Evidence for the independent evolution of a rectal complex within the beetle superfamily Scarabaeoidea

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A B S T R A C T

Rectal or cryptonephridial complexes have evolved repeatedly in arthropods, including in beetles where they occur in ~190,000 species of Cucujiformia + Bostrichoidea, and Lepidoptera where they occur in ~160,000 species. Sections of the Malpighian/renal tubules coat the outer surface of the rectum, acting as powerful recycling systems of the gut contents, recovering water and specific solutes. There are hints that a rectal complex evolved independently within another beetle group, Scarabaeoidea. Here we report our observations of rectal complexes in Scarabaeoidea, which support this view. We did not find a rectal complex in the related group, Staphylinoidea, or in Lucanidae, a basal group of Scarabaeoidea. We did observe rectal complexes in *Melolontha melolontha* (Melolonthini), *Pachnoda marginata* and *Cetonia aurata* (Cetoniinae), consistent with previous reports from these groups. Intriguingly we found that rectal complexes occur in adult, but not *M. melolontha* larvae, and larvae but not adults within Cetoniinae, indicating dramatic pupal remodelling of these organ systems. Insights into the structure of the rectal complexes of Scarabaeoidea are compared with the well-studied rectal complexes of Cucujiformia. Finally, we discuss possible functions of the rectal complexes of beetles within Scarabaeoidea, and future approaches to address this question.

1. Introduction

Cryptonephridial complexes (CNC), also known as rectal complexes, are remarkably widespread organ systems which appear to have evolved multiple times in arthropods. They result from an evolutionary reorganisation of the renal Malpighian tubules (MpTs) in relation to the rectum. MpTs function in maintaining water and solute homeostasis, and removal of nitrogenous waste (Wessing and Eichelberg, 1978; Dow et al., 1994; Denholm, 2013). Their ancestral organisation is as blind ended tubes, the proximal ends of which open into the gut at the junction of the midgut and hindgut, allowing the primary urine they produce to be expelled. In the derived, cryptonephridial organisation, their distal ends form a secondary association with the gut, having highly elaborated courses over the surface of the rectum, where they function in the

retrieval of water or solutes from the rectal contents, to be returned to the haemolymph (Ramsay, 1964; Kolosov and O'Donnell, 2019; Beaven et al., 2023; Beaven and Denholm, 2024). The CNC is ensheathed in a tissue, the perinephric membrane, which insulates the complex to enable its efficient function (Grimstone et al., 1968; Koefoed, 1971). There are also some cases of more rudimentary complexes which lack a perinephric membrane (Ullman et al., 1989; Dallai et al., 1991; Arab and Caetano, 2002). We will use rectal complex as a broader term, with CNC reserved for complexes with a perinephric membrane.

Beetle species from the Cucujiformia infraorder + Bostrichoidea superfamily possess CNCs, with seemingly a single evolutionary origin (Beaven and Denholm, 2024). Cucujiformia + Bostrichoidea contain ~190,000 species (Hunt et al., 2007) making this the most species rich clade to possess CNCs. The structure and physiology of the CNC has been long studied and extensively characterised in beetles from Tenebrionidae (infraorder Cucujiformia) (Ramsay, 1964; Grimstone et al., 1968; Noble-Nesbitt, 1970; Marshall and Wright, 1972; Dunbar and Winston, 1975; Machin, 1975; Coutchié

and Machin, 1984; Machin and O'Donnell, 1991; O'Donnell and Machin, 1991; Hansen et al., 2004). More recently a molecular understanding of CNC function has been gained in the tenebrionid beetle *Tribolium castaneum* (King and Denholm, 2014; Naseem et al., 2023; Beaven and Denholm, 2024; Beaven et al., 2024).

A remarkably similar system seems to have evolved independently within Lepidoptera, where it is present in the larval stage. CNCs have been observed in the clade Ditrysia (Metalnikov, 1908; Ito, 1921; Henson, 1937; Saini, 1964; Judy, 1968; Irvine, 1969; Wigglesworth, 1974; Ramsay, 1976; O'Donnell and Ruiz-Sanchez, 2015; Beaven and Denholm, 2024), which contains most species (~160,000; Regier et al., 2009), and only seems to be absent in the most basal, species-poor groups (Kristensen, 2003). Interestingly, although CNCs can return water to the body in Lepidoptera (Reynolds and Bellward, 1989), it is considered that their main function is in the return of solutes from the rectal contents, in order to maintain correct acid-base balance. This could be an adaptation to allow rapid digestion of leaves during the larval stage, as well as to maintain water and ion balance during this stage of rapid growth (Ramsay, 1976; Kolosov and O'Donnell, 2019). Therefore CNCs appear to be powerful recycling systems, which have evolved to meet the diverse environmental and dietary challenges faced by different insect groups. For an extensive review of CNC biology, see Beaven and Denholm (2024).

A rectal complex has also been reported in some beetle species within Scarabaeoidea, with the most detailed description being for adults of the common cockchafer, *Melolontha melolontha* (tribe Melolonthini; Fig. 1). In this species the rectal complex is reported to be ensheathed in a very fine cellular layer which may represent a perinephric membrane (Lison, 1938; Saini, 1964). The picture of the presence of rectal complexes across Scarabaeoidea remains extremely unclear, as reported differences in morphologies may reflect evolutionary changes between species, but also developmental changes between stages studied (larval or adult). Furthermore there has been no attempt to look systematically across this group, and the quality and detail of previous reports varies significantly. Details of previous reports are contained in Table S1.

No rectal complex was observed in adults of related superfamilies Staphylinoidea or Hydrophiloidea (Dufour, 1824). These are considered the most closely related superfamilies to Scarabaeoidea (McKenna et al., 2019; Fig. S1). Rectal complexes also appear to be lacking in several families and subfamilies within Scarabaeoidea (Fig. 1). This includes in Lucanidae, the stag beetles, which is a basal family (Ahrens et al., 2014, Fig. 1). In Lucanidae a rectal complex is reportedly absent in adults of the European stag beetle, *Lucanus cervus* (Dufour, 1824; Saini, 1964), and larvae and adults of the lesser stag beetle, *Dorcus parallelipipedus* (Dufour, 1842; Edwards, 1930; Saini, 1964).

A rectal complex is also reportedly absent in adults of Passalidae (Baker, 1968), Geotrupidae (Saini, 1964), Scarabaeinae (Dufour, 1842; Verma, 1969), and Aphodiinae (Verma, 1969), relatively basal groups within Scarabaeoidea (Ahrens et al., 2014, Fig. 1). A rectal complex has been reported in larvae in Glaphyridae (Areekul, 1957), although in this case the evidence remains particularly weak. This is another relatively basal family (Fig. 1). Together these observations suggest that a rectal complex was likely absent in the last common ancestor of the Scarabaeoidea + Staphylinoidea + Hydrophiloidea clade.

Melolontha melolontha (tribe Melolonthini) falls within the Pleurosticti lineage (Fig. 1). Within this lineage there is a complex picture of rectal complexes being reportedly present or absent in different species and life stages (Dufour, 1824; Dufour, 1842; Gérard, 1942; Sirodot, 1858; Werner, 1926; Fletcher, 1930; Swingle, 1930; Jones, 1940; Schäfer, 1954; Areekul, 1957; Menees, 1958; Berberet and Helms, 1972; Bayon, 1981; Sheehan et al., 1982; Nagae et al., 2013; Wang et al., 2024; Table S1). Reports include members

of the subfamily Cetoniinae where a rectal complex appears absent in adult *Cetonia aurata* (Dufour, 1824, 1842) but present in larval *Protaetia cuprea* (Werner, 1926). A putative evolutionary distribution of rectal complexes is shown in Fig. 1. Note that a more recent phylogeny based on transcriptomic data gave somewhat different relationships, for example placing Glaphyridae in a more basal position (Dietz et al., 2023). This distribution may be explained by multiple gains and/or losses of rectal complexes in Scarabaeoidea, although the limited sampling and variable quality of previous reporting adds to the uncertainty. A comprehensive understanding of the evolutionary distribution of rectal complexes, and their precise structure and functional role(s), remain unknown in Scarabaeoidea. Our study aimed to shed light on these questions.

2. Materials and methods

Throughout the text, taxonomic classification is based upon (Bouchard et al., 2011), including the Cucujiformia series/infra-order, which following the phylogenetic relationships proposed by McKenna et al. (2019), is taken to include the superfamilies Cucujoidea, Tenebrionoidea, Coccinelloidea, Cleroidea, Chrysomeloidea and Curculionoidea. Phylogenetic relationships are based on the findings of McKenna et al. (2019), Ahrens et al. (2014) and Dietz et al. (2023).

The insects studied in this research were sourced from wild populations, reared, or purchased, as detailed in Table 1.

In the case of *C. aurata* and *Pachnoda marginata*, larvae were kept in leaf mould with added rotten wood, both from deciduous trees, in plastic boxes with drilled airholes, at 25 °C. The substrate was kept moist by spraying with water every few days. Adults were kept in the same conditions and fed with chopped apple and banana.

Live beetles were decapitated with scissors, and where possible the central nervous system removed with forceps. They were then dissected in phosphate buffered saline (PBS) and imaged on a Leica S9i microscope with integrated camera. Fixation of the dissected *M. melolontha* gut and MpTs was in 4 % formaldehyde in PBS at 4 °C overnight, before washing in PBT + BSA (PBS + 0.3 % Triton X-100 + 0.5 % bovine serum albumin). Staining was with anti-α-Tubulin (mouse, 1:20, AA4.3, DSHB) for 4 h at room temperature, washing in PBT + BSA followed by staining with anti-mouse-488 (1:200, Jackson ImmunoResearch), phalloidin-568 (1:200, A12380, Molecular Probes) and DAPI (1:1000, Molecular Probes) for 5 h at room temperature. The sample was placed on a coverslip, covered in mounting media (85 % glycerol, 2.5 % propyl gallate), and imaged on a Nikon A1R confocal microscope.

3. Results

3.1. Rectal complexes are not observed in Staphylinoidea and basal Scarabaeoidea

To revisit the reported absence of rectal complexes within the superfamily Staphylinoidea (Dufour, 1824), the sister group to Scarabaeoidea (McKenna et al., 2019; Fig. S1), we firstly dissected final instar larvae of the burying beetle *Nicrophorus vespilloides*. We found that a looped midgut sits ventral to the hindgut (Fig. 2A). The MpTs are highly elaborated and lie alongside the hindgut, but are not applied to its surface (Fig. 2B and C). This supports the lack of a rectal complex in Staphylinoidea, and is the first observation we are aware of in the larval stage. The MpTs insert laterally into either side of the gut (Fig. 2D). For this species, and for all species subsequently reported, the point of insertion is assumed to be the junction of the midgut and hindgut, which is consistently the case across insects. Two MpTs were observed (Fig. 2C and D, Table 2). In Coleoptera the number of MpTs generally ranges from four to eight
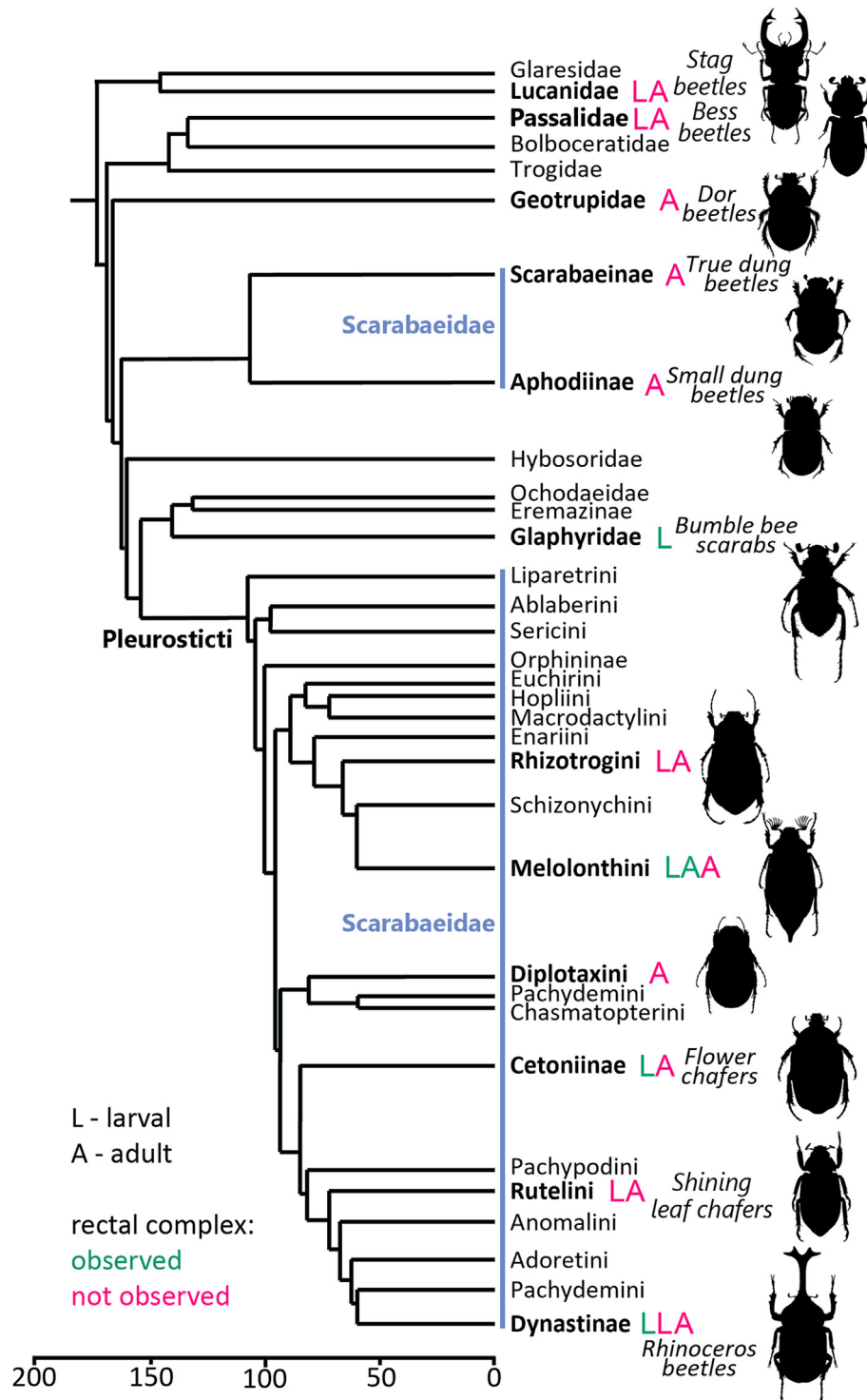
Fig. 1. Phylogenetic tree of the beetle superfamily Scarabaeoidea, with previous reports of occurrence of rectal complex. The tree is drawn according to Ahrens et al. (2014), and represents an ~180 million year evolutionary period. Reports of CNC or rectal complex presence (green) or absence (magenta) are indicated, along with the life stage reported (L − larval stage, A − adult stage). Note that subfamilies/tribes belonging to Scarabaeidae are indicated, which have more recently been recovered as monophyletic, with Scarabaeinae and Aphodiinae being the most basal subfamilies (Dietz et al., 2023). Also note that parts of Pachydemini appear in two different branches. Scale bar shows estimated evolutionary time in millions of years.

(Beutel and Haas, 2000). Staphylinoidea have mostly been considered to have four (Lawrence and Newton, 1982), although members of the tribe Nanosellini were reported to have only two. This was speculated to represent a reduced number due to miniaturisation of body size in this group (Polilov, 2008), but this question should be revisited in light of our observation in this larger species (*N. vespilloides*).

We also revisited the reported absence of a rectal complex in Lucanidae, a basal family within Scarabaeoidea (Fig. 1). In final (3rd) instar larvae of *Platycerus caraboides* we found caeca in three

Table 1
Specimens analysed in study.

family or tribe	species	stage	source	number of individuals analyses
Staphylinoidea				
Silphidae	*Nicrophorus vespilloides*	larval	laboratory bred, from wild Lincoln (UK) population	2
Scarabaeoidea				
Lucanidae	*Platycerus caraboides*	larval	Vicenza province, Italy (Scaccini, 2022; Scaccini et al., 2024)	2
	Dorcus parallelipipedus	adult	Colchester, UK (Scaccini et al., 2023)	1
Melolonthini	*M. melolontha*	larval	Colchester, UK, direct collection	1
		adult	Suffolk, UK, direct collection	1
Cetoniinae	*Pachnoda marginata*	larval	Livefood UK Ltd	5
		adult		2
	Cetonia aurata	larval	Colchester, UK (Fremlin, 2018)	1
		adult		2

regions of the gut (Fig. 3A and B). There are some caeca like extensions at the anterior end of the midgut (Fig. 3B). There is also a central row of caeca within the midgut, and posterior caeca within the hindgut (Fig. 3A and B). MpTs wrap around the ventral caeca from the posterior row (Fig. 3C). There are four MpTs, with two inserting into the gut on the dorsal side (Fig. 3D), and two on the ventral side, which was revealed when the MpT regions enwrapping the caeca were teased away (Fig. 3E). Highly elaborated MpTs coat the surface of part of the midgut on the dorsal side (Fig. 3F). The most anterior part of the hindgut is wide, and is followed posteriorly by a narrow ileum. Posterior to this is a dilated colon (Fig. 3A). This is likely to be a smaller equivalent of the fermentation chamber which exists in larvae of Scarabaeidae (Chapman, 1998). MpTs are associated with the ileum and colon, although this does not appear to represent a rectal complex, as MpTs are only loosely associated and are not extensively elaborated (Fig. 3G–Table 2). The observed morphology looks broadly comparable to that reported for larval *D. parallelipipedus* by Edwards (in which the posterior caeca are referred to as diverticulum of the ileum) (Edwards, 1930).

We also dissected an adult female lesser stag beetle (*D. parallelipipedus*), to compare with previous reports. We observed elaborated MpTs loosely surrounding the midgut and hindgut (Fig. 4A and B). Four MpTs insert into the gut (Fig. 4C). There is only a very loose association between the MpTs and the rectum (Fig. 4B–Table 2). Together with our findings from *P. caraboides*, this supports previous suggestions that a rectal complex is not present in species within Lucanidae. The absence of a rectal complex in Staphylinoidea, as well as in Lucanidae (which is a basal family of Scarabaeoidea), support the view that the rectal complex of Scarabaeoidea has an independent evolutionary origin from the CNC of Cucujiformia + Bostrichoidea (see Fig. 1 and Fig. S1).

3.2. The rectal complex of Melolontha melolontha likely forms during pupal metamorphosis

Although a rectal complex has been reported in adults of the cockchafer *M. melolontha* (Lison, 1938; Saini, 1964), the gut and MpT morphology had not been reported in the larval stage, which we therefore decided to analyse. In a final (3rd) instar larva, we observed two morphologically distinct rows of caeca in the midgut (Fig. 5A–C). The hindgut is folded into an S-shape. There is a very
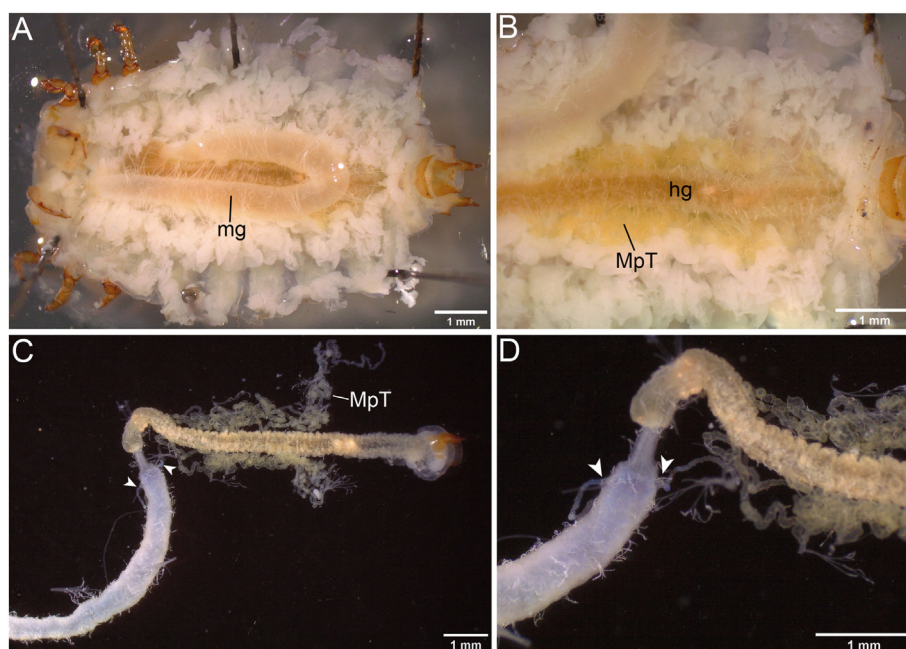

Fig. 2. Gut and Malpighian tubules of final instar larva of the burying beetle, *Nicrophorus vespilloides*. In all images anterior is to the left. **(A)** Ventral view in which the looped midgut (mg) can be seen lying on top of the hindgut. **(B)** Ventral view in which the midgut has been lifted to reveal the hindgut (hg), and the mass of MpTs (MpT) lying on either side of it. **(C)** Gut removed from body, revealing two MpTs lying on either side of the hindgut, with insertion points into the gut indicated (arrowheads). **(D)** An enlarged view with MpT insertion into the gut marked (arrowheads).

Table 2
Summary of gut and Malpighian tubule features observed in this study.

species		*Nicrophorus vespilloides*	*Platycerus caraboides*	*Dorcus parallelipipedus*	*Melolontha melolontha*		*Pachnoda marginata*		*Cetonia aurata*	
stage		L	L	A	L	A	L	A	L	A
number of MpTs		2	4	4	4	4	4	4	4	4
association of MpTs with:	**mg**	–	elaborated, on mg dorsal surface	close to mg	elaborated, on + close to mg	branched MpTs on ant. mg surface	elaborated, on ventral/lateral mg surface	elaborated, close to ant. mg	on dorsal/lateral mg surface	small amount close to mg
	ant. hg	elaborated, close to ant. hg	encircling caeca	close to ant. hg	–	branched MpTs on + wide MpTs close to ant. hg	–	–	–	elaborated, close to ant. hg
	re./post. hg	**elaborated, close to re.**	**some on co. surface**	**close to re.**	**some on FC re. surface**	**sinuous, bound to post. section of hg**	**sinuous, on re. surface (encircles re.)**	**elaborated, close to re.**	**sinuous, on re. surface (encircles re.)**	**elaborated, close to re.**
MpT regions		uniform	uniform	clear + yellow	uniform	clear (narrow) + white (wide) + yellow (branched)	uniform	white + clear/yellow	uniform	white + clear/yellow
rows of caeca:	**mg**	0	2	0	2	0	3	0	3	0
	hg	0	1	0	0	0	0	0	0	0
hg morphology:	**ant.**	narrow il., smooth surface	narrow il.	hg with variable diameter, sub-divisions unclear	narrow il.	narrower il.	narrow il.	narrower il.	narrow il.	narrower il.
	cent.	long wider section,	dilated co.		FC	post. hg with variable diameter (both ends wider), sub-divisions unclear	FC	dark wide region	FC	post. hg with variable diameter (both ends wider), sub-divisions unclear
	re./post.	irregular surface	narrow re.		2 lobed re.		narrow re., post. enclosed by FC	narrower re. getting wider at posterior	narrow re., post. enclosed by FC	

Abbreviations: L – larval, A – adult, MpT – Malpighian tubule, mg – midgut, hg – hindgut, re. – rectum, co. – colon, FC – fermentation chamber, il. – ileum, ant. – anterior, cent. – central, post. – posterior.

narrow ileum (i.e. the anterior portion of the hindgut), which opens into a huge fermentation chamber (Fig. 5C). The fermentation chamber is folded back to lie dorsally upon the ileum (Fig. 5A and B). As reported for other beetles from Scarabaeidae (Kucuk et al., 2023; Sheehan et al., 1982), the bases of papillae can be seen on the surface of the fermentation chamber (Fig. 5A and B). The rectum can be observed as a narrow section of the most posterior hindgut, forming two lobes, and it runs over the surface of the fermentation chamber to the anus (Fig. 5A and B). There are elaborated MpTs over some of the surface of the midgut (Fig. 5D). There are also some elaborated MpTs sandwiched between the rectum and fermentation chamber, revealed when lifting the rectum away from the fermentation chamber (Fig. 5E–Table 2); however, this does not appear to represent a rectal complex, as there is a relatively small amount of MpT, and this has very limited coverage of the rectal surface.

Two MpTs insert into the gut side by side on its ventral surface, close to the posterior row of caeca (Fig. 5F). Two more MpTs insert dorso-laterally on either side of the gut (Fig. 5G). Curiously the dorsal pair lie some way from the row of caeca. It appears that all four MpTs insert at the boundary between lighter and darker gut regions, likely to be the midgut and hindgut respectively. This boundary is not straight, forming a chevron on the ventral side of the gut, meaning the row of caeca does not follow the midgut-hindgut boundary (Fig. 5F and G).

Regarding the rectal complex, our observations were unexpected, as while there are some elaborated MpTs associating closely with the hindgut (Fig. 5E), this does not resemble a rectal complex, despite the presence of a rectal complex having been reported in adults. Even though there are indications that rectal complexes may be present in larvae of other species within Melolonthini, the detail in these reports is not sufficient to make clear comparisons with our findings for *M. melolontha*, and further studies would be

required to clarify the occurrence of rectal complexes in larvae of other species of Melolonthini.

To confirm the apparent difference between larvae and adults, we dissected an adult male *M. melolontha*. This showed a dramatically remodelled gut and MpTs, when compared to the larva of the same species. Convolutions in the gut bring a section of the hindgut into close proximity to the rectum (Fig. 6A). Three morphologically distinct MpT regions were observed. There are pale yellow MpTs with a branched morphology, and some of these are sandwiched between the rectum and the loop of the hindgut (Fig. 6A). There are also unbranched MpT regions, which are either white, and of a variable and generally wider diameter, or colourless and of a generally narrower diameter (Fig. 6A–C).

We observed that both white and colourless MpTs are incorporated into a rectal complex, having elaborate sinuous courses over the surface of the rectum (Fig. 6A–C). Furthermore, much of the gut has branched MpTs running along its length, intimately associated with its surface. This includes both midgut (Fig. 6D) and hindgut (Fig. 6E) regions. The branched MpTs have short regularly spaced branches along their length, which often arranged into opposite pairs (Fig. 6D and E). There are four MpTs which insert separately into the gut (Fig. 6F). For an overview of the gut and associated MpT organisation, see also Fig. S2 and Table 2.

To gain a more detailed view of the rectal complex of adult *M. melolontha*, and assess structural similarities and differences with the CNCs studied in Cucujiformia and + Bostrichoidea species (Saini, 1964; Grimstone et al., 1968; Beaven and Denholm, 2024), we fixed and fluorescently stained the gut and MpTs. This revealed a number of interesting features of rectal complex structure. The overall organisation is as follows. Bands of circular muscle encircle the epithelial layer of the rectum (Fig. 7A and B). Overlaying this are the sinuous wide and narrow MpT subtypes, which are both coated in nets of muscle (Fig. 7A and B). The branched MpT regions, lying
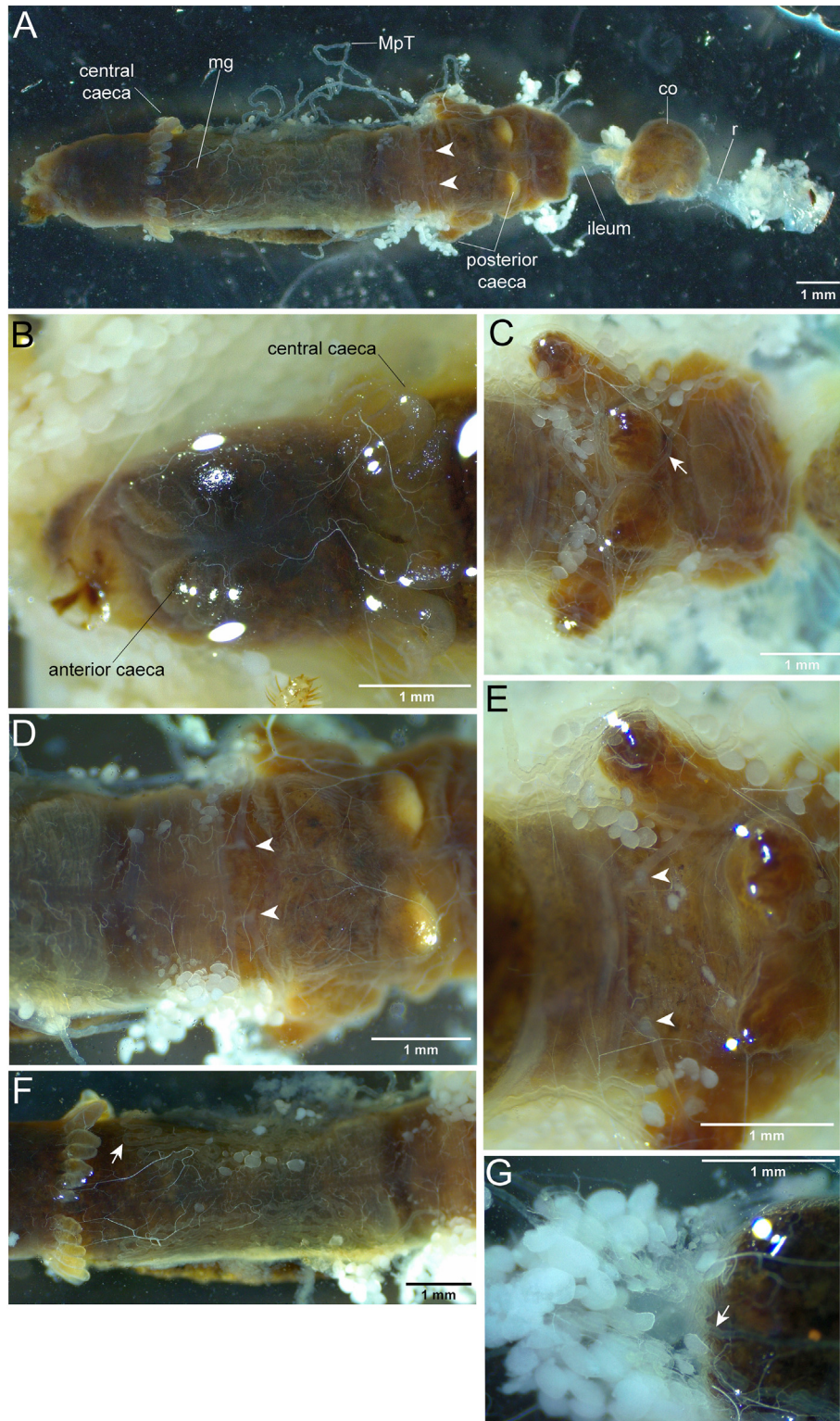
Fig. 3. Gut and Malpighian tubules of final instar larvae of a stag beetle, *Platycerus caraboides*. In all images anterior is to the left. **(A)** Dorsal view. Overview of the dissected gut with midgut (mg), ileum, colon (co), rectum (r), central and posterior rows of caeca, and insertion point of two of the MpTs (arrowheads) indicated. **(B)** Ventral view. Anterior and central caeca are indicated. **(C)** Ventral view. MpTs enwrap posterior caeca on ventral side of gut (arrow). **(D)** Dorsal view. Two MpTs insert into dorsal side of gut (arrowheads). **(E)** Ventral view. Two MpTs insert into ventral side of gut (arrowheads). **(F)** Dorsal view. Highly elaborated MpTs cover part of the dorsal midgut (arrow). **(G)** Ventral view. Elaborated MpTs (arrow) loosely associate with the ileum and colon.
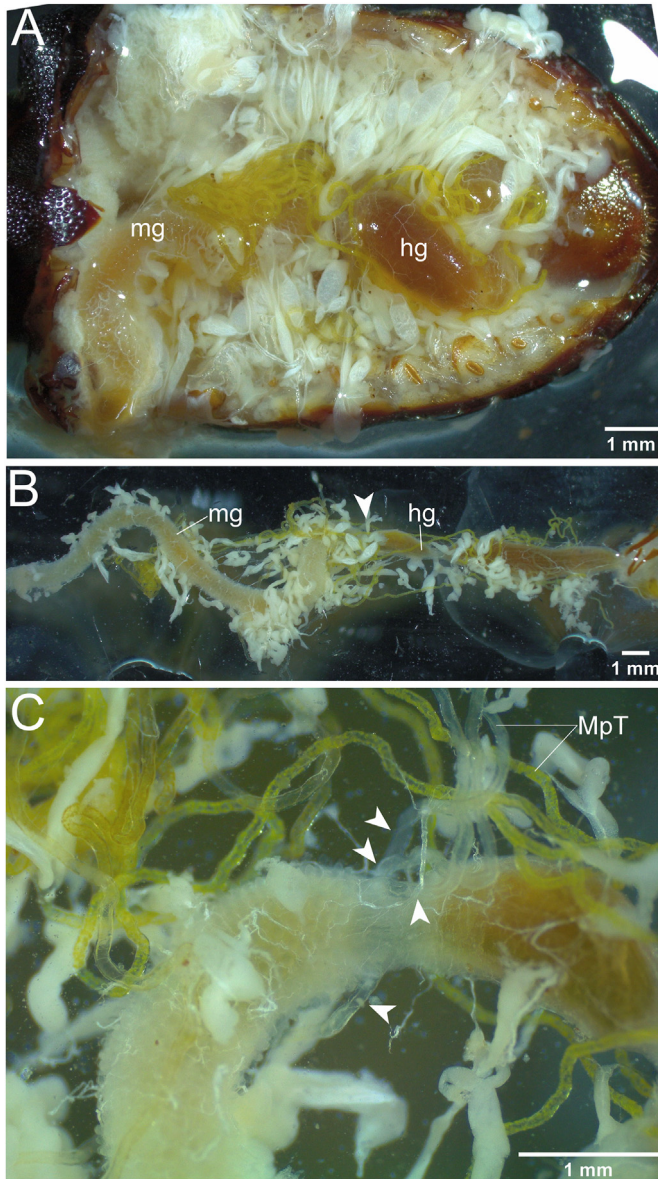
Fig. 4. Gut and Malpighian tubules of an adult lesser stag beetle, *Dorcus parallelipipedus*. In all images anterior is to the left. **(A)** Dorsal view indicating position in the body cavity of midgut (mg) and hindgut (hg). **(B)** Dissected gut and associated MpTs (which have yellow and transparent regions), with midgut and hindgut indicated as in A, along with the position of the midgut-hindgut boundary (arrowhead). **(C)** Close up view of the midgut-hindgut boundary, with the four MpTs indicated (arrowheads) close to their point of entry into the gut.

outside the rectal complex, are also netted by muscle cells (Fig. 7C). There appear to be a number of other cell types associated with the rectal complex. There is a cellular layer surrounding it (Fig. 7A and B), which was just discernible as an extremely thin transparent membrane, in the unstained preparation. This does not appear to form a continuous sheet coating the complex (Fig. 7A and B), and would therefore not be expected to act as an insulating barrier. Trachea could be seen extending into the membrane (Fig. 7B), and the membrane and trachea may be part of a common tissue. Additionally other muscle cells were noted, which form connections with the thin MpTs and with the membrane tissue (Fig. 7A and B).

3.3. A rectal complex is integrated into the hindgut fermentation complex of Cetoniinae larvae, but is lost by the adult stage

We then investigated the morphology of the gut and MpTs in Cetoniinae, another Scarabaeoidea subfamily, for which there were previous reports of a rectal complex in larvae but not adults (Dufour, 1824, 1842; Werner, 1926). Firstly we investigated intestinal morphology in the rose chafer, *C. aurata*. In a final (3rd) instar larva we observed that there are three rows of caeca within the midgut (Fig. 8A and B). Much like for *M. melolontha* larvae, the hindgut has an S-shaped course, with the rectum running ventrally over the surface of the fermentation chamber (Fig. 8A and B). However, unlike *M. melolontha*, the most posterior part of the rectum is enclosed by infoldings of the fermentation chamber from either side (Fig. 8B). A membranous tissue appears to overlay the seam made by the folded fermentation chamber. By pulling apart each side of the folded fermentation chamber, and lifting up the rectum, a remarkable structure was revealed. There are four rows of sinuous, tightly packed MpTs, which had entirely surrounded the rectum, and had been sandwiched between the rectum and fermentation chamber (Figs. 8C and 9A). These four MpTs enter the structure as two pairs, with a pair on either side of the rectum at its anterior end (Fig. 8C).

There also appears to be a transparent membrane structure; however, this is sandwiched between the MpTs and the rectum, unlike the membrane seen in *M. melolontha* which resides outside the MpTs, as does the perinephric membrane seen in species of Tenebrionidae. As in the *M. melolontha* larva, the anterior hindgut is a narrow ileum (Fig. 8D and E). The shape of the midgut-hindgut boundary, its position relative to the posterior row of caeca, and the insertion points of the four MpTs, also seems very similar between these species (Fig. 8D−F). There are also some MpTs which associate with the midgut surface (Fig. 8E, Table 2).

Dissecting adult *C. aurata* revealed dramatic remodelling of the gut and MpTs between larval and adult stages, as seen for *M. melolontha*. There are distinct MpT regions, with most being clear or pale yellow, along with a second type which are white (Fig. 10A−C). Some MpT regions are free within the body cavity, whilst others are loosely associated with the gut, particularly a central portion of the hindgut (Fig. 10A−F). Whilst there is clearly association of MpT regions with the rectum, these do not have the regular sinuous course, or tight association typical of a rectal complex (Fig. 10C and D, Table 2). The insertion points of four MpTs are as a pair close together on the ventral gut surface, and another pair on either side of the gut (Fig. 10G).

Finally, we investigated the gut and MpT morphology in *P. marginata*, which is another member of the subtribe Cetoniina, within the Cetoniinae subfamily. Dissecting final (3rd) instar larvae revealed a broadly similar morphology to *C. aurata* (Fig. 11A−F). The morphology at the adult stage also looked very similar between the two species (Fig. 12A−G), although in *P. marginata* there is a more extensive association of MpTs with the anterior part of the midgut (Fig. 12A, Table 2). These findings show that an unusual rectal complex, integrated into the fermentation chamber, is more widespread in the subtribe Cetoniina. Investigation of further groups within and beyond the subfamily Cetoniinae will be required to reveal how widespread and variable this structure is.

4. Discussion

4.1. The independent evolution of a rectal complex in Scarabaeoidea

In arthropods, digestive and renal organs form part of a single integrated system, which underpins acquisition of nutrients, water and ion homeostasis, and the removal of nitrogenous waste. They
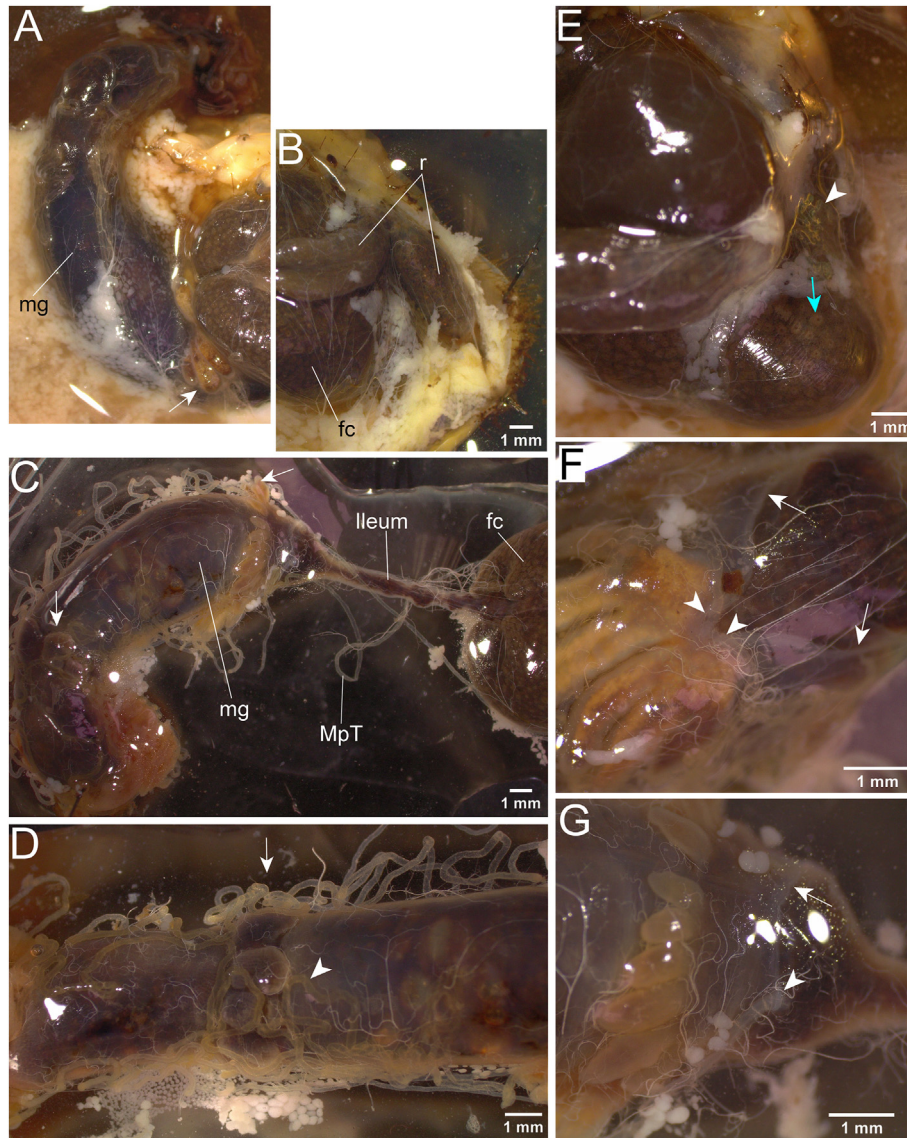
Fig. 5. Gut and Malpighian tubules of a final instar larva of the cockchafer, *Melolontha melolontha*. In all images anterior is to the left. **(A,B)** Panels together forming an overview of the gut. Ventral view with midgut (mg), hindgut fermentation chamber (fc) and two lobes of rectum (r) indicated, along with the posterior row of caeca (white arrow) which is partially beneath the fermentation chamber. **(C)** Dorsal view in which the hindgut has been unfolded, with the midgut (mg), ileum, fermentation chamber (fc) and two rows of caeca (white arrows) indicated. The posterior row of caeca are close to the midgut-hindgut junction. **(D)** Dorsal view of a midgut portion, showing the anterior row of caeca (arrow), and convoluted MpTs associated with the midgut (arrowhead). **(E)** Ventral view with most posterior lobe of rectum lifted, revealing a region of elaborated MpTs (arrowhead) which had been sandwiched between the rectum and the fermentation chamber. The bases of papillae dapple the fermentation chamber surface (blue arrow). **(F)** Ventral view showing junction of two MpTs with the gut (arrowheads), and the boundary between the lighter midgut and darker hindgut (arrows). **(G)** Dorso-lateral view showing the insertion into the gut of one of the lateral pair of MpTs (arrowhead) and the midgut-hindgut boundary (arrow).

play a central role in survival, and have been modified at molecular as well as morphological levels during evolution, enabling shifts in diet and habitat. They have therefore played critical roles in the remarkable success of many arthropod groups.

The cryptonephridial or rectal complexes are examples of the evolutionary reorganisation of arthropod digestive/renal organs, and act as powerful recycling systems of water or solutes (Kolosov and O'Donnell, 2019; Beaven et al., 2023; Beaven and Denholm, 2024). The importance of rectal complexes is indicated by the multiple times they have evolved, and their prevalence, existing in several hundred thousand species, mainly within Lepidoptera and Coleoptera (Beaven and Denholm, 2024). From assessing the phylogenetic distribution of rectal complexes observed in this and previous studies, it seems likely that a rectal complex evolved

independently within Scarabaeoidea, possibly more than once. Rectal complexes are restricted to part of the Scarabaeoidea tree, and are seemingly absent in more basal families, and in the related superfamily Staphylinoidea. Furthermore the structural differences we see in the rectal complexes of Scarabaeoidea, compared to those of Cucujiformia + Bostrichoidea, are also consistent with an independent evolutionary origin(s). These include incorporation of a rectal complex into the fermentation chamber in larval Cetoniinae, and the nature of the membrane tissue surrounding the rectal complex in adult *M. melolontha*. Within Scarabaeoidea, rectal complexes are seemingly more variable for example comparing *M. melolontha* (Melolonthinae) with Cetoniinae species, and plastic through insect life stages, than the rectal or cryptonephridial complexes found in Cucujiformia + Bostrichoidea (Fig. 9). Rectal
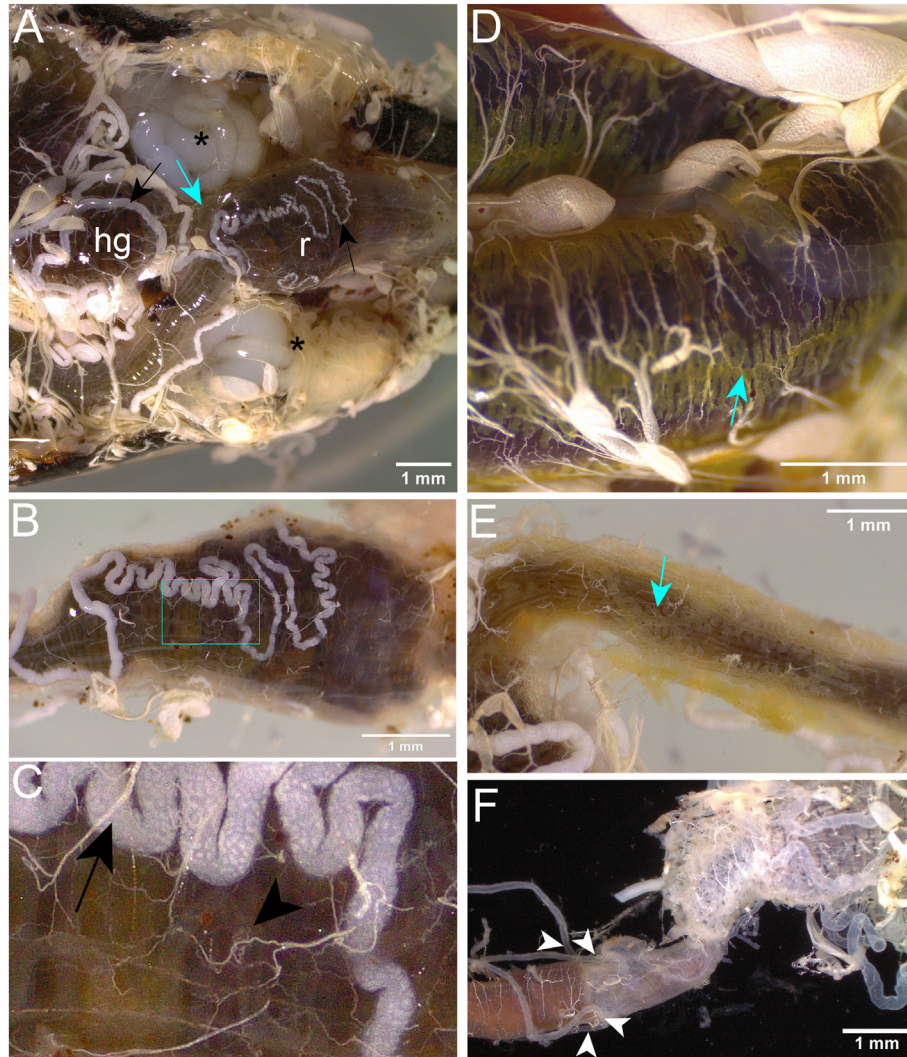
Fig. 6. Gut and Malpighian tubules of an adult male cockchafer, *Melolontha melolontha*. All images are dorsal view, with anterior to the left. **(A)** Part of the hindgut (hg) is looped back into close proximity with the rectum (r) and highly branched MpTs are packed between the two (blue arrow). There are also white regions of MpT (black arrows). Part of male reproductive organs (*) are also indicated. **(B)** The rectal complex. **(C)** Enlarged region corresponding to blue boxed rectangle in B. A thick white MpT (black arrow) and a narrower clear MpT (black arrowhead) are indicated. **(D)** Anterior section of midgut, along the surface of which run branched MpTs (blue arrow). **(E)** Central region of hindgut, with associated branched MpTs (blue arrow). **(F)** The four MpTs indicated (white arrowheads) close to their insertion points into the gut. This image is from fixed tissue, which has altered its colouration.

complexes in the Scarabaeoidea superfamily are therefore fertile ground for understanding the evolution and functions of these organ systems.

4.2. Structure and remodelling of Scarabaeoidea rectal complexes and MpTs

Dramatic remodelling of the gut during pupal metamorphosis has been documented in *Oryctes rhinoceros* (subfamily Dynastinae), as well as *Thaumastopeus shangaicus* and *Protaetia* species (subfamily Cetoniinae), which are all members of the Scarabaeidae family. It has been proposed that this is an adaptation to the radically different diets of larvae and adults (Chiang and Shelomi, 2023). Our observations reveal that there are also dramatic reorganisations of the MpTs and their relationship to the gut in Scarabaeoidea. In the case of *M. melolontha*, the rectal complex is likely to form during pupal metamorphosis. This is very different from the situation in tenebrionid beetles, where the complex is formed during embryonic development and appears to maintain a largely

unchanged morphology between larval and adult stages (Ramsay, 1964; King and Denholm, 2014). The rectal complexes that we found in beetles from Cetoniinae are present in the larvae, and our preliminary findings suggest they form during embryonic development (Beaven unp. obs.), as for the CNCs of tenebrionid beetles.

In larval Cetoniinae, we observed that sinuous MpTs coat the surface of the rectum, and are themselves enveloped within a fold in the hindgut fermentation chamber. It could therefore be considered that they from a unique structure; a hindgut fermentation chamber complex. The posterior rectum being enclosed by infoldings of the fermentation chamber has also been noted in larval *Protaetia* species and *T. shangaicus* (Cetoniinae) (Chiang and Shelomi, 2023), and in the case of *P. cuprea*, sinuous MpTs surrounding the rectum have been described (Werner, 1926), consistent with our findings. The morphology and arrangement of the MpTs around the rectum appear strikingly similar to those of the CNC of tenebrionid beetles (Ramsay, 1964; Grimstone et al., 1968; King and Denholm, 2014; Naseem et al., 2023), whilst its enclosure in the fermentation chamber is very different from what has been
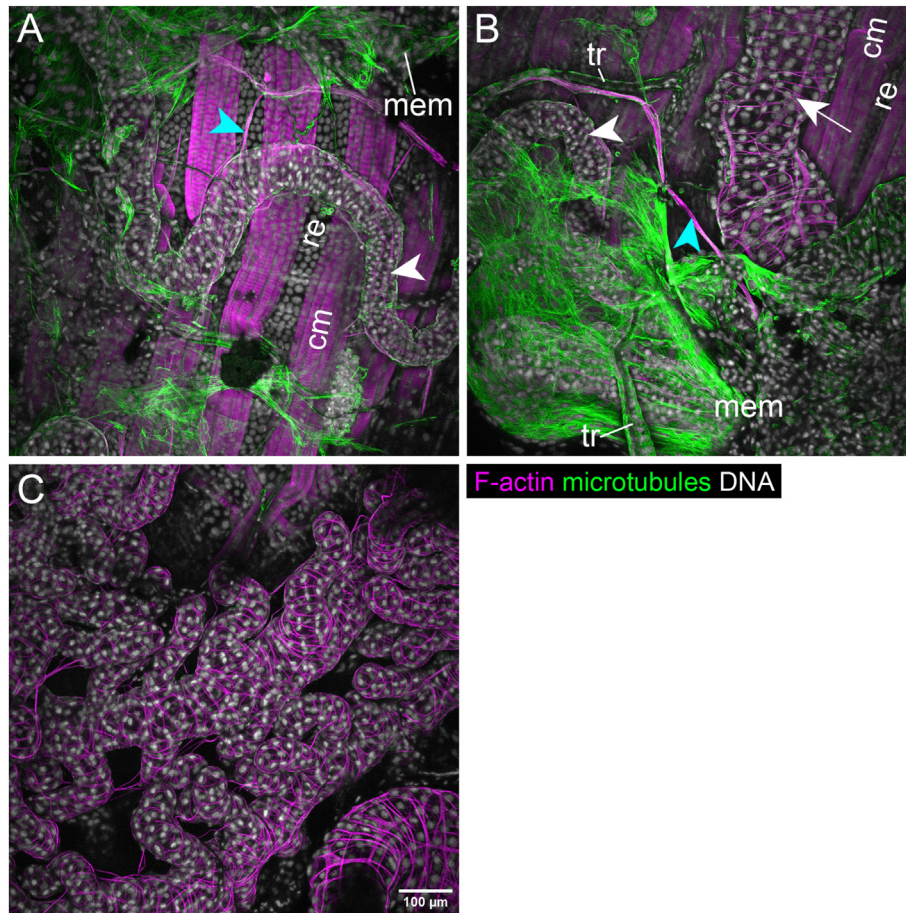
Fig. 7. Fluorescent images of the rectal complex and branched MpTs from an adult male cockchafer, *Melolontha melolontha*. F-actin (phalloidin stain, magenta), microtubules (anti-alpha-Tubulin, green), and nuclei/DNA (DAPI stain, white). **(A)** The rectal epithelium (re) is encircled in bands of circular muscle (cm) around the circumference of the rectum. F-actin enrichment makes the muscle clearly visible. The thin MpTs (white arrowhead) are coated in a mesh of muscle, and have a sinuous course over the rectum. The complex is enwrapped by a cellular membrane layer (mem) although this does not appear to be a continuous sheet. This cellular layer is rich in microtubules. There are also more muscles (blue arrowhead), that form connections with the membrane tissue and the thin MpTs. **(B)** Labelled as in A, but also showing a region of a thick MpT (white arrow), and parts of the tracheal network (tr) which appear to connect to, or be part of, the membrane tissue. **(C)** Region showing the branched MpTs, netted in muscle.

observed in tenebrionids.

We also find evidence that this rectal/hindgut fermentation chamber complex is dramatically remodelled, being lost by adulthood. This is akin to the developmental remodelling of rectal complexes in Lepidoptera, occurring during pupal metamorphosis (Ito, 1921; Ishimori, 1924; Judy, 1968 Barbehenn and Kristensen, 2003), which is considered to reflect the different diets of larvae compared to adults, as well as the rapid growth during larval life which would make maintaining water and ion balance more challenging (Kolosov and O'Donnell, 2019; Beaven and Denholm, 2024). There also appears to be significant remodelling of the MpTs in Lucanidae, for example elaborated MpTs cover much of the midgut surface in *D. parallelipipedus* and *P. caraboides* larvae, but are not tightly associated with the midgut in adult *D. parallelipipedus* (Edwards, 1930; Figs. 3 and 4).

In the rectal complex of adult *M. melolontha* the MpTs are surrounded by a membrane tissue, as has been previously reported (Lison, 1938; Saini, 1964). The perinephric membrane has been characterised in most detail in *Tenebrio molitor* (Lison, 1937; Grimstone et al., 1968), and *T. castaneum* (Naseem et al., 2023; Beaven et al., in prep). In these species the perinephric membrane entirely coats the CNC, except for small windows overlying specialised MpT cells (the leptophragmata) where it is reduced to

blisters of extracellular material. In contrast, our observations suggest that the rectal complex membrane of *M. melolontha* is far from a continuous sheet, and may serve a structural role, rather than insulating the complex from the haemolymph as in tenebrionids, where it is considered to prevent the aberrant movement of water and ions (Ramsay, 1964). These structural differences indicate functional differences of the rectal complex of *M. melolontha*, compared to the CNCs of tenebrionid beetle species, and also provides a further indication of their independent evolution. Furthermore, the differences we see in the rectal complexes of adult *M. melolontha*, compared to Cetoniinae larvae, point towards rectal complexes potentially having evolved more than once within Scarabaeoidea. Future analysis of other groups should help clarify rectal complex evolutionary distribution and variability.

In adult *M. melolontha*, we also saw evidence of major remodelling of the MpT regions not associated with the rectum, particularly in the occurrence of a branched form of MpT, which was previously described by Lison (1938). These changes to MpT morphology between larvae and adults have also been reported in a related species, *Phyllophaga anxia*. In this species the larval MpTs are morphologically uniform but in the adult transform to a morphology with three distinct regions, including a branched region referred to as diverticular (Berberet and Helms, 1972). The role
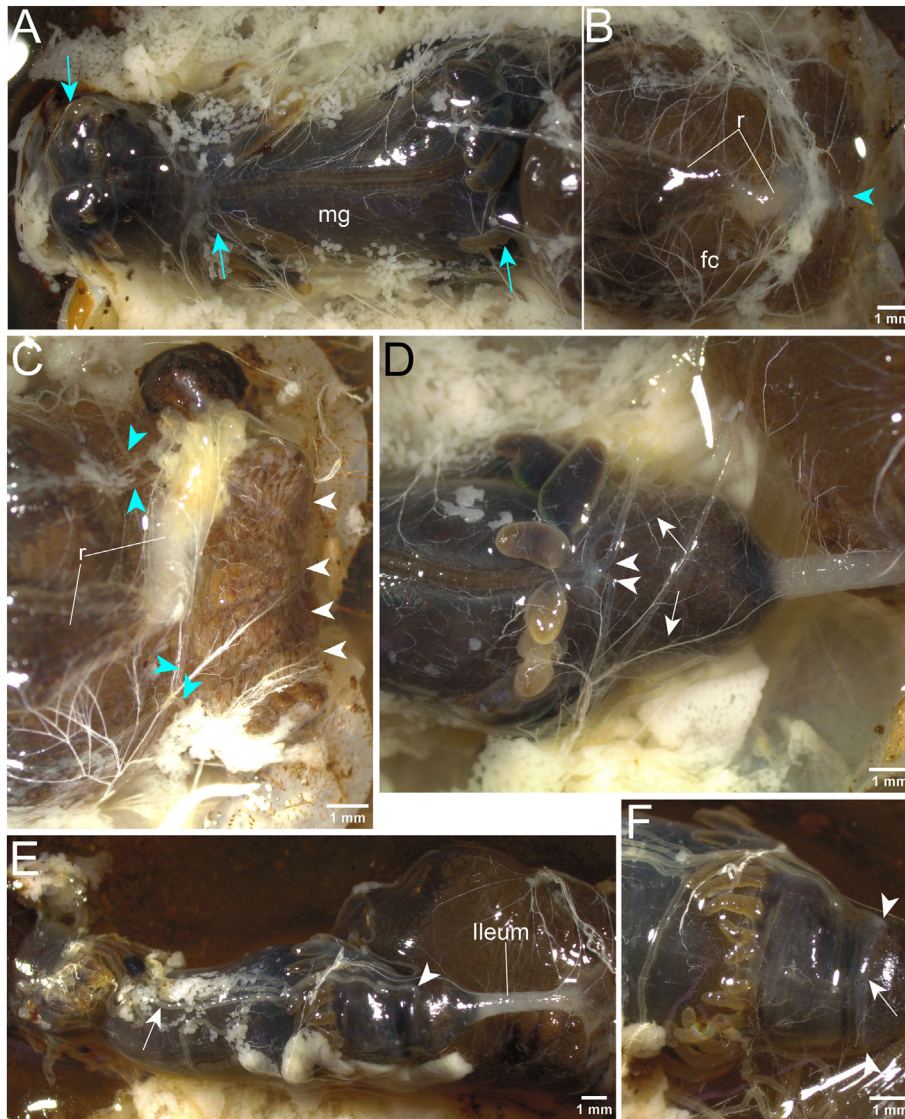
Fig. 8. Gut and Malpighian tubules of a third instar larva of the rose chafer, *Cetonia aurata*. In all images anterior is to the left. **(A,B)** Panels together forming an overview of the gut. Ventral view, showing three rows of caeca (blue arrows) in the midgut (mg), as well as the fermentation chamber (fc), rectum (r) and the seam between the two infoldings of the fermentation chamber (blue arrowhead). **(C)** Ventral view. Upon opening the fold in the fermentation chamber and pulling aside part of the rectum (r), the sinuous course of four MpTs (white arrowheads) can be seen. A pair of MpTs enter on either side of the rectum (blue arrowheads). **(D)** Ventral view. Moving aside the fermentation chamber reveals the insertion points of two of the MpTs (white arrowheads), and the midgut-hindgut boundary (white arrows). **(E)** Dorsal view, showing ileum, one of the laterally inserted pair of MpTs (arrowhead) and a MpT associated with the midgut (arrow). **(F)** Dorsal view, showing insertion points of lateral pair of MpTs (arrowheads), at the midgut-hindgut boundary (arrow).

of this curious morphology is unclear. The branching would dramatically increase the surface area of the tubule available for molecular transport. These regions tend to associate with the midgut and hindgut surface, and branching could be to maximise coverage of the gut surface. The MpT branches are reminiscent of the diverticula or regenerative crypts seen in the midguts of some beetles such as adult *Tribolium*. These harbour stem cells which replenish the epithelium (Sinha, 1958; Ameen and Rahman, 1973; Snodgrass, 1994; Parthasarathy and Palli, 2008). Similar structures are seen in diverse arthropod groups including arachnids (Lipovšek et al., 2014, 2018, 2022). Bugs of the superfamilies Coreoidea and Lygaeoidea also have structures termed caeca or crypts at the posterior end of their midguts, which appear to provide microenvironments to harbour symbiotic bacteria (Glasgow, 1914; Itoh et al., 2019; Takeshita et al., 2015; Takeshita and Kikuchi, 2020). Branches from the main MpT axis, observed in adult *M. melolontha*,

may also create distinct microenvironments, potentially harbouring stem cells or symbiotic bacteria.

4.3. Putative functions of Scarabaeoidea rectal complexes

In Tenebrionoidea the CNC appears to primarily function in water conservation (Beaven and Denholm, 2024). There is currently little evidence for whether rectal complexes fulfil a similar role in Scarabaeoidea. Within Lucanidae, which lack rectal complexes, there are indications of a requirement for very specific microhabitat conditions, including relatively high humidity (Scaccini, 2016, 2022). Further investigation will be required to determine the relative ability of groups with and without rectal complexes to retain water, and to tolerate periods of lower humidity. The rectum is an important site of water reabsorption, even in insect groups which lack rectal complexes (Wigglesworth, 1932, 1974; Ramsay,
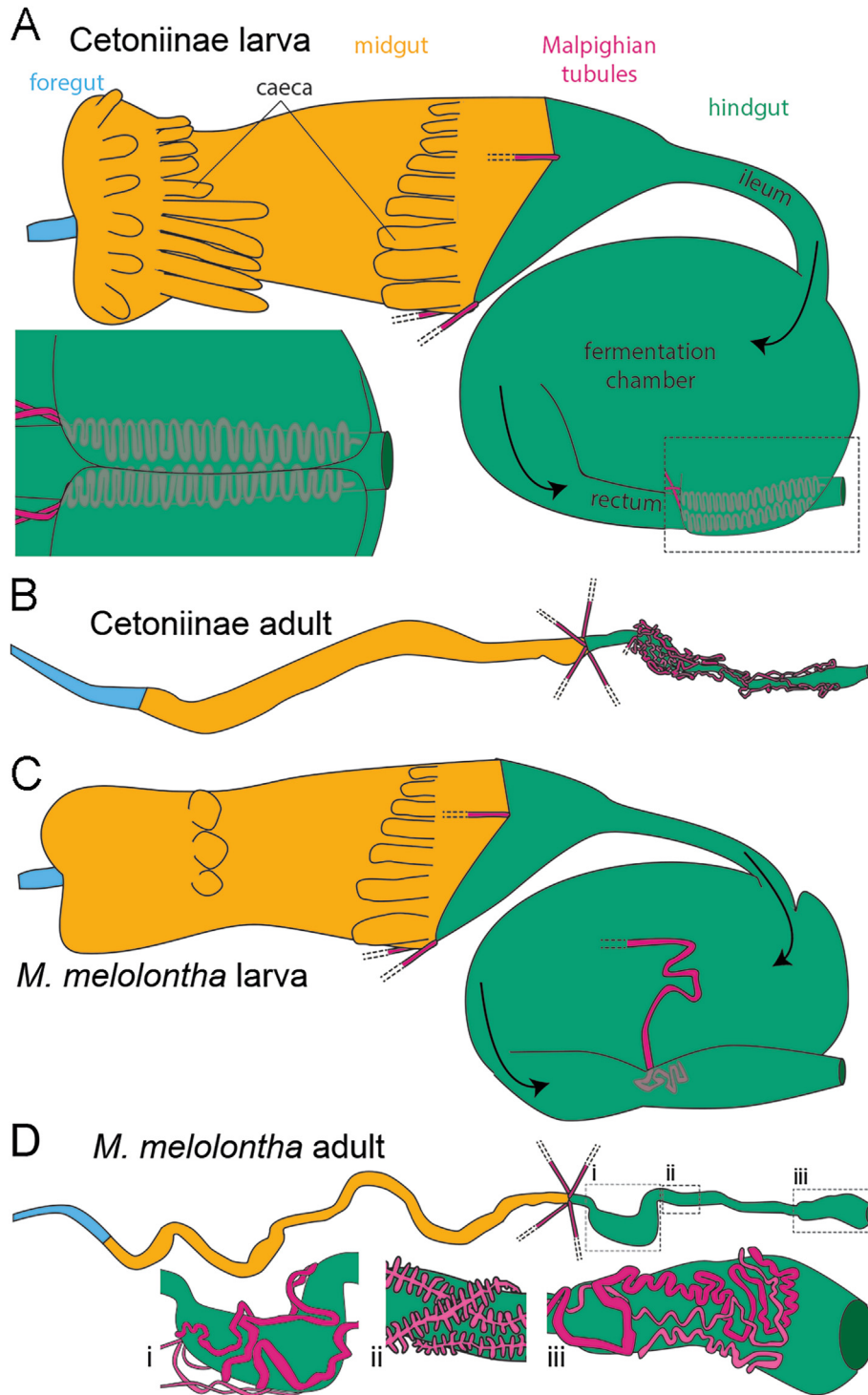
Fig. 9. Approximate morphology of the gut and Malpighian tubules observed in larvae and adults of Cetoniinae species and *Melolontha melolontha*. **(A)** Lateral view, with insertion points of MpTs shown, as well as MpTs surrounding rectum. Arrows indicate the direction of flow of the gut contents. Enlarged region is a ventral view, corresponding to the boxed area. **(B)** Cetoniinae adult, with insertion points of MpTs, and MpTs associated with hindgut, shown. **(C)** *M. melolontha* larva, with MpT insertion points, and association with fermentation chamber and rectum, shown. **(D)** *M. melolontha* adult with MpT insertion points shown. Lower panels (i-iii) illustrate association of MpTs with regions of the hindgut, corresponding to boxed areas. All images are coloured according to labels in A.

1950, 1955; Phillips, 1964; Phillips et al., 1987). In larvae of some Scarabaeoidea species, the rectal contents can be expelled as a startle reflex, and such contents can be very wet and unformed. This is the case in species of Cetoniinae (*Cremastocheilus armatus* and *P. marginata*) as well as Lucanidae (*Platycerus* species,

D. parallelipipedus and *L. cervus*) (Puker et al., 2015; our observations). These findings support the notion that drying of the gut contents would normally occur in the recta of larvae both with and without rectal complexes, and it remains to be clarified whether a rectal complex enhances this ability.
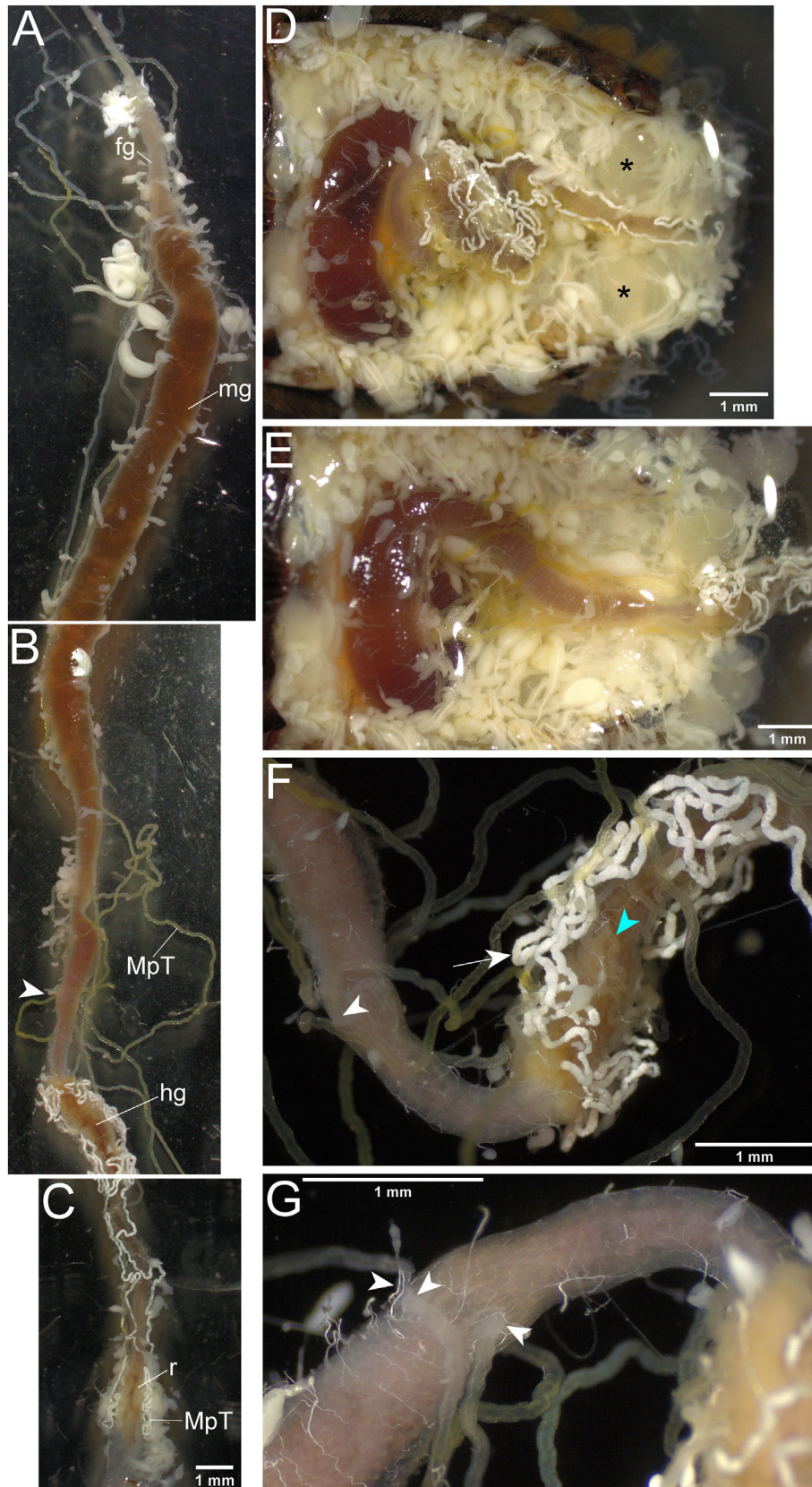
Fig. 10. Gut and Malpighian tubules of an adult rose chafer, *Cetonia aurata*. (A–C) Dorsal views, anterior at the top, with foregut (fg), midgut (mg), hindgut (hg) and rectum (r) indicated. **(D)** Dorsal view to show position of gut within the abdomen. Part of male reproductive organs are indicated (*). **(E)** Dorsal view in which the hindgut has been unfolded to reveal the ileum. **(F)** Dorsal view, showing a region of the hindgut with elaborated, loosely associated MpTs. These are of a white subtype (white arrow) or transparent/yellow subtype (blue arrowhead). The insertion of one of the MpTs is indicated (white arrowhead). **(G)** Ventro-lateral view, showing a pair of MpTs which insert close together on the ventral side of the gut, and one of the laterally inserted pair of MpTs (arrowheads). Anterior is to the left in D-G.
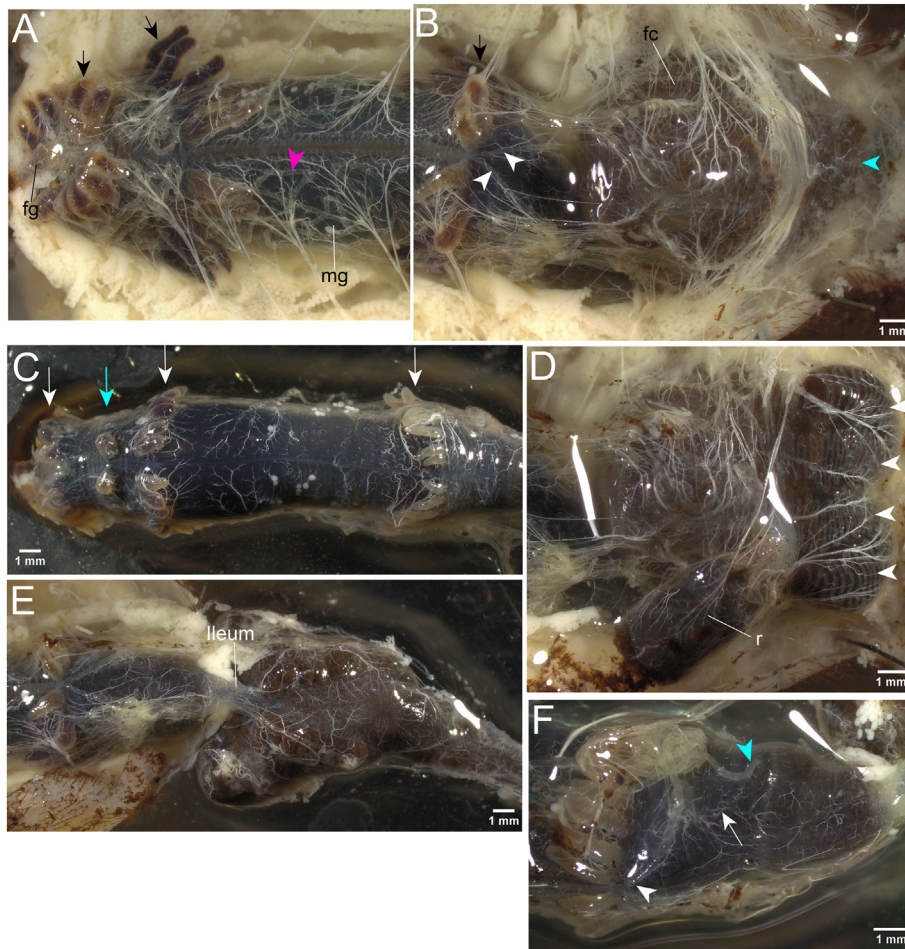
Fig. 11. Gut and Malpighian tubules from third instar larvae of the sun beetle, *Pachnoda marginata*. In all images anterior is to the left. **(A,B)** Panels together forming an overview of the gut. Ventral view, showing foregut (fg), three rows of caeca (black arrows) in the midgut (mg), convoluted MpTs on the midgut surface (magenta arrowhead), the fermentation chamber (fc), and the seam between the two infoldings of the fermentation chamber (blue arrowhead). **(C)** Dorsal view of midgut with three rows of caeca indicated (white arrows) along with an additional pair of caeca between the anterior and central rows (blue arrow). **(D)** Ventral view. Upon pulling apart the folded sides of the fermentation chamber, and lifting back the rectum (r), four sinuous MpTs are revealed (arrowheads). **(E)** Unfolding the hindgut reveals the narrow ileum which had been obscured by the fermentation chamber. **(F)** Ventro-lateral view. Showing the insertion point of one of the laterally inserted pair of MpTs (blue arrowhead), one of the pair of MpTs which insert together on the ventral side of the gut (white arrowhead) and the midgut-hindgut boundary (white arrow).

A second possible function of rectal complexes in Scarabaeoidea relates to the availability of dietary nitrogen. For some termite species which have a diet very low in protein, there is evidence they utilise hindgut bacteria for recycling of nitrogen from excreted uric acid, and fixation of N_2 (Benemann, 1973; Breznak et al., 1973; Potrikus and Breznak, 1981; Ohkuma et al., 1996, 1999; Yamada et al., 2007; Desai and Brune, 2012). There is also evidence that the midgut of larval Lepidoptera can absorb ammonia, enabling rapid growth on a low protein diet (Hirayama et al., 1996; Weihrauch, 2006; Blaesse et al., 2010).

Scarabaeoid beetles face similar challenges in accessing nitrogen, and nitrogen fixation has been reported including in adult *Odontotaenius disjunctus* (Passalidae) (Ceja-Navarro et al., 2014), and larval *Dorcus rectus* and *Ceruchus piceus* (Lucanidae) (Kuranouchi et al., 2006; Mifsud et al., 2023), although for lucanids it remains contested whether this contributes to their nitrogen budgets (Tanahashi et al., 2018; Mifsud et al., 2023; our unpublished results). Interestingly the gut microbiome of *O. rhinoceros*, a beetle from Scarabaeoidea that feeds on rotting wood, is reported to be similar to that of wood feeding termites (Shelomi et al., 2019).

As MpTs in Diptera and Lepidoptera can transport nitrogenous molecules, including ammonia (Browne and O'Donnell, 2013; Weihrauch and O'Donnell, 2021), it is conceivable that their association with the rectum, for example in larvae of Cetoniinae species, functions to transport nitrogenous molecules from the gut contents into the body. Such a role could also suggest why a rectal complex has been incorporated into the larval hindgut fermentation chamber. This is the major site of symbiotic bacteria, i.e. the presumptive site of nitrogen fixation or conversion of nitrogenous waste, in larvae of Scarabaeidae species (Zheng et al., 2012; Ebert et al., 2021), including *P. marginata* (Vidal-Verdú et al., 2024).

A third possibility is that the rectal complexes of Scarabaeoidea function in recycling bases from the gut contents. There is considerable variation in the reported midgut content pH in Scarabaeoidea species (Table 3; Huang et al., 2010), with some being very alkaline, for example larvae of *Oryctes nasicornis* (Dynastinae) at pH 12.2 (Table 3). In this case the alkalinity has been proposed to function in precellulolysis, or chemically modifying cellulose to render it accessible to subsequent enzymatic breakdown in the hindgut (Bayon, 1980). In larval *Pachnoda ephippiata* (Cetoniinae) which have alkaline midgut contents (pH 10.7, Table 3), there is evidence that macromolecules, particularly humic acid bound
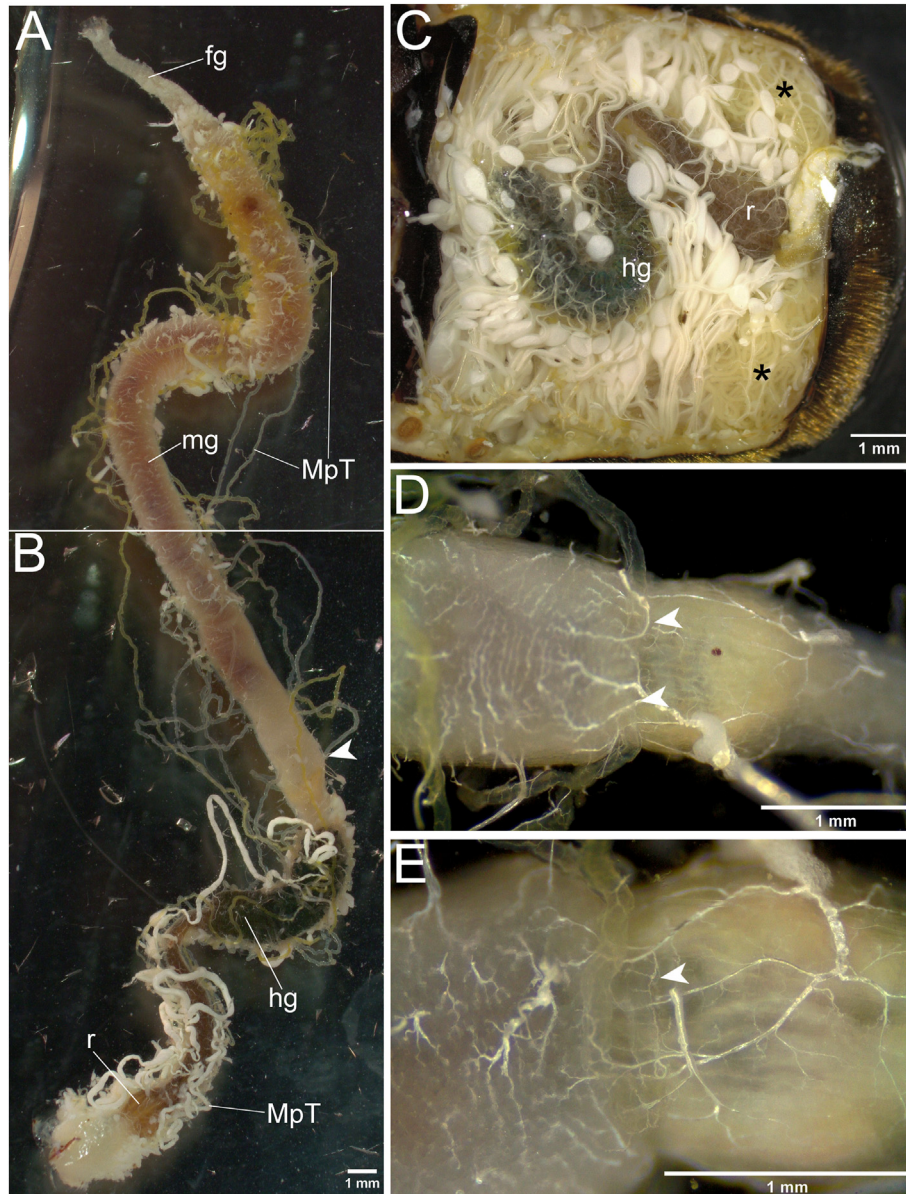
Fig. 12. Gut and Malpighian tubules from adult sun beetles, *Pachnoda marginata*. (A,B) Dorsal view with anterior at the top, showing foregut (fg), midgut (mg), hindgut (hg), rectum (r), Malpighian tubules (MpTs) and midgut-hindgut boundary (arrow). **(C)** Showing position of gut in the body cavity, labelled as in B, and part of the male reproductive organs indicated (*). **(D)** A pair of MpTs insert into the gut in close proximity (arrowheads). **(E)** One of a pair of MpTs which insert laterally into the gut. Anterior is to the left in C-E.

peptides and polysaccharides, are rendered soluble in the midgut. This enables their depolymerisation by enzymes (Li and Brune, 2005a; Li and Brune, 2005b; Li and Brune, 2007). A highly alkaline midgut environment is also thought to enable the enzymatic activity of certain proteinases which can release amino acids from soil humic acid, as suggested by studies in this species (Zhang and Brune, 2004). High midgut pH may also be an adaptation to dietary tannins, although the possible effects of tannins on insects, such as inhibiting feeding, toxic effects or inhibiting digestion, is a long contested issue (Feeny, 1968; Bernays, 1978; Berenbaum, 1980; Forkner et al., 2004; Barbehenn and Constabel, 2011).

In Lepidoptera, high midgut pH is generated by transport of potassium and bicarbonate ions into the lumen (Dow, 1992), and their CNCs are considered to reabsorb these ions downstream in the ileum and rectum. This would allow the larvae to maintain their acid-base balance (Ramsay, 1976; Moffett, 1994; Kolosov and

O'Donnell, 2019; Kolosov and O'Donnell, 2020).

A function of recycling ions also points to a possible explanation for why a rectal complex is present in Scarabaeoidea in some species and life stages, and absent in others. From the current reports existing for Scarabaeoidea, the most alkaline midguts are from larvae with a rectal complex, whilst larval *M. melolontha* (which we find to lack a rectal complex) has a less alkaline midgut (Table 3). This may relate to dietary properties, such as how resistant their food is to digestion, or how rich it is in tannin.

Lucanidae larvae may also be adapted to digest highly resistant materials such as rotting wood, and also have a relatively high midgut pH of ~10.3 (Table 3). They are likely to have evolved alternative strategies to efficiently recover bases from their gut contents, or to maintain homeostasis of other ions and water, or access dietary nitrogen. This could include re-ingestion of their own faeces (Hendriks and Fremlin, 2017; Tanahashi et al., 2018),

Table 3
Diet, midgut pH and presence of rectal complex reported for members of the superfamily Scarabaeoidea. For midgut pH column, value is represented by colour, from green (mildly alkaline) to purple (highly alkaline). For rectal complex presence column, green indicates presence and magenta, absence. Ordered as in the phylogenetic tree in Fig. 1. (Bayon, 1980, 1981; Biggs and McGregor, 1996; Cazemier et al., 1997; Ceja-Navarro et al., 2014; Egert et al., 2005; Gérard, 1942; Lemke et al., 2003; Mishima and Araya, 2016; Sirodot, 1858; Soo Hoo and Dudzinski, 1967; Tanahashi and Hawes, 2016; Tanahashi and Kubota, 2013; Wada et al., 2014; Wang et al., 2024).

species	stage	diet	reported midgut pH	rectal complex presence
Dorcus rectus (Lucanidae)	larva	rotting wood and associated fungi (Tanahashi and Kubota, 2013)	**10.2–10.4** (Tanahashi and Hawes, 2016) or **9.9** (Mishima and Araya, 2016)	unlikely
Odontotaenius disjunctus (Passalidae)	adult	rotting wood	**8.38** (Ceja-Navarro et al., 2014)	unlikely
Sericesthis geminata. (Scitalini, part of Liparetrini in Fig. 1)	larva	roots	**9.0** (Soo Hoo and Dudzinski, 1967)	?
Costelytra zealandica (Liparetrini)	larva	roots	**10.8** (Biggs and McGregor, 1996)	?
Melolontha melolontha (Melolonthini)	larva	roots	**8.2** (Egert et al., 2005)	no (this study)
Pachnoda marginata (Cetoniinae)	larva	rotting leaves	**11.7** (Cazemier et al., 1997)	yes (this study)
Pachnoda ephippiata (Cetoniinae)	larva	rotting leaves	**10.7** (Lemke et al., 2003)	likely
Oryctes nasicornis (Dynastinae)	larva	rotting wood	**12.2** (Bayon, 1980)	yes (Bayon, 1981; Gérard, 1942; Sirodot, 1858)
Trypoxylus dichotomus (Dynastinae)	larva	rotting wood and leaves	**10.7** (Wada et al., 2014)	no (Wang et al., 2024)

which Cetoniinae larvae do not appear to do (Tanahashi and Fremlin, 2013), and potentially reliance on fungi growing on dead wood as an important dietary component (Tanahashi et al., 2009; Tanahashi and Kubota, 2013; Mishima and Araya, 2016; Fukasawa, 2021; Scaccini, 2022).

4.4. Evolutionary and ecological implications of the rectal complexes of Scarabaeoidea

Adoption of phytophagy (eating leaves) by insects is considered an evolutionary hurdle (Southwood, 1972; Strong et al., 1984; Mitter et al., 1988), that once surmounted has enabled major species radiations. This is particularly the case for external leaf eaters, which being highly exposed, may require greater resistance to desiccation (Southwood, 1972). Although rectal complexes or CNCs have been found in insect species with very diverse diets, it is noteworthy that many of the major evolutionary radiations of phytophagous insects appear to have occurred in groups with rectal complexes/CNCs. These include Phytophaga in the Cucujiformia infraorder of beetles (Dufour, 1824; Saini, 1964; Crowson, 1981; Farrell, 1998), and within Lepidoptera (Powell et al., 1998; Barbehenn and Kristensen, 2003). There has also been a significant radiation of phytophagous beetles in the Pleurosticti lineage within Scarabaeoidea (Ahrens et al., 2014; Fig. 1). More extensive sampling of digestive/renal system anatomies could inform whether rectal complexes are likely to have contributed to the adoption of phytophagy within Scarabaeoidea.

Scarabaeoidea is a very large beetle superfamily, comprising ~35,000 species (Hunt et al., 2007). These beetles play critical roles in many ecosystems, for example in the breakdown of resistant plant materials, such as dung and wood, rendering their components accessible in the soil (Stokland, 2012; Ulyshen, 2018; Stanbrook et al., 2021). These functions must rely on adaptations of their digestive/renal systems. The ability of Scarabaeoidea species to digest highly resistant material such as lignocellulose is central to this, and relies on the symbiotic bacteria and yeasts within their guts (Vargas-Asensio et al., 2014; Mishima and Araya, 2016; Tanahashi et al., 2017; Shelomi et al., 2019; Ebert et al., 2021). For this reason Protaetia brevitarsis, a member of Cetoniinae that is farmed as a food source in East Asia, is being investigated for its ability to convert organic waste into fertiliser (Li et al., 2019; Wang et al., 2022). There is evidence from P. ephippiata (Cetoniinae) that their fresh faeces are rich in ammonia, which is a useable nitrogen source for plants (Li and Brune, 2007). Whilst microbial activity and the availability of useable nutrients is high in fresh faecal pellets of several Cetoniinae species, this may be relatively short lived; the older pellets are highly resistant to further degradation, and may then fulfil other ecosystem services such as sequestration of carbon (Jönsson et al., 2004; Li and Brune, 2007; Micó et al., 2011; Sánchez et al., 2017; Kulikova and Perminova, 2021). The faeces of Cetoniinae larvae can remain undegraded for many years (Pawlowski, 1961; Martin, 1997; Ranius and Nilsson, 1997; Jönsson et al., 2004; our observations). There is also evidence that Scarabaeoidea larvae can act as ecosystem engineers, for example the presence of Cetonia aurataeformis appears to benefit the larvae of hover fly species, also living on rotting wood (Sánchez-Galván et al., 2014). Shedding light on the rectal complexes found in Scarabaeoidea therefore has important implications for understanding the ecological roles played by its members.

5. Conclusion

By studying gut and MpT morphology in Scarabaeoidea and Staphylinoidea, we have found evidence supporting the independent evolution of a rectal complex within Scarabaeoidea, rather than sharing an evolutionary origin with the CNCs present in Cucujiformia + Bostrichoidea. We have also uncovered evidence that rectal complexes of Scarabaeoidea can be present during larval life but absent in the adult stage, or vice versa, which presumably reflect differences in diet or habit. Finally, we investigated the structure of the rectal complex of adult M. melolontha in greater detail, and found that its enveloping membrane is unlikely to be an insulating tissue, like the perinephric membrane of tenebrionid beetles. Studies with more extensive sampling of gut and MpT morphologies, from species across the Scarabaeoidea tree, could shed further light on when a rectal complex arose in this superfamily, and the likely pattern of its gain(s) and/or secondary losses. Relating such an understanding to the diets and habitats of species, as well as physiological insights including gut pH, could provide a clearer picture of the adaptive roles played by rectal complexes in Scarabaeoidea. Our study could also pave the way for testing rectal complex function in this superfamily, using a combination of molecular and physiological approaches.

CRediT authorship contribution statement

Robin Beaven: Writing − original draft, Visualization, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Barry Denholm:** Writing − review & editing, Funding acquisition. **Maria Fremlin:** Writing − review & editing, Resources, Conceptualization. **Davide Scaccini:** Writing − review & editing, Visualization, Resources.

Declaration of competing interest

We have nothing to declare.

Abbreviations

MpT   Malpighian tubule
CNC   cryptonephridial complex

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.asd.2024.101406.

References

Ahrens, D., Schwarzer, J., Vogler, A.P., 2014. The evolution of scarab beetles tracks the sequential rise of angiosperms and mammals. Proc. Biol. Sci. 281.
Ameen, M., Rahman, M.F., 1973. Larval and adult digestive tracts of Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Int. J. Insect Morphol. Embryol. 2, 137−152.

Arab, A., Caetano, F.H., 2002. Segmental specializations in the Malpighian tubules of the fire ant *Solenopsis saevissima* Forel 1904 (Myrmicinae): an electron microscopical study. Arthropod Struct. Dev. 30, 281−292.

Areekul, S., 1957. The Comparative Internal Larval Anatomy of Several Genera of Scarabaeidae (Coleoptera), vol. 50. Annals of the Entomological Society of America, pp. 562−577.

Baker, W.V., 1968. The gross structure and histology of the adult and larval gut of *Pentalobus barbatus* (Coleoptera: Passalidae). Can. Entomol. 100, 1080−1090.

Barbehenn, R.V., Constabel, C.P., 2011. Tannins in plant-herbivore interactions. Phytochemistry 72, 1551−1565.

Barbehenn, R.V., Kristensen, N.P., 2003. 6. Digestive and excretory systems. In: Kükenthal, W. (Ed.), Teilband/Part 36 Vol 2: Morphology, Physiology, and Development. De Gruyter, Berlin, pp. 165−188.

Bayon, C., 1980. Volatile fatty acids and methane production in relation to anaerobic carbohydrate fermentation in *Oryctes nasicornis* larvae (Coleoptera: Scarabaeidae). J. Insect Physiol. 26, 819−828.

Bayon, C., 1981. Ultrastructure de l'epithelium intestinal et flore parietale chez la larve xylophage d'*Oryctes nasicornis* L. (Coleoptera : Scrabaeidae). Int. J. Insect Morphol. Embryol. 10, 359−371.

Beaven, R., Denholm, B., 2024. The cryptonephridial/rectal complex: an evolutionary adaptation for water and ion conservation. Biol. Rev. 99. https://doi.org/10.1111/brv.13156.

Beaven, R., Halberg, K.V., Denholm, B., 2023. The insect cryptonephridial complex. Curr. Biol. 33, R748−R749.

Beaven, R., Koyama, T., Naseem, M.T., Halberg, K.V., Denholm, B., 2024. Something old, something new: the origins of an unusual renal cell underpinning a beetle water-conserving mechanism. Development 151, dev202994.

Benemann, J.R., 1973. Nitrogen fixation in termites. Science 181, 164−165.

Berberet, R.C., Helms, T.J., 1972. Comparative Anatomy and Histology of Selected Systems in Larval and Adult *Phyllophaga anxia* (Coleoptera: Scarabaeidae), vol. 65. Annals of the Entomological Society of America, pp. 1026−1053.

Berenbaum, M., 1980. Adaptive Significance of Midgut pH in Larval Lepidoptera. Am. Nat. 115, 138−146.

Bernays, E.A., 1978. Tannins: An Alternative Viewpoint. Entomol. Exp. Appl. 24, 244−253.

Beutel, R.G., Haas, F., 2000. Phylogenetic Relationships of the Suborders of Coleoptera (Insecta). Cladistics 16, 103−141.

Biggs, D.R., McGregor, P.G., 1996. Gut pH and amylase and protease activity in larvae of the New Zealand grass grub (*Costelytra zealandica*; Coleoptera: Scarabaeidae) as a basis for selecting inhibitors. Insect Biochem. Mol. Biol. 26, 69−75.

Blaesse, A.K., Broehan, G., Meyer, H., Merzendorfer, H., Weihrauch, D., 2010. Ammonia uptake in *Manduca sexta* midgut is mediated by an amiloride sensitive cation/proton exchanger: Transport studies and mRNA expression analysis of NHE7, 9, NHE8, and V-ATPase (subunit D). Comparative biochemistry and physiology. Part A, Molec. Integrat. Physiol. 157, 364−376.

Bouchard, P., Bousquet, Y., Davies, A.E., Alonso-Zarazaga, M.A., Lawrence, J.F., Lyal, C.H., Newton, A.F., Reid, C.A., Schmitt, M., Slipiński, S.A., et al., 2011. Family-group names in Coleoptera (Insecta). ZooKeys 88, 1−972.

Breznak, J.A., Brill, W.J., Mertins, J.W., Coppel, H.C., 1973. Nitrogen fixation in termites. Nature 244, 577−580.

Browne, A., O'Donnell, M.J., 2013. Ammonium secretion by Malpighian tubules of *Drosophila melanogaster*: application of a novel ammonium-selective microelectrode. J. Exp. Biol. 216, 3818−3827.

Cazemier, A.E., Op den Camp, H.J., Hackstein, J.H.P., Vogels, G.D., 1997. Fibre digestion in arthropods. Comp. Biochem. Physiol. 118A, 101−109.

Ceja-Navarro, J.A., Nguyen, N.H., Karaoz, U., Gross, S.R., Herman, D.J., Andersen, G.L., Bruns, T.D., Pett-Ridge, J., Blackwell, M., Brodie, E.L., 2014. Compartmentalized microbial composition, oxygen gradients and nitrogen fixation in the gut of *Odontotaenius disjunctus*. ISME J. 8, 6−18.

Chapman, R.F., 1998. Alimentary canal, digestion and absorption. In: Chapman, R.F. (Ed.), The Insects: Structure and Function. Cambridge University Press, Cambridge, pp. 38−68.

Chiang, M.R., Shelomi, M., 2023. Anatomical changes of the beetle digestive tract during metamorphosis correspond to dietary changes. J. Morphol. 284, e21575.

Coutchié, P.A., Machin, J., 1984. Allometry of water vapor absorption in two species of tenebrionid beetle larvae. Am. J. Physiol. 247, R230−R236.

Crowson, R.A., 1981. Chapter 18 - Herbivorous Beetles. In: The Biology of the Coleoptera. Academic Press, pp. 584−618.

Dallai, R., Del Bene, G., Marchini, D., 1991. The ultrastructure of Malpighian tubules and hindgut of *Frankliniella occidentalis* (Pergande) (Thysanoptera : Thripidae). Int. J. Insect Morphol. Embryol. 20, 223−233.

Denholm, B., 2013. Shaping up for action: the path to physiological maturation in the renal tubules of *Drosophila*. Organogenesis 9, 40−54.

Desai, M.S., Brune, A., 2012. Bacteroidales ectosymbionts of gut flagellates shape the nitrogen-fixing community in dry-wood termites. ISME J. 6, 1302−1313.

Dietz, L., Seidel, M., Eberle, J., Misof, B., Pacheco, T.L., Podsiadlowski, L., Ranasinghe, S., Gunter, N.L., Niehuis, O., Mayer, C., et al., 2023. A transcriptome-based phylogeny of Scarabaeoidea confirms the sister group relationship of dung beetles and phytophagous pleurostict scarabs (Coleoptera). Syst. Entomol. 48, 672−686.

Dow, J.A., 1992. pH Gradients in Lepidopteran Midgut. J. Exp. Biol. 172, 355−375.

Dow, J.A., Maddrell, S.H., Gortz, A., Skaer, N.J., Brogan, S., Kaiser, K., 1994. The Malpighian tubules of *Drosophila melanogaster*: a novel phenotype for studies of fluid secretion and its control. J. Exp. Biol. 197, 421−428.

Dufour, M.L., 1824. Recherches anatomiques sur les Carabiques et sur plusieurs autres insectes Coléoptères. Ann. des Sci. Naturelles. Atlas des Tomes 1 (2), 27−42.

Dufour, M.L., 1842. Histoire comparative des métamorphoses et de l'anatomie des *Cetonia aurata* et *Dorcus parallelipipedus*. Ann. Des Sci. Naturelles 18, 162−181.

Dunbar, B.S., Winston, P.W., 1975. The site of active uptake of atmospheric water in larvae of *Tenebrio molitor*. J. Insect Physiol. 21, 495−500.

Ebert, K.M., Arnold, W.G., Ebert, P.R., Merritt, D.J., 2021. Hindgut microbiota reflects different digestive strategies in dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). Appl. Environ. Microbiol. 87, e02100−20.

Edwards, E.E., 1930. On the Morphology of the Larva of *Dorcus parallelopipedus*, L. (Coleoptera). Zool. J. Linn. Soc. 37, 93−108.

Egert, M., Stingl, U., Bruun, L.D., Pommerenke, B., Brune, A., Friedrich, M.W., 2005. Structure and topology of microbial communities in the major gut compartments of *Melolontha melolontha* larvae (Coleoptera: Scarabaeidae). Appl. Environ. Microbiol. 71, 4556−4566.

Farrell, B.D., 1998. "Inordinate Fondness" Explained: Why Are There So Many Beetles? Science 281, 555−559.

Feeny, P.P., 1968. Effect of oak leaf tannins on larval growth of the winter moth *Operophtera brumata*. J. Insect Physiol. 14, 805−817.

Fletcher, F.W., 1930. The Alimentary Canal of *Phyllophaga gracilis* Burm. Ohio J. Sci. 30, 109−119.

Forkner, R.E., Marquis, R.J., Lill, J.T., 2004. Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*. Ecol. Entomol. 29, 174−187.

Fremlin, M., 2018. The Rose Chafer C*etonia aurata* L. (Coleoptera: Scarabaeidae: Cetoniinae) in Essex: distribution and some aspects of its ecology. Essex Nat. (Lond.) 35, 167−178.

Fukasawa, Y., 2021. Ecological impacts of fungal wood decay types: A review of current knowledge and future research directions. Ecol. Res. 36, 910−931.

Glasgow, H., 1914. The Gastric Cæca and the Cæcal Bacteria of the Heteroptera. Biol. Bull. 26, 101−170.

Grimstone, A.V., Mullinger, A.M., Ramsay, J.A., 1968. Further studies on the rectal complex of mealworm *Tenebrio molitor*, L. (Coleoptera, Tenebrioidae). Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 253, 343−382.

Gérard, P., 1942. Les tubes de Malpighi de la larve d'*Oryctes nasicornis* L. Ann. Soc. R. Zool. Belg. 73, 122−133.

Hansen, L.L., Ramløv, H., Westh, P., 2004. Metabolic activity and water vapour absorption in the mealworm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae): real-time measurements by two-channel microcalorimetry. J. Exp. Biol. 207, 545−552.

Hendriks, P., Fremlin, M., 2017. How stag beetle *Lucanus cervus* larvae feed. http://maria.fremlin.de/stagbeetles/larva_feeding/index.html.

Henson, H., 1937. The structure and post-embryonic development of *Vanessa urticse* (Lepidoptera)−II. The larval Malpighian tubules. Proc. Zool. Soc. Lond. 107, 161−174.

Hirayama, C., Konno, K., Shinbo, H., 1996. Utilization of ammonia as a nitrogen source in the silkworm, *Bombyx mori*. J. Insect Physiol. 42, 983−988.

Huang, S.-W., Zhang, H.-Y., Marshall, S., Jackson, T.A., 2010. The scarab gut: A potential bioreactor for bio-fuel production. Insect Sci. 17, 175−183.

Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O.S., Wild, R., Hammond, P.M., Ahrens, D., Balke, M., Caterino, M.S., et al., 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. Science 318, 1913−1916.

Irvine, H.B., 1969. Sodium and potassium secretion by isolated insect Malpighian tubules. Am. J. Physiol. 217, 1520−1527.

Ishimori, N., 1924. Distribution of the Malpighian vessels in the wall of the rectum of lepidopterous larvae. Ann. Entomol. Soc. Am. 17, 75−86.

Ito, H., 1921. On the metamorphosis of the Malpighian tubes of *Bombyx mori* L. J. Morphol. 35, 195−211.

Itoh, H., Jang, S., Takeshita, K., Ohbayashi, T., Ohnishi, N., Meng, X., Mitani, Y., Kikuchi, Y., 2019. Host-symbiont specificity determined by microbe-microbe competition in an insect gut. Proc. Natl. Acad. Sci. U.S.A. 116, 22673−22682.

Jones, C.R., 1940. The alimentary canal of *Diplotaxis liberta* Germ. (Scarabaeidae: Coleoptera). Ohio J. Sci. 40, 94−103.

Judy, K., 1968. Studies on the Metamorphosis of the Alimentary Canal of *Hyalophora cecropia* (L.). ProQuest Dissertations Publishing.

Jönsson, N., Méndez, M., Ranius, T., 2004. Nutrient richness of wood mould in tree hollows with the Scarabaeid beetle *Osmoderma eremita*. Anim. Biodivers. Conserv. 27, 79−82.

King, B., Denholm, B., 2014. Malpighian tubule development in the red flour beetle (*Tribolium castaneum*). Arthropod Struct. Dev. 43, 605−613.

Koefoed, B., 1971. Ultrastructure of the cryptonephridial system in the meal worm *Tenebrio molitor*. Z. für Zellforsch. Mikrosk. Anat. 117, 487−501.

Kolosov, D., O'Donnell, M.J., 2019. Chapter Five - The Malpighian tubules and cryptonephric complex in lepidopteran larvae. Adv. Insect Physiol 56, 165−202.

Kolosov, D., O'Donnell, M.J., 2020. Mechanisms and regulation of chloride transport in the Malpighian tubules of the larval cabbage looper *Trichoplusia ni*. Insect Biochem. Mol. Biol. 116, 103263.

Kristensen, Niels P., 2003. Lepidoptera, Moths and Butterflies, Volume 2: Morphology, Physiology, and Development Handbook of Zoology: A Natural History of the Phyla of the Animal Kingdom Volume IV Arthropoda: Insecta, Part 36. Walter de Gruyter, Berlin, New York, p. 564.

Kucuk, R.A., Campbell, B.J., Lyon, N.J., Shelby, E.A., Caterino, M.S., 2023. Gut bacteria of adult and larval *Cotinis nitida* Linnaeus (Coleoptera: Scarabaeidae) demonstrate community differences according to respective life stage and gut region.

Front. Microbiol. 14, 1185661.

Kulikova, N.A., Perminova, I.V., 2021. Interactions between Humic Substances and Microorganisms and Their Implications for Nature-like Bioremediation Technologies. Molecules 26, 2706.

Kuranouchi, T., Nakamura, T., Shimamura, S., Kojima, H., Goka, K., Okabe, K., Mochizuki, A., 2006. Nitrogen fixation in the stag beetle, Dorcus (Macrodorcus) rectus (Motschulsky) (Col., Lucanidae). J. Appl. Entomol. 130, 471—472.

Lawrence, J.F., Newton, A.F., 1982. Evolution and Classification of Beetles. Annu. Rev. Ecol. Systemat. 13, 261—290.

Lemke, T., Stingl, U., Egert, M., Friedrich, M.W., Brune, A., 2003. Physicochemical conditions and microbial activities in the highly alkaline gut of the humus-feeding larva of Pachnoda ephippiata (Coleoptera: Scarabaeidae). Appl. Environ. Microbiol. 69, 6650—6658.

Li, X., Brune, A., 2005a. Digestion of microbial biomass, structural polysaccharides, and protein by the humivorous larva of Pachnoda ephippiata (Coleoptera: Scarabaeidae). Soil Biol. Biochem. 37, 107—116.

Li Brune, A., X., 2005b. Selective digestion of the peptide and polysaccharide components of synthetic humic acids by the humivorous larva of Pachnoda ephippiata (Coleoptera: Scarabaeidae). Soil Biol. Biochem. 37, 1476—1483.

Li Brune, A., X., 2007. Transformation and mineralization of soil organic nitrogen by the humivorous larva of Pachnoda ephippiata (Coleoptera: Scarabaeidae). Plant Soil 301, 233—244.

Li, Y., Fu, T., Geng, L., Shi, Y., Chu, H., Liu, F., Liu, C., Song, F., Zhang, J., Shu, C., 2019. Protaetia brevitarsis larvae can efficiently convert herbaceous and ligneous plant residues to humic acids. Waste Manag. 83, 79—82.

Lipovšek, S., Janžekovič, F., Novak, T., 2014. Autophagic activity in the midgut gland of the overwintering harvestmen Gyas annulatus (Phalangiidae, Opiliones). Arthropod Struct. Dev. 43, 493—500.

Lipovšek, S., Novak, T., Dariš, B., Hofer, F., Leitinger, G., Letofsky-Papst, I., 2022. Ultrastructure of spherites in the midgut diverticula and Malpighian tubules of the harvestman Amilenus aurantiacus during the winter diapause. Histochem. Cell Biol. 157, 107—118.

Lipovšek, S., Novak, T., Janžekovič, F., Brdelak, N., Leitinger, G., 2018. Changes in the midgut diverticula epithelial cells of the European cave spider, Meta menardi, under controlled winter starvation. Sci. Rep. 8, 1—13.

Lison, L., 1937. Sur la structure de la region cryptosoleniee chez les Coleopteres Tenebrio molitor L. et Dermestes lardarius L. Bullet. Classe Sci., Acad. Roy. Belgique/Mededeelingen van de Afdeeling Wetenschappen, Koninklije Belgische Acad. 23, 317—327.

Lison, L., 1938. Contribution à l'étude morphologique et histophysiologique du système malpighien de Melolontha melolontha Linn. (Coleoptera). Ann. Soc. Royal Zoologiq. Belgiq. 69, 195—233.

Machin, J., 1975. Water balance in Tenebrio molitor, L. Larvae; the effect of atmospheric water absorption. J. Comp. Physiol. 101, 121—132.

Machin, J., O'Donnell, M.J., 1991. Rectal complex ion activities and electrochemical gradients in larvae of the desert beetle, Onymacris: Comparisons with Tenebrio. J. Insect Physiol. 37, 829—838.

Marshall, A.T., Wright, A., 1972. Detection of diffusible ions in insect osmoregulatory systems by electron probe X-ray microanalysis using scanning electron microscopy and a cryoscopic technique. Micron 4, 31—45.

Martin, O., 1997. Fredede insekter i Danmark. Del 2: Biller knyttet til skov. Entomol. Meddelelser 61, 63—76.

McKenna, D.D., Shin, S., Ahrens, D., Balke, M., Beza-Beza, C., Clarke, D.J., Donath, A., Escalona, H.E., Friedrich, F., Letsch, H., et al., 2019. The evolution and genomic basis of beetle diversity. Proc. Natl. Acad. Sci. U.S.A. 116, 24729—24737.

Menees, J.H., 1958. The anatomy and histology of the larval alimentary canal of the European chafer, Amphimallon majalis Razoumowsky (Scarabaeidae). J. N. Y. Entomol. Soc. 66, 75—86.

Metalnikov, S., 1908. Recherches expérimentales sur les Chenilles de Galleria mellonella. Arch. Zool. Exp. Gen. 4, 489—588.

Micó, E., Juárez, M., Sánchez, A., Galante, E., 2011. Action of the saproxylic scarab larva Cetonia aurataeformis (Coleoptera: Scarabaeoidea: Cetoniidae) on woody substrates. J. Nat. Hist. 45, 41—42.

Mifsud, I.E.J., Akana, P.R., Bytnerowicz, T.A., Davis, S.R., Menge, D.N.L., 2023. Nitrogen fixation in the stag beetle, Ceruchus piceus (Coleoptera: Lucanidae): could insects contribute more to ecosystem nitrogen budgets than previously thought? Environ. Entomol. 52, 618—626.

Mishima, T., Araya, K., 2016. Are the larvae of stag beetles xylophagous or mycophagous? - Analysis of polysaccharide digestive enzymatic systems of the larvae of Dorcus rectus rectus. KOGANE 18, 94—100.

Mitter, C., Farrell, B., Wiegmann, B., 1988. The Phylogenetic Study of Adaptive Zones: Has Phytophagy Promoted Insect Diversification? Am. Nat. 132, 107—128.

Moffett, D.F., 1994. Recycling of K+, Acid-Base Equivalents, and Fluid between Gut and Hemolymph in Lepidopteran Larvae. Physiol. Zool. 67, 68—81.

Nagae, T., Miyake, S., Kosaki, S., Azuma, M., 2013. Identification and characterisation of a functional aquaporin water channel (Anomala cuprea DRIP) in a coleopteran insect. J. Exp. Biol. 216, 2564—2572.

Naseem, M.T., Beaven, R., Koyama, T., Naz, S., Su, S.Y., Leader, D.P., Klaerke, D.A., Calloe, K., Denholm, B., Halberg, K.V., 2023. NHA1 is a cation/proton antiporter essential for the water-conserving functions of the rectal complex in Tribolium castaneum. Proc. Natl. Acad. Sci. U.S.A. 120, e2217084120.

Noble-Nesbitt, J., 1970. Water uptake from subsaturated atmospheres: its site in insects. Nature 225, 753—754.

O'Donnell, M.J., Machin, J., 1991. Ion Activities and Electrochemical Gradients in the Mealworm Rectal Complex. J. Exp. Biol. 155, 375—402.

O'Donnell, M.J., Ruiz-Sanchez, E., 2015. The rectal complex and Malpighian tubules of the cabbage looper (Trichoplusia ni): regional variations in Na+ and K+ transport and cation reabsorption by secondary cells. J. Exp. Biol. 218, 3206.

Ohkuma, M., Noda, S., Kudo, T., 1999. Phylogenetic diversity of nitrogen fixation genes in the symbiotic microbial community in the gut of diverse termites. Appl. Environ. Microbiol. 65, 4926—4934.

Ohkuma, M., Noda, S., Usami, R., Horikoshi, K., Kudo, T., 1996. Diversity of Nitrogen Fixation Genes in the Symbiotic Intestinal Microflora of the Termite Reticulitermes speratus. Appl. Environ. Microbiol. 62, 2747—2752.

Parthasarathy, R., Palli, S.R., 2008. Proliferation and differentiation of intestinal stem cells during metamorphosis of the red flour beetle, Tribolium castaneum. Dev. Dynam. Off. Publ. Am. Asso. Anat. 237, 893—908.

Pawlowski, J., 1961. Próchnojady blazkorozne w biocenozie leśnej Polski — Lamellicornes cariophages in forest biocenosis of Poland. Ekol. Pol. Ser. A 9, 355—437.

Phillips, J.E., 1964. Rectal Absorption in the Desert Locust, Schistocerca Gregaria Forskål I. Water. J. Exp. Biol. 41, 15—38.

Phillips, J.E., Thomson, B., Hanrahan, J., Chamberlin, M., 1987. Mechanisms and Control of Reabsorption in Insect Hindgut. Adv. Insect Physiol 19, 329—422.

Polilov, A.A., 2008. Anatomy of the smallest coleoptera, featherwing beetles of the tribe nanosellini (Coleoptera, Ptiliidae), and limits of insect miniaturization. Entomol. Rev. 88, 26—33.

Potrikus, C.J., Breznak, J.A., 1981. Gut bacteria recycle uric acid nitrogen in termites: A strategy for nutrient conservation. Proc. Natl. Acad. Sci. U.S.A 78, 4601—4605.

Powell, J.A., Mitter, C., Farrell, B., 1998. 20. Evolution of Larval Food Preferences in Lepidoptera. In: Kükenthal, W. (Ed.), Teilband/Part 35 Volume 1: Evolution, Systematics, and Biogeography. De Gruyter, Berlin, Boston, pp. 403—422.

Puker, A., Rosa, C.S., Orozco, J., Solar, R.R.C., Feitosa, R.M., 2015. Insights on the association of American Cetoniinae beetles with ants. Entomol. Sci. 18, 21—30.

Ramsay, J.A., 1950. Osmotic regulation in mosquito larvae. J. Exp. Biol. 27, 145.

Ramsay, J.A., 1955. The excretory system of the stick insect, Dixippus morosus (Orthoptera, Phasmidae). J. Exp. Biol. 32, 183—199.

Ramsay, J.A., 1964. The rectal complex of the mealworm Tenebrio molitor, L. (Coleoptera, Tenebrionidae). Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 248, 279—314.

Ramsay, J.A., 1976. The rectal complex in the larvae of Lepidoptera. Philos. Trans. R. Soc. Lond. B Biol. Sci. 274, 203—226.

Ranius, T., Nilsson, S.G., 1997. Habitat of Osmoderma eremita Scop. (Coleoptera: Scarabaeidae), a beetle living in hollow trees. J. Insect Conserv. 1, 193—204.

Regier, J.C., Zwick, A., Cummings, M.P., Kawahara, A.Y., Cho, S., Weller, S., Roe, A., Baixeras, J., Brown, J.W., Parr, C., et al., 2009. Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. BMC Evol. Biol. 9.

Reynolds, S.E., Bellward, K., 1989. Water balance in Manduca sexta caterpillars: Water recycling from the rectum. J. Exp. Biol. 141, 33.

Saini, R.S., 1964. Histology and Physiology of the Cryptonephridial System of Insects, vol. 116. Transactions of the Royal Entomological Society of London, pp. 347—392.

Scaccini, D., 2016. First record of oviposition scars in two European Platycerus species: P. caprea (De Geer, 1774) and P. caraboides (Linnaeus, 1758) (Coleoptera: Lucanidae). Bull. Soc. R. Belge Entomol. 152, 142—151.

Scaccini, D., 2022. Habitat and microhabitat suitability for Italian Platycerus species (Coleoptera: Lucanidae): elevation, slope aspect and deadwood features. Scand. J. For. Res. 37, 172—181.

Scaccini, D., Bartolozzi, L., Zilioli, M., Di Giulio, A., Ruzzier, E., 2024. Distribution of the genus Platycerus Geoffroy (Coleoptera, Lucanidae) in Italy. Biodivers. Data J. 12, e127088.

Scaccini, D., Hendriks, P., Fremlin, M., 2023. Morphometric traits of Dorcus parallelipipedus (Coleoptera: Lucanidae) larvae and adults: can differences between populations be ruled out? Ann. Soc. Entomol. Fr. 59, 249—262.

Schäfer, R., 1954. Zur Kenntnis der Anatomie, Physiologie und Ökologie des Brachkäfers, Rhizotrogus aestivus Oliv. (Col. Lam.). Z. Angew. Entomol. 35, 381—424.

Sheehan, C.M., Crawford, A.M., Wigley, P.J., 1982. Anatomy and histology of the alimentary canal of the black beetle, Heteronychus arator. N. Z. J. Zool 9, 381—385.

Shelomi, M., Lin, S.S., Liu, L.Y., 2019. Transcriptome and microbiome of coconut rhinoceros beetle (Oryctes rhinoceros) larvae. BMC Genom. 20.

Sinha, R.N., 1958. The Alimentary Canal of the Adult of Tribolium castaneum Herbst (Coleoptera, Tenebrionidae). J. Kans. Entomol. Soc. 31, 118—125.

Sirodot, M.S., 1858. Recherches sur les sécrétions chez les insects. Ann. Sci. Nat. 10, 141—189.

Snodgrass, R.E., 1994. Principles of Insect Morphology. Cornell University Press, Ithaca, NY.

Soo Hoo, C.F., Dudzinski, A., 1967. Digestion by the larva of the pruinose scarab Sericesthis geminata. Entomol. Exp. Appl. 10, 7—15.

Southwood, T.R.E., 1972. The Insect/plant Relationship — an Evolutionary Perspective, vol. 6. Symposia of the Royal Entomological Society of London, pp. 3—30.

Stanbrook, R., Harris, E., Jones, M., Wheater, C.P., 2021. The effect of dung beetle size on soil nutrient mobilization in an Afrotropical forest. Insects 12, 141.

Stokland, J., 2012. Wood decomposition. In: Stokland, J., Siitonen, J., Jonsson, B. (Eds.), Biodiversity in Dead Wood. Cambridge University Press, Cambridge, pp. 10—28.

Strong, D.R., Lawton, J.H., Southwood, R., 1984. 2 - The evolution of phytophagous insects. In: Insects on Plants: Community Patterns and Mechanisms. Blackwell Scientific Publications, pp. 15—45.

Swingle, M., 1930. Anatomy and Physiology of the Digestive Tract of the Japanese

Beetle. J. Agric. Res. 41, 181.

Sánchez, A., Micó, E., Galante, E., Juárez, M., 2017. Chemical transformation of *Quercus* wood by *Cetonia* larvae (Coleoptera: Cetoniidae): An improvement of carbon and nitrogen available in saproxylic environments. Eur. J. Soil Biol. 78, 57–65.

Sánchez-Galván, I.R., Quinto, J., Micó, E., Galante, E., Marcos-García, M.A., 2014. Facilitation among saproxylic insects inhabiting tree hollows in a Mediterranean forest: the case of cetonids (Coleoptera: Cetoniidae) and syrphids (Diptera: Syrphidae). Environ. Entomol. 43, 336–343.

Takeshita, K., Kikuchi, Y., 2020. Genomic comparison of insect gut symbionts from divergent *Burkholderia* subclades. Genes 11, 744.

Takeshita, K., Matsuura, Y., Itoh, H., Navarro, R., Hori, T., Sone, T., Kamagata, Y., Mergaert, P., Kikuchi, Y., 2015. *Burkholderia* of plant-beneficial group are symbiotically associated with bordered plant bugs (Heteroptera: Pyrrhocoroidea: Largidae). Microb. Environ. 30, 321–329.

Tanahashi, M., Fremlin, M., 2013. The mystery of the lesser stag beetle *Dorcus parallelipipedus* (L.) (Coleoptera: Lucanidae) mycangium yeasts. Bull. Amateur Entomologists' Soc. 72, 146–152.

Tanahashi, M., Hawes, C.J., 2016. The presence of a mycangium in European *Sinodendron cylindricum* (Coleoptera: Lucanidae) and the associated yeast symbionts. J. Insect Sci. 16, 76.

Tanahashi, M., Ikeda, H., Kubota, K., 2018. Elementary budget of stag beetle larvae associated with selective utilization of nitrogen in decaying wood. Sci. Nat. 105, 1–14.

Tanahashi, M., Kim, J.K., Watanabe, K., Fukatsu, T., Kubota, K., 2017. Specificity and genetic diversity of xylose-fermenting *Scheffersomyces* yeasts associated with small blue stag beetles of the genus *Platycerus* in East Asia. Mycologia 109, 630–642.

Tanahashi, M., Kubota, K., 2013. Utilization of the nutrients in the soluble and insoluble fractions of fungal mycelium by larvae of the stag beetle, *Dorcus rectus* (Coleoptera: Lucanidae). Eur. J. Entomol. 110, 611–615.

Tanahashi, M., Matsushita, N., Togashi, K., 2009. Are stag beetles fungivorous? J. Insect Physiol. 55, 983–988.

Ullman, D.E., Westcot, D.M., Hunter, W.B., Mau, R.F.L., 1989. 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. 18, 289–310.

Ulyshen, M.D., 2018. Saproxylic Insects–Diversity, Ecology and Conservation, 1 edn. Springer, Cham.

Vargas-Asensio, G., Pinto-Tomas, A., Rivera, B., Hernandez, M., Hernandez, C., Soto-Montero, S., Murillo, C., Sherman, D.H., Tamayo-Castillo, G., 2014. Uncovering the cultivable microbial diversity of costa rican beetles and its ability to break down plant cell wall components. PLoS One 9.

Verma, P.S., 1969. The alimentary canal and associated organs of two saprophagous beetle, *Onthophagus catta* Fabr. and *Aphodius moestus* Fabr. (Coleoptera: Scarabaeidae). Bull. Entomol. 10, 4–11.

Vidal-Verdú, À., Torrent, D., Iglesias, A., Latorre-Pérez, A., Abendroth, C., Corbín-Agustí, P., Peretó, J., Porcar, M., 2024. The highly differentiated gut of *Pachnoda marginata* hosts sequential microbiomes: microbial ecology and potential applications. npj Biofilms Microbiom. 10, 65.

Wada, N., Sunairi, M., Anzai, H., Iwata, R., Yamane, A., Nakajima, M., 2014. Glycolytic Activities in the Larval Digestive Tract of *Trypoxylus dichotomus* (Coleoptera: Scarabaeidae). Insects 5, 351–363.

Wang, K., Gao, P., Geng, L., Liu, C., Zhang, J., Shu, C., 2022. Lignocellulose degradation in *Protaetia brevitarsis* larvae digestive tract: refining on a tightly designed microbial fermentation production line. Microbiome 10, 90.

Wang, M.-J., Sun, X.-Y., Jiang, L., 2024. Ultrastructural comparison of the larval midguts between *Trypoxylus dichotomus* (Linnaeus, 1771) and *Anomala corpulenta* (Motschulsky, 1854) (Coleoptera: Scarabaeidae). Tissue Cell 90, 102521.

Weihrauch, D., 2006. Active ammonia absorption in the midgut of the Tobacco hornworm *Manduca sexta* L.: transport studies and mRNA expression analysis of a Rhesus-like ammonia transporter. Insect Biochem. Mol. Biol. 36, 808–821.

Weihrauch, D., O'Donnell, M.J., 2021. Mechanisms of nitrogen excretion in insects. Curr. Opin. Insect Sci. 47, 25–30.

Werner, E., 1926. Die ernährung der larve von *Potosia cuprea* fbr. (*Cetonia floricola* hbst.). Zeitschrift für Morphologie und Ökologie der Tiere 6, 150–206.

Wessing, A., Eichelberg, D., 1978. Malpighian tubules, rectal papillae and excretion. Genet. Biol. Drosoph. 2c, 1–42.

Wigglesworth, V.B., 1932. Memoirs: On the Function of the so-called 'Rectal Glands' of Insects. Q. J. Microsc. Sci. s2–75, 131–150.

Wigglesworth, V.B., 1974. The Principles of Insect Physiology, seventh ed. Chapman & Hall, London: London.

Yamada, A., Inoue, T., Noda, S., Hongoh, Y., Ohkuma, M., 2007. Evolutionary trend of phylogenetic diversity of nitrogen fixation genes in the gut community of wood-feeding termites. Mol. Ecol. 16, 3768–3777.

Zhang, H., Brune, A., 2004. Characterization and partial purification of proteinases from the highly alkaline midgut of the humivorous larvae of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). Soil Biol. Biochem. 36, 435–442.

Zheng, W., Zhao, Y., Zhang, H., 2012. Morphology and ultrastructure of the hindgut fermentation chamber of a melolonthine beetle *Holotrichia parallela* (Coleoptera: Scarabaeidae) during larval development. Micron 43, 638–642.
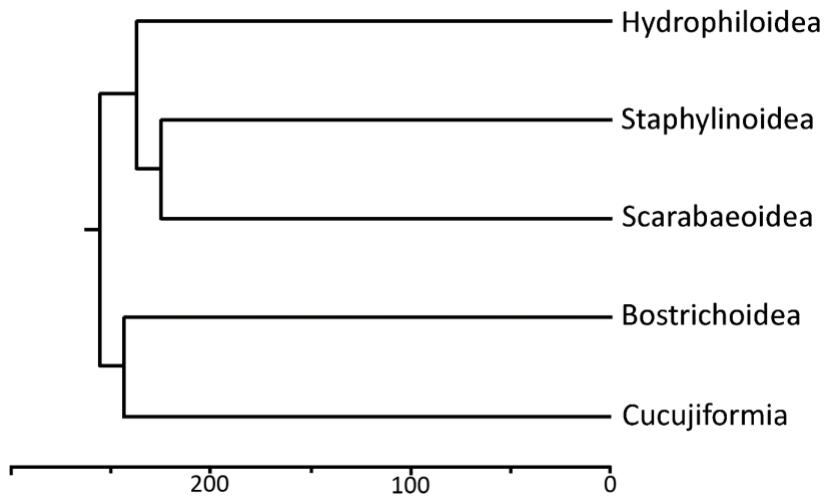
Figure S1. Phylogenetic tree of Polyphaga suborder of beetles. CNCs occur in Bostrichoidea and Cucujiformia. The tree is modified from McKenna et al., 2019, with estimated evolutionary time shown (millions of years).
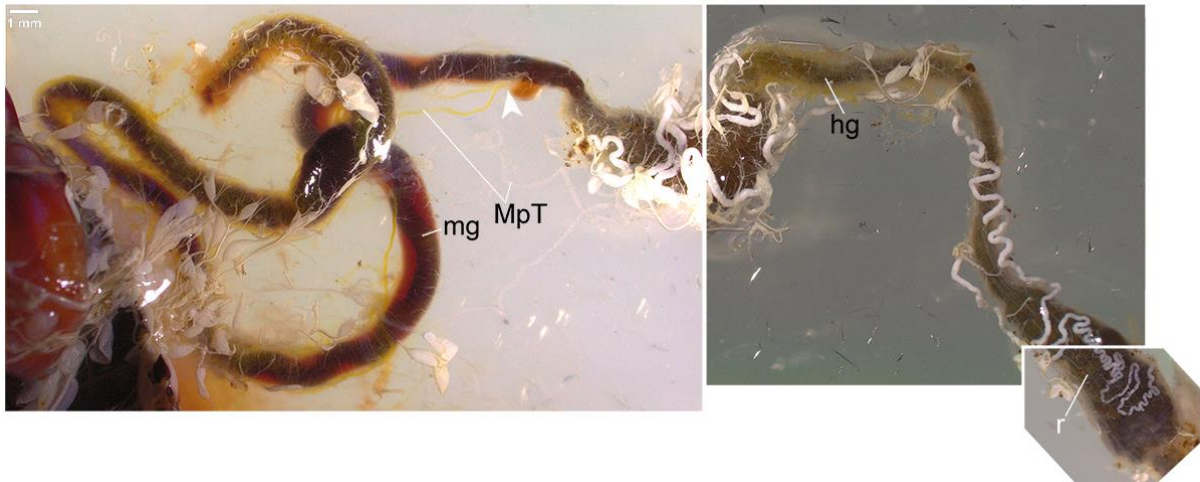

Figure S2. Gut and MpTs of an adult male cockchafer, *Melolontha melolontha*. Montage of images in which the midgut (mg) and hindgut (hg) are indicated, along with the boundary between the two (arrowhead), as well as the rectum (r). Note that the most anterior end of gut is missing, from dissection.

Table S1. Summary of reports of the presence or absence of rectal complexes in species within Scarabaeoidea and the related superfamilies, Staphylinoidea or Hydrophiloidea, and diets of these species.

	family, subfamily or tribe	species	rectal complex presence	stage	diet
Scarabaeoidea	Lucanidae	*Lucanus cervus*	**no** (Dufour, 1824; Saini, 1964)	adult	tree sap? (Fremlin and Hendriks, 2011)
		Dorcus parallelipipedus	**no** (Dufour, 1842; Edwards, 1930)	larval	rotting wood and associated fungi?
			no (Saini, 1964; this study)	adult	tree sap (Fremlin and Hendriks, 2011)
		Platycerus caraboides	**no** (this study)	larval	rotting wood and associated fungi? (Scaccini, 2022)
	Geotrupidae	*Geotrupes stercorarius*	**no** (Saini, 1964)	adult	dung or rotting fungi
	Scarabaeinae	*Copris lunaris*	**no** (Dufour, 1824)	adult	dung
		Digitonthophagus catta	**no** (Verma, 1969)	adult	dung
	Aphodiinae	*Neocalaphodius moestus*	**no** (Verma, 1969)	adult	dung
	Glaphyridae	*Lichnanthe rathvoni*	**yes?** (Areekul, 1957)	larval	decaying leaves (Ritcher, 1966)
	Rhizotrogini	*Rhizotrogus aestivus*	**no** (Schäfer, 1954)	larval	roots
				adult	non-feeding
	Melolonthini	*Melolontha melolontha*	**no** (this study)	larval	roots (Ritcher, 1958)
			yes (Lison, 1938; Saini, 1964; this study)	adult	leaves, flowers
		Amphimallon majale	**yes?** (Menees, 1958)	larval	grass roots
		Polyphylla decemitheata	**yes?** (Areekul, 1957)	larval	roots
		Phyllophaga anxia	**no** (Berberet and Helms, 1972)	larval	roots (Berberet and Helms, 1972)
				adult	leaves
		Phyllophaga gracilis	**yes?** (Areekul, 1957)	larval	roots
			no (Fletcher, 1930)	adult	leaves
	Diplotaxini	*Diplotaxis liberta*	**no** (Jones, 1940)	adult	leaves (Vaurie, 1958)
	Cetoniinae	*Cetonia aurata*	**yes** (this study)	larval	rotting leaves
			no (Dufour, 1824; Dufour, 1842; this study)	adult	nectar, pollen, fruit (Karolyi et al., 2009;

	Subfamily	Species		Stage	Food
					Šípek et al., 2016)
		Protaetia cuprea	**yes** (Werner, 1926)	larval	rotting leaves
		Pachnoda marginata	**yes** (this study)	larval	rotting leaves
			no (this study)	adult	fruit
	Rutelinae	*Anomala cuprea*	**no** (Nagae et al., 2013)	larval	roots and organic matter (Tsunoda et al., 2017)
		Anomala corpulenta	**no** (Wang et al., 2024)	larval	roots
		Popillia japonica	**no** (Swingle, 1930)	larval	roots (Swingle, 1930)
				adult	leaves (Swingle, 1930)
	Dynastinae	*Heteronychus aratar*	**no** (Sheehan et al., 1982)	larval	roots and organic matter
				adult	plant stems
		Trypoxylus dichotomus	**no** (Wang et al., 2024)	larval	rotting leaves
		Oryctes nasicornis	**yes** (Sirodot, 1858; Gérard, 1942; Bayon, 1981)	larval	rotting wood
	Passalidae	*Pentalobus barbatus*	**no** (Baker, 1968)	larval	wood, frass, invertebrates? (Baker, 1968; Schuster, 2004)
				adult	
	Pleocomidae	*Pleocoma crinita*	**yes?** (Areekul, 1957)	larval	roots (Ellertson and Ritcher, 1959)
Staphylinoidea	Staphylinidae	*Staphylinus erythropterus*	**no** (Dufour, 1824)	adult	invertebrates
		Paederus riparius	**no** (Dufour, 1824)	adult	invertebrates
	Silphidae	*Silpha obscura*	**no** (Dufour, 1824)	adult	carrion
		Nicrophorus vespilloides	**no** (this study)	larval	vertebrate carcasses
Hydrophiloidea	Histeridae	*Hister*	**no** (Dufour, 1824)	adult	invertebrates

References

Areekul, S. 1957. The Comparative Internal Larval Anatomy of Several Genera of Scarabaeidae (Coleoptera). Annals of the Entomological Society of America. 50, 562-577.

Baker, W. V. 1968. The gross structure and histology of the adult and larval gut of *Pentalobus barbatus* (Coleoptera: Passalidae). The Canadian Entomologist. 100, 1080-1090.

Bayon, C. 1981. Ultrastructure de l'epithelium intestinal et flore parietale chez la larve xylophage d'*Oryctes nasicornis* L. (Coleoptera : scrabaeidae). International Journal of Insect Morphology and Embryology. 10, 359-371.

Berberet, R. C. and Helms, T. J. 1972. Comparative Anatomy and Histology of Selected Systems in Larval and Adult *Phyllophaga anxia* (Coleoptera: Scarabaeidae). Annals of the Entomological Society of America. 65, 1026-1053.

Dufour, M. L. 1824. Recherches anatomiques sur les Carabiques et sur plusieurs autres insectes Coléoptères. Annales des Sciences Naturelles. Atlas des Tomes 1,2,3, 27-42.

---- 1842. Histoire comparative des métamorphoses et de l'anatomie des *Cetonia aurata* et *Dorcus parallelipipedus*. Annales Des Sciences Naturelles. 18, 162-181.

Edwards, E. E. 1930. On the Morphology of the Larva of *Dorcus parallelopipedus*, L. (Coleoptera). Zoological journal of the Linnean Society. 37, 93-108.

Ellertson, F. E. and Ritcher, P. O. 1959. Biology of rain beetles, P*leocoma s*pp., associated with fruit trees in Wasco and Hood River counties. Agricultural Experiment Station, Oregon State College, Corvallis OR. Technical Bulletin. 44, 1-42.

Fletcher, F. W. 1930. The Alimentary Canal of *Phyllophaga Gracilis* Burm. The Ohio Journal of Science. 30, 109-119.

Fremlin, M. and Hendriks, P. 2011. Sugaring for stag beetles - different feeding strategies of *Lucanus cervus* and *Dorcus paralellipipedus*. Biology, Agricultural and Food Sciences. 70, 57-67.

Gérard, P. 1942. Les tubes de Malpighi de la larve d'*Oryctes Nasicornis* L. Annales de la Société royale zoologique de Belgique. 73, 122-133.

Jones, C. R. 1940. The alimentary canal of *Diplotaxis liberta* Germ. (Scarabaeidae: Coleoptera). The Ohio Journal of Science. 40, 94-103.

Karolyi, F., Gorb, S. N. and Krenn, H. W. 2009. Pollen grains adhere to the moist mouthparts in the flower visiting beetle C*etonia aurata* (Scarabaeidae, Coleoptera). Arthropod-Plant Interactions. 3, 1-8.

Lison, L. 1938. Contribution à l'étude morphologique et histophysiologique du système malpighien de M*elolontha melolontha* Linn. (Coleoptera). Annales de la Société royal zoologique de Belgique. 69, 195-233.

Menees, J. H. 1958. The Anatomy and Histology of the Larval Alimentary Canal of the European Chafer, *Amphimallon majalis* Razoumowsky (Scarabaeidae). Journal of the New York Entomological Society. 66, 75-86.

Nagae, T., Miyake, S., Kosaki, S. and Azuma, M. 2013. Identification and characterisation of a functional aquaporin water channel (*Anomala cuprea* DRIP) in a coleopteran insect. The Journal of Experimental Biology. 216, 2564-2572.

Ritcher, P. O. 1958. Biology of Scarabaeidae. Annual Review of Entomology. 3, 311-334.

Ritcher, P. O. 1966. White grubs and their allies: a study of North American scarabaeoid larvae. Corvallis, Oregon: Oregon State University Press.

Saini, R. S. 1964. Histology and physiology of the cryptonephridial system of insects. Transactions of the Royal Entomological Society of London. 116, 347-392.

Scaccini, D. 2022. Habitat and microhabitat suitability for Italian *Platycerus* species (Coleoptera: Lucanidae): elevation, slope aspect and deadwood features. Scandinavian Journal of Forest Research. 37, 172–181.

Schuster, J. 2004. Bess Beetles (Coleoptera: Passalidae). In Encyclopedia of Entomology. Dordrecht: Springer.

Schäfer, R. 1954. Zur Kenntnis der Anatomie, Physiologie und Ökologie des Brachkäfers, *Rhizotrogus aestivus* Oliv. (Col. Lam.). Zeitschrift für Angewandte Entomologie. 35, 381-424.

Sheehan, C. M., Crawford, A. M. and Wigley, P. J. 1982. Anatomy and histology of the alimentary canal of the black beetle, *Heteronychus arator*. New Zealand Journal of Zoology. 9, 381-385.

Šípek, P., Fabrizi, S., Eberle, J. and Ahrens, D. 2016. A molecular phylogeny of rose chafers (Coleoptera: Scarabaeidae: Cetoniinae) reveals a complex and concerted morphological evolution related to their flight mode. Molecular phylogenetics and evolution. 101, 163-175.

Sirodot, M. S. 1858. Recherches sur les sécrétions chez les insects. Annales des sciences naturelles. 10, 141-189.

Swingle, M. 1930. Anatomy and Physiology of the Digestive Tract of the Japanese Beetle. Journal of Agricultural Research. 41, 181.

Tsunoda, T., Suzuki, J.-I. and Kaneko, N. 2017. Fatty acid analyses to detect the larval feeding preferences of an omnivorous soil-dwelling insect, *Anomala cuprea* (Coleoptera: Scarabaeidae). Applied Soil Ecology. 109, 1-6.

Vaurie, P. 1958. A revision of the genus Diplotaxis (Coleoptera, Scarabaeidae, Melolonthinae). Part 1. Bulletin of the American Museum of Natural History. 115, 263-396.

Verma, P. S. 1969. The alimentary canal and associated organs of two saprophagous beetle, *Onthophagus catta* Fabr. and *Aphodius moestus* Fabr. (Coleoptera: Scarabaeidae). Bulletin of entomology. 10, 4-11.

Wang, M.-J., Sun, X.-Y. and Jiang, L. 2024. Ultrastructural comparison of the larval midguts between *Trypoxylus dichotomus* (Linnaeus, 1771) and *Anomala corpulenta* (Motschulsky, 1854) (Coleoptera: Scarabaeidae). Tissue and Cell. 90.

Werner, E. 1926. Die ernährung der larve von *Potosia cuprea* fbr. (*Cetonia floricola* hbst.). Zeitschrift für Morphologie und Ökologie der Tiere. 6, 150-206.