Phytoparasitica (2021) 49:317–326
https://doi.org/10.1007/s12600-020-00855-y

SHORT COMMUNICATION

Mirid bug, *Helopeltis cinchonae* Mann: a new pest of economically important horticultural crops in Northeast India

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Received: 25 July 2020 / Accepted: 5 October 2020 / Published online: 14 October 2020

Abstract The mirid bug, *Helopeltis cinchonae* Mann (Order: Hemiptera) has been recorded for the first time on six economically important horticultural crops in India. The infestation of *H. cinchonae* was considerably higher during June–July on guava, geranium and chrysanthemum plants; whereas during August–September on brinjal and chili plants. Mirid bug, *H. cinchonae* was found causing significant yield losses to guava fruits and chili plants in Meghalaya. This study provides basic knowledge on biology, feeding damage and morphological characters of *H. cinchonae* supported with its DNA barcode and phylogeny.

Keywords Brinjal · Chili · Chrysanthemum · Guava · Geranium · Ginger · Tea mosquito bug

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Introduction

The mirid bugs of the genus *Helopeltis* (Hemiptera) are serious pests of many plant species in the world including India. Apart from its major host 'tea and cashew', numerous species of plants have been reported yet as hosts for *Helopeltis* spp., including black pepper *(Piper nigrum),* cashew (*Anacardium occidentale*), cinchona (*Cinchona* spp.), cocoa (*Theobroma cocoa*), and tea (*Camellia sinensis*) (Stonedahl et al. 1995; Mann 1907). Previously, three species of *Helopeltis* (*viz., H. theivora, H. bradyi* and *H. antonii*) are known to infest guava (*Psidium guajva*), chili (*Capsicum annum*) and brinjal (*Solanum melongena*) from India (Ganga Visalakshy et al. 2019; Kalita et al. 2016; Sivakumar and Yeshwanth 2019). We recorded a significant infestation of *Helopeltis cinchonae* (Hemiptera: Miridae) on chili plants and guava fruits at Umiam, Meghalaya. Also, this species was found developing on four more host plants *viz*., brinjal, geranium (*Pelargonium* spp.), chrysanthemum and ginger (*Zingiber officinale*) in Meghalaya.

Mann (1902) described *H. cinchonae* infesting cinchona trees at Munsong, British Bhutan and also at Mangpu (erstwhile Mungpoo), Darieeling (India). Besides this, no other information about *H. cinchonae* is available hitherto from India. Nevertheless, *H. cinchonae* is known to feed on over 60 plants including *Capsicum frutescens* and *Solanum* spp. in Malaysia (Miller 1941; Lever 1949). According to Stonedhal (1991), *H. cinchonae* is distributed in Bhutan, south-east Asia, Java, Taiwan and Assam province (which presently includes Assam, Meghalaya, Mizoram, Nagaland and Arunachal Pradesh states of India). Besides record of six new host plants, we report for the first time the feeding damage of *H. cinchonae* to chili and guava fruits in India. Externally, *H. cinchonae* are close to *H. theivora* (Mann 1902). Therefore besides morphological diagnosis, we characterized *H. cinchonae* for the first time at molecular level including its phylogenetic aspects.

Materials and methods

The experimental site was located at Umiam (25^{0}41' N latitude, 91^{0}55′ E longitude), Meghalaya in the North-Eastern Hill Region of India, representing the mid-altitude of 1010 m above the (MSL) with the agro-climatic zone of the mixed subtropical hills.

Observations and species confirmation

Mirid bugs were collected from six newly recorded host plants (*viz*., chili, guava, brinjal, ginger, chrysanthemum and geranium) planted in entomology farm and office premises of Institute during 2016–2018. The specimens of this unidentified mirid bug were examined and identified based on the external morphological characters. For species level confirmation, the genitalia characters of both males and female specimens were examined. The voucher specimens of *H. cinchonae* have been deposited in University of Agricultural Sciences, Bengaluru (UASB), India. Due to taxonomic complexities in the genus '*Helopeltis*', we have developed DNA barcode based on COI gene of the mitochondrial DNA to facilitate its rapid and reliable identification. The genomic DNA (gDNA) was extracted from the adults by using DNeasy® Blood and Tissue Kit (Qiagen, Valencia, USA) as described in (Behere et al. 2015). The standard barcoding region of the Cytochrome oxidase I (COI) gene of mtDNA was amplified using LepF1 and LepR1 primers as per procedure described for rose sawfly (Firake et al. 2013). For molecular studies, we extracted DNA from two *H. cinchonae* specimens collected from each host. The PCR amplicons of *H. cinchonae* from all the six hosts were sequenced commercially from M/S Eurofins Genomics India Pvt. Ltd., Bengaluru, India. The samples were sequenced from both the ends (5′ and 3′) for accuracy purposes. We used the Staden Molecular Biology analysis software for analysis of sequences (Staden et al. 1998). Sequence identity was determined by BLASTN search (Altschul et al. 1997) against the nr DNA database in GenBank.

Phylogenetic analysis and evolutionary divergence between *Helopeltis* species

In order to establish the evolutionary relationships of *H. cinchonae* with other *Helopeltis* species, we retrieved the text sequences of *H. antonii, H. bradyi* and *H. theivora* available at NCBI GenBank. All the nucleotide sequences were trimmed to equal length of 560 bp and were aligned with the sequence alignment program Clustal X (Thompson et al. 1997). The evolutionary relationship of *Helopeltis* species was inferred by the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993) in MEGA X (Kumar et al. 2018). The tree with the highest log likelihood (−1986.10) is shown as Fig. 2. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.2927)). The estimates of evolutionary divergence between four *Helopeltis* species were conducted using the Tajima-Neo model (Tajima and Nei 1984). The rate variation among sites was modeled with a gamma distribution. Evolutionary analysis was conducted in MEGA X (Kumar et al. 2018).

Occurrence and feeding damage of *H. cinchonae*

The incidence of *H. cinchonae* was studied during *kharif* 2016–2017 on brinjal and chili plants in experimental farms of entomology section at Umiam. Soon after the pest appearance, the plants of chili, *Capsicum annum* (variety: Guntur Hope) and brinjal, *Solanum melongena* (variety: Ri Bhoi local) were observed from the 25m^2 area at randomly selected three locations in the field and the infestation level (number of nymphs and adults per plants) was recorded. Incidence and feeding damage of *H. cinchonae* was also recorded from 3 randomly selected guava trees, 9 geranium plants and 13 chrysanthemum plants grown in office premises of the Institute. Observations were made at weekly basis from June to December.

To study the biology of this species, five pairs of *H. cinchonae* were exposed to the potted chili plants inside the specially developed cages (45x45x45cm) and maintained under laboratory conditions (25 ± 1 °C temperature, 75 ± 5% relative humidity and 12:12, light:

dark period). Observations on oviposition, incubation period, nymphal period, nymphal instars and adult longevity were recorded regularly.

Results

Identification of the mirid bug based on morphological and anatomical features

The characters of new mirid pest, *Helopeltis* sp. recorded in this study was found different than other three common species of *Helopeltis* in India (*viz.*, *H. antonii, H. bradyi* and *H. theivora*), this species is having the first antennal segment I subequal to or slightly longer than width of head including eyes, whereas in other three species it's greatly elongate. Morphologically, the *Helopeltis* sp. recorded in this study confirmed to be *H. cinchonae*, which differs from other related *Helopeltis* species such as the bright red colouration of the cuneus in *H. cinchonae* is another external morphological character which is absent (usually brown) in other three species. In male genitalia, the vesical with lobal sclerite is a very important structure in differentiating three species of *H. antonii*, *H. bradyii*, and *H. theivora* but the vesica is without the lobal sclerite in *H. cinchonae* (Plate 1a and b) and thus it can be easily distinguished form other three species. The structure of the female genital chamber is simple without out transverse and medial sclerities (Plate 1c and d), whereas the other three species have very prominent medial sclerites. *H. cinchonae* also lacks the sclerotized ribs at the lateral oviductus, but in the other three species it is present.

DNA barcode development of the mirid bug, *H. cinchonae* and phylogenetic analysis

DNA was successfully extracted and COI fragment was PCR amplified from multiple specimens of *H. cinchonae* from each host. After trimming the ambiguous 5′ and 3′ ends of the sequences, final 560 to 572 bp good quality sequences were also obtained for all specimens, which was further trimmed to 560 bp for phylogenetic analysis. *H. cinchonae* sampled from chilli, brinjal, guava, geranium, ginger and chrysanthemum were 100% identical. A BLASTn search of representative *H. cinchonae* as a query in NCBI shown 86.97 to 88.25% identity with *H. theivora* sequences deposited from India, Indonesia, Bangladesh and iBOL. Since no matching molecular data was available for *H. cinchonae* in NCBI Genbank, the identity of *H. cinchonae* was established on the basis of distinct taxonomic characters without any ambiguity. Since there was 100% similarities among the *H. chinchonae* sequences collected from six different hosts, the representative COI sequence of *H. cinchonae* has been deposited in the NCBI GenBank vide accession number 'MT775884'.

Maximum Likelihood tree derived from partial COI sequence shown clear clustering of *H. antonii*, *H. bradyii*, and *H. theivora* sequences deposited from different hosts and locations across the world. *H. bradyi* and *H. antonii* were monophylotic with 99% boostrap support value and shared common ancestor with *H. theivora* with 96% bootstrap support value. As shown in ML tree (Fig. 2), *H. cinchonae* appeared to be phylogenetically different from *H. bradyi* and *H. antonii* and shown 86.97 to 88.25% similarities with *H. theivora*.

Estimates of genetic divergence of *H. cinchonae* with *H. antonii*, *H. theivora* and *H. bradyi* was 0.196, 0.162, 0.195, respectively. Lower level of genetic distance were detected between *H. antonii* and *H. bradyi* (0.088). However, genetic divergence between *H. theivora* and *H. antonni* was 0.123 and *H. theivora* and *H. bradyi* was 0.133 (Table 1).

Occurrence and feeding damage of *H. cinchonae* on newly recorded host plants in Meghalaya

Chili

Incidence of *H. cinchonae* started since early June (vegetative growth stage). The population of *H. cinchonae* ranged from 2.33 to 6.67 nymphs & adults per plant over the season, although the population buildup was highest during August–September (Fig. 1). Both nymphs and adults preferred to stay on young shoots of dense humid areas of the plants (Plate 2a). Due to toxic salivary secretions, the feeding portion of the plants becomes tarnished and typical necrotic lesions appeared on the affected plant tissues leading to deformed tips and dieback of young shoots (Plate 2b). Nymphs and adults were also found feeding on immature fruits of chili during evening and morning hours. The infested fruits occasionally become shriveled with scabby appearance during later stages, losing their market value.

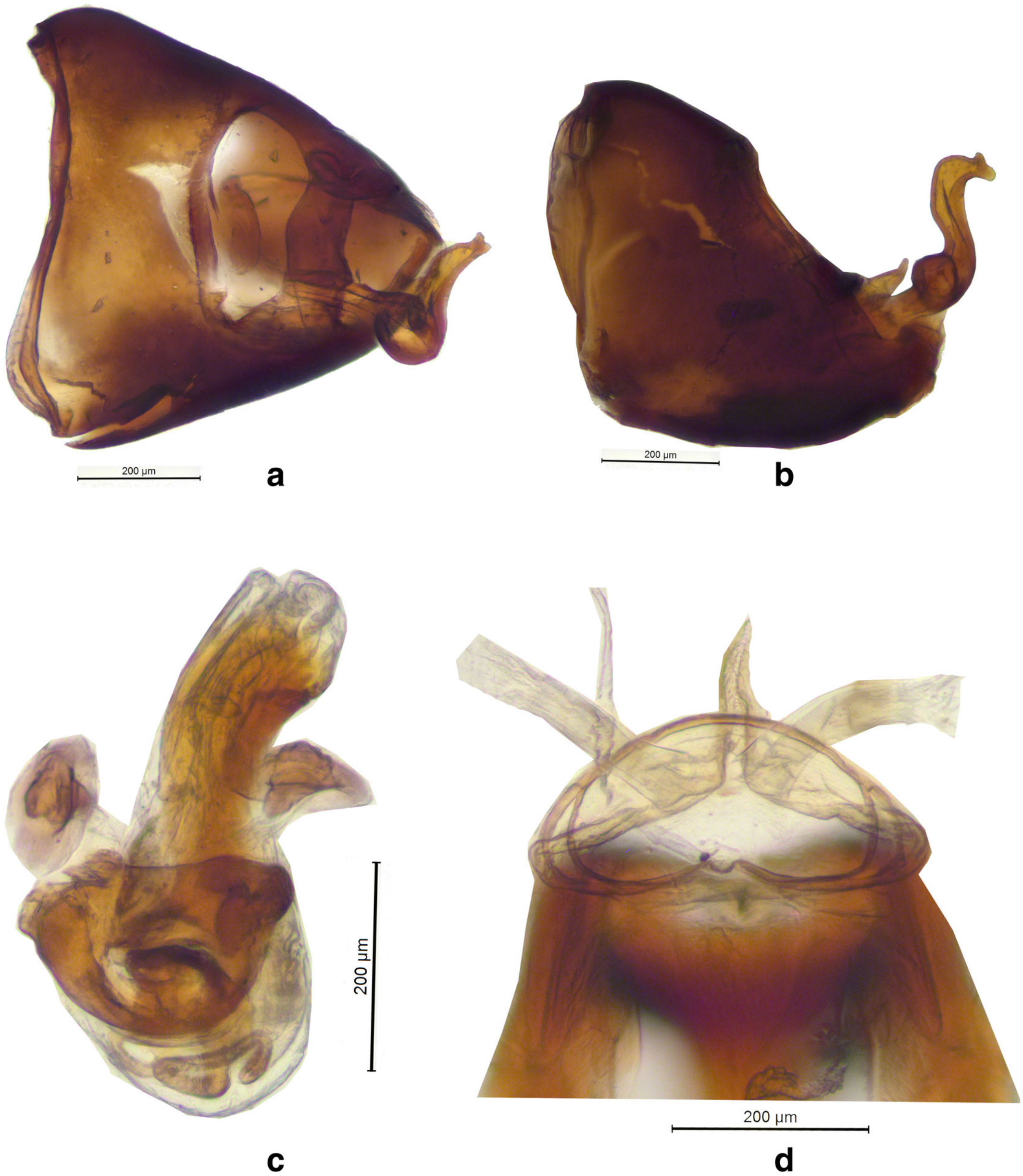

Plate 1 Genitalia structure of male and female *H. cinchonae*. Male genital capsule with parameres dorsal view (**a**), Male genital capsule with parameres lateral view (**b**), Aedeagus dorsal view (**c**), Female genital chamber (**d**)

Brinjal

Incidence of *H. cinchonae* was noticed from June, being higher during August (1.3–6.0 nymphs & adults per plant) (Fig. 1). Similar to chili, both nymphs and adults preferred to feed on young shoots of the plants and sometimes on leaves (Plate 2c and d) and occasionally damage to the young tender fruits. Unlike chili, the feeding spots and damage symptoms were less evident on brinjal plants.

Guava

Mixed population of *H. cinchonae* and *H. bradyi* were found sucking the sap from the marble sized guava fruits during June–July, resulted into corky outgrowth, scabby

Table 1 Estimates of evolutionary divergence between partial (560 bp) COI sequences of four species of the genus *Helopeltis*

	H. cinchonae	*H. antonii*	*H. theivora*	*H. bradyi*
H. cinchonae	–			
H. antonii	0.196	–		
H. theivora	0.162	0.123	–	
H. bradyi	0.195	0.088	0.133	–

Evolutionary divergence analysis was conducted using the Tajima-Nei model. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The differences in the composition bias among sequences were considered in evolutionary comparisons

lesions (black spots) on the fruits (Plate 3a). A total of 41.44% of total fruits develop black spots and scabby appearance by the end of the July due to *Helopeltis* spp. (Plate 3b). Sometimes the entire surface of immature fruits changed into black and such fruits drop prematurely, although the moderate to big size fruits often develops scabby appearance making them unmarketable.

Geranium and Chrysanthemum

Nymphs and adults of *H. cinchonae* were residing in new flush of the plants and suck the sap from the leaves especially during morning and evening hours (Plate 3c), making the characteristic transparent spots on the developing leaves (Plate 3d). The population was recorded to be higher during last week of June (3.67 nymph & adults per plant). *H. cinchonae* was also found feeding on new flush and leaves of chrysanthemum (Plate 3e).

Ginger

Nymphs and adults were occasionally found harboring newly formed ginger leaves (Plate 3f) and suck the sap from the tender portion of the plants, making the prominent scars on the leaves. Incidence of *H. cinchonae* was recorded during July–August.

Biology of mirid bug, *H. cinchonae* on chili plants

The oviposition period of female *H. cinchonae* was found to be 10–15 days. Adult females were found ovipositing near developing buds and young shoots of chili. Incubation period was found to be 14–20 days. After hatching, newly emerged nymphs were found harboring young shoots and feed on developing leaves, shoots and occasionally on fruits. Nymphal period was found completing in 33–44 days with five instars. Female longevity was found to be ranged from 16 to 20 days.

Discussion

Mirid bugs of genus '*Helopeltis*' including *H. cinchonae* are important pests of many cultivated

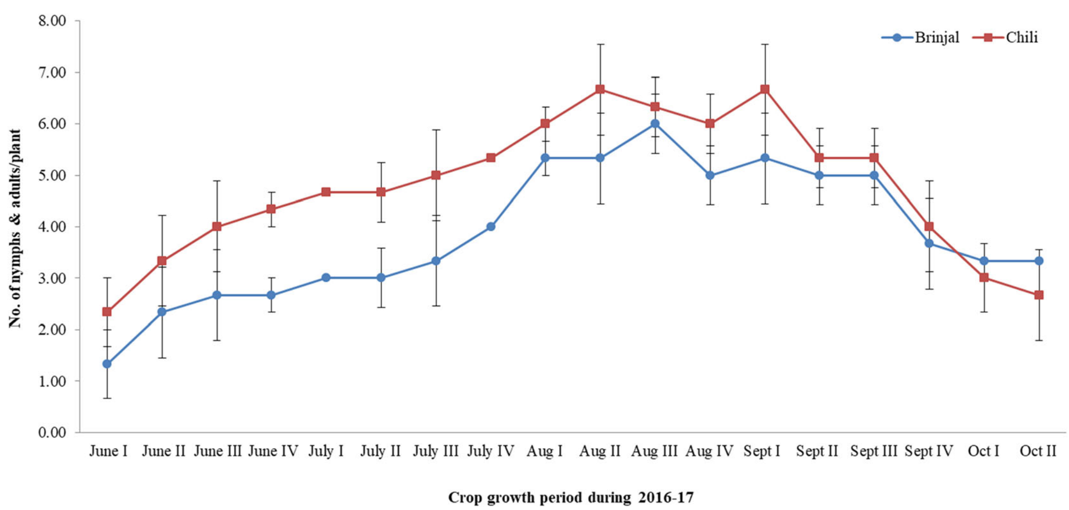

Fig. 1 Seasonal incidence of *H. cinchonae* on brinjal and chili at Umiam, Meghalaya during 2016–17. Error bar represents standard error of means

Plate 2 Nymphs of *H. cinchonae* are sucking the sap from flush and young shoots (**a**); Damage symptoms of *H. cinchonae* to chlli plants (**b**); *H. cinchonae* is feeding on young shoots near floral buds of brinjal (**c**) and on brinjal leaf (**d**)

plant species in Bhutan, south-east Asia, Java and Taiwan (Miller 1941; Lever 1949; Stonedahl 1991). Despite of three common species of *Helopeltis* in India (*viz*., *H. antonii, H. theivora* and *H. bradyi*), we recorded a large population of only *H. cinchonae* on chili, brinjal, geranium, chrysanthemum and ginger; while *H. bradyi* was found together with *H. cinchonae* on guava. This study is the first record of *H. cinchonae* on six new host plants.

We strongly believe that the *H. cinchonae* would also be available in other states of eastern India, but their presence was remained unreported probably due to misidentification with other related species. During survey work in Sikkim, we found nymphs and adults of *H. cinchonae* feeding on red cherry paper (*Capsicum annum*) and large cardamom (*Amomum subulatum*). However, the *Helopeltis* species damaging red cherry paper and large cardamom in Sikkim has been reported as *H. theivora* (Kalita et al. 2016; Kalita et al. 2018; Gopi et al. 2018). Since *H. theivora* looks apparently similar to *H. cinchonae*, a systematic investigation is necessary to determine the correct diagnosis of *Helopeltis* species in Sikkim and other parts of eastern India.

This study provides diagnostic morphological characters of *H. cinchonae* supported with DNA barcode for its reliable identification. *H. antonii* and *H. theivora* could easily be identified at molecular level using species specific markers Asokan et al. (2012). *H. cinchonae* differs from other three species externally in having first antennal segment short, the bright red coloration of the apex and inner margins of cuneus and the differently shaped scutellar horn and the male vescia without lobal sclerite which makes this species different from other three closely related species (Mann 1907). Stonedahl (1991) also mentioned two distinct species-groups of *Helopeltis* based on the structure of the female genital

Plate 3 *H. cinchonae* damage to marble sized guava fruits (**a**) and medium sized guava fruit (**b**); Nymph and adult of *H. cinchonae* are sucking the sap from geranium leaf (**c**); Feeding damage of *H. cinchonae* on geranium leaves (**d**); *H. cinchonae* adult feeding on chrysanthemum leaf (**e**) and on ginger leaf (**f**)

chamber and *H. cinchonae* does not fit any either species group. Therefore, *H. cinchonae* is clearly different species than that of other known *Helopeltis* species in India which is also evident from the high level of genetic distance (0.162 to 0.196) between *H. cinchonae* and other three species (*H. antonni*, *H. theivora* and

H. bradyi). As compare to other *Helopeltis* spp. the genetic distance between *H. antonni and H. bradyi* is only 0.09. Similar trend is also observed in Maximum Liklihood (ML) tree generated based on partial COI gene, where *H. antonni and H. bradyi* were monophyletic with 99% bootstrap support value (Fig. 2). Chandrashekara et al. (2015) also demonstrated the close relationships between *H. antonni* and *H. bradyi* based on partial COI gene. We sequenced twelve specimens of *H. cinchonae* collected from six different hosts but all the sequences were 100% identical. This sequence similarity could be attributed due to the fact that our *H. cinchonae* specimens were sampled from different hosts that were planted in closely located experimental farms/premises. Host associated genetic variation has been recorded in the *H. theivora* population collected from widely separated geographic locations in India (Chandrashekara et al. 2015). However, phylogenetic analysis based on partial COI gene (658 bp) did not show any geographic or host-associated genetic differences in *H. antonii* in India (Asokan et al. 2012). Therefore, analysis involving more *H. cinchonae* samples collected from different hosts and geographically separated locations would provide more insight in terms of host-associated genetic differences (if any) in *H. cinchonae.*

Studies on biology and life cycle of mirid bugs including their alternate host plants are very much useful

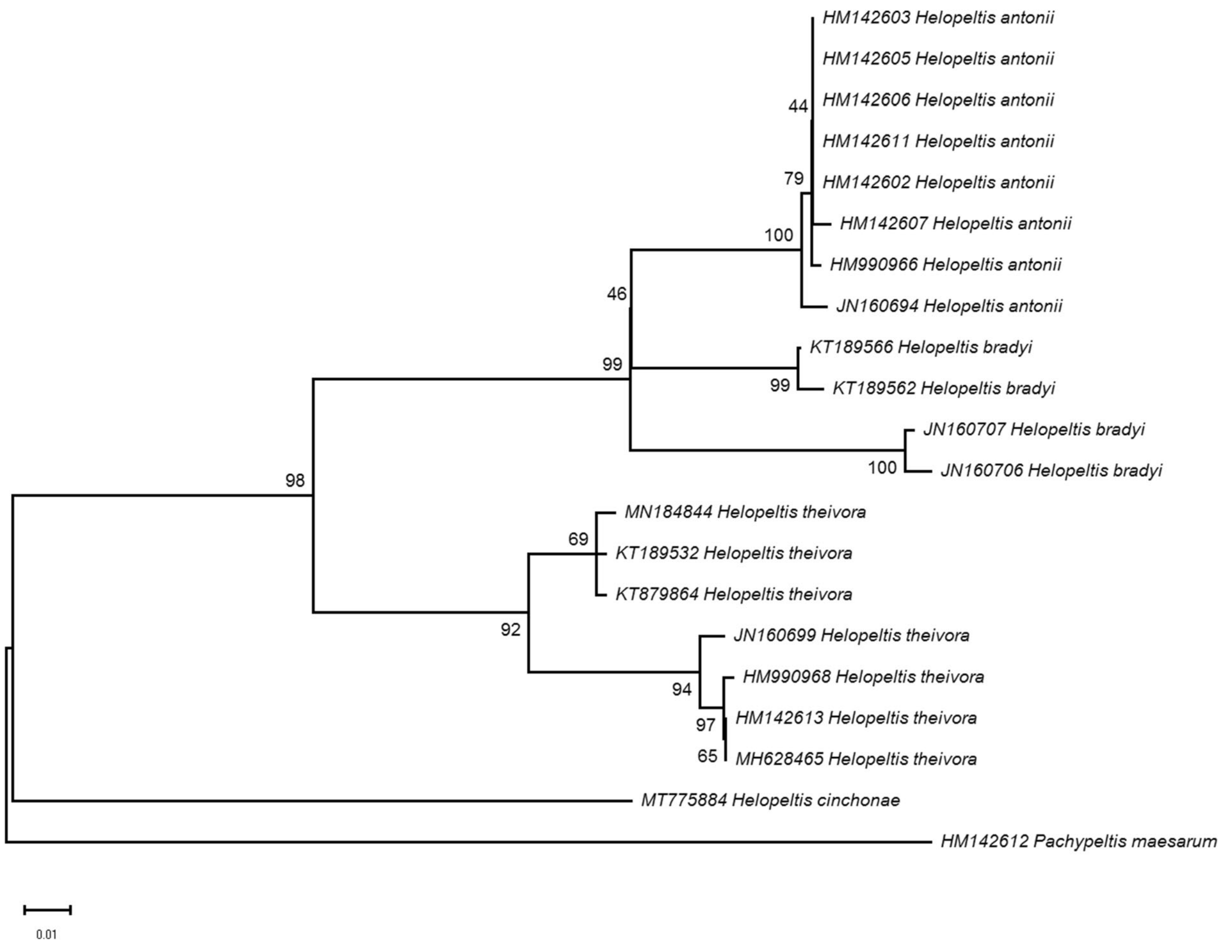

Fig. 2 Maximum Liklihood (ML) tree based on partial COI sequence (560 bp) of *Helopeltis cinchonae, H. antonii, H. bradyi* and *H. theivora*, with bootstrap support (1000 replicates), *Pachypeltis maesarum* (HM142612) were used as out groups. ML analysis involved 21 nucleotide sequences and there were a total of 560 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

for their better management decisions (Srikumar and Bhat 2013). Unlike other species of *Helopeltis*, information is very limited with respect to bioecology of *H. cinchonae* from India. According to Lever (1949), the activity of *H. cinchonae* found throughout the year in Malaysia and the biological attributes including oviposition choices of this species on other host plants are more or less similar in Malaysia as observed in our study. Higher incidence of *H. cinchonae* on guava and chili during June–September attributed to availability of favorable climatic conditions (sufficient moist weather) and appropriate growth stages of the host plants in the Meghalaya state. Damage of *H. cinchonae* is known to be severe during dull, calm and misty weather in tea fields of Cameron Highlands (Malaysia) and the activity of nymphs and adults is less during heavy rain, wind and bright sunshine (Lever 1949).

Conclusion

This study reports mirid bug, *H. cinchonae* as a new pest of chili and guava and provides basic diagnostic characters along with DNA barcode for quick and reliable identification. Besides its two major hosts, *H. cinchonae* was also found breeding on brinjal, geranium, chrysanthemum and ginger plants in Meghalaya. Therefore, these alternate host plants should be avoided growing near guava and chili plants to reduce pest multiplication.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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