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RESEARCH ARTICLE

Redescription of *Cryptophlebia ombrodelta* (Lower, 1898) (Lepidoptera, Totricidae) an emerging pest of leguminous medicinal plants with new host record from India

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Abstract

Cryptophlebia ombrodelta (Lower, 1898) (Olethreutinae: Tortricidae: Lepidoptera) larvae were recorded for the first time from the fruits of *Saraca asoca* (Roxb.) Willd. (Fabaceae) a culturally and medicinally important tree at Thiruvananthapuram, Kerala, India. The larvae were reared to the adult stage at Butterfly House, Tamil Nadu Agricultural University Insect Museum. Pupae and adults of *C. ombrodelta* are described in detail. The pupal morphology is described for the first time. Adults are redescribed in detail and special emphasis is given on Male and Female genital morphology. Mitochondrial DNA was extracted from adult specimens, DNA sequence was compared with other related sequences on the NCBI database for additional confirmation. A phylogenetic tree was constructed using sequences submitted worldwide which show more than 98% similarity.

Keywords

DNA barcode; genitalia; litchi nut borer; pupa; *Saraca asoca*; Tortricidae

Introduction

As part of our study on the Lepidoptera diversity in the medicinal plants of the family Fabaceae, *Cryptophlebia ombrodelta* (Lower, 1898) (Olethreutinae: Tortricidae) larvae were collected from fruits of *Saraca asoca* (Roxb.) Willd. (Fabaceae). Previously *C. ombrodelta* has been recorded on 29 species of Fabaceae (1).

Genus *Cryptophlebia* Walsingham consists of 53 species that are mostly distributed in the Indo-Pacific region (2). The taxonomic position of the genus *Cryptophlebia* has been much worked by phylogenetic approaches and morphological studies (3) and has been assigned within sub-tribe of Grapholitini. *Cryptophlebia* spp. are medium-sized moths and are often borers as larvae feeding on plants of Fabaceae. *Cryptophlebia* spp. are frequently confused with *Thaumatotibia* Zacher. In the era of invasive insects across the globe, it is essential to determine the correct identity of the insect. Of the species that have been described under *Cryptophlebia*, *C. ombrodelta* is a polyphagous pest that is considered as a significant threat to biosecurity across the globe. This insect is widely distributed in tropical regions (4). It was first described by Oswald Bertram Lower as *Arotrophora ombrodelta* in 1898 from Sydney, Australia (5). The taxonomy of *C. ombrodelta* has been studied elsewhere (4). In India except for the studies on the biology of *C. ombrodelta* (6), a description based on genitalia from India is wanting. Since, *Saraca asoca* is a revered medicinal plant in India owing to its high medicinal value (7, 8). It is necessary to document the insects feeding on *S. asoca* and establish the correct identity. In view of the above said, this study was undertaken to describe the

pupa and adult morphology in detail along with DNA barcoding of the insect for additional confirmation.

Materials and Methods

Insect collection and rearing

Asoka trees *Saraca asoca* (Roxb.) Willd. (Fabaceae) in the fruiting stage at Vellayani, Thiruvananthapuram, Kerala, India showed symptoms of damage on its fruits by having pepper corn-sized holes on the surface. Pods showing such symptoms were collected. The collected pods were brought to the Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India and were placed in separate containers and kept in the butterfly house of TNAU Insect Museum at a relative humidity of 80% and temperature of 26 °C, for the herbivore inside to mature properly. After a few days when head capsules of several pupae started protruding from the holes, one such pod was taken and incised to extract the pupae (2 nos.) which were then preserved in 70% ethyl alcohol in 2 mL vials and used for further study. Upon emergence, adult moths (2 males and 2 females) were killed using chloroform and spread and used in morphological studies.

Morphological characterization

Adult specimens were scrutinized for morphological characteristics such as antennae, labial palpi, wings, legs, wing venation and genitalia. Appendages were processed by soaking in 10% KOH, descaling, staining with 5% acid fuchsin dye and mounting on slides with DPX mountant. Pupal nomenclature followed Mosher (9) and Wing venation was studied using Zimmerman's (10) methods and Comstock and Needham's (11) nomenclature. Genitalia dissection followed Robinson's (12) protocol and Klots (13) nomenclature, with specimens preserved in glycerol. Imaging was performed with the image analyzers (LAS V4.12 and LAS X) attached to Leica M205 A and M205 C microscopes. Specimens were deposited in the TNAU Insect Museum.

Genetic analysis

Mitochondrial DNA was isolated from adult moths using the CTAB method (14). Three adult moths were used for DNA isolation. The quality of DNA was evaluated through 0.8% agarose gel electrophoresis and DNA concentration was quantified with a Nanodrop spectrophotometer (Nanodrop One, Thermo Scientific). The cytochrome oxidase I (COI) gene was amplified with primers LCO 1490 and HCO 2198 (15) using an Eppendorf Mastercycler-Nexus thermocycler. The amplification products were analyzed via 1.2% agarose gel electrophoresis and PCR programme was followed (16). Sequencing of PCR products was conducted using the Sanger dideoxy method at Genespec Pvt. Ltd. Kochi. The DNA sequence of *C. ombrodelta* was compared with GeneBank entries using NCBI BLAST search. Sequence alignment was carried out with CLUSTALW and a phylogenetic tree was constructed using the neighbour-joining method and Bayesian inference in MEGA X (17). A phylogenetic tree was constructed using kimura-2-parameter with 1000 bootstrap values. Sequence data and specimen details were submitted to the NCBI database and BOLD database for DNA barcode generation.

Results

Morphological description

Cryptophlebia ombrodelta (Lower, 1898)

Distribution: Australia, China, Guam, India, Indonesia, Japan, Malaysia, Nepal, New Guinea, Sri Lanka, Taiwan, Thailand, the Caroline Islands, the Hawaiian Islands, the Philippines and Vietnam (18).

Material examined: Thiruvananthapuram, India, 22.04.2024, 2 pupae; adults 2 ♀♀, 2 ♂♂ leg. Amrit Sekhar Mallick.

Host plant: *Saraca asoca* (Roxb.) Willd.

Feeding behaviour (Fig. 1): Larvae feed inside pods and eat the seed kernels by burrowing inside. Burrows or tunnels are irregular and lined with frass. Peppercorn-sized burrow holes can be seen from outside of pods with few faecal pellets. After pupation, the pupal head capsule can be seen protruding out of the burrow holes.

Pupa (Fig. 2): Obtect, pupation inside infested pods, a part of anterior portion of pupa protrudes out of the tunnel holes lined with frass. Dark, bright reddish brown in colour.

Dorsal: Division between vertex and fronto clypeus is not very clear. Antennal bases flank this region on both the sides. Pronotum narrow, fusiform in shape, finely rugose. Mesonotum largest among the thoracic segments, highly rugose, gibbous, with an expanded oval caudad, expands lateroventrally towards the posterior end on both sides as the fore wings. Metanotum very narrow, rugose, expands lateroventrally towards the posterior as hindwings under forewings, only part of anal region of hind wing visible, this region appears M shaped. Abdominal segments are very finely rugose and slightly rough. A1 and A2 trapezoidal between hind wing expansions, A1 with a raised, prominent, transverse rugose line at the midportion. A2 spiracle prominent, with dark brown peritreme. A2-A7 with two transverse rows of spines parallel to each other, first row at cephalad of A2-A7, with larger spines in comparison to second row, size of spines gradually increasing from A2-A7, black in colour. Second row at caudad of A2 and little posterior to midportion on A3-A7, with spines of near equal size on all the five segments, black in colour. A8 and A9 with single a row of spines towards the cephalad, spines fewer in number but very large, prominent and darker in comparison to spines on other segments. A10 with four stout spines. Segmental division of A8, A9 and A10 clear.

Lateral: Part of the compound eye, maxilla, prothoracic leg and antenna visible. Maxillary palpus is very small and present towards the lateral corner of maxilla, below the compound eye. Wing expansions reach cephalad of A4, rugose. Spiracles from A2 -A7 are prominent, slightly raised with dark brown peritreme. Spiracle of A8 vestigial, appear as a scar. Transverse rows of spines arising from the dorsum end near the spiracles.

Ventral: Frontoclypeus dark brown, labrum indistinct, small and dark brown, compound eyes globular, blackish brown, mostly glabrous, slightly rugose at the basal corners. Labial palpi are large, prominent, lanceolate, present between maxillae. Maxillae, legs, antennae and wing expansions rugose. Maxillae are two times the length of labial palpi and ends near prothoracic coxae. Besides maxillae, prothoracic coxae and femur are present, followed by prothoracic leg which ends at same level

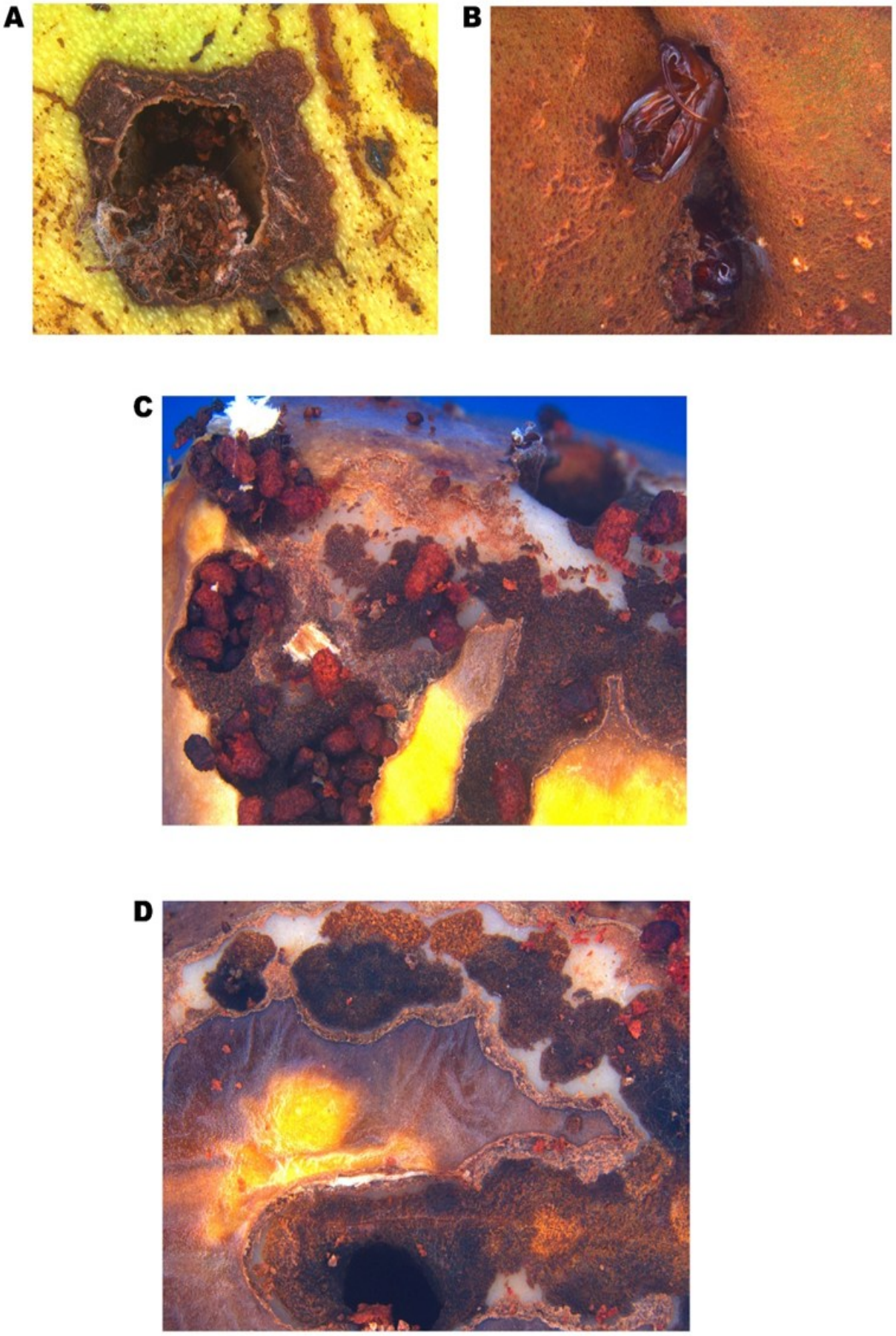

Fig. 1. A-burrow hole, B-pupal head capsule protruding out of burrow hole, C-feeding tunnels lined with frass, D-feeding galleries.

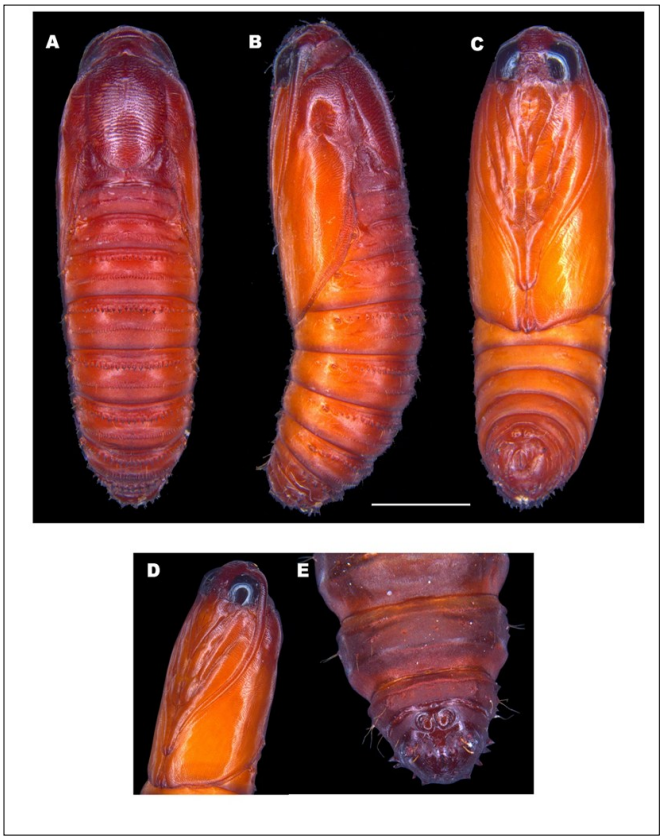

Fig. 2. A-pupa dorsal, B-pupa lateral, C-pupa ventral, D-pupa anterolateral, E-ventral details of abdominal segments 8,9 and 10 scale bar- 1 mm.

where prothoracic coxae start. Proximal part of prothoracic legs is separated by prothoracic coxae. Mesothoracic coxae present posterad to prothoracic coxae, mesothoracic legs present next to prothoracic legs, join each other and end slightly anterad from the apex of fore wing expansions. Antenna starts behind compound eyes, present close to mesothoracic legs and ends slightly anterad to their proximal end. Metathoracic legs and wing expansions reach cephalad of A4, only proximal part of metathoracic legs is visible between forewing expansions separating them from each other. Abdominal segments appear uniformly, finely rugose and non-glossy on the ventrum, devoid of any spinous structure. Cremaster rudimentary, four short spine-like perianal setae with hooked tips present on A10, with two spines on each side of the anal slit.

Adult (Male) (Fig. 3 A-B): Body length: 8.55 mm. Wingspan: 17.4 mm. Head: vertex and frontoclypeal region clothed in fuscous grey and dull purple scales. Antennae are long, filiform, scape and flagellum clothed in brown scales. Compound eyes globose, dark brown, with brown fringes. Small black ocellus present behind antenna. Labial palpi (Fig. 4 A-B) three-segmented, porrect, very conspicuous, covered in brown scales, terminal segment and basal segment subequal in length, middle segment nearly four times the length of terminal segment. Proboscis golden brown, devoid of scales. Thorax: patagia covered in fuscous grey and dull purple scales, a tuft of chalky grey scales presents towards the base, tegula covered in fuscous brown scales. Wings (Fig. 5): FW ups: quadrate with rounded apex, apical margin straight. Costal fold absent. Major portion of wing area is covered in fuscous brown scales. Dorsum with a prominent dark brown band-like fascia. Pre terminal region at the tornus comparatively pale with light grey scales. A dark maroon pre tornal spot somewhat pointed or triangular with white margin present. FW: uds: Uniformly grey brown. Apical margin fringed with creamy cilia. HW: ups: oblong with rounded apex, costal margin and outer margin slightly sinuate. uniformly covered in cream-coloured scales with light brown speckling towards the apical region. Androconia is present on the jugal fold, covered in dark grey scales. HW:uds: covered uniformly in cream-coloured scales. Vannal fold with long, creamy, piliform scales. Cilia with creamy, grey-coloured scales.

Adult (Female) (Fig. 3 C-D): Almost identical to males, dark brown band-like fascia on the dorsum is mostly absent or very faint and indistinct if present, the dark maroon pre-tornal spot is very prominent and more rounded. Androconia on the hindwings is absent.

Wing Venation (Fig. 5 C-D): FW: Sc arises from the basal region and terminates at half the length of the costal margin. Discal cell is a narrow-elongated triangle. R1 arises at the midpoint of anterior side of the discal cell and terminates at two-thirds of length of the costal margin. R2 free, arising at three fourth length of anterior side of costal margin. R3 and R4 free, arising separately near the anterior angle of discal cell and terminating near the apical angle, parallel to each other. R5 arises close to the base of R4 and terminates at the apical angle. M1 arises from the lateral side of the discal cell and terminates at one-third length of the outer margin. M2 and M3 arise close to each other near the posterior angle of the discal cell, M2 parallel to M1 and M3 parallel to M2. CuA1 arises from the posterior angle of the discal cell, parallel to M3. CuA2 arises at two-third length of posterior side of discal cell and terminates at tornal angle. 1A+2A arises from the base, close but free till one-fourth of their length then completely fused, terminates near the tornal angle. HW: discal cell in the shape of a short triangle. Sc+R1 arises near the base from anterior side of discal cell terminating near the apical angle. Rs arises from the anterior angle of discal cell, parallel to Sc+R1

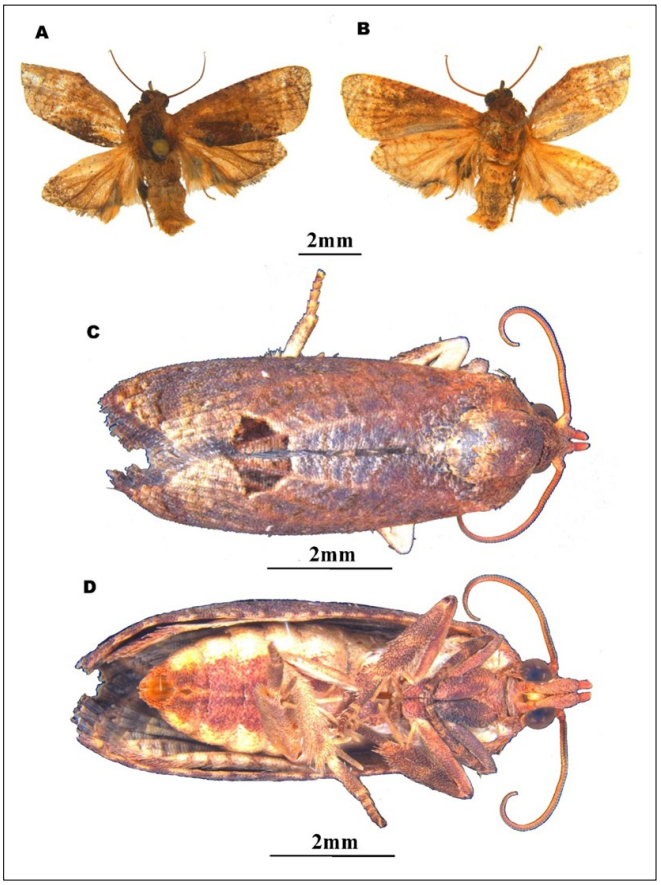

Fig. 3. A-Male dorsal, B- Male ventral, C- female dorsal, D-female ventral.

and terminates at apical angle. M1 arises from anterior angle of discal cell close to base of Rs. M2 and M3 arise close to each other from posterior angle of discal cell. CuA1 free, arises from the posterior angle of the discal cell. CuA2 free, anatomises at half the length of posterior side of the discal cell. 1A free, arises from the wing base, terminates near anal angle. 2A and 3A free, arise together from the wing base and terminate at anal angle.

Legs (Fig. 4 C-H): All three pairs are covered in brown scales on both surfaces, except hind tibia with long, cream coloured and dark grey scales on the dorsum along with a bare, highly sclerotised, oval region bearing the sex scales. Fore femur is three times the length of fore tibia. Fore tibia with epiphysis half its length. Mid femur is subequal in length to mid tibia. Mid tibia with two spurs near the proximal end, one spur is two times the length of the other spur. Hind tibia with two pairs of spurs, one at mid-point and one at proximal end, in each pair one spur is two times the length of the other spur.

Abdomen: Dorsum and ventrum uniformly covered in fuscous brown scales, lateral sides with creamy brown scales. Paired coremata (Fig. 6 A) are present on A8 of males.

Male genitalia (Fig. 6 B-C): Uncus absent. Gnathos and socii absent. Tegumen is slightly sclerotized. Transtilla was slightly sclerotised, the terminal connection forming a bridge. Juxta sclerotized and fused with aedeagus. Valva cup-like with setose, rounded cuculla, with three spines, one on costal margin and two on basal margin. Harpe and fibula are absent. Sacculus is slightly sclerotized, fused to form the intersaccular bridge. Vinculum sclerotized; short, inverted triangle shaped. Adeagus long, slightly sinuate, with broadened base. cornuti with spines as an oval shaped patch towards the tip.

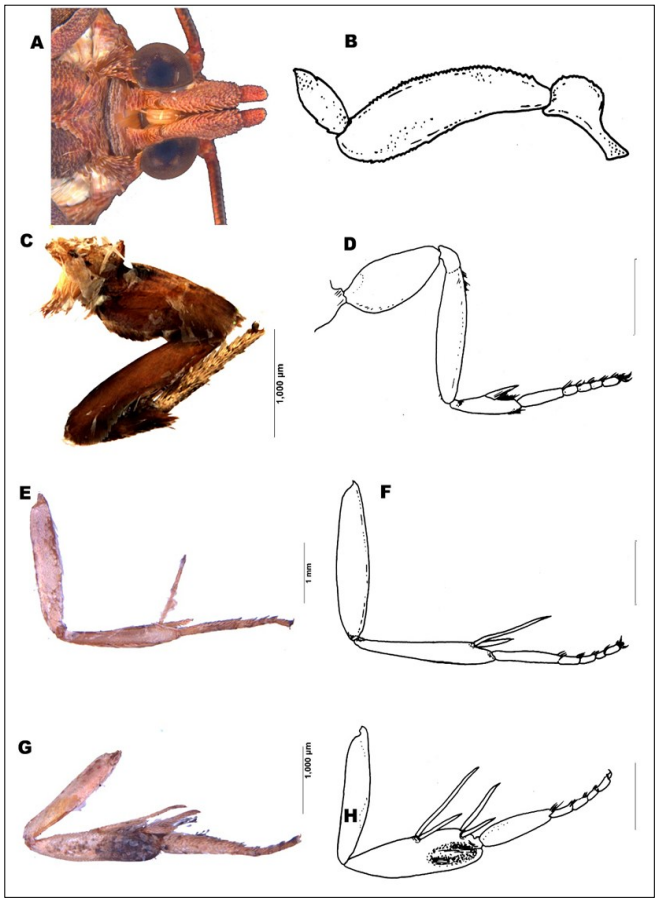

Fig. 4. A and B-labial palpi, C and D-fore leg, E and F-mid leg, G and H-hindleg (scale bar for D, F and H is 1 mm).

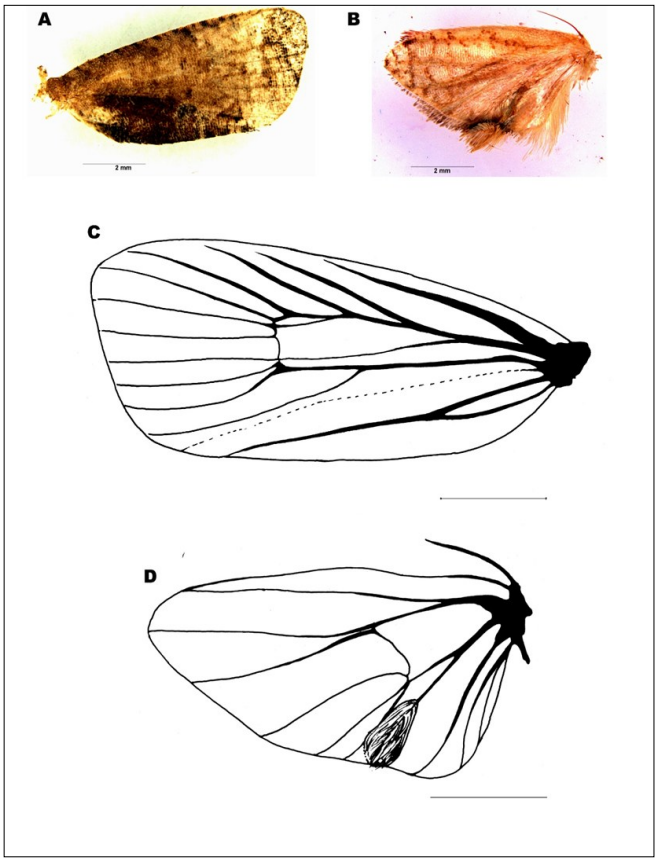

Fig. 5. A-forewing, B- hindwing, C- forewing venation, D- hindwing venation (scale bar for C and D is 2mm).

Female genitalia (Fig. 6 D): Papillae anales parallel sided, highly setose, goat hoof shaped in appearance. Apophyses anteriores and apohyses posteriors slightly sclerotized, apophyses posteriores and apophyses anteriores subequal in length. sterigma prominent, sclerotised and deep V shaped. Antrum cup shaped, membranous. Ductus bursae membranous, tubular, sub equal in length to corpus bursae. Corpus bursae membranous, globose, rough on the inside. Signa as two cat claw shaped projections, with oval tips on opposite lateral sides towards the base of corpus bursae. Ductus seminalis present at the mid portion of ductus bursae.

Genetic analysis

Our study sequence with accession no. PQ345542 showed 99% similarity to several *Cryptophlebia ombrodelta* sequences submitted to the NCBI database. The closest sequence was OQ836338 submitted from the Netherlands using specimen collected from Vietnam. However, submissions from India showed less similarity (around 98%) with sequences ON965798 (submitted from New Delhi) and KX150511, KX150514, KX150513 (submitted from Jharkhand). The South Indian population of *C. ombrodelta* radiated separately from the North Indian population of *C. ombrodelta* (Fig. 7).

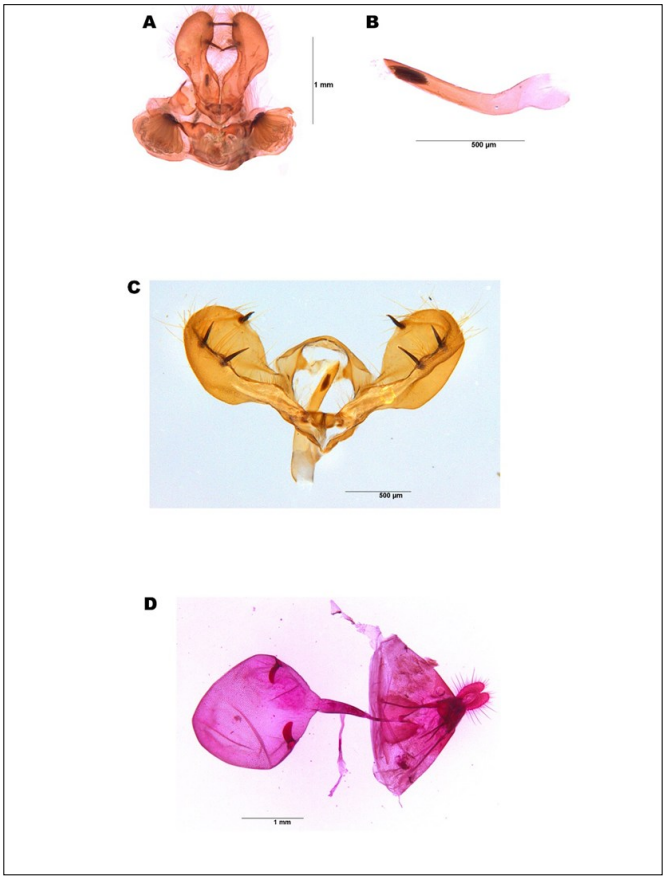

Fig. 6. A- coremata on 8th sternite, with closed genital capsule above (Male), B -Aedeagus, C-Male genitalia, D-Female genitalia.

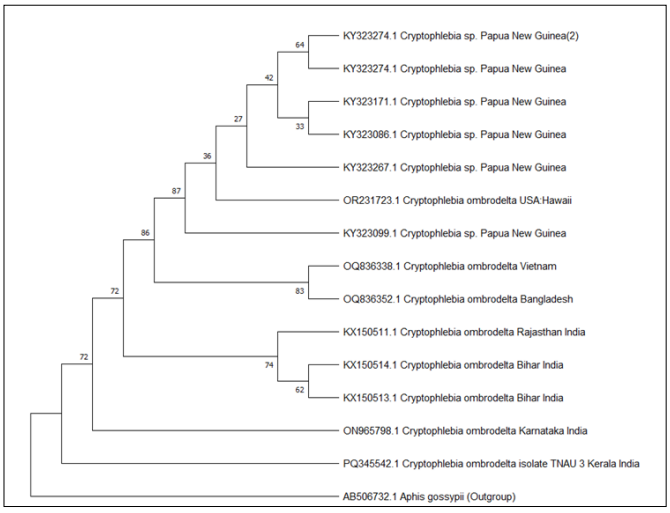

Fig. 7. The phylogenetic tree of *C. ombrodelta* constructed using neighbor joining method.

Discussion

Most features are common to that of typical Tortricidae pupa descriptions like labial palpi, prothoracic femur and mesothoracic coxae exposed as stated by Common (19) and Patočka & Turčáni (20). Metatarsus tip visible and alar furrow on mesonotum present as stated by Patočka & Turčáni (20). Two transverse rows of uniseriate spines on the abdominal segments are present as given by Patočka & Turčáni (20), Bradley *et al.* (21), Common (19), Scoble (22), Komai (23), Razowski (24). Abdominal segments are movable as stated by Komai (23) and Razowski (24). On A10 four hooked short perianal setae are present as given by Patočka & Turčáni (20) and Mosher (9). Cremaster is rudimentary, like most Olethreutinae (19, 21-24). The prominent pretornal spot in female forewing and brown band like fascia on dorsum of male forewing are in accordance with the description given by Bradley (25) and Sohn *et al.* (4). The presence of a bare sclerotised oval patch on hind tibia is a distinguishing feature of *C. ombrodelta* (26). The absence of uncus and three sclerotised spines on cucullus are characteristic features of *C. ombrodelta* (4, 25). The divergence in the grouping of South Indian population of *C. ombrodelta* from the North Indian population of *C. ombrodelta* indicate the genetic diversity soundly. This may be due to the change in habitat, climatic conditions and the host plants. Being a polyphagus pest, *C. ombrodelta* will be subjected to genetically diversified population within the same geographical area.

Conclusion

With differences observed in Indian specimens, molecular analysis reveals a 99% genetic resemblance to other *C. ombrodelta* populations distributed worldwide. Overall, several characteristics, are consistent with descriptions of the family Tortricidae such as those of the pupal stage but unique morphological features like the sclerotized patch on the hind tibia and distinctive genital structures, are important distinguishing marks for *C. ombrodelta.* Proper identification is very crucial for determination and implementation of control measures for emerging pest species such as *C. ombrodelta.* The first record of *Saraca asoca* being an alternate host for this species paves way for further research regarding its status as a pest on other medicinal plants and its potential invasiveness.

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Authors' contributions

ASM collected insect specimens from Kerala and conducted the major research at TNAU insect museum like rearing, killing and pinning, photography, dissections, microscopic studies, illustrated the line diagrams and drafted the manuscript. CN conceived the research topic and guided other authors throughout the research process, she gave the format layout for research result presentation and finally reviewed and corrected the manuscript. SRP, ET and VM reviewed the manuscript and made important corrections. DN conducted the molecular characterisation work. MH helped analyzing the molecular results and constructed the phylogenetic tree.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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