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Cover: Green Sea Turtle *Chelonia mydas* watercolour by Elakshi Mahika Molur.

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COMMUNICATION

Report of *Bathycoelia indica* Dallas, 1851 (Hemiptera: Heteroptera: Pentatomidae) as a pest of pomegranate *Punica granatum* L. cultivated in Maharashtra State

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Abstract: A pentatomid bug *Bathycoelia indica* Dallas, 1851 is reported as a pest of pomegranate plantation in Maharashtra (India). Brief re-description of the species, including that of male and female genitalia, eggs, some nymphal stages and live adults, with many digital illustrations, is provided.

Keywords: Bathycoeliini, DNA Barcode, eggs, genetic divergence, male and female genitalia, nymphs, pest, stink bug.

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Author contributions: PSK did field work and worked on lab population. GGG did molecular analysis. HVG dissected the specimens and prepared images. All contributed to writing and checked the text.

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INTRODUCTION

There was a plantation of pomegranate *Punica granatum* L. over an area of about 3 acres in Shindodi (District Ahmednagar), Maharashtra, India, until January 2023. The trees in this plantation were seven years old; the plants were about 3 m tall (Image 1A).

On 6 August 2022, one of us (PSK) noticed a few nymphs of a pentatomid bug feeding on fruits and tender shoots (Image 1C,F). Within a few days, on 15 August, there were many adult bugs affecting practically every plant (Image 1B), some pairs were mating. A couple of fifth instar nymphs were observed, along with adults, on 5 September. Total number of bugs on the farm reached several hundred specimens within 15 days. The trees started showing effects of this infestation by bugs, such as: curling and yellowing of young leaves and puncture marks that led to black spotting of all affected fruits (Image 1D). By mid-September approximately 70–80 % of trees showed stunted and spotted fruits, no control measures were applied, no pesticides were sprayed. Eventually, the entire produce of the farm lost its value as all pomegranates were damaged by bugs. The plantation was cut down in February 2023 for other cultivation.

This pentatomid bug was subsequently identified using keys in Salini & Viraktamath (2015) as *Bathycoelia indica* Dallas, 1851. Additionally, species confirmation was also made by sequencing ~550 nucleotide bases of the mitochondrial barcoding region [cytochrome c oxidase subunit gene (COI)], which was aligned with other related species of the family Pentatomidae and subjected to phylogenetic analyses.

Brief morphology of the bug and comments on the structure of the male and the female genitalia, eggs, and nymphs are provided here.

MATERIALS AND METHODS

Field observations were carried out every 15 days from August to October 2022. Heavy rains disturbed the population of bugs. So, a few bugs were collected in August 2022 for laboratory rearing to observe mating and subsequent life history. Five males and five females, which are easily identifiable because of external morphology, were kept in large 5 l plastic jar and provided with fresh tender stems and small fruits of pomegranate.

Dissections of male and female genitalia were done as per established methods. Briefly, the male was treated with warm 10% KOH for five minutes and the pygophore was pulled out with fine forceps. The dissected male was then washed with 5% acetic acid and then 70% alcohol and absolute alcohol before mounting on card. The pygophore was further boiled in 10% KOH for 8–10 min. Parameres were removed with fine forceps and the pygophore was carefully opened from dorsal side to free the phallus from attachment. The phallus was then treated with 10% lactic acid for 15 minutes and then carefully everted with forceps. For female genitalia the abdomen was boiled in 10% KOH for 10 minutes and washed with water. The female genitalia, including spermatheca, were stained with dilute methylene blue for contrast. Terms used broadly follow Morariu (2012), Salini (2015), and Schuh & Weirauch (2020).

In the field, photographs were taken on mobile camera Moto G Plus which is equipped with 16-megapixel camera, while in the laboratory photographs were taken on Leica stereozoom microscope MZ-6 with attached Canon Powershot S50. Multiple photos taken under microscope were stacked using Combine ZM freeware. The images were processed in Photoshop CS5.

Total genomic DNA was extracted from two legs of a single specimen which was further subjected to COI amplification and sequencing following the protocols as mentioned in Tembe et al. (2014); the work was outsourced this time to Barcode Biosciences, Bangalore, who provided sequence data.

Sequence alignment

The reverse and the forward sequences were aligned in MEGA v.6 (Tamura et al. 2013) and a consensus sequence was generated with the help of chromatograms visualized in Chromas v.2.6.5 (Technelysium Pty. Ltd. 2018). Sequences of related species from the family Pentatomidae available on GenBank® (Benson et al. 2017) were downloaded, including a sequence of *Bathyoelia indica* (HQ236463) and were aligned with the newly generated sequence using MUSCLE incorporated in MEGA v.6. Low quality ends were trimmed and the resultant 467 base pair (bp) long alignment was used for molecular phylogenetic analyses. Other sequences included in the alignment are listed in the Table 1.

Genetic divergence (p-distance)

The p-distances were calculated for the mitochondrial COI in MEGA v.6. The substitution type was set as nucleotide, the model was kept as *p*-distance and the substitutions included were d: transitions + transversions. Uniform rates were kept for analysis. Missing data were partially deleted and the site cut-off was set as 95%. All three codon position sites were

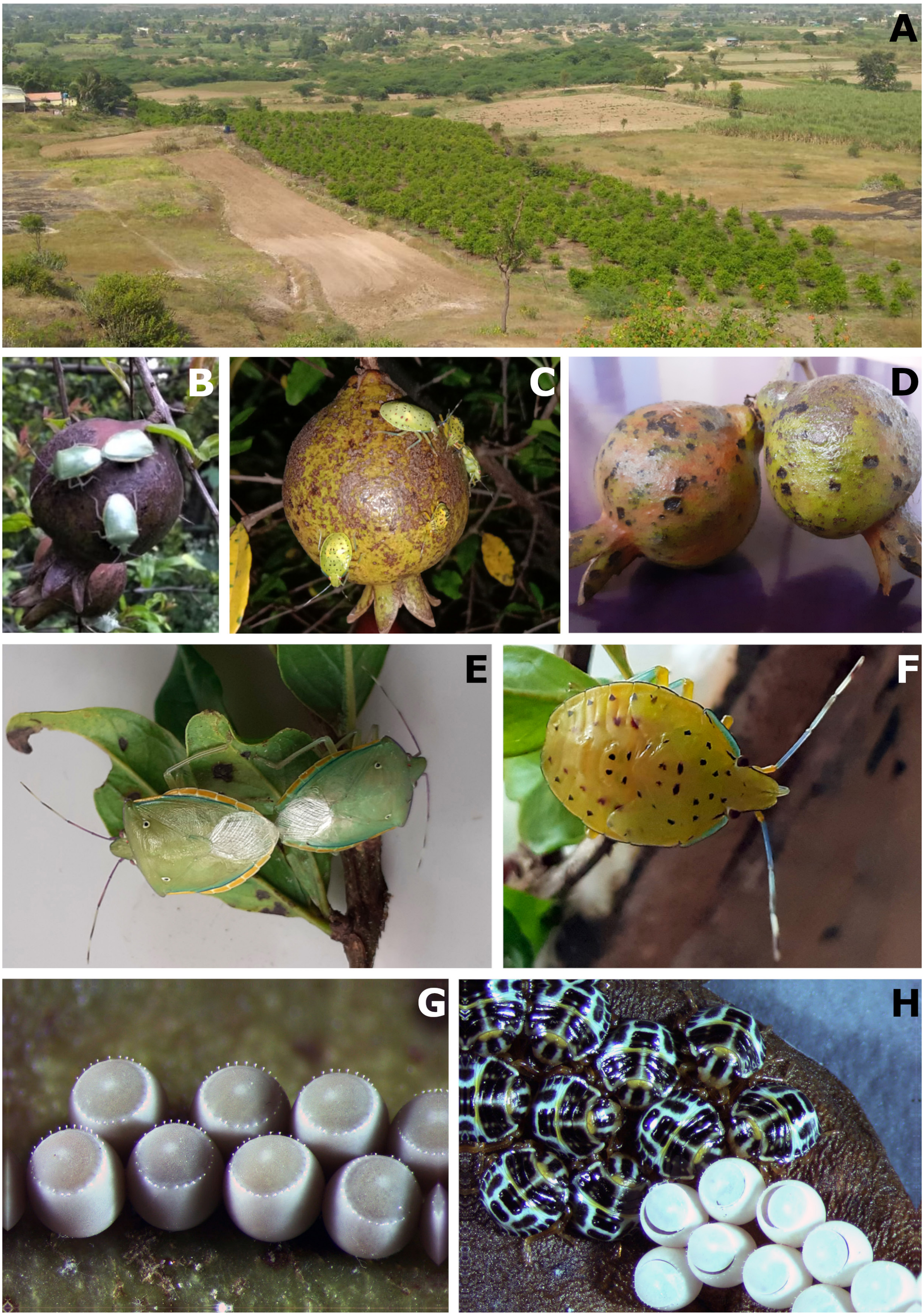

Image 1. A—Pomegranate farm | B–H *Bathycoelia indica* eggs, nymphs and adults: B— mating pair on fruit | C—nymphs on fruit | D—fruits showing black spots | E—mating pair | F—fifth instar nymph | G—eggs | H—hatched eggs with nymphs. © 1A—P.S. Kudnar | 1B–H—H.V. Ghate.

Table 1. A list of sequences used in the molecular phylogenetic analyses along with their accession numbers.

Accession Number	Species	Family
MW983247	*Dysdercus fasciatus*	Pyrrhocoridae
MG838358	*Dysdercus evanescens*	Pyrrhocoridae
MG838360	*Dysdercus koenigii*	Pyrrhocoridae
HQ236463	*Bathycoelia indica*	Pentatomidae
PP177471 This study	*Bathycoelia indica*	Pentatomidae
OM263631	*Bathycoelia distincta*	Pentatomidae
MT253050	*Piezodorus punticeps*	Pentatomidae
MG838405	*Piezodorus hybneri*	Pentatomidae
MW535996	*Nezara viridula*	Pentatomidae
KY835350	*Nezara viridula*	Pentatomidae
MG838340	*Catacanthus incarnatus*	Pentatomidae
HQ236459	*Catacanthus incarnates*	Pentatomidae
KX051838	*Catacanthus viridicatus*	Pentatomidae

selected (the p-distances are mentioned in Table 2).

Molecular phylogenetic analyses

Maximum Likelihood (ML) method of phylogenetic analyses was implemented. Maximum Likelihood analysis was performed using the web implementation of IQ-tree (Nguyen et al. 2015) web server (Trifinopoulos et al. 2016) under the HKY+F+R2 for position 1, TN+F+G4 for position 2 and HKY+F+I for position 3 models of sequence evolution, which were determined using ModelFinder (Kalyaanamoorthy et al. 2017) on the IQ-tree web platform. Branch support was tested using 1000 non-parametric rapid ultrafast bootstrap pseudo-replicates (Hoang et al. 2018). Members of the genus *Dysdercus* Guérin-Méneville, 1831 (Pyrrhocoridae) were used to root the alignment.

RESULTS

Classification (as per website: Pentatomoidea web page, Rider 2024):

Heteroptera, Pentatomoidea, Pentatomidae, Pentatominae, Bathycoeliini

Bathycoelia Amyot & Serville, 1843

= *Jurtina* Stål, 1868 (syn. by Bergroth 1913)

Bathycoelia indica Dallas, 1851

Material examined for morphology and dissection: three males and three females of *Bathycoelia indica* collected by P.S. Kudnar from Shindodi, near Sangamner (19.3748N and 74.3797E), District Ahmednagar, Maharashtra State, India, 15 August 2022. Specimens are deposited in Modern College. Two males are numbered MASCZ Het 153 and 154. One female is numbered MASCZ 155.

Table 2. p-distances between the sequences used in the molecular phylogenetic analyses

	Sequence	1	2	3	4	5	6	7	8	9	10	11	12
1	HQ236463.1_*Bathycoelia_ indica*												
2	PP177471 this study *Bathycoelia_indica*	0.00%											
3	OM263631.1_*Bathycoelia_ distincta*	5.14%	5.14%										
4	MT253050.1_*Piezodorus_ punctipes*	10.71%	10.71%	10.71%									
5	MG838405.1_*Piezodorus_ hybneri*	14.35%	14.35%	14.13%	12.21%								
6	MW535996.1_*Nezara_viridula*	14.99%	14.99%	13.49%	14.13%	14.35%							
7	KY835350.1_*Nezara_viridula*	13.49%	13.49%	12.21%	12.42%	13.92%	6.42%						
8	MG838340.1_*Catacanthus_ incarnatus*	17.77%	17.77%	17.56%	14.99%	18.63%	13.70%	13.70%					
9	KX051838.1_*Catacanthus_ viridicatus*	19.06%	19.06%	17.56%	15.85%	19.06%	16.49%	16.06%	13.28%				
10	HQ236459.1_*Catacanthus_ incarnatus*	17.77%	17.77%	17.56%	14.99%	18.20%	13.70%	13.70%	0.43%	13.28%			
11	MG838358.1_*Dysdercus_ evanescens*	15.63%	15.63%	14.56%	14.13%	17.34%	16.92%	16.27%	15.63%	16.92%	16.06%		
12	MW983247.1_*Dysdercus_ fasciatus*	16.06%	16.06%	13.92%	15.20%	17.99%	17.99%	16.49%	17.56%	18.20%	17.56%	8.78%	
13	MG838360.1_*Dysdercus_ koenigii*	16.70%	16.70%	14.78%	14.13%	18.20%	16.49%	16.27%	15.20%	16.92%	15.63%	4.93%	7.71%

Brief comments on bionomics

The males and females kept under laboratory condition were found feeding and surviving well under lab conditions and were also observed mating (28 & 30 August 2022). Mating lasted for several hours. In nature as well as in lab the bugs were observed mating in typical end-to-end position (Image 1B,E). In field, mating was observed on shoots, above and under the leaves and even on fruits. Both, nymphs and adults were found feeding on tender shoots and fruits under natural conditions and the feeding marks on fruits turned black after two or three days.

In laboratory one female laid 12 eggs on 5 September 2022, these eggs were arranged in two rows but actual egg laying behaviour was not observed. These eggs hatched on 8 September (Image 1 G,H); on 13 September, second instar nymphs were observed; subsequent instars did not survive. In natural condition also nymphs were washed away by rains, so no details on other nymphal instars are available. The two other egg clutches observed in field showed 14 and 16 eggs (i.e., an average of 14 eggs / female). Since late fourth and fifth instar nymphs were observed in field during early August, the September generation was likely to be a second generation.

Each egg is barrel shaped, pale green, about 1.4 mm in height, somewhat broader in the middle than at both ends. Each egg showed about 25 tiny micropyles around "the lid or cap" at cephalic end; proximal end of egg is glued to leaf surface. Hatching was 100% successful. First instar nymphs resembled rounded buttons, about 2.5 mm long, with a pattern of black blotches or spots. These nymphs remained together for two days around the empty egg shells and moulted; the second instar nymphs were initially about 4.5 mm long but measured about 6 mm after two days of feeding.

Due to some unknown factor (probably the fruits brought from other farm and supplied as food were sprayed with pesticide), all the adults and nymphal stages of the bug in laboratory-maintained population did not survive and the observations on egg-laying behaviour and nymphal development under lab conditions also remained incomplete. A few fifth instar nymphs were again observed in field 20 days after the heavy rains which had wiped out most of the nymphs and adults. These fifth instar nymph showed many black spots on a green or yellowish green body (see Image 1F) with well-developed wing pads reaching third abdominal segment. The dorsal abdominal glands were prominent in this stage.

Brief redescription of adults

Male

Overall colour green, lateral margins of head and pronotum violaceous or magenta. Eyes red. Antennomeres 1, 2 and proximal half of antennomere 3 violaceous. Basal angles of scutellum with black spots which are surrounded by cream coloured, slightly elevated callose rim. Posterolateral angles of abdominal segments with minute black spine. All legs green, tibiae and tarsi paler than femora (Image 2A). Ventral side pale green.

Head triangular but truncate at apex. Mandibular plates and clypeus of equal length. Mandibular plates transversely rugulose dorsally. Ocelli closer to eye than to each other. Bucculae well developed (Image 2E). Labium very long, reaching posterior margin of sixth abdominal ventrite and fitting in shallow, median, longitudinal, abdominal groove (Image 2D). Antennae long, first antennomere just reaching apex of head. Ventrally head finely punctured and finely rugulose.

Pronotum trapezoidal, finely and superficially punctured, finely rugulose in anterior half, pronotal calli indistinct; pronotal anterior margin concave behind head, anterolateral margin straight, posterior margin straight. Pronotal anterior angles obtuse, width at anterior angles only slightly shorter than width of head including eyes (Image 2B). Scutellum triangular, slightly convex or tumescent in basal half, slightly longer than broad, passing middle of abdomen, rugulose punctate, distinctly narrowed in distal one third of its length, its basal angles depressed.

Pro-, meso- and metasterna finely and sparsely punctured, discal area medially smooth and shallowly sulcate. Metathoracic scent gland peritreme transverse, evaporatorium very small. Legs mostly smooth, only distal half of tibia with short setae.

Hemelytra long, extending beyond tip of abdomen in both sexes; corium finely punctured and dull, broadest in middle; membrane translucent, with multiple veins. Connexivum narrowly exposed.

Abdomen ventromedially sulcate, third ventrite anteriorly with minute median tubercle. Segmental sutures curved; spiracles closer to anterior border than lateral border of segment. Pygophore not visible externally in dorsal as well as ventral view.

Detached pygophore rhomboidal in shape, narrow at base but wide at apex, with deeply emarginate dorsal and ventral rims producing prominent caudo-lateral angles; dorsal rim smooth but ventral rim with long setae along its entire length. Small knob-like, black and sclerotized dorsal sclerites visible on either side in dorsal

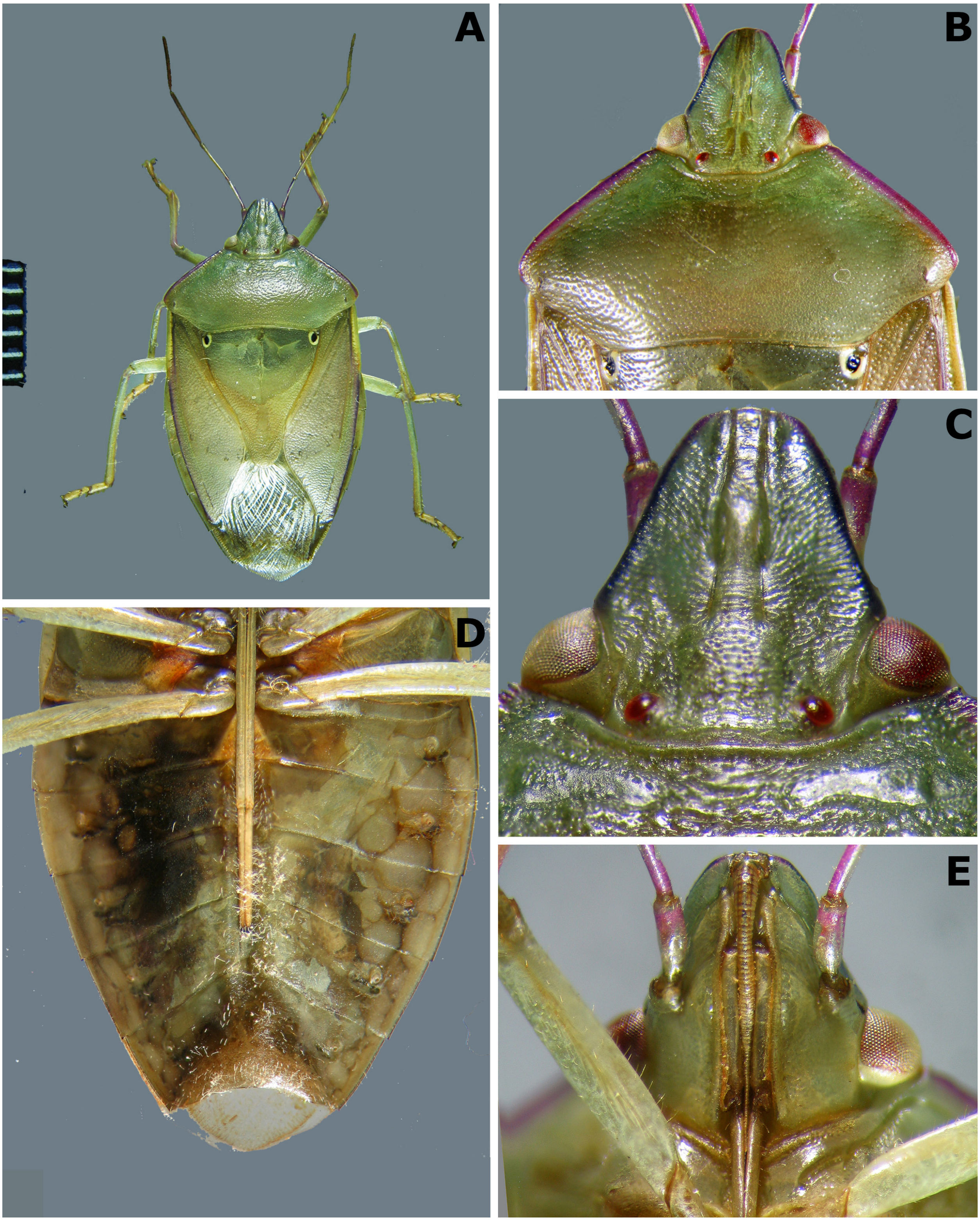

Image 2. *Bathycoelia indica* structure: A—dorsal habitus, scale 5 mm | B—head and pronotum | C—head in dorsal view | D—abdomen in ventral view | E—head in ventral view. © H.V. Ghate.

view. In dorsal view proctiger and moderately large, projecting parameres, with black, sclerotized distal margin of crown, are visible (Image 3A). In ventral view caudo-lateral angles and median portion of emargination show some black spots (Image 3B). Parameres of characteristic shape, as shown in Image 3C, with a T-like crown, stem and a short basal apodeme, with few but long setae on crown and median part. Phallus with short but well-developed articulating apparatus; phallotheca partly sclerotized, more or less cylindrical, without any processes; a pair of membranous conjunctival processes with three lobes at apex are present, most apical part of some of these lobes are black (Image 3E,F); processes of aedeagus short, sclerotized, encircling median aedeagus, as seen in Image 3D.

Female

Female is very similar to male but is slightly larger than male. The female terminalia are shown here in ventral view (Image 4A). The female genitalia include two pairs of valvifers (= gonocoxites) and two pairs of associated valvulae (= gonapophyses); valvifer VIII (labelled vf 8) and valvifer IX (labelled vf9); valvifer VIII is larger, triangular, visible externally and covered with sparse but long setae while valvifer IX is small and covered with setae; valvulae are very small and not seen externally; laterotergites (= paratergites) VIII and IX are also seen posterior to valvifers (labelled 8 lt and 9 lt). Spermatheca is large with elongate balloon-like and membranous proximal dilation (MD) and small distal pumping region of peculiar shape, with proximal (PF) and distal flange (DF); with proximal (PSD) and distal spermathecal ducts (DSD) with the connection of PSD to genital chamber. Spermathecal bulb (SB) is small with a long lateral appendage (Image 4B,C).

Molecular analysis

The ML analysis (see Image 5) placed the newly generated sequence of *Bathycoelia indica* as sister to the other sequence of the species available on GenBank® (HQ236463), with very strong ultrafast bootstrap support (97), and no intraspecific divergence (p-distance 0%) for the 467 bp COI sequences. *Bathycoelia distincta* was recovered as sister to its congener *B. indica*, with strong ultrafast bootstrap support of 93. The interspecific genetic divergence (p-distance) between the two species stood at 5.1%. All the representatives of the family Pentatomidae included in this study were observed to be monophyletic, with a very strong ultrafast bootstrap support (100). Molecular phylogenetic analysis confirms the specimens included in this study as *Bathycoelia indica*.

Measurements (in mm. Males (n = 2, separated by /). Total length 18.0 /17.5; head length 2.9 / 2.9; head width at eye 3.4 / 3.3; interocular distance 2.0 / 2.0; labium segment I 2.7 / 2.8, segment II 3.25 / 3.0, segment III 4.5 / 4.5, segment IV 2.5 /2.25; antennomere one 1.0 / 1.0, two 1.6 / 1.8, three 2.5 / 2.8, four 4 2.7 / 3.2, five 2.5/ 2.7; pronotal median length 3.25 / 3.25, pronotal width at humerus 9.0 / 9.0; scutellum width at base 5.5 / 5.70, scutellum median length 6.2 / 6.3; fore leg coxa 0.5 / 0.5, femur 4.0 / 4.0, tibia 3.25 / 3.5, tarsus 2.0/2.0; middle leg coxa 0.6 / 0.6, femur 4.6 / 4.7, tibia 4.0/4.0, tarsus 2.0 / 2.0; hind leg coxa 0.7 / 0.7, femur 5.5 / 5.5, tibia 5.0 / 5.0 ; tarsus 2.75 / 2.75.

Females (n = 2, separated by /).Total length 19.0 / 19.0; head length 3.4 / 3.2; head width at eye 3.75 / 3.70; interocular distance 2.25 / 2.20; labium segment I 2.75 / 2.8, segment II 3.0 / 3.0, segment III 5.0 / 5.1, segment IV 3.0 /2.9; antennomere one 1.2 / 1.0, two 1.75 / 1.75, three 2.0 / 2.5, four 2.75 / 2.8, five 2.75/ 2.75; pronotal median length 4.0 / 4.0, pronotal width at humerus 9.5 / 9.4; scutellum width at base 7.5 / 7.4, scutellum median length 4.0 / 4.0; fore leg coxa 0.5 / 0.5, femur 4.0 / 4.1, tibia 3.75 / 3.7, tarsus 2.0 / 1.9; mid leg coxa 0.6 / 0.5, femur 5.5 / 5.4, tibia 4.25 / 4.25, tarsus 2.1 / 2.2; hind leg coxa 0.7 / 0.6, femur 6.25 / 6.20, tibia 5.75 / 5.75 ; tarsus 2.8 / 2.9.

DISCUSSION

Some species of the genus *Bathycoelia,* especially species *Bathycoelia thalassina* (Herrich-Schaffer, 1844), are known as a serious pest on Cocoa plant *Theobroma cacao,* L., (Malvaceae) in tropical Africa, causing considerable damage to cocoa beans (e.g., Lodos 1967; Linnavuori 1982; Nwana 1983). The other species, namely *Bathycoelia distincta* Distant, 1878 is known to affect and severely damage Macadamia *Macadamia integrifolia* Maiden & Betche and *Macadamia tetraphylla* L. Johnson, (Proteaceae) plantations in South Africa (Schoeman 2018). *Bathycoelia indica* has been recorded from pomegranate (Balikai et al. 2011 and additional references cited there) but not regarded as a serious pest in any recent publications (e.g., Elango et al. 2021). However, our observations indicate that this species has a potential of becoming serious pest of pomegranate if control measures are not taken. These bugs seriously damage pomegranate fruits by puncturing that leads to formation of black necrotic spot at the place of puncture; the fruits are also smaller on bug affected plants. At present *B. indica* is the only species under this genus in India.

The egg morphology and even nymphs are remarkably similar to those of *Bathycoelia thalassina*, a species that

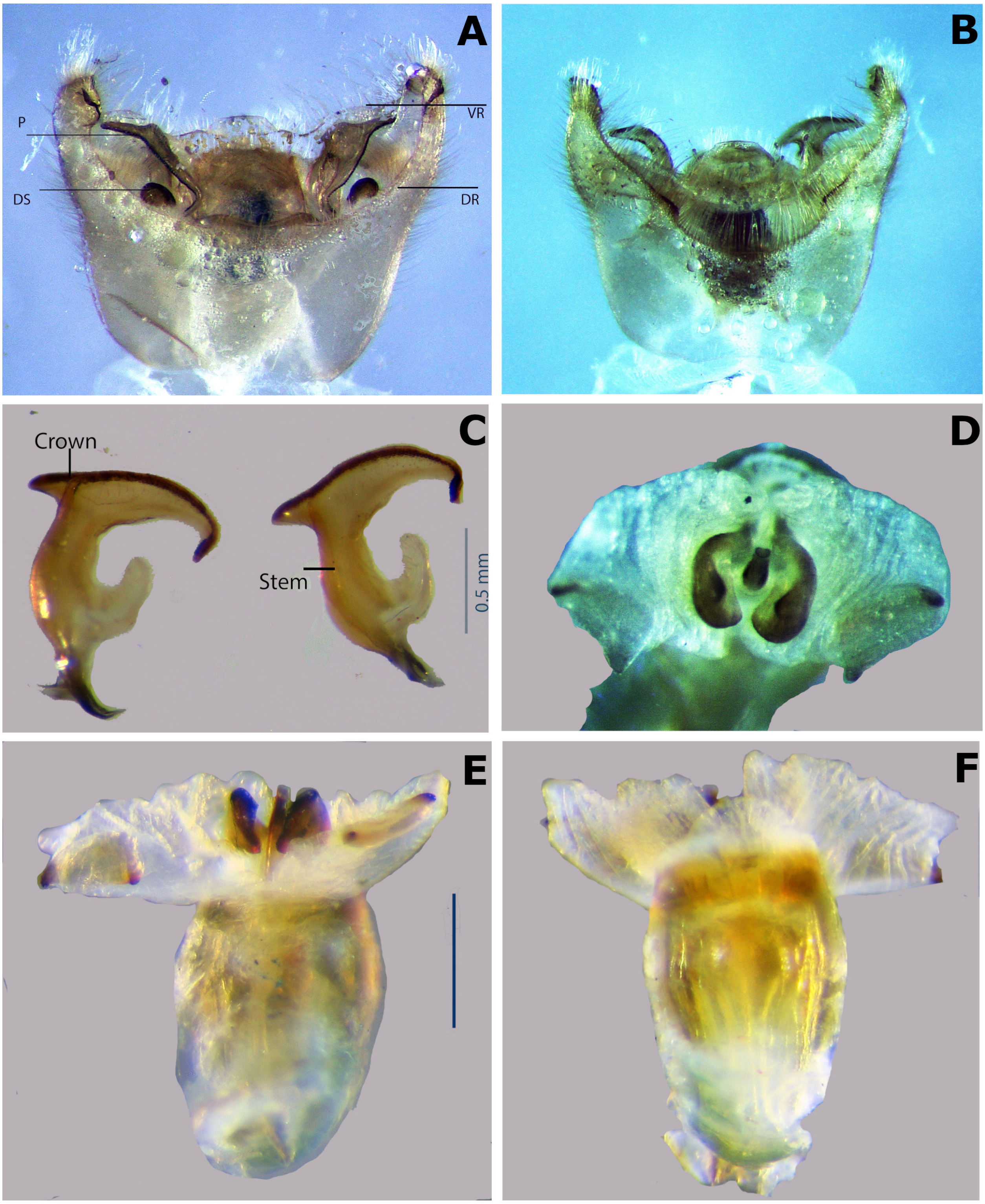

Image 3. *Bathycoelia indica* male genitalia: A & B—pygophore dorsal and ventral view respectively | C—parameres | D—phallus posterior view | E & F—phallus dorsal and ventral view respectively, scale bar 1mm. Abbreviations: DR—dorsal rim | DS—dorsal sclerite | VR—ventral rim | P—parameres. © H.V. Ghate.

damages cocoa pods, studied by Lodos (1966); the eggs however have about 25 micropyles in *B. indica* while in *B. thalassina* there are about 110 micropyles. According to Lodos (1966) the tissue around the site of puncture

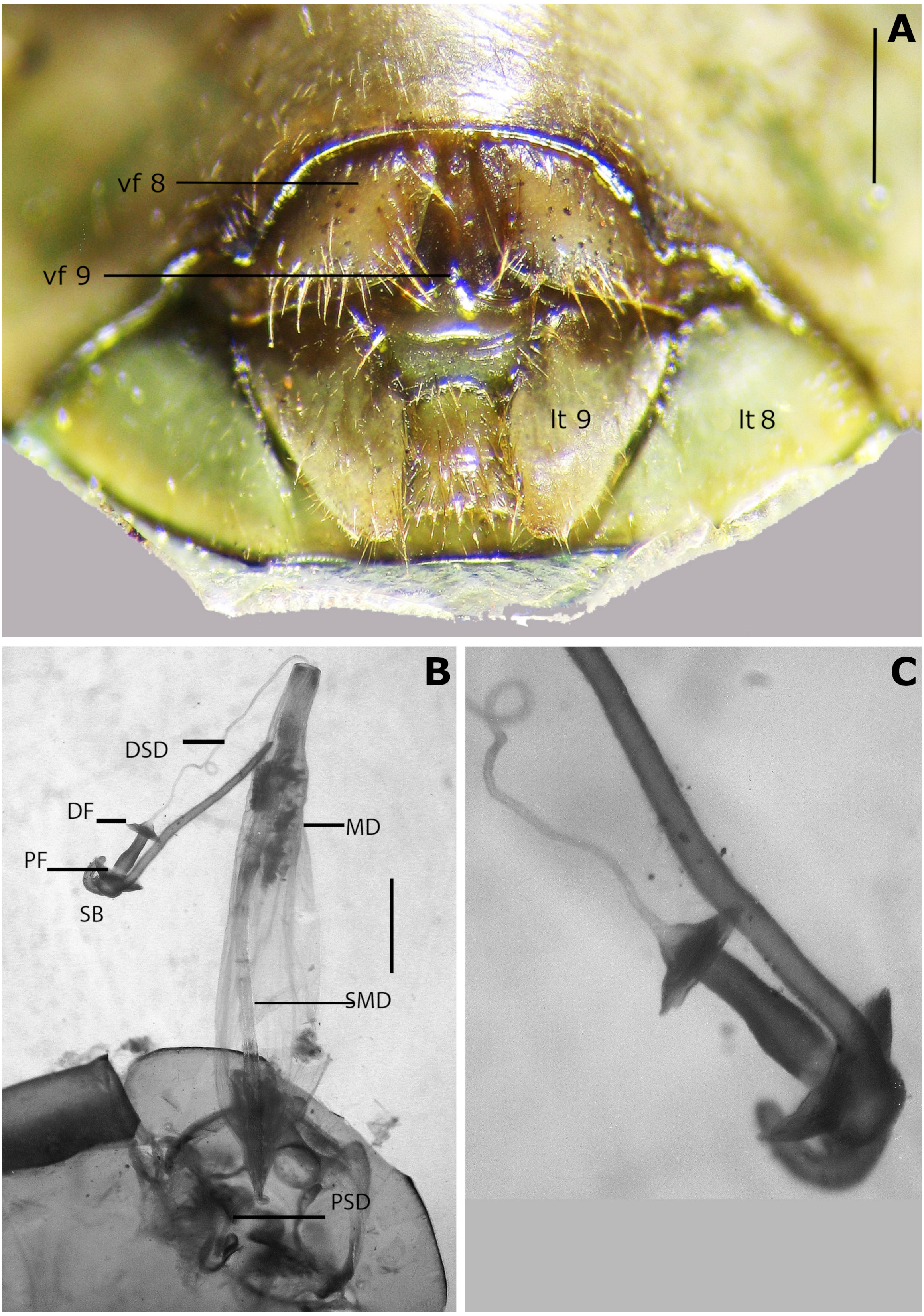

Image 4. *Bathycoelia indica* female genitalia: A—terminalia in ventral view, scale bar 1 mm vf8 and vf9 are valvifers; lt8 and lt9 are laterotergites| B—dissected view of female genitalia, scale 1 mm | C—spermatheca details. Abbreviations: DF—distal flange | DSD—distal spermathecal tube | MD—median dilation | PF—proximal flange | PSD—proximal spermathecal duct | SB—spermathecal bulb | SMD—sclerotized median dilation. © H.V. Ghate.

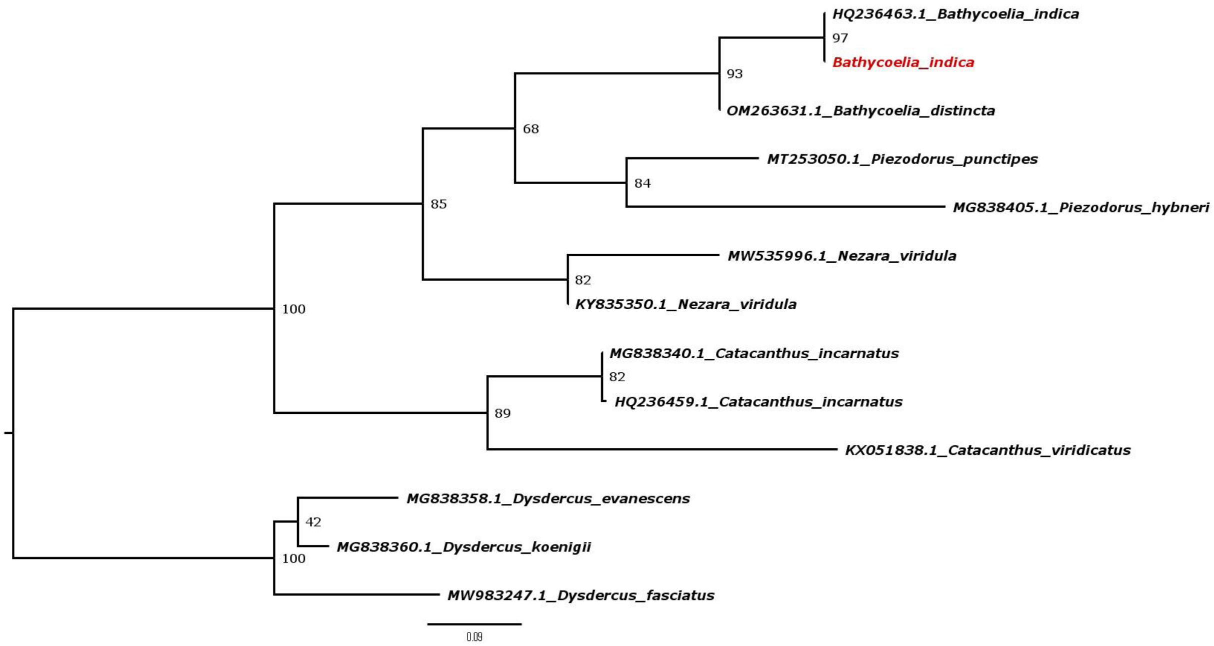

Image 5. Maximum likelihood tree based on COI gene.

in pods dies due to injected fluid and turns yellow to brown to black. This is similar to black spots observed on pomegranate fruits punctured by *B. indica*.

Here we have given photographic documentation of *B. indica* and also the damage caused by it. In addition, we have provided brief information on morphology, including that of male / female genitalia. Earlier Ahmad (1996) gave some diagrams of *B. indica* and Linnavuori (1982) discussed about male genitalia of several *Bathycoelia* species from western Africa, with many figures, and found male genitalia to be relatively similar in species studied. Detailed morphology of *B. indica* has been previously described by Salini (2015, Unpublished PhD Thesis), who also reviewed previous work on this genus.

Fan & Liu (2009) studied *Bathycoelia sinica* Zheng & Liu, 1987 and provided details of male genitalia which are also comparable with our images. Another species in which comparable structure of male genitalia can be observed is *Bathycoelia chlorospila* Walker, 1867 collected from New Guinea (Gross 1978), however, based on figures provided in these papers, their pygophore and parameres are distinctly different from *B. indica*. Gross (1978) also gives detailed diagnosis of the genus *Bathycoelia*, comments on its distribution and also suggests that it is closely related to '*Pentatoma*' group. Tsai & Rédei (2014), who revised the genus *Amblycara* Bergroth, 1891 from the Oriental and Austro-Pacific areas, found close relationship between *Bathycoelia* and *Amblycara* and suggested that both these genera could belong to the tribe Pentatomini; an isolated position in a separate tribe Bathycoeliini is unnecessary for *Bathycoelia*, but more studies on related genera are required. Some of these aspects and general characters of the tribe Bathycoeliini, including pest status of some species, are also discussed by Rider et al. (2018).

We have also sequenced COI gene and shown that the sequence is similar to Pune population that was sequenced earlier (Tembe et al. 2014). It is also apparent that the African species *Bathycoelia distincta* is genetically very close to Indian species. Pal et al. (2022) studied in detail the genetic diversity of *B. distincta* affecting macadamia in three different areas from South Africa and found very low pairwise mean genetic distance among different populations. Based on comparison of COI sequences it appears that *Piezodorus* and *Nezara* are closely related to *Bathycoelia*. Unfortunately, there are no sequences of *Amblycara*, a genus which is suggested close to *Bathycoelia*, as stated above.

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