Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Stethorini (Coleoptera: Coccinellidae: Coccinellinae) of South India: their associated mite species and barcode gap analysis

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Abstract

Background: Stethorini are specialized mite predators in the family Coccinellidae, which are small with black body and brown or yellow colored appendages. This study aimed to survey species of Stethorini associated with phytophagous mites of South India and their prey range.

Results: Eight species of Stethorini, viz., *Parastethorus* sp. *Stethorus* (*Allostethorus*) *forficatus*, *S.* (*A.*) *pauperculus*, *S.* (*A.*) *tetranychi*, *S.* (*A.*) sp., *S. keralicus*, *S.* (*S.*) *rani* and *S.* (*S.*) sp., each associated with distinct species of mite were identified in this study and part of mitochondrial cytochrome c oxidase subunit I sequences of seven of these species are provided. Barcode gap analysis and distance summary analysis in the BOLD system as well as phylogenetic analysis of these seven species were given.

Conclusions: This study revealed the species of Stethorini in South India along with their prey range and associated plants. As all the species look similar with black body and yellow appendages, DNA barcodes generated can be used as an effective bio-identification tool for this group.

Keywords: *Stethorus*, *Parastethorus*, Mite predators, COI gene, Barcode gap analysis

Background

Stethorini sensu stricto Dobzhansky is a group of coccinellid predators that are highly specialized feeders on mites belonging to the family Tetranychidae (spider mite) and Tenuipalpidae (false spider mite or flat mite). They pierce the prey, siphon prey juices and regurgitate them back and finally imbibe the juices, leaving the exoskeleton (Houck 1991). Stethorini occur in diverse habitats and many feed on different prey species, while some are more specialized, preferring to feed exclusively on certain species. Species of Stethorini have been used as potential biological control agents of spider mites and many species have been intentionally introduced throughout the world (Biddinger et al. 2009).

Members of Stethorini are grouped into two genera, viz., *Stethorus* Weise and *Parastethorus* Pang & Mao under the tribe Coccidulini of subfamily Coccinellinae (Seago et al. 2011). Earlier, Dobzhansky (1924) erected Stethorini as a tribe under the subfamily Scymninae to place a single genus *Stethorus*, which was earlier included under the tribe Scymnini. Kapur (1948) stated that the generic nomen *Stethorus* was originally proposed by Weise in 1885 as a subgenus of *Scymnus* and was later elevated to generic status by Weise & Casey in 1899. *Stethorus* was further divided into three subgenera, viz., *Allostethorus* Iablokoff-Khnzorian, *Parastethorus* and *Stethorus* based on the characters of genitalia and later, Slipinski (2007) raised subgenus *Parastethorus* to generic status. Based on morphology and a multilocus molecular dataset, Seago et al. (2011) revised the sub-familial classification of the family Coccinellidae and included the tribe

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Stethorini as part of the tribe Coccidulini. Members of Stethorini are cosmopolitan and the world fauna consists of more than 100 species (Biddinger et al. 2009) of which ten species are reported from India (Poorani 2002).

Adults of all species of Stethorini are small (< 2 mm), pubescent, black with brown or yellow colored antennae, mouth parts and legs. As these are cryptic in nature, misidentifications are commonly reported and therefore this group was a candidate for further diagnostic taxonomy using molecular barcoding methods (Biddinger et al. 2009).

Mitochondrial gene cytochrome c oxidase subunit I (COI) has been used widely as a 'barcode' for bio-identification in animals (Hebert et al. 2003). This has been used as a tool for the identification of many insects including beetles (Zhao et al. 2017). Huang et al. (2020) used mini barcoding primers to generate DNA barcode of 104 coccinellid species.

The objectives of this study were to identify species of Stethorini associated with phytophagous mites of South India, understand their prey range and provide barcodes of the species in order to facilitate identification of this otherwise cryptic group of beetles.

Methods

Sample collection and preservation

Extensive surveys were carried out in 27 locations covering three South Indian states, viz., Kerala, Karnataka and Tamil Nadu during 2015–2017 for the collection of Stethorini associated with phytophagous mites. Field crops, plantation crops, vegetables, fruits, spices, ornamentals and weeds were examined and the beetles were collected using aspirators. The grubs and pupae were also collected during the survey and were reared to adults. The beetles were preserved in 99% alcohol at − 80 °C for DNA extraction. For morphological characterization and identification of species, the beetles were either dry preserved by mounting on paper points or wet preserved in 70% alcohol. During the survey, the associated prey mites were also collected along with the infested leaf samples in polythene bags. In the laboratory, slides were prepared by mounting adult female and male mites separately on dorsal position in Hoyer's medium on glass slide. Morphological characters, viz., chaetotaxy of hysterosoma and legs, structure of empodium were used for genus level identification. Males of spider mites were also mounted in lateral position (Henderson 2001) for observing the shape of the aedeagus to determine the species.

Identification of Stethorini and prey mites

Species of Stethorini were identified based on available literature and taxonomic keys (Kapur 1948, 1961 and Poorani 2017). Mite specimens were identified based on available species descriptions and taxonomic keys provided by Gupta (1985), Gupta and Gupta (1994), Ehara (1995), Srinivasa et al. (2012) and Zeity et al. (2016).

DNA extraction, PCR amplification

DNA was extracted using Qiagen DNeasy® blood and tissue kit following manufacturer's protocol. The quantity and quality of DNA samples were assessed using Nanodrop spectrophotometer by measuring the absorbance at wavelength of 260 nm and 280 nm (JenwayGenova Nano). The AE buffer in the extraction kit, in which the isolated DNA was stored, was used as blank to measure the absorbance.

The DNA extracts were subjected to polymerase chain reaction (PCR) in order to amplify 658 bp region near the 5′ terminus of the mitochondrial protein coding gene, mitochondrial cytochrome c oxidase subunit I, by following standard protocols. The reaction was carried out in 20 µl reaction volume containing 10 µl EmeraldAmp® GT PCR Master Mix, 9 µl of molecular water + template DNA and 0.5 µl each of forward primer and reverse primer. The forward and reverse primers used were LCO 1490: 5′-GGTCAACAAATC ATAAAGATATTGG-3′ and HCO 2198: 5′-TAAACT TCAGGGTGACCAAAAAATCA-3′ respectively (Folmer et al. 1994). The PCR was performed using Eppendorf Master Cycler gradient thermal cycler. Thermal cycling included an initial denaturation of 94 °C for 4 min, followed by 30 cycles of denaturation of 94 °C for 30 s, annealing 60.1 °C for 1 min and extension of 72 °C for 2 min, followed by final extension of 72 °C for 10 min. The amplified product was analyzed by Agarose Gel Electrophoresis (AGE) in 1.2 per cent agarose gel.

Sequencing and data analysis

PCR products were bidirectionally sequenced using Sanger technology at AgriGenome Labs Pvt Ltd, Kochi, Kerala, India. Sequencing was done by using Big Dye 3.1 cycle sequencing kit and the instrument used was sequencing machine ABI 3730 XL DNA Analyzer. The forward and reverse sequences were aligned to check for nucleotide mismatches at specific positions. The final alignment was assembled in CAP 3 and was checked for stop codons using MEGA7 (Molecular Evolutionary Genetics Analysis) (Kumar et al. 2016). Homology search with the available database was done using NCBI BLAST. The sequences generated were submitted to GenBank (NCBI) and BOLD (Barcode of Life Data Systems).

BOLD analysis

Seventeen sequences of seven species of Stethorini were subjected to barcode gap analysis and distance summary analysis in BOLD. Barcode gap analysis provides the distribution of distance within species and distance to the nearest neighbor. The distance summary gives the distribution of within species divergence to between species divergence for the samples used in the study. The distance model used in both analysis was Kimura 2 Parameter. K2P was used as the model as it has been used widely in DNA barcoding literature and hence it facilitates comparisons with earlier works (Candek and Kuntner 2014). BOLD aligner (Amino Acid-based HMM) was used for the alignment of sequences.

Phylogenetic analysis

Fifteen COI sequences from India comprising 6 putative species of *Stethorus* and three from one species of *Parastethorus* were generated as part of this study. Nine sequences of *Stethorus* were obtained from NCBI that belong to the species *S. punctillum* Weise, *S. pusillus* (Herbst) and *S. punctum* (LeConte). Representatives of the genus *Scymus* (4 sequences) were used as out-group according to the phylogeny published by Giorgi et al. (2009). The sequences used for phylogenetic analyses are listed in Table 1.

The maximum likelihood (ML) algorithm was used for phylogenetic inference as implemented in the online version of IQ-TREE (Trifinopoulos et al. 2016), where branch support was evaluated using ultrafast bootstraps (Hoang et al. 2018). The sequence type was set to Invertebrate mitochondrial (CODON 5 in IQ-TREE) and data were partitioned using the edge linked method by codon position. The substitution models for each partition recommended and used by IQ-TREE are listed in Table 2. Ultrafast bootstrapping and single branch tests were performed using 1000 replicates (alignments) and a correlation coefficient of 0.99. Parameters for tree search were left at default values.

Table 1 Sequences used for phylogenetic analysis

Name of species	NCBI accession number
Parastethorus sp.	MG672475.1
	MG672476.1
	MG672477.1
Stethorus (*Allostethorus*) *forficatus*	MG672471.1
	MG672472.1
	MG672473.1
	MG744262.2
S. (*A.*) *pauperculus*	MG672468.1
S. (*A.*) sp.	MG744264.2
	MG744265.2
S. (*Stethorus*) *rani*	MG744266.2
	MG744267.2
	MG744268.2
S.(*S.*) sp.	MG744261.2
	MG744263.2
S. keralicus	MF592697.1
	MG672466.1
	MG672467.1
S. punctillum	MG055530
	JF889549
	KM446280
	MG055530
	KM448697
	MF594675
S. punctum	MG059221
	HQ582511
S. pusillus	KJ963340
Scymnus scapanulus	MK 802,060.1
Scymnus pinguis	MK 802,059.1
Scymnus trisulicus	MK 802,062.1
Scymnus sufflavus	MK 802,061.1

Table 2 Substitution model recommended for each partition

Partition No	Model	BIC score
1	TIM2e+G4	1877.231
2	F81+F+I	798.367
3	TPM3u+F+G4	5490.732

Results

Species of Stethorini and their prey range

Eight distinct lineages of Stethorini belonging to two genera, viz., *Parastethorus* and *Stethorus* associated with seven species of mites of families Tetranychidae and Tenuipalpidae were identified in this study. Genus *Stethorus* was represented by two subgenera, *Allostethorus* and *Stethorus*. Of the eight lineages, five were identified as nominal species, two up to the subgenus and one at the generic level. Species identified included *Stethorus* (*Allostethorus*) *forficatus* Poorani, *S.* (*A.*) *pauperculus* (Weise), *S.* (*A.*) *tetranychi* Kapur, *S. keralicus* Kapur and *S.* (*Stethorus*) *rani* Kapur. The prey and associated host plants of Stethorini observed during this study are given in Table 3.

Sequence analysis and DNA barcoding

Eighteen sequences were generated for seven species and homology analysis of these sequences in BLAST showed a similarity that ranged from 88 to 84% with

Table 3 Stethorini species associated with different mite species

Species	Associated prey	Host plant
Parastethorus sp.	*Eutetranychus orientalis* (Klein)	Castor, plumeria
S. (*A.*) *forficatus*	*Tetranychus truncatus* Ehara	*Amaranthus*, cosmos, cowpea, papaya, tapioca
	T. okinawanus Ehara	Cowpea
	Oligonychus biharensis (Hirst)	*Bauhinia*
	No prey found	Jack, mulberry, *Butea monosperma*
S. (*A.*) *pauperculus*	*Oligonychus indicus* (Hirst)	Bajra, maize, sorghum
	Oligonychus sp.	Arecanut, banana
	Tetranychus truncatus	Okra, cowpea
	T. macfarlanei Baker and Pritchard	*Amaranthus*
	T. okinawanus	Cowpea
	No prey found	Banana, coconut, ridge gourd, soyabean, sugarcane
S. (*A.*) *tetranychi*	*Tetranychus* sp.	Coconut
	O. biharensis	*Bauhinia*
	No prey found	*Helicteres isora*
S. (*A.*) sp.	*Tetranychus* sp.	Coconut
S. (*S.*) *rani*	*Tetranychus* sp.	Cardamom
S. (*S.*) sp.	No prey found	Pomegranate
S. keralicus	*Raoiella indica* Hirst	Arecanut, banana, coconut

the sequences in NCBI database, except for *Parastethorus* which showed 95 per cent similarity with a species of coccinellid, whose species identity is not mentioned. Generated sequences were submitted in GenBank and BOLD and the details of NCBI accession number and Barcode index number are given (Table 4).

BOLD analysis

The distribution of sequence divergence at each taxonomic level using distance summary analysis and mean intraspecific distance, maximum intraspecific and distance to nearest neighbor using Barcode gap analysis in BOLD are given (Tables 5 and 6). The mean distance

Table 4 NCBI accession number and Barcode index numbers for species of Stethorini

Species	Locality	Prey/host plant	NCBI accession number	Barcode index number
Parastethorus sp.	Madathukulam, TamilNadu	*Eutetranychus orientalis* on castor	MG672475	BOLD: ADK9009
	Madathukulam, TamilNadu	*Eutetranychus orientalis* on castor	MG672476	BOLD: ADK9009
	Madathukulam, TamilNadu	*Eutetranychus orientalis* on castor	MG672477	BOLD: ADK9009
Stethorus (*Allostethorus*) *forficatus*	Thavanur, Kerala	*Tetranychus truncatus* on *Cosmos*	MG672471	BOLD: ADK8741
	Vellanikkara, Kerala	*Tetranychus truncatus* on Papaya	MG672472	BOLD: ADK8741
	Avinissery, Kerala	on *Butea monosperma* (no prey found)	MG672473	BOLD: ADK8741
	Bengaluru, Karnataka	on *Bauhinia* (no prey found)	MG744262	BOLD: ADK8741
S. (*A.*) *pauperculus*	Raichur, Karnataka	*Oligonychus indicus* on bajra	MG672468	BOLD: ADK8742
S. (*A.*) sp.	Tiruchirappalli, Tamil Nadu	*Tetranychus* sp. on coconut	MG744264	BOLD: ADW9988
	Ambalavayal, Kerala	*Tetranychus* sp. on coconut	MG744265	
S. (*Stethorus*) *rani*	Pampadumpara, Kerala	*Tetranychus* sp. on cardamom	MG744266	BOLD: ADW4083
	Pampadumpara, Kerala	*Tetranychus* sp. on cardamom	MG744267	BOLD: ADW4083
	Pampadumpara, Kerala	*Tetranychus* sp. on cardamom	MG744268	BOLD: ADW4083
S. (*S.*) sp.	Ambalavayal, Kerala	on pomegranate (no prey found)	MG744261	BOLD: ADW4271
	Ambalavayal, Kerala	on pomegranate (no prey found)	MG744263	BOLD: ADW4271
S. keralicus	Vandazhy, Kerala	*Raoiella indica* on arecanut	MF592697	BOLD: ADI4772
	Vadakkenchery, Kerala	*Raoiella indica* on arecanut	MG672466	BOLD: ADI4772
	Vellanikkara, Kerala	*Raoiella indica* on arecanut	MG672467	BOLD: ADI4772

Table 5 Distribution of sequence divergence

	Minimum distance (%)	Maximum distance (%)	Mean distance (%)	SE distance (%)
Intraspecific	0	2.73	0.68	0.05
Interspecific	13.67	23.54	19.10	0.03
Intergeneric	16.62	23.10	19.34	0.05

within species was 0.68%, whereas that of within genus and within family was found to be 19.10 and 19.34%, respectively. The minimum and maximum divergences within species were recorded as zero and 2.73. The maximum intraspecific distance varied from 0 to 2.73, whereas the distance to the nearest neighbor varied from 13.67 to 19.27.

Phylogenetic inference

The phylogenetic reconstruction presents a strong support for all branches leading to species-level lineages, but not for branches distending from deeper nodes in the tree (Fig. 1).

Nevertheless, the monophyly of *Stethorus* + *Parastethorus* was strongly supported (BS 100). However, the composition of the two broad clades within it and the phylogenetic position of *Parastethorus* cannot be inferred due to the lack of robust branch support. The monophyly of all species-level lineages is recovered with strong support (BS 98–100), reinforcing the taxonomic validity of the species from a phylogenetic perspective. Phylogenetic inference suggests that the sequence of *S. pusillus* was nested within the *S. punctillum* clade. It was understood that *S. pusillus* and *S. punctillum* were synonyms.

Discussion

Species of Stethorini and their prey range

Stethorini consists of two genera, viz., *Parastethorus* and *Stethorus*. The present study included one species of *Parastethorus* and seven species of *Stethorus*. There are 14 species of *Parastethorus* reported around the world (Li et al. 2015). *Parastethorus indira* (Kapur) is the only species of *Parastethorus* reported from India so far. Genus *Stethorus* is represented by the subgenera *Allostethorus* and *Stethorus*. World fauna of *Stethorus* consists of approximately 94 species, of which 38 belong to *Stethorus* (*Allostethorus*). Ten species belong to two genera, viz., *Parastethorus indira*, *S. gilvifrons* (Mulsant), *S. keralicus*, *S. parcepunctatus* (Kapur), *S. pauperculus*, *S. rani*, *S. tetranychi* (Poorani 2002), *S. forficatus* (Poorani 2017), *S. aptus* (Govindasamy and Khursheed 2018) and *S. gangliiformis* Li, Chen & Ren (Janakiraman and Thangjam 2019) were reported from India so far.

Stethorini is the only mite specialist predator under the family Coccinellidae (Biddinger et al. 2009) feeding on tetranychid or tenuipalpid mites. Stethorini usually feed on different species of tetranychid mites, but a few are specialised on some species. For instance, *S. punctillum* and *S. gilvifrons* do not prefer to feed on or reproduce on the tetranychid mites of the genus *Bryobia* (Kaylani 1967). There are reports on the utilization of non-tetranychid or non-tenuipalpid mite as prey, especially during starvation (Biddinger et al. 2009).

Prey ranges of different species of coccinellids reported from India were catalogued by Pervez (2004), in which they also mentioned non-mites as prey of *Stethorus*. Biddinger et al. (2009) opined that reports of Stethorini feeding on non-acarid prey and non-prey foods challenge the argument that Stethorini are specialized mite predators. However, they believed that superficial similarity of *Stethorus* to some species of *Delphastus*, *Scymnus* and *Telsimia* might have accounted for most reports of Stethorini feeding on non-mites like scale or aphids. During the present study, we could observe Stethorini feeding only on tetranychid and tenuipalpid mites. In this study, *S.* (*A.*) *pauperculus* recorded a wider host range included *Oligonychus indicus*, *Tetranychus macfarlanei*, *T. truncatus* and *T. okinawanus*. *Stethorus keralicus* was found to

Table 6 Comparison of species with nearest-neighbor species

Species	Mean intraspecific distance	Max. intraspecific distance	Nearest-neighbor species	Distance to nearest neighbor
Parastethorus sp.	0.42	0.64	*S.* (*A.*) *pauperculus*	16.62
S. (*A.*) *forficatus*	0.42	0.64	*S.* (*A.*) *pauperculus*	13.67
S. (*A.*) *pauperculus*	N/A	0	*S.* (*A.*) *forficatus*	13.67
S. (*A.*) sp.	N/A	0	*S.* (*A.*) *forficatus*	14.30
S. (*S.*) *rani*	1.82	2.73	*S.* (*A.*) sp.	15.04
S. (*S.*) sp.	0	0	*S.* (*S.*) *rani*	15.70
S. keralicus	0.53	0.80	*S.* (*A.*) sp.	19.27

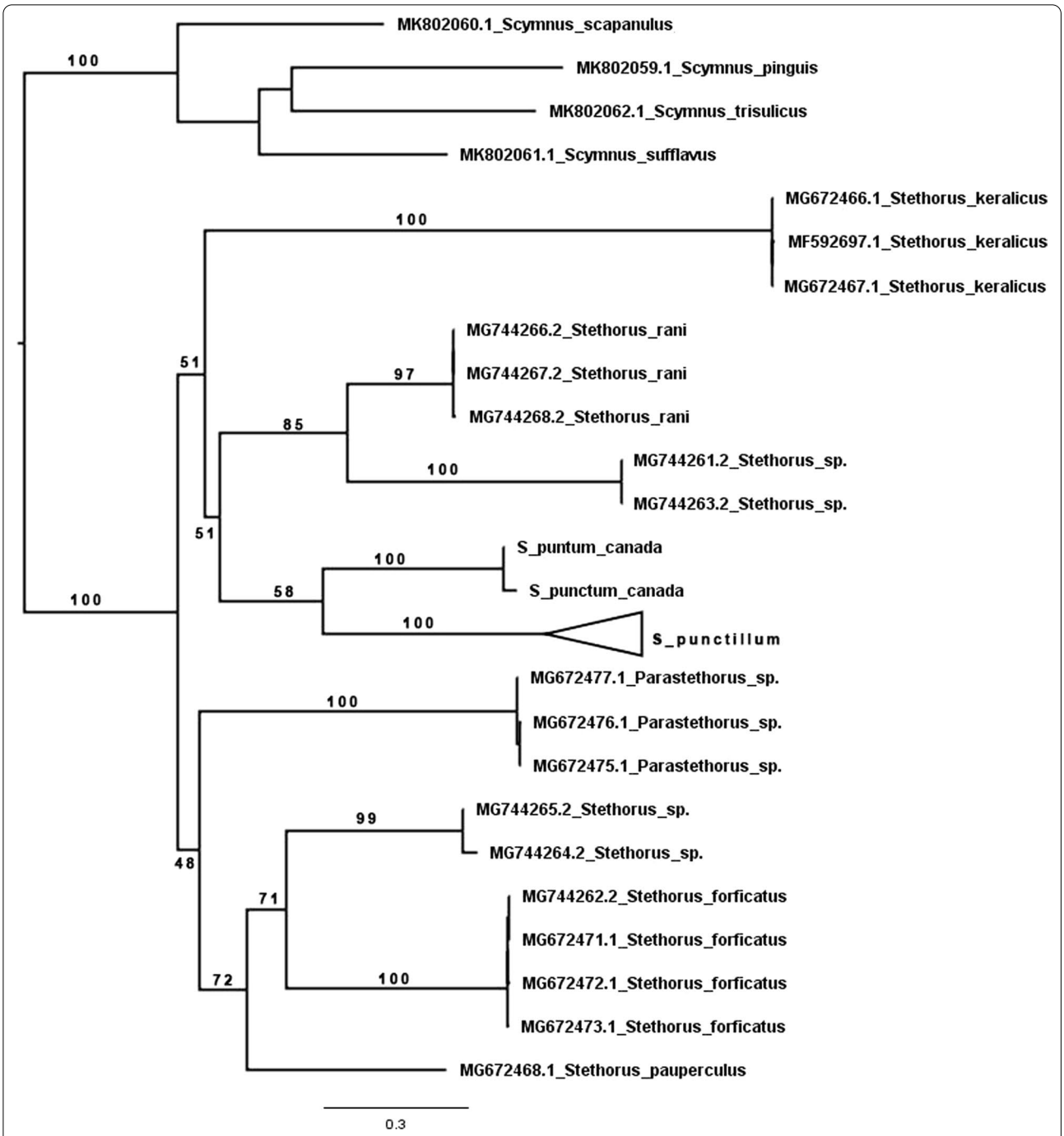

Fig. 1 A maximum-likelihood-based phylogenetic reconstruction of the Indian species of *Stethorus* and *Parastethorus*. The numbers represent bootstrap support for the respective branches as evaluated based on the online version of IQ-TREE

be more specialized and recorded only on a tenuipalpid mite, *Raoiella indica.* Carrillo et al. (2012) considered *S. keralicus* specific to the mites of the genus *Raoiella*. Interestingly, whenever an infestation of *R. indica* was observed on arecanut, *S. keralicus* was found in association with the mites. Daniel (1981) considered *S. keralicus* as the most important predator of *R. indica* in Kerala. *Stethorus keralicus* was a voracious predator of all stages of *R. indica* and was found throughout the year (Puttaswamy and Rangaswamy 1976).

DNA barcoding

Among the 17 sequences (seven species under two genera) generated during the present study, there was no overlap between intraspecific and interspecific variations. In this dataset, the maximum intraspecific distance was relatively low (0–2.73) and the nearest-neighbor distance between species was found to be higher (13.67–19.27). This may be due to the smaller number of samples in the study. There was a significant positive correlation between the number of specimens sampled for a species and its maximum intraspecific divergence (Pentinsaari et al. 2014). However, among the species studied the maximum intraspecific distance was lower than the minimum interspecific distance.

The analysis showed that the nearest-neighbor species of *Parastethorus* sp. was *S.* (*A.*) *pauperculus*, *S.* (*A.*) sp. was *S.* (*A.*) *forficatus*, and *S.* (*S.*) sp. was *Stethorus* (*Stethorus*) *rani*. Meier et al. (2008) suggested that in order to check the barcode gap the overlap between the lowest interspecific and highest intraspecific genetic distance has to be considered. They estimated that for Coleoptera, the mean intraspecific variability (%), mean interspecific variability (%) and smallest interspecific variability (%) were 2.0 ± 2.3, 11.2 ± 4.3 and 7 ± 5.4, respectively. The study of North European beetle fauna by Pentinsaari et al. (2014) showed that the nearest-neighbor divergences among species of Coleoptera were higher than those detected in other groups such as Lepidoptera and the overall average K2P divergence between nearest-neighbor species for Coleoptera was 11.99%. They also reported that for Coccinellidae the minimum, mean and maximum divergences within species were (zero, 1.34 and 7.83), respectively. The minimum, mean and maximum distances to nearest-neighbor species were estimated as (zero, 11.91 and 21.12), respectively.

In the present study, it was observed that maximum interspecific distance was more than maximum intergeneric distance. This means species within a particular genus was found to be more divergent than species within two different genera. This is more evident when the species were compared with nearest-neighbor species. Genetic distance of *S. keralicus* to its nearest neighbor (*S.* (*Allostethorus*) sp.—19.27) was larger than the genetic distance of *Parastethorus* sp. to its nearest neighbor (*S. pauperculus*—16.62). Among the species studied, *S. keralicus* was the most genetically distant species. *Stethorus keralicus* was found to be distinct from other species of Stethorini with 10 antennomeres and variation in shape of male genitalia and spermatheca. Hence, the question arises whether *S. keralicus* has to be treated separately, for which further studies are needed.

Phylogenetic analysis

The position of *Parastethorus* in the phylogeny presented here causes paraphyly within *Stethorus*. In order to maintain cladistic correctness, *i.e.,* the monophyly of *Stethorus*, *Parastethorus* must be subsumed under the former. However, this phyletic relationship must be tested further using nuclear DNA to be able to make a tangible inference, and therefore, we refrain from making any taxonomic changes at this stage. Given the morphological similarities between *Stethorus* and *Parastethorus*, we posit that the latter might just be a divergent lineage of the broader *Stethorus* radiation. Similarly, the species *S. keralicus* was sister related by a well-supported (BS 100) long branch to a broad clade comprising *S. rani*, *S. punctum*, *S. punctillum* and an unidentified species. Given its morphological dissimilarities with other *Stethorus* sp., this relationship was expected to change with better sampling. *Stethorus* were poorly sampled for genetic studies from across the world, with very few sequences publicly available for use. Further phylogenetic studies that incorporate near-complete sampling in terms of species and a larger genetic dataset will be able to resolve relationships within this cosmopolitan group of beetles.

Conclusion

Eight species of Stethorini belonging to two genera were identified along with their prey and associated host plants from South India. The sequences of seven species of Stethorini were submitted to NCBI and BOLD for the first time. Cryptic characters of adults of *Stethorus* might have led to species misidentification in the literature. As all the species look similar with black body and yellow appendages, sequences of COI will be used for easy and reliable identification. Thus, DNA barcoding and estimation of genetic distance can be used as an effective tool in separating the species and hence complementary to conventional taxonomy.

Abbreviations

COI: Cytochrome C oxidase subunit I; PCR: Polymerase chain reaction; AGE: Agarose Gel Electrophoresis; MEGA: Molecular Evolutionary Genetics Analysis; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information; BOLD: Barcode of Life Data Systems; K2P: Kimura 2 Parameter; ML: Maximum likelihood.

Acknowledgements

The first author is grateful to Kerala Agricultural University, India, for the financial and physical support extended during the period of study. We thank Dr. Miko Pentinsaari (Finland) for his critical comments in barcoding part of the paper, Dr. J. Poorani (India) for her help in confirmation of species and Dr. D. Girija & Dr. Mani Chellappan (KAU) for the laboratory facilities.

Author contributions

This is a part of CVV's PhD research project. CVV and HB conceptualized and designed the experimental work. CVV and HB did the field collection of specimens. CVV performed the laboratory works. CVV identified the coccinellids. HB identified the mites. CVV and DM performed the sequence analysis and

barcode analysis. CR performed phylogenetic analysis. CVV and CR wrote the manuscript with substantial input of all other authors. All authors read and approved the final manuscript.

Funding
The work was funded by Kerala Agricultural University, Kerala, India.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

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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Received: 13 June 2022 Accepted: 26 October 2022
Published online: 30 October 2022

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