ± 3.97
Adult male	34.67 ± 4.33

Male adult longevity was 17.6 ± 1.44 days. Female adult longevity was 20.2 ± 2.18 days. Preoviposition, oviposition and post oviposition period were 5.2 ± 0.37, 3.8 ± 0.37, 7.6 ± 0.51 days, respectively. Fecundity, hatching and survival of *S. bifidus* were 83 ± 5.94% , 63.4 ± 4.52% and 46.4 ± 3.78%, respectively. Egg development and hatching eggs of reduviid *S. bifidus* was similar to reduviid *S. collaris* reared on two different preys *S. litura* and *C. cephalonica* (Rajan et al., 2017). Usually the lonfivity of *Sycanus* is very high, but *S. bifidus* longevity was extremely less especially in adults in the current study, this might be due to variation in the rearing conditions. The development period of I and II nymphal instars reduviid *S. falleni* were similar to the current study (Abdul et al., 2018). The longevity of adult predatory insects may be significantly affected by their prey (Srikumar et al., 2014). In the present study, the female longevity was longer than male when they were reared by two different preys which was a common feature of most reduviids (Srikumar et al., 2014; Shanker et al., 2016; Nitin et al., 2017). The ability to lay eggs of reduviid *S. bifidus* reared on two different preys was similar as reduviids *S. dichotomus, S. galbanus, S. annulicornis and S. collaris* (Nitin et al., 2017; Abdul et al., 2018; Rajan et al., 2017).

Life-table parameters of S. bifidus

Age specific life table vital statistics was computed for the S. bifidus reared on the laboratory prey S. litura. The life-table parameters, net rate of increase (R_0) 391.3 number of female/generation, generation time (GT) was 21.26 days, capacity for increase (Tc) was 0.281, intrensic rate of increase (r_m) was 0.229, finite rate of increase (λ) 1.26 number/day, mean generation time (T) of S. bifidus was 26.08 days and population doubling time (T_2) was 3.03 days (Table 3). Surviviorship curves for the S. bifidus was also computed and it follows Type I type of surviviorship curve which indicates that there is a little loss in the population in the early stages of the S. bifidus as population older high loss of the population was observed (Fig. 3).

Table 3 : Life table statistics of *S. bifidus under laboratory conditions* on *Spodoptera litura*

Life table parameters	*S. bifidus*
Net rate of increase (R_0)	391.3 number of female/generations
Generation time (GT)	21.26 days
Capacity for increase (Tc)	0.281
Intrinsic rate of increase (r_m)	0.229
Finite rate of increase (λ)	1.26 number/day
Population doubling time (T_2)	3.03 days

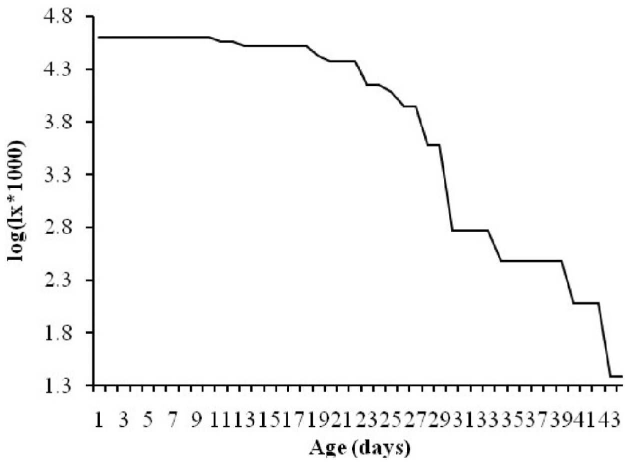

Fig. 3 : Survivioushiр curve for *S. bifidus*

Egg development and hatching eggs of reduviid *S. bifidus* was similar to reduviid *S. collaris* reared on two different preys *S. litura* and *C. cephalonica* (Rajan et al., 2017). The development period of I and II nymphal instars reduviid *S. falleni* were similar to the current study (Abdul et al., 2018). The longevity of adult predatory insects may be significantly affected by their prey (Srikumar et al., 2014). In the present study, the female longevity was longer than male when they were reared by two different preys which was a common feature of most reduviids (Srikumar et al., 2014; Shanker et al., 2016; Nitin et al., 2017). The ability to lay eggs of reduviid *S. bifidus* reared on two different preys was similar as reduviids *S. dichotomus, S. galbanus, S. annulicornis and S. collaris* (Nitin et al., 2017; Abdul et al., 2018; Rajan et al., 2017).

Predatory Behaviour: *Sycanus bifidus* exhibited pin and jab mode of predation after sight of their prey, bugs gets aroused, stay motionless till the prey come close then approaches, bugs capture their prey with the help of their elongated raptorial forelegs and tranquilise/paralise by trusting their rostrum and injecting the toxin. After paralysing, bugs suck the haemolymph which changes the colour of larvae to blackish brown. After predation, bugs brush their antenna with combs present on forelegs. Theis's behaviour was similar to the behaviour of other reduviids *viz.*, *Endochus albomaculatus* Stål, *Epidaus bicolor* Distant, *Euagoras plagiatus* Burmeister, *Irantha armipes* Stål, *Panthous bimaculatus* Distant, and *Sphedanolestes signatus* Distant (Srikumar et al., 2014).

Copulatory behaviour: *Sycanus bifidus* had showed pre-oviposition period of 5.2 days and they followed sequential pattern of arousal, approach, riding over and mating for successful copulation. Male bugs aroused after seeing their female partner and approaches with their antenna and an erected rostrum then place their forelegs over female form posterior side. Male clasp the female, ride over her body dorsoventrally then copulate by extending their pygophore. Mating pairs remain motion less during copulation. Bugs took nearly 2 to 6 hours for copulation. After successful mating, bugs laid eggs in cluster covered by gummy secretions on mango twigs and leaves.

In conclusion, this multidimensional study offers a comprehensive overview of the predator *S. bifidus*, combining morphological, molecular, ecological, behavioural and functional aspects. The findings contribute not only to the scientific understanding of this intriguing species but also have practical implications for pest management in mango ecosystem and ecological conservation efforts.

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