Lumen anatomy and localization of *Wolbachia* sp. in the thrips, *Plicothrips apicalis* (Bagnall)

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A detailed anatomical study of digestive system of insects has been done in some of the major insect orders such as Lepidoptera, Diptera, Coleoptera and Hemiptera. For other insects such as thrips, their emergence as an important agricultural pest has brought order Thysanoptera to focus. We describe the alimentary canal of *Plicothrips apicalis* (Bagnall) (Phlaeothripidae) and localize *Wolbachia* sp. in *P. apicalis*. The digestive tract of *P. apicalis*, a grass feeding species, is observed to be well demarcated in the foregut, midgut and hindgut. We evince a new finding in this study that the midgut opens into a globular chamber containing the mycetome. It is from the globular chamber that the hindgut follows and at their juncture, two pairs of malphigian tubules originate. The presence of mycetome has been reported in order Hemiptera and Isoptera that bears a structure similar to mycetome, known as paunch that harbours protists which are known to be involved in cellulose digestion. Presence of mycetome has not been reported in other thrip species till date except in *Bactothrips buffai*. The finding of an additional structure in the alimentary canal of thrips is suggestive of the significance it might hold in the evolutionary linking with other insect orders. We also tried localizing endosymbiont in *P. apicalis* and detected *Wolbachia* sp. in the abdominal region of both the larva and the adult.

Keywords: Gut, mycetome, thrips, termites, *Wolbachia* sp.

WIDELY distributed and occupants of vast habitats, insects are taxonomically important organisms. These insects have well-developed physiological systems, but occurrence of slight modifications in the anatomical structures within these systems during evolution, categorizes different orders distinctly. The insect digestive system is a key system of fundamental processes such as providing nutrition, maintaining a balance of water and solutes and acting as a first line of defense[1]. The alimentary canal of insects is a long, tubular and muscular structure extending from the mouth to the anus. It is differentiated into three regions the foregut, the midgut and the hindgut. Constituted with different cell types, the foregut and hindgut have ectodermal origin whereas the midgut has endodermal origin[2]. Allotted with different and specific functions, the foregut helps in storage and partial digestion; midgut serves to completely digest and absorb the nutrients; and the hindgut helps in final absorption of useful nutrients and water[3].

It is evident from earlier reports that the insect digestive system brings in distinctness not only between different orders but at species level as well. Anatomical variations have been observed among orders such as Lepidoptera, Diptera, Coleoptera, Orthoptera, and Hymenoptera and within the order Hemiptera in different species such as aphid, kissing bug, alydid bean bug, and paltaspid stinkbug, as well as in order Isoptera between lower and higher termites. The differences in some features of alimentary canal of scolytinae beetles, *Scolytus multistriatus*[4], *Dendroctonus* group[5–7], *Gnathotrichus retusus*[8] were reported to be useful for the taxonomy of these beetles[9]. The noted variations in the alimentary canal have been ascribed to the varied feeding habits and food types of the insects. Earlier work focused only on major orders, and with availability of limited literature for particular species from order Thysanoptera[10–14], we were interested in detailing the gut structure of these species as they are emerging as a major pest group at an alarming rate.

Thysanoptera, commonly called thrips, are minute and slender insects with fringed wings. Thrips cause direct damage by puncturing plants and sucking up the sap and indirect damage by vectoring and transmitting viruses. Of the 7700 well-described thrip species, nearly 5000 species are categorized as economically important[15]. For a clear understanding of the digestive system in thrips, we previously worked on a terebrantian species, *Thrips tabaci* Lindeman (unpublished manuscript). Therefore, in our current work we studied *Plicothrips apicalis* (belonging to suborder Tubulifera), as an experimental model. *P. apicalis* is a primary pollen feeder of grasses (*Cynodon dactylon*). Previously known by the name *Hindsiana apicalis* Bagnall, *P. apicalis* belongs to the subgenus Trybomiella. These insects have a single sense cone on third antennal segment; all the prothoracic major setae are developed; their micropterous forms have three basal wing setae with expanded apices and the maxillary bridge is long and slender[16]. Commonly referred to as grass thrips, recently an association of *P. apicalis* with groundnut bud necrosis virus (GBNV)-infected plants of *Vigna* spp. has been reported by Akram *et al.*[17]. Their association with leaf curl virus was reported as the presence of *P. apicalis* was

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recorded on the foliage of Urd bean[18]. Priesner[19] showed presence of *P. apicalis* adults and second instar larval stage in onion fields in Indonesia. The presence of *P. apicalis* on different leguminous plants and in onion field, and their association with GBNV and leaf curl virus is indicative of the potential it holds to infest leguminous and onion plants with a probability to turn out as a major pest species and vector of plant viruses. By minding the possibility of *P. apicalis* emerging as a pest and a plant virus vector and with availability of limited literature on gut anatomy of Tubulifera group of Thysanoptera, the present study aims to provide an update on the description of gut anatomy of *P. apicalis* using modern imaging tools.

Materials and methods

P. apicalis collection

Larvae and adults of *Plicothrips apicalis* were collected from the Department of Zoology, University of Delhi (India), by vigorously tapping the grass in a collecting tray. Samples were collected in 70% ethanol for DNA isolation. For studying the gut structure of the specimens, thrips were collected in 1X PBS and immediately dissected.

Dye feeding experiment

To visualize the complete structure of the gut, thrips were fed with bromophenol blue and eosin dye separately. Thrips were collected and kept for feeding in small transparent cages made with plastic sheets (2.5 cm diameter and 2.5 cm height), sealed at one end. The cage was covered with parafilm after placing the larvae and adults separately in the cages. Feeding solution was prepared by mixing 20% sucrose with bromophenol blue or eosin and was placed over the parafilm. The solution was sealed with another layer of parafilm.

Detection of bacterial endosymbiont

DNA isolation: Single larva and single adult of *P. apicalis* were washed briefly with 70% ethanol and subsequently with autoclaved sterile water. Samples were then homogenized in 14 µl lysis buffer (1 M *Tris*-Cl pH 8.0, 100 mM NaCl, 100 mM EDTA pH 8.0, 1% SDS and 1% proteinase K). Homogenates were incubated at 70°C for 45 min. Further, 28 µl of pre-chilled buffer containing 6 M lithium chloride and 5 M potassium acetate was added to the homogenates and incubated on ice for 15 min. Samples were centrifuged at 12,000 rpm for 10 min. Supernatant was transferred to a fresh microfuge tube discarding the pellet. To the supernatant 60 µl of isopropanol was added and incubated at –20°C for 1 h. DNA was precipitated by centrifuging at 12,000 rpm for 10 min. DNA pellet so obtained was washed with 50 µl of 70% ethanol. Pellet was air-dried and dissolved in MQ water and treated with RNase at 37°C for 40 min. DNA extract was stored at –20°C for further use.

Diagnostic PCR for detection of Wolbachia sp. in thrips: To detect *Wolbachia* sp. in *P. apicalis*, specific bacterial primers for 16S rRNA and *Wolbachia* sp. were used. 16S rRNA gene was amplified using universal primers, 27F and 1492R (ref. 20). PCR reaction mix (25 µl) contained 20 ng DNA template, 1 unit of *Taq* polymerase, 2.5 µl of 10 × PCR buffer, 2.5 mM of DNTP mix, and 7.5 pmoles of each primer. DNA fragment was amplified using thermal cycler (Thermo Applied Biosystem, USA) with initial denaturation at 94°C for 30 sec followed by 24 cycles of 94°C for 30 sec, 56°C for 1 min and 72°C for 1 min 30 sec. *Wolbachia* sp. specific primer with sequence F-5′CGGGGGAAAATTTATTGCT3′ and R-5′AGCTGTAATACAGAAAGGAAA3′ (ref. 21) was used. For each PCR mix, 20 ng DNA template, 1 unit of *Taq* polymerase, 2.5 µl of 10 × PCR buffer, 2.5 mM of DNTP mix, and 4 pmoles of each primer were added. PCR conditions were as follows: denaturation at 94°C for 30 sec followed by 43 cycles at 94°C for 30 sec, 52°C for 30 sec and 72°C for 40 sec and final extension at 72°C for 5 min.

For negative control, MQ water instead of DNA template was taken in the reaction mix. Amplified PCR products were run on 0.8% agarose gel and observed.

Fluorescent in situ Hybridization (FISH) with LNA probe: For further confirmation of *Wolbachia* sp. in *P. apicalis* FISH was performed. *Wolbachia* sp. sequence specific LNA (Locked Nucleic Acid) probe of 10 nmoles/ml concentration was used. The *Wolbachia* sp. LNA probe with sequence-CTTCTGTGAGTACCGTCATTATC, TEX615 (ref. 22) was supplied by Exiqon A/S. FISH analysis was performed as follows: *P. apicalis* larvae and adults ($n = 20$, 3 replicates) were fixed in Carnoy's fixative (ethanol : chloroform : glacial acetic acid, 6 : 3 : 1) overnight. Samples were then incubated in 6% H_2O_2 for 48 h for decolourization. After the samples were completely decolourized, they were incubated in 50 µl of hybridization buffer (20 mM *Tris*-Cl, pH 8, 1% sodium dodecyl sulphate, 0.9 M sodium chloride, 30% formamide) containing *Wolbachia* sp. specific LNA probe overnight at 42°C. The samples were then thoroughly washed twice with washing buffer (0.03 M sodium citrate, 0.3 M sodium chloride, 0.01% SDS-sodium dodecyl sulphate) for 15 min each. The thrips were mounted on slides with fluoroshield (sigma). Fluorescence imaging was done using Nikon A1 si-Ti eclipse laser-scanning confocal microscope on a Nikon Ti inverted microscope (4×, 0.13 NA and 10×, 0.45 NA). For positive

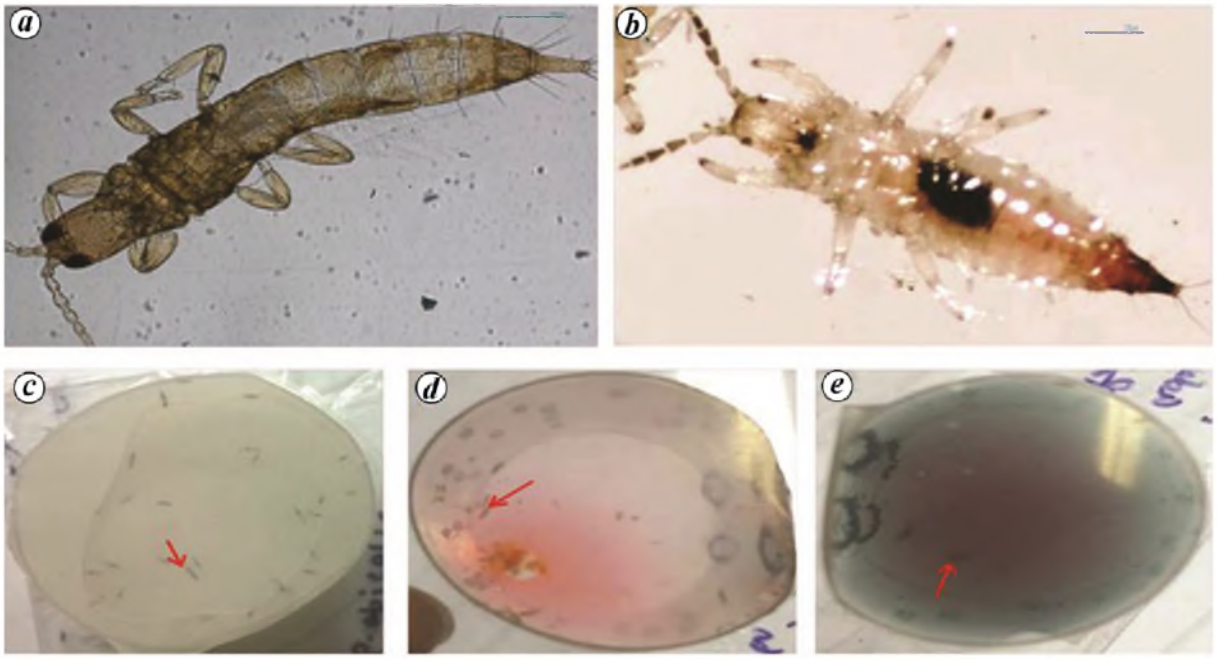

Figure 1. *Plicothrips apicalis*: ***a***, Adult female; ***b***, Larva. Image taken 10× magnification. Scale bar, 100 µm. ***c–e***, Thrips feeding on Sucrose solution (***c***), eosin red (***d***), and bromophenol blue (***e***) in plastic cages (indicated by red arrow) covered with layer of parafilm.

control, in our earlier work we had used the same *Wolbachia* sp. probe in *Bemisia tabaci*[23].

Microscopy and imaging

Guts of larval and adult stages were dissected in 1X PBS (phosphate buffered saline). The guts were observed and images were captured using Nikon SMZ745T stereomicroscope, objective C-W10xB/22. DIC and FISH imaging were done using Nikon A1 si-Ti Eclipse laser-scanning confocal microscope on a Nikon Ti inverted microscope (4×, 0.13 NA and 10×, 0.45 NA).

Results

Morphology and biology of P. apicalis

Plicothrips apicalis (Bagnall) (Phlaeothripidae) are pollen feeding species that breed on *Cynodon dactylon*. The body is distinctly bi-coloured, brown and yellow, where the females are 1.5–2 mm in length and males are 1.3–1.8 mm in length. The females of this species lack an ovipositor (Figure 1 *a*). Their life cycle consists of the egg stage, 2 larval stages (Figure 1 *b*), 2 pre-pupal stages, 1 pupal stage and adult stage. The adult and larval stages are found on grass blades from where the second larval stage drops down into the soil to undergo pupation, and emerge as adults. In our work we tried rearing *P. apicalis* on grass without placing soil, but could not obtain the eggs. Also, on placing the larval stages either on grass blades or on tissue paper provided in the jar, pupation did not occur and pupal stages were not obtained.

Dye feeding and morphology of the alimentary canal

The larval as well as adult stages were fed with eosin (red) and bromophenol (blue) dye separately (Figure 1 *d* and *e*). On observing the live stages under light microscope, uptake of dye in both stages was visible as it clearly outlined the structure of the gut (Figure 2). *P. apicalis* larva and adult share a similar appearance and arrangement of gut. The alimentary canal of *P. apicalis* running from mouth to anus is an easily distinguishable short linear tube varying in diameter along the length. The gut is well demarcated and differentiated into a small foregut, long midgut and hindgut.

The foregut originates with a pre-oral or buccal cavity, leading into a tubular and muscular pharynx that is followed by oesophagus downward. The anterior foregut branches into two pairs of translucent pouches, the salivary glands. The oesophagus at the distal end enlarges into a small pouch-like structure, the crop that further leads into the lumen of the midgut. Midgut constitutes major part of the alimentary canal. Originating in the first (I) thoracic segment, it extends till the sixth (VI) abdominal segment. Midgut originates as a wide cylindrical translucent structure (constituting major portion of the midgut), but further extends posteriorly, returning into its tubular form (constituting minor portion of the midgut).

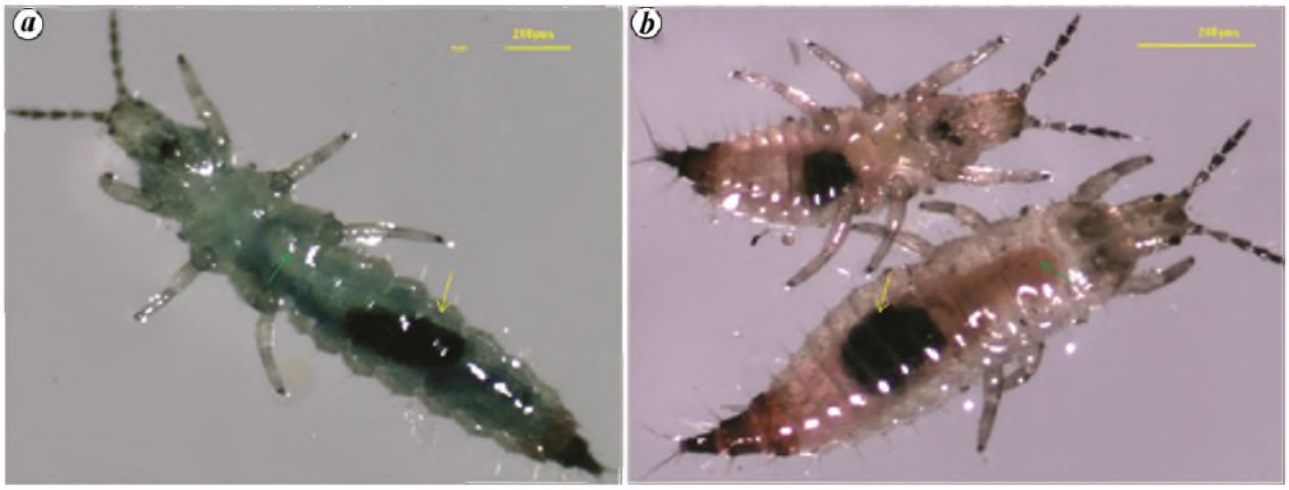

Figure 2. Outline of *Plicothrips apicalis* Gut: ***a***, 2nd instar larva; ***b***, 1st and 2nd instar larvae. The alimentary canal appears blue and red in larvae in (***a*** and ***b*** respectively) after feeding on the bromophenol blue and eosin red dye respectively. Starting with the oral cavity, foregut leads into the midgut. Midgut starts from I thoracic segment and continues till the VI abdominal segment. The green arrow indicates the cylindrical shaped midgut in both the adult and the larval stages. Following the midgut, the accessory organ mycetome (indicated by yellow arrow) within the globular chamber appears as a dark coloured sac. The chamber extends into the hindgut that is flanked by two pairs of malphigian tubules.

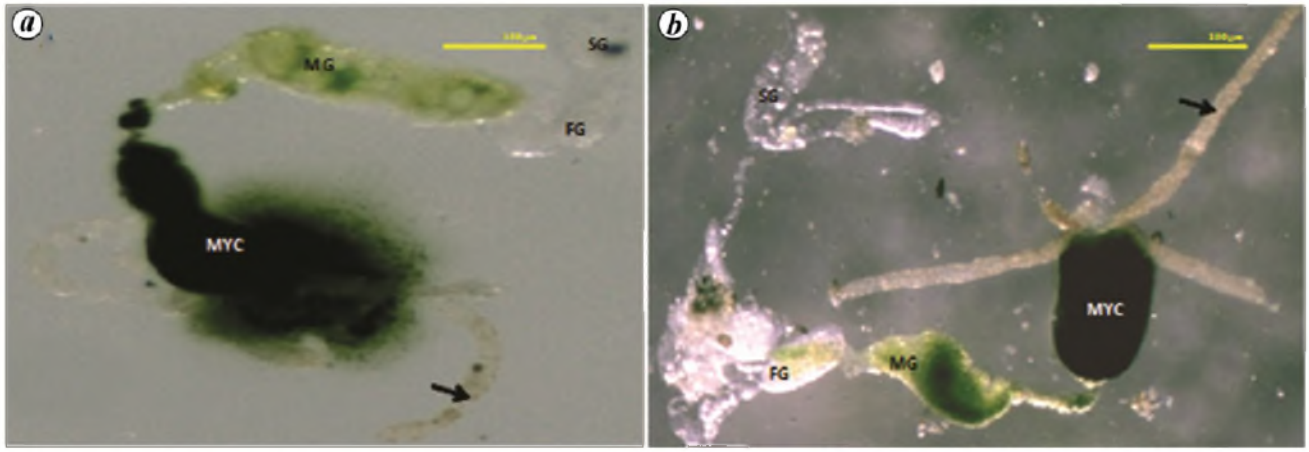

Figure 3. *Plicothrips apicalis* Gut Structure: ***a***, Larval gut; ***b***, Adult gut. A translucent oesophagus (Oe) and sac like crop (C) constitute the foregut (FG) is surrounded by a pair of salivary glands (SG). FG leads into midgut (MG) which is a long cylindrical structure that appears light green in colour. The midgut is later constricted in a narrow tube opening into the globular chamber containing mycetome (MYC) that appears dark green in colour. Hindgut emerges immediately after the globular chamber and 2 pairs of malphigian tubules (indicated by black arrow) are observed at their juncture.

Furthermore, posteriorly the midgut opens in a wide globular chamber. The chamber appears as a dark green coloured sac that contains mycetome, which on puncturing is detected to harbour protists in it. The globular chamber finally leads into the narrow tubular hindgut that ends in the terminal anal opening. At the juncture of globular chamber and the hindgut, both the larvae and the adults have two pairs of short and stout malphigian tubules (Figure 3).

We present as a diagram the observed gut structure (Figure 4) for a clear and better understanding of the gross morphological features of the digestive system in *P. apicalis*.

Wolbachia sp. detection in P. apicalis

We extended our work to probe the presence of *Wolbachia* sp. in *P. apicalis*, as presence of secondary endosymbionts has been linked to provide various advantages to its host species; with helping its host turn into a pest species an important advantage[24].

Genomic DNA isolated from single larval and adult stages, was used to detect *Wolbachia* sp. by diagnostic PCR. The 16SrRNA PCR product of 1500 bp indicated the presence of bacterial diversity (Figure 5 *a*) and presence of *Wolbachia* sp. was further confirmed with a

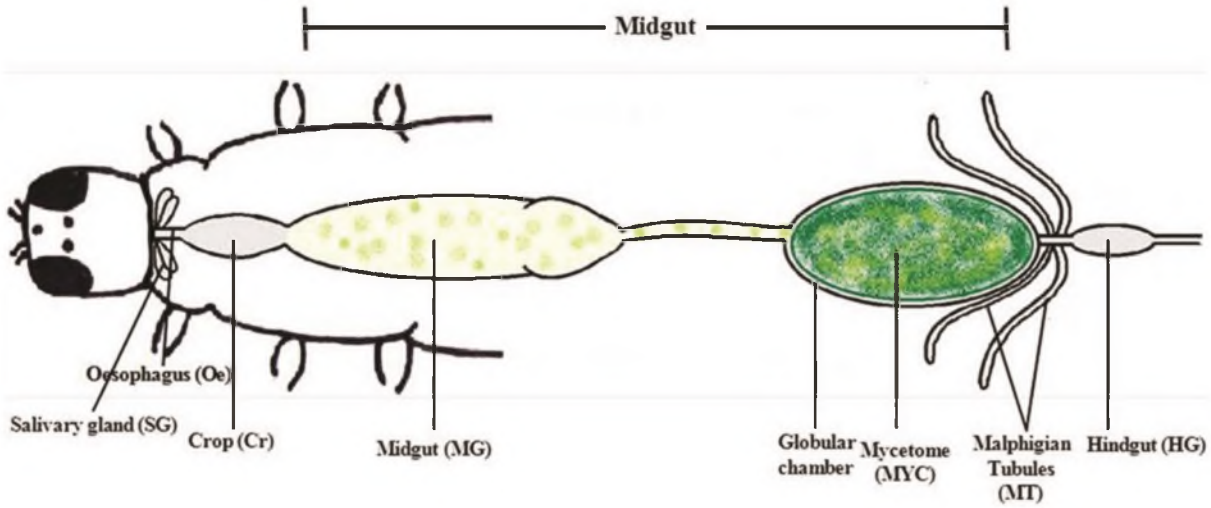

Figure 4. Diagrammatic representation of *P. apicalis* gut. Dorso-ventral view of gut is similar in both the larva and the adult. Presentation of internal organs – foregut (FG) consitituted by oesophagus (Oe) and crop (Cr), salivary glands (SG), midgut (MG), globular chamber, mycetome (MYC), malphigian tubules (MT) and hidgut (HG). The midgut shows linear arrangement within the abdomen.

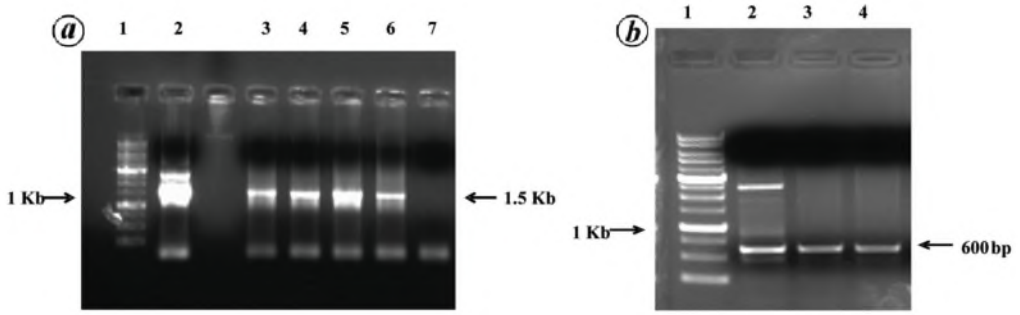

Figure 5. Agarose gel electrophoresis. ***a***, 16S rDNA PCR product amplified from genomic DNA of *Plicothrips apicalis*. Lane 1, 1 Kb DNA ladder; lane 2, positive control; lanes 3,4, 16S rDNA amplified from *P. apicalis* adult; lanes 5, 6, 16S rDNA amplified from *P. apicalis* larva; lane 7, negative control. ***b***, Diagnostic PCR to detect *Wolbachia* from *P. apicalis* genomic DNA using *Wolbachia* specific primers. Lane 1, 1 kb DNA ladder; lane 2, positive control; lane 3, PCR product from single larva; lane 4, PCR product from single adult with band size 600 bp.

600 bp PCR product with *Wolbachia* sp. specific primers (Figure 5 *b*). Positive and negative controls gave the intended results (number of replicates = 10). Moreover, to confirm the presence of endosymbiont *Wolbachia* sp., FISH using fluorescent LNA oligo was carried out. Representative results of whole mount of *P. apicalis* larva and adult are presented (Figure 6), that indicate and confirm the presence of *Wolbachia* sp. in the abdominal region, not in the mycetome.

Discussion

The gastrointestinal (GI) tract or the digestive system aids in various functions ranging from digestion, osmoregulation to providing primary protection (immune response or homeostasis). In invertebrates, the anatomical and functional characteristics of the alimentary canal is importantly a reflection of their taxon, developmental stage, feeding behaviour, food sources (detritus, phloem sap, leaves, other insects and blood)[25,26], the biomolecular composition (protein, carbohydrates, fats) and bacterial symbionts. Previous studies showed that factors such as different developmental stages, feeding behaviour and food sources steer diversification in morphology, biochemistry and physiology of the digestive system in insects[27]. Studies show that insects share a similar basic plan of the digestive system, with one or more noticeable modifications in the structural composition and arrangement of the alimentary canal. Thus, the alimentary canal of insects is indicative of the life history traits and phylogenetic relationships within the species and to some extent between different orders such as Diptera, Coleoptera, Lepidoptera, Hemiptera and Isoptera.

Key observations in the past few years show that the huge economic losses incurred due to thrips, have made this order emerge as a serious pest of various crops. Thrips, belonging to order Thysanoptera, are broadly classified in two suborders – Terebrantia and Tubulifera. These minute insects show specific host associations;

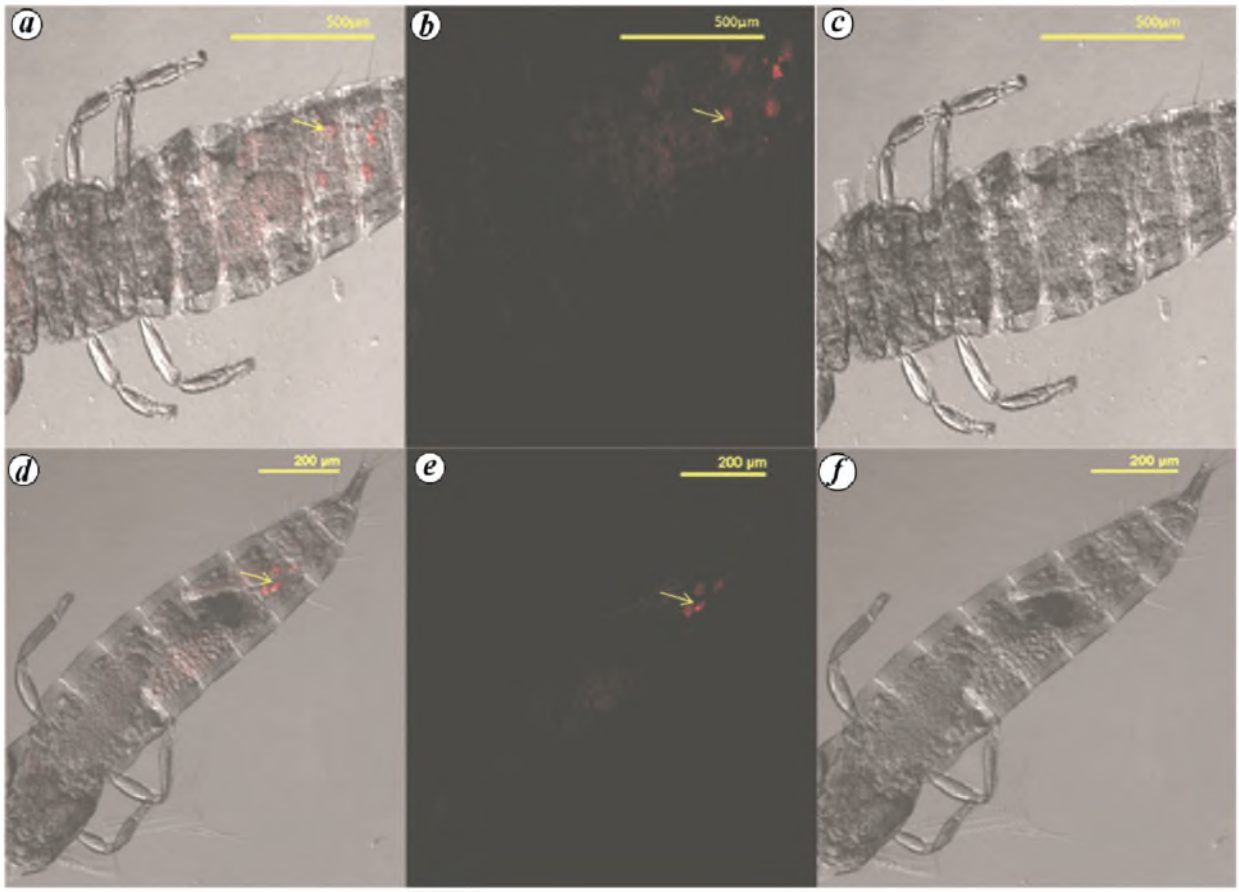

Figure 6. Localization of *Wolbachia* in *P. apicalis* using FISH: ***a***, Merged; ***b***, Texas red; ***c***, TD view showing presence of *Wolbachia* in abdomen of larval stage (indicated by yellow arrow); ***d***, Merged; ***e***, Texas red; ***f***, TD view of adult stage detected with endosymbiont *Wolbachia* (indicated by yellow arrow). Images captured at 20× (***a***–***c***) and at 10× (***d***–***f***). Scale bar 500 µm (***a***–***c***) and 200 µm (***d***–***f***).

nearly 95% of terebrantia are associated with green plants, whereas 60% of tubuliferans are fungivorous[28]. The successful exploitation of a variety of niches and cropping systems, and ability to easily move from crops to weeds and vice versa are some of the key points of thrips bionomics[29]. Till date, nearly 5000 species of thrips have been listed as economically important. They have high efficacy in causing direct damage by feeding on the plants phloem sap and indirectly by serving as a vector of *Tospovirus*. Using thrips considerable molecular studies have been carried out[30–32], whereas studies on internal anatomy of thrips have been less studied[10–14]. In our current study, we studied *Plicothrips apicalis* to delineate its gut structure for two basic reasons. First, because it is a tubuliferan and no anatomical work was earlier taken up in the tubuliferan group except in *Haplothrips distinguendus*[12,14]. Secondly its association with GBNV and leaf curl virus, as well as its expanding host range from grass to onion plants[19], *Vigna* spp.[17] and urd bean[18] is drawing attention to its pestiferous potential and (or) vectoral ability.

In the present study we have made an attempt to describe the morphology of the alimentary canal of *P. apicalis*. On detailing the structure we found resemblance in morphology and its arrangement between larval and adult stages, with slight modifications, that are uncommon to order Thysanoptera. Sharga[14] in his work on *Aptinothrips rufus* displayed the length of alimentary canal to be twice the length of insect that was present in convoluted form within the body cavity. The crop in *A. rufus* was three times wider than oesophagus. Jordan[10] showed the whole tube running behind the pharynx till midgut as oesophagus in *Heliothrips darcaenae*, whereas Uzel[12] described the hindpart as dilation of oesophagus. Further, Uzel described the alimentary canal in *Haplothrips distinguendus* as a shorter and wider structure, unlike that of *A. rufus* and other terebrantian species. The alimentary canal in *P. apicalis* constituted the pharynx, oesophagus, two pairs of salivary glands and a crop that extends into a combinatorial cylindrical (saccular) and tubular midgut. A morphological subdivision in the midgut has also been reported in *Rhynchosciara* fly by Ferreira *et al.*[33]. As observed by Sharga[14] in *H. distinguendus*, the morphology and arrangement of the midgut were similar to *P. apicalis*. The anterior midgut is long and cylindrical, whereas the posterior midgut is marked by the presence

of globular chamber that is viewed to contain mycetome. Further the passage from midgut to hindgut is surrounded with two pairs of malphigian tubules. Mycetome is a specialized structure that comprises a group of either gut cells or fat cells[34]. In some hemipterans such as whiteflies and aphids the mycetome or bacteriocytes are present as a separate organ within the abdomen, but not as a part of the alimentary canal. They are considered to play a role in the metabolism of vitamins and other essential substances[35,36]. On dissecting the mycetome we observed that it was inhabited by a large number of protists. Further studies are required to characterize and classify these protists. Observing mycetome inhabited with protists in *P. apicalis*, we assume they might have some similarity with the paunch found in higher termites. In termites the hindgut is always enlarged and is known as a paunch, a residence for numerous symbionts, in particular the protists. As paunch in termites has been linked with the digestion of lignocellulose[37], similarly, the mycetome might be involved in bolstering an efficient digestive process in *P. apicalis*, thereby making digestion of cellulose or similar components an easy task. They might also provide some nutritional benefits to *P. apicalis*, as earlier reports in other insects such as hemipterans suggest that symbionts provide nutritional requirements such as need of amino acids or other biomolecules to their insect hosts[38].

Sighting of *P. apicalis* in onion field[19] and noting an expansion in their host plant range such as, *Vigna* spp[17]. and urd bean[18], as well as their developing association with GBNV and leaf curl virus is marking its candidature to become a potential pest and plant virus vector. Further in our work, we tried detecting the presence of *Wolbachia* sp. in *P. apicalis* to confirm if *P. apicalis* displays any association with a secondary endosymbiont, *Wolbachia*. Findings from earlier reports on insects such as stinkbugs (*Megacopta cribraria*) suggest the important role being played by obligate endosymbionts in helping its host to turn into a pest[24]. *Wolbachia*, one of the most common intracellular bacteria, is found in numerous arthropods and has been known to alter their host's biology in various ways. In arthropods, *Wolbachia* mainly acts as a reproductive parasite as it manipulates the reproductive biology of its hosts to increase its own transmission. *Wolbachia* primarily aids in feminization in insects, as the process doubles the potential of its transmission in following generations[39]. So far, detection of *Wolbachia* even in thrips species has been put forward as a model for mediated reproductive modes[40]. By using FISH and diagnostic PCR techniques, our results confirm the presence of *Wolbachia* sp. in *P. apicalis*. In both larval and adult stages *Wolbachia* sp. was detected in the posterior part of the abdomen (not specifically in the mycetome). On detecting *Wolbachia* sp. we can hypothesize that its presence might confer *P. apicalis* the status of pest species by acting as reproductive parasite causing feminization or parthenogenesis or male killing in the near future, thereby broadening the range of host plants by turning *P. apicalis* into pests. These observations open the field for further work on *Wolbachia–P. apicalis* interaction.

In conclusion, we can assign our work on the internal anatomy of *P. apicalis* to three broad points: (1) Overall, the alimentary canal of *P. apicalis* is a simple and linear structure and differs from the other thrips (as observed in *Thrips tabaci*; manuscript under consideration) species in structural organization. (2) The presence of crop and symbiont filled mycetome brings in variation between *P. apicalis* and its terebrantian counterparts and draws some evolutionary similarity between *P. apicalis* and hemipterans/isopterans. (3) Localization of *Wolbachia* sp. confirms the association of *P. apicalis* with a secondary endosymbiont, where the presence of *Wolbachia* sp. might provide its host some beneficial services, for which further studies are required.

Competing interests: The authors have declared that no competing interests exist.

1. Sansonetti, P. J., War and peace at mucosal surfaces. *Nat. Rev. Immunol.*, 2004, **4**, 953–964.
2. Chapman, R. F., *The Insects: Structure and Function*, Cambridge University Press, Cambridge, 1998.
3. Klowden, M. J., *Physiological Systems in Insects*, Elsevier Incorporation, Academic Press, London, Second edn, 2007.
4. Baker, W. V. and Estrin, C. L., The alimentary canal of *Scolytus multistriatus* (Coleoptera-Scolytidae). A histological study. *Can Entomol.*, 1974, **106**, 673–686.
5. Diaz, E., Cisneros, R., Zuniga, G. and Uria-Galicia, E., Comparative anatomical and histological study of the alimentary canal of *Dendroctonus parallelocollis*, *D. rhizophagus* and *D. valens* (Coleoptera-Scolytidae). *Ann. Entomol. Soc. Am.*, 1998, **91**, 479–487.
6. Diaz, E., Arciniega, O., Sanchez, L., Cisneros, R. and Zuniga, G., Anatomical and histological comparison of the alimentary canal of *Dendroctonus micans*, *D. ponderosae*, *D. pseudotsugae pseudotsugae*, *D. rupifenis* and *D. terebrans* (Coleoptera-Scolytidae). *Ann. Entomol. Soc. Am.*, 2003, **96**, 144–152.
7. Silva-Olivares, A., Diaz, E., Shibayama, M., Tsutsumi, V., Cisneros, R. and Zuniga, G., Ultrastructural study of the midgut and hindgut in eight species of the genus *Dendroctonus* Erichson (Coleoptera-Scolytidae). *Ann. Entomol. Soc. Am.*, 2003, **96**, 883–900.
8. Schneider, I. and Rudinsky, A., Anatomical and histological changes in internal organs of adult *Trypodendron lineatum*, *Gnathotichus retusus* and *G. sulcatus* (Coleoptera-Scolytidae). *Ann. Entomol. Soc. Am.*, 1969, **62**, 995–1003.
9. Lopez-Buenfil, J. A., Valdez, J., Equihau, A. and Burgos, A., El proventrículo como estructura para identificar géneros Mexicanos de Scolytidae (Coleoptera). *Folia Entomol. Mex.*, 2001, **40**, 325–372.
10. Jordan, K., Anatomie und Biologie der physapoda, Z. Wiss. *Zool.*, 1888, **47**, 541–620.
11. Buffa, P., Contributo allo studio anatomico della *Heliothrips haemorrhoidalis*. *Revista di Patologia Vegetale.*, 1899, **7**, 94–108, 129–142.
12. Uzel, H., Monographie der Ordnung Thysanoptera. Uzel, Königgrätz., 1895, p. 473.
13. Cary, L. R., The grass thrips (*Anaphothrips striata* Osborn). *Bull. Maine Agric. Exp. Stat.*, 1902, **83**, 97–128.

14. Sharga, US, On the internal anatomy of some thysanoptera. *Ecol. Entomol.*, 1933, **81**, 185–204.
15. Zur Strassen, R., Key to and catalogue of the known species of *Chirothrips* Haliday, 1836 (Thysanoptera: Thripidae). *J. Entomol. Soc. South. Afr.*, 1960, **23**, 144–176.
16. Mound, L. A., A review of R.S. Bagnall's Thysanoptera collections. *Bull. Brit. Mus. (Nat. Hist) Entomol.*, 1968, **11**, 1–181.
17. Akram, M., Naimuddin, Pratap, A., Malviya, N. and Yadav, P., First report of *Groundnut bud necrosis virus* infecting wild species of *Vigna*, based on *NP* gene sequence characteristics. *Phytopathol. Mediterr.*, 2013, **52**, 532–540.
18. Annual Report 2010–2011, by Indian Institute of Pulses Research, Kanpur.
19. Priesner, H., On the Haplothrips from the Oriental region. *Rec. Indian Mus.*, 1933, **35**, 347–369.
20. Weisburg, W. G., Barns, S. M., Pelletier, D. A. and Lane, D. J., 16S ribosomal DNA amplification for phylogenetic study. *J. Biotechnol.*, 1991, **173**, 697–703.
21. Singh, S. T., Kumar, J., Thomas, A., Ramamurthy, V. V. and Rajagopal, R., Detection and localization of *Rickettsia* sp. in mealybug. *Environ. Entomol.*, 2013, **42**, 711–716.
22. Skaljac, M., Zanic, K., Ban, S. G., Kontsedalov, S. and Ghanim, M., Co-infection and localization of secondary symbionts in two whitefly species. *BMC Microbiol.*, 2010, **10**, 142.
23. Raina, H. S., Singh, A., Popli, S., Pandey, N. and Rajagopal, R., Infection of bacterial endosymbionts in insects: a comparative study of two techniques, viz. PCR and FISH for detection and localization of symbionts in whitefly, *Bemisia tabaci*. *PLOS ONE*, 2015, **10**(8), e0136159.
24. Hosokawa, T., Kikuchi, Y., Shimada, M. and Fukatsu, T., Obligate symbiont involved in pest status of host insect. *Proc. R. Soc. B*, 2007, **274**, 1979–1984.
25. Wigglesworth, V. B., Digestion and nutrition. In *The Principles of Insect Physiology*, Chapman and Hall, London, UK, 7th edn, 1972.
26. Chapman, R. F., Structure of the digestive system. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, Pergamon Press, Oxford, UK, 1985.
27. Karasov, W. H. and Douglas, A. E., Comparative digestive physiology. *Compr. Physiol.*, 2013, **3**, 741–783.
28. Mound, L. A., Thrips and their host plants; New Australian records (Thysanoptera; Terebrantia). *Aust. Entomol.*, 2002, **29**, 49–60.
29. Ananthakrishnan, T. N. and Annadurai, R. S., Thrips tospovirus interaction: biological and molecular implications. *Curr. Sci.*, 2007, **92**, 1083–1086.
30. Moritz, G., Kumm, S. and Mound, L., Tospovirus transmission depends on thrips ontogeny. *Virus Res.*, 2004, **100**, 143–149.
31. Ullman, D. E., Westcot, D. M., Hunter, B. W. and Mau, R. F. L., Internal anatomy and morphology of *Frankliniella occidentalis* (pergande) (thysanoptera: thripidae) with special reference to interactions between thrips and tomato spotted wilt virus. *Int. J. Insect Morphol. Embryol.*, 1989, **18**, 289–310.
32. Ullman, D. E., Westcot, D. M., Mau, R. F. L., Cho, J. J. and Custer, D. M., Tomato spotted wilt virus and one thrips vector: *Frankliniella occidentalis* (Pergande) internal morphology and virus location. Virus-Thrips-Plant Interaction of tomato spotted wilt virus. Proceedings of the USDA Workshop. Agricultural Research Serv ARS-87 (eds Hsu, H. and Lawson, R.), 1991.
33. Ferreira, C., Ribeiro, A. F. and Terra, W. R., Fine structure of the larval midgut of the fly Rhynchoscira and its physiological implications. *J. Insect. Physiol.*, 1981, **27**, 559–570.
34. Musgrave, A. J., Insect mycetomes. *Can. Entomol.*, 1964, **96**, 377–389.
35. Baumann, P., Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu. Rev. Entomol.*, 2005, **59**, 155–189.
36. Buchner, P., Symbiosis in animals which suck plant juices. In *Endosymbiosis of Animals with Plant Microorganisms, Interscience* (ed. Buchner, P.), New York, 1965, pp. 210–432.
37. Brune, A., Symbiotic digestion of lignocelluloses in termite guts. *Nat. Rev. Microbiol.*, 2014, **12**, 168–180.
38. Douglas, A. E., Phloem-sap feeding by animals: problems and solutions. *J. Exp. Bot.*, 2006, **57**, 747–754.
39. Werren, J. H., Baldo, L. and Clark, M. E., *Wolbachia*: master manipulators of invertebrate biology. *Nat. Rev. Microbiol.*, 2008, **6**, 741–751.
40. Nault, B. A. *et al.*, Reproductive modes in onion thrips (Thysanoptera: Thripidae) populations from New York fields. *Enviorn. Entomol.*, 2006, **35**, 1264–1271.

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