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Journal of Threatened Taxa

10.11609/jott.2023.15.3.22771-22926

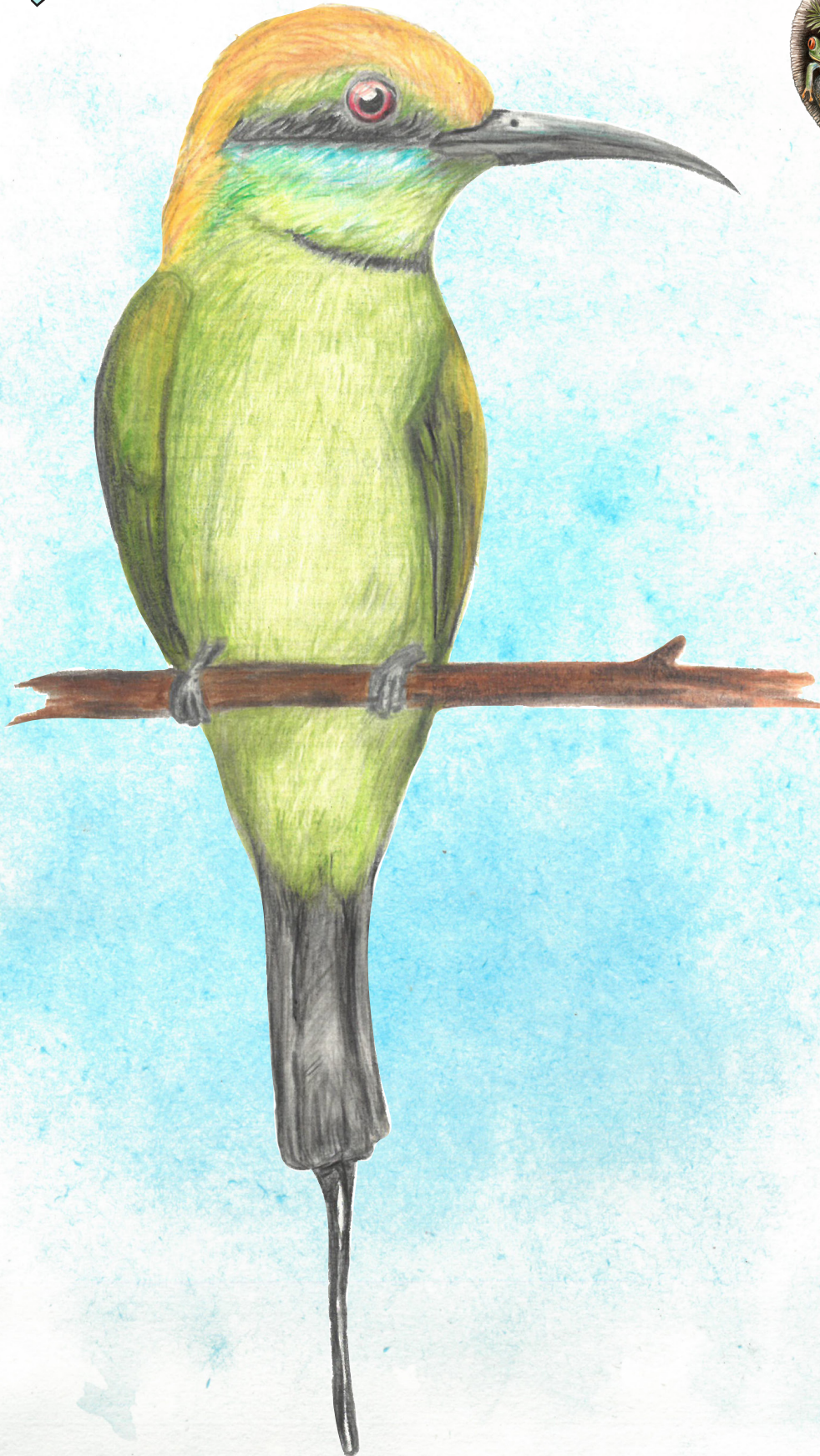
www.threatenedtaxa.org

26 March 2023 (Online & Print)

15(3): 22771-22926

ISSN 0974-7907 (Online)

ISSN 0974-7893 (Print)



Open Access



ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

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Cover: Green Bee-eater with colour pencils and watercolor wash by Elakshi Mahika Molur.



First report of the beetle *Henosepilachna nana* (Kapur, 1950) (Coleoptera: Coccinellidae) from Maharashtra with special reference to molecular phylogeny and host plants

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Abstract: A ladybird beetle, collected from different localities of Kolhapur and Satara districts (Maharashtra) was identified as *Henosepilachna nana* (Kapur, 1950). The presence of this species in Maharashtra considerably extends its range to the north-west by about 700 km. Since this species is found on vegetables, Pumpkin (*Cucurbita* spp.), Cucumber (*Cucumis* spp.), and Karit fruit plant (*Cucumis* spp.). It is being recorded for the first time that this species is a pest of these vegetables. Also, molecular phylogeny has been studied for the first time in this species in which, this species is the sister taxon of *Henosepilachna boisduvali*. This species has been described briefly with colour photographs of male genitalia, female coxites and the 6th sternal plate of female, tarsi, & pronotum. *Henosepilachna nana* is being reported for the first time from Maharashtra and now the molecular data of this species is available.

Keywords: Epilachna, Epilachnini, GenBank, genitalia, first record, ladybug, phylogenetic tree, vegetable pest.

Editor: Mandar Paingankar, Government Science College Gadchiroli, Maharashtra, India.

Date of publication: 26 March 2023 (online & print)

Citation: Patil, P.B. & S.M. Gaikwad (2023). First report of the beetle *Henosepilachna nana* (Kapur, 1950) (Coleoptera: Coccinellidae) from Maharashtra with special reference to molecular phylogeny and host plants. *Journal of Threatened Taxa* 15(3): 22859–22865. <https://doi.org/10.11609/jott.7664.15.3.22859-22865>

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Funding: Shivaji University, Kolhapur.

Competing interests: The authors declare no competing interests.

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Author contributions: SMG involved in design of the research and write up of the manuscript. PBP collected and dissected specimens and participated in design of the research, digital photography and map preparation.

Acknowledgements: The authors would like to thank the Head, Department of Zoology, Shivaji University, Kolhapur for providing laboratory facilities and financial assistance under the Golden Jubilee Research Fellowship.



INTRODUCTION

The tribe Epilachnini (Mulsant 1846) comes under the subfamily Coccinellinae (Slipinski 2013; Seago et al. 2011) but previously it was considered as a separate subfamily Epilachninae of the family Coccinellidae (Szawaryn & Tomaszewska 2014). The members of this tribe are economically important and distributed throughout the world (Szawaryn et al. 2015), comprising 27 genera (Tomaszewska & Szawaryn 2016). There are about 1,000 herbivorous species of this tribe that feeds on plant tissues and fluids (Giorgi et al. 2009; Bustamante-Navarrete et al. 2018).

The genus *Epilachna* is one of the genera of the subfamily Epilachninae in which hundreds of species from all over the world have been described and later many species were subsequently removed to other genera (Jadwiszczak & Węgrzynowicz 2003). Based on basal toothed tarsal claw and longitudinally divided sixth female abdominal sternite, Li & Cook (1961) raised a separate genus *Henosepilachna* from *Epilachna*. To date, there are about 110 described species of the genus *Henosepilachna* from Asia and Australia (Szawaryn 2011). There are 33 species of *Henosepilachna* in India (Poorani 2012; Poorani et al. 2021).

The male specimen collected from Nilgiri Hills, India has been described by Kapur (1950) as *E. nana* and its holotype is in the Zoological Survey of India (No. 3426/13). In the world catalogue of Coccinellidae, Jadwiszczak & Węgrzynowicz (2003) placed *E. nana* in the genus *Henosepilachna*, which is followed by Poorani (2012) in the updated checklist of Coccinellidae from Indian subregion and Borowski (2020) in the inventory of world Epilachninae. In the present study, we describe the detailed morphology of the male genitalia, 6th sternal plate, female genitalia and tarsi of *H. nana* along with coloured photographs. In addition, this species is being reported for the first time in Maharashtra through this communication and its molecular phylogeny has been given.

MATERIAL AND METHODS

Specimens of *H. nana* were collected on different vegetable crops by handpicking method from Halondi (Hatkanangale), Kanthewadi (Radhanagari) and Parite (Karveer) of Kolhapur district and Sajjangad and Yerad (Patan) of Satara district of Maharashtra, India (Image 1). Specimens were photographed on stereo zoom microscope LYNX LM-52-3621 using TCapture software

and preserved dry and wet and deposited at the Department of Zoology, Shivaji University, Kolhapur. Captured images stacked in Helicon Focus 7 software. Measurements were taken in ImageJ software. The genitalia was dissected and photographed using the same microscope and software. Species was identified and confirmed based on the detailed taxonomic description provided by Kapur (1950) along with genitalia description.

Molecular analysis

Genomic DNA extraction was done by the manual CTAB method (Boopathi et al. 2020). PCR of extracted DNA was carried out using the primers LepF1-5'-ATTCAACCAATCATAAAGATATTGG-3' and LepR1-5'-TAAACTTCTGGATGTCCAAAAAATCA-3' (Hebert et al. 2004; Wilson 2012). PCR amplified mitochondrial COI gene was sequenced by the Sanger sequencing method. The obtained sequence were submitted to GenBank. To construct the phylogenetic tree, sequences for the COI gene of *Henosepilachna* were downloaded from NCBI. A total of 54 sequences for eleven species of *Henosepilachna* and one for *Epilachna* were downloaded from NCBI GenBank (Table 1) of which *Epilachna* sp. is an outgroup.

Generated COI gene sequences were edited in BioEdit Sequence Alignment Editor Software. Multiple sequence alignment was carried out using MUSCLE (Edgar 2004) in MEGA 7 (Kumar et al. 2016). Phylogenetic analysis was done for COI sequences for 13 taxa.

Phylogenetic analysis was carried out using Maximum Likelihood (ML) method (Felsenstein 1981). MEGA 7 was used to find out the best-fit nucleotide model for the dataset under the corrected Akaike Information Criterion (AICc). The best nucleotide substitution found was T92+G, which is not implemented in the RAxML program, so we used the default model which was GTR + G (Lanave et al. 1984; Gu et al. 1995). ML analysis was performed on RAxML HPC2 (Stamatakis 2014) at the CIPRES portal (Miller et al. 2010). Bootstrap support values were obtained with the rapid bootstrap algorithm with 1000 bootstrap replicates.

RESULTS

Material examined: 1 male, SUKDZLB94, Halondi, Kolhapur (16.7348°N, 74.3016°E) 27.ii.2018, Priyanka Patil; 2 females, SUKDZLB314, SUKDZ317, 3 males, SUKDZLB311, SUKDZLB312, SUKDZLB313, Kanthewadi, Kolhapur (16.4693°N, 74.0329°E), 26.x.2018, coll.-

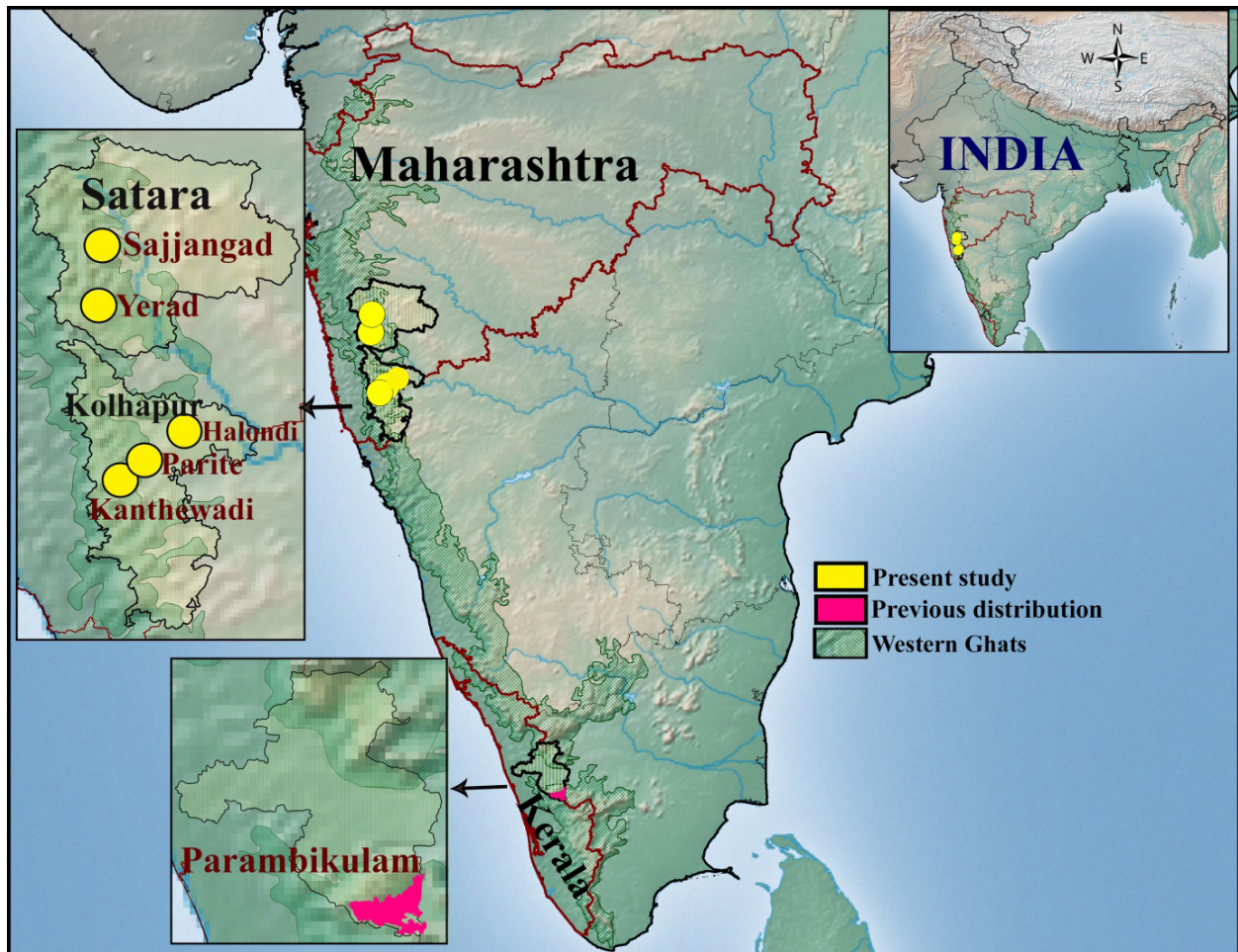


Image 1. Distribution map for *Henosepilachna nana*.

Priyanka Patil; 1 female, SUKDZLB450, 1 male, SUKDZLB453, Yerad, Satara (18.3614°N, 73.3502°E) 15.ix.2019, coll. Priyanka Patil.

Henosepilachna nana (Kapur, 1950)

Diagnosics: Length 6.0–6.6 mm, width 4.6–5.2 mm.

The body is oval with 6 black elytral spots over grey background (Image 2a). The black elytral spots are surrounded by a brown ring. The dorsal body shows grey pubescence except for black spots. Brick red coloured pronotum with four black spots (Image 2m). In many examples, pronotal spots on the same side join to form a quadrate spot, which is nearer to the base than the apex and does not touch to anterolateral margins and pronotal median line (Image 2l). Scutellum brown, triangular, pointed at the elytral side (Image 2i). Ventrally brown except dark patches on metasternum and abdominal sternites. Tarsi are pseudotrimerous having bifid tarsal claws (Image 2o). The tarsal claw is with a basal tooth (Image 2p). Sixth abdominal sternite is divided in females

(Image 2h) and emarginated in males (Image 2j).

Male genitalia: Penis is thin, with a slightly broad penis capsule (Image 2f). Penis apex is with V-shaped notch and a small projection at the notch base (Image 2d,e). Tegmen with paramere tip rounded having many setae at the apex and fewer setae on the distal half. Penis guide is uniformly curved at the distal half, curved and pointed at the apex (Image 2b,c).

Female genitalia: Female coxites quadrate with blunt corners, inner margin is straight except for a shallow notch at the base and with a pear-shaped small stylus with numerous setae (Image 2g).

Host plants: The specimens of *Henosepilachna nana* were found to be associated with Cucumber (*Cucumis* spp.), Pumpkin (*Cucurbita* spp.), and Karit fruit plant (*Cucumis* spp.). On the underside of leaves, in a bunch, approximately 20 elliptical, yellow, sparsely laid eggs were observed. Both the larvae and adults of *H. nana* skeletonize the leaves of the above vegetables by eating the chlorophyll and can act as a serious pest

Table 1. Taxa used in phylogeny analysis with their GenBank accession numbers.

	Taxa	GenBank accession numbers
1	<i>Henosepilachna niponica</i>	LC228599, LC228594, LC228597, AB002185, LC228600
2	<i>Henosepilachna pustulosa</i>	AB300448, LC228587, AB300446, LC228586, AB495213, AB495212, AB495210, AB300447, AB300454, AB300457, AB495211, LC228585, AB300452, LC228588, AB300456, AB002183, AB300451, AB300453, AB300450, AB300449, LC228582
3	<i>Henosepilachna vigintioctopunctata</i>	KU234209, KU234206, KU234207, KU234208, KU234204, KU234205, KU234199, KU234210, KU234203, KU234201, KU234200, AB002180, KU234211, KU234202
4	<i>Henosepilachna yasutomii</i>	LC228592, AB002184
5	<i>Henosepilachna enneasticta</i>	AB002173
6	<i>Henosepilachna pusillanima</i>	MH395854, AB002177, MT985168
7	<i>Henosepilachna implicata</i>	MT985166
8	<i>Henosepilachna</i> sp. 1	MH395855
9	<i>Henosepilachna</i> sp. 2	AB002174
10	<i>Henosepilachna boisduvali</i>	AB002175
11	<i>Henosepilachna septima</i>	KT693136, AB002176, KX503056, MT985165
	<i>Epilachna</i> sp.	AB002179

of cucurbitaceous plants. Under laboratory conditions, conspecific egg predation by this species was observed (Image 2n).

Phylogeny (Figure 1): COI gene sequences for *Henosepilachna nana* generated during this study were first time submitted to NCBI GenBank under accession numbers ON220741 (for SUKDZLB94) and ON220742 (for SUKDZLB311). The phylogenetic tree is rooted with outgroup *Epilachna* sp. The larger part of the tree is a nested clade that comprises four main sub-clades. Within the first sub-clade, *H. septima* and *Henosepilachna* sp.1 are the sister taxon to all remaining species with high support (bootstrap (BS) 99). The second sub-clade, *H. boisduvali* is sister taxa to *H. nana* with a high bootstrap value (BS 88). Within the second sub-clade, *Henosepilachna* sp.2 and *H. implicata* are sisters to *H. pusillanima* with high support (BS 98). The third sub-clade shows three different *Henosepilachna* sp. named *H. vigintioctopunctata* showing its variation among its congeners. The sub-clade at the top of the tree shows two nodes, one for *H. enneasticta* and the other for *H. vigintioctopunctata* complex consisting of *H. niponica*, *H. pustulosa*, *H. yasutomii*, and *H. vigintioctopunctata*. The species *H. nana* is genetically closer to *H. boisduvali* than other congeners.

DISCUSSION

Kapur (1950) wrote a note on *Epilachna ocellata* with a description of three species, viz., *E. nana*, *E. anita*, and *E. manipurensis*. It includes details of identification and

genitalia description along with images of all four species. After 25 years, Gordon (1975) revised Epilachninae of the Western hemisphere in which he described one new species named *E. nana*. However, the species which was described by Gordon (1975) is different from *E. nana* having different characteristics than *E. nana* which was described by Kapur (1950). Later, Kapur informed Gordon that the nomenclature *E. nana* is already given to one ladybird species and then Gordon (1985) replaced the *E. nana* name with *E. minuta*. The Holotype of *E. nana* was collected in 1892 and paratypes were collected in 1914 from Nilgiri Hills and Parambikulam (Kerala), respectively (Kapur 1950). The species is distributed over the Nilgiri Hills of southern India (Kapur 1950; Poorani 2012; Borowski 2020). The localities in Maharashtra, which are being reported in the present article, will add *H. nana* to the Fauna of Maharashtra, proving that the range extension of this species towards the north-west by about 700 km.

According to the illustrations and description given by Kapur (1950), the specimens recorded during the study are treated here as *H. nana*. Every specimen of *H. nana* collected during the study show 12 elytral spots without any variation in the count. Based on the number of elytral spots, *H. nana* differs from the other *Henosepilachna* species. The species *H. boisduvali* is yellowish-red having a median black pronotal spot and 12 elytral spots (Li & Cook 1961; Li 1993). The species *H. nana* is varying from *H. boisduvali* in having two spots present on each side of the median line, there is no median pronotal spot and the median area is always spotless. In the male genitalia, the penis tip of

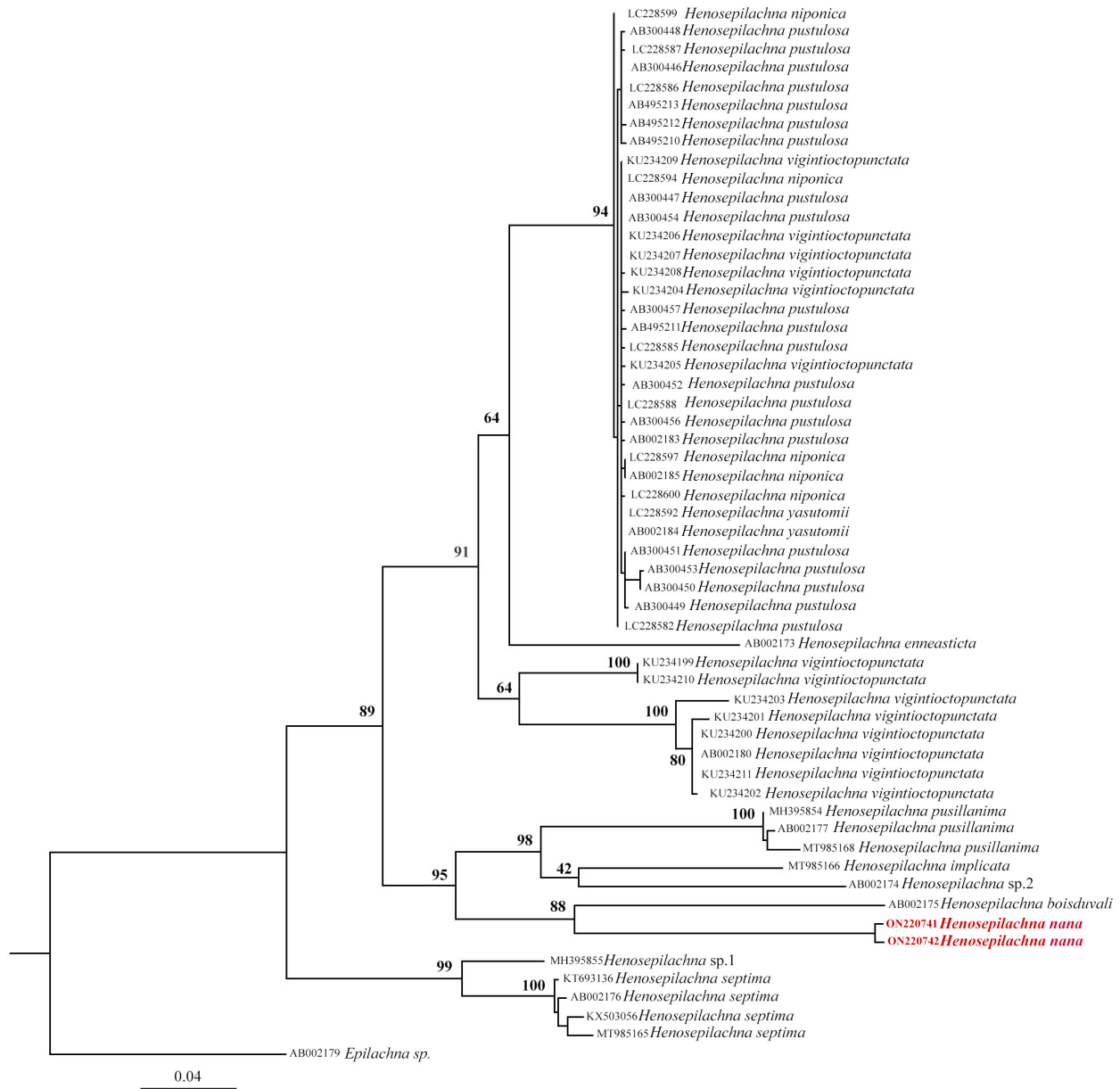


Figure 1. Maximum likelihood phylogenetic tree for *Henosepilachna* species based on COI sequences.

H. nana is similar to *H. boisduvali* but differs in having a small projection at the base of the notch. Poorani et al. (2021) have also mentioned 5–7 pronotal spots in *H. implicata*. In *H. nana*, the total number of pronotal spots is 2–4 and elytral black spots are 12 (6 on each elytron) which agrees with the observations of Kapur (1950) and confirms the identification of *H. nana*. The species *H. implicata* is with 26–28 black spots on the elytra, resembles other 28-spotted species of *Henosepilachna* such as *H. vigintioctopunctata*, *H. septima*, and *H. pusillanima* (Poorani et al. 2021). Although the tegmen of *H. implicata* and *H. nana* look a bit similar but it differs in their penis structure. The penis apex in *H. implicata* is

with a shallow notch (Poorani et al. 2021) while the penis apex in the species under study is with a deep notch having a central small projection at the base of the notch which is similar to the penis illustration of *H. nana* given by Kapur (1950). So far, no information is available about the host plant of *H. nana*. Kapur (1950) wrote a note on *E. ocellata* including *H. nana* in which he mentioned that *E. ocellata* is found on potato but he does not mention the host of *H. nana*. According to Katoh et al. (2014), adults and larval stages of all Epilachnini species show phytophagous feeding nature. The record of this species on the Cucurbitaceae plant is providing new host records for the species under study and related species. In the

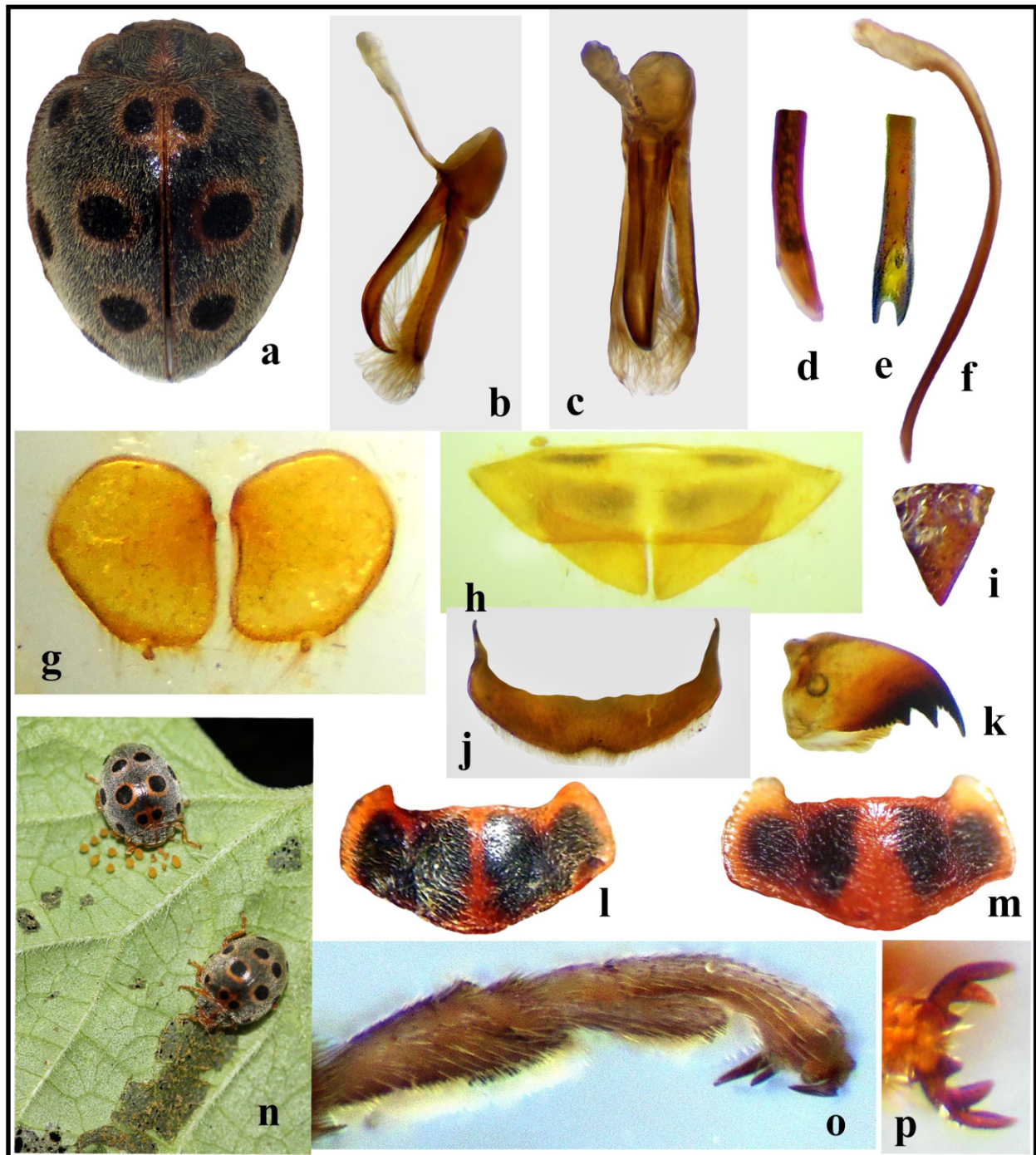


Image 2. a—*Henosepilachna nana* habitus | b—male genitalia: tegmen lateral view | c—tegmen ventral view | d—penis tip- lateral view | e—penis tip- ventral view | f—penis | g—female coxites | h—6th abdominal sternite of female | i— scutellum | j—6th abdominal sternite of male | k—mandible | l,m—pronotum | n—feeding adults | o—tarsi | p—tarsal claw. © Priyanka Patil.

present study, *H. nana* specimens were observed on the leaves of pumpkin, cucumber, and karit fruit plant which are additional host records.

A recent study on DNA sequence data and phylogenetic tree of *Henosepilachna* species includes five species (Poorani et al. 2021) excluding species *H.*

nana. Therefore, the COI gene sequence of *H. nana* is becoming available in the NCBI database for the first time. The present consensus tree arrangement shows the co-evolution of species from common ancestors. When the phylogenetic tree of *H. nana* was constructed using COI gene sequences of eleven species of the

Henosepilachna genus and one outgroup *Epilachina* sp. from the same family from NCBI's database. The species *H. nana* shows variation among its congeners.

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NAAS rating (India) 5.64

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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

March 2023 | Vol. 15 | No. 3 | Pages: 22771–22926

Date of Publication: 26 March 2023 (Online & Print)

DOI: 10.11609/jott.2023.15.3.22771-22926

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