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A parasitic nematode induces dysbiosis in susceptible but not resistant gastropod hosts

Laura Sheehy[1†], Kerry MacDonald-Howard [1†], Chris D. Williams [1], Gareth D. Weedall [1], Hayley Jones [2], Robbie Rae [1*]

[1] Liverpool John Moores University, School of Biological and Environmental Sciences, Byrom Street, Liverpool, L3 3AF, U.K.
[2] Royal Horticultural Society Garden, Wisley, Woking, Surrey, GU236QB, U.K.

*__Correspondence__:
Corresponding author
r.g.rae@ljmu.ac.uk
†These authors have contributed equally to this work and share first authorship
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Abstract

Animals' gut microbiomes affect a wide array of biological processes including the immunity and protection from pathogens. However, how the microbiome changes due to infection by parasites is still largely unknown, as is how the microbiome changes in hosts that differ in their susceptibility to parasites. To investigate this, we exposed two slug species of differing susceptibility to the parasitic nematode *Phasmarhabditis hermaphrodita* (*Deroceras reticulatum* is highly susceptible and *Ambigolimax valentianus* resistant to the nematode) and profiled the gut microbiota after 7 and 14 days. Prior to infection, both slug species' microbiota was dominated by similar bacterial genera: *Pseudomonas* (by far the most abundant), *Sphingobacterium*, *Pedobacter*, *Chryseobacterium* and *Flavobacterium*. In the resistant host *A. valentianus* there was no significant change in the bacterial genera after infection but in *D. reticulatum*, the bacterial profile changed, with a decrease in the abundance of *Pseudomonadaceae* and an increase in the abundance of *Flavobacteriaceae* and *Sphingobacteriaceae* after 7 days post infection. This suggests nematode infection causes dysbiosis in hosts that are susceptible to infection, but the microbiome of resistant species remains unaltered. In summary, the regulation of the immune system is tightly linked with host survival and nematode infection can alter the microbiome structure.

Introduction

Bacteria are ubiquitous across the world, often sharing habitats with multicellular life, even colonising animals either as pathogens or as symbionts [1]. Developments in next-generation sequencing has enabled the widespread application of genomics to investigate animal hosts and their bacterial associations, revolutionising the understanding of these interactions at the level of the microbiome [2]. It is well established that the microbiome plays an important role in the health of the animal [3-5]. The immune system of the animal host continuously interacts with the microbiome, it is known that the microbiome plays a crucial role in the development of a host's immune system [6].

A host and its microbiome have complex interactions at many levels often undergoing co-evolutionary pressures, as such it is not correct to truly consider them separate entities. Instead, it is preferable to consider the host and its microbial community as a holobiont recognising its diversity and dynamic association [7]. Brealey et al (2022) suggested that parasites can also be considered holobionts, as they are residing within the microbial community of their holobiont host. Brealey et al demonstrated that an infection by the intestinal cestode *Eubothrium* spp. is associated with dysbiosis of the Atlantic salmon gut microbiome. The cestode *Eubothrium* spp. was shown to select for bacteria belonging to the family mycoplasmas when infecting Atlantic salmon, this highlights the importance of considering the parasite holobiont when studying parasitic infections [8].

Currently most research is focused on vertebrate holobiont systems, yet invertebrate systems have the potential to become tractable laboratory models for holobiont research due to their relatively simple gut communities and ease of laboratory culture [9]. Cardoso (2012), Landry (2015), and Jackson (2021) investigated the effects of environment and diet on invertebrate microbiomes showing how microbiome can shift due to external factors [10-12]. Cardoso et al demonstrated that the microbiome of the land snail *Achatina fulica* can be altered by their diet, with a high-sugar diet let to an increase in the abundance of phyla Bacteroidota and Bacillota [10]. Landry et al discovered that the majority of bacteria found in the spruce budworm (*Chroistoneura fumiferana*) microbiome belonged to the phylum Pseudomonadota, and their experiments showed species diversity was significantly affected by environment and diet [11]. Jackson et al identified and determined that the

core microbiome of the slug *Ambigolimax valentianus* can be influenced by diet (sterile and non-sterile) and environment (garden or lab-reared) [12]. It has also been shown that parasitic infections can alter the balance of the host's microbiome, causing dysbiosis [8] but more research is needed to fully understand this effect. Furthermore, while many studies have concentrated on freshwater parasites and hosts there is little information about the role the gut microbiome plays in host immunity in terrestrial environments.

One such host/parasite system that could provide insight is that of slugs and their nematode parasites. Several slug species are global pests of agricultural crops, responsible for millions of pounds of damage each year [13]. Slugs are parasitised by flies, trematodes, viruses, and microsporidia but the most prevalent group are the nematodes [14], with 108 species using gastropods as intermediate, definitive and paratenic hosts [15]. One species, *Phasmarhabditis hermaphrodita*, is a lethal parasite that has been developed as a biological control agent (Nemaslug®) to control pestiferous slugs on gardens and farms in the UK and northern Europe by BASF Agricultural Specialities [16]. Infective stage nematodes (Fig 1 A) are applied to soil and are attracted to chemical cues such as slug mucus and faeces [17, 18], they then enter through the back of the mantle proliferate and kill the slug in 4-21 days [19, 20]. Self-fertilizing adult nematodes (Fig 1 B) reproduce on the cadaver of the slug and once the food supply is depleted, they develop into new infective juveniles where they search for new slug hosts in the soil. *P. hermaphrodita* has successfully been used to control slugs in many crops including lettuce [21] and in floriculture e.g. orchids [22]. *P. hermaphrodita* is not the only nematode to kill slugs, there are several other species e.g. *P. californica* and *P. neopapillosa* that can kill susceptible slugs [23]. There is natural variation in the pathogenic ability of *P. hermaphrodita* [24] and crucially, *P. hermaphrodita* (and other *Phasmarhabditis* species) are facultative parasites that can be cultured under laboratory conditions allowing infection experiments to be carried out (see Cutler and Rae, 2020 [24]).

P. hermaphrodita has been shown to infect and kill several species of pestiferous slug [18, 20] though the mechanism of pathogenesis is debated. It was thought the nematodes vectored the bacterium *Moraxella osloensis* into the haemocoel of the slug and this was responsible for host death [19, 25, 26] however, recent research by Sheehy et al (2022) failed to find this bacterium in the next generation of *P. hermaphrodita* (and two other *Phasmarhabditis* species, *P.*

1 *californica* and *P. neopapillosa*) after killing a slug using 16S rRNA metagenomic
2 sequencing [23]. Furthermore, these authors showed using 16S rRNA amplicon
3 sequencing that *M. osloensis* is a *Psychrobacter* spp. Therefore, the role bacteria play
4 in causing death to slugs is currently unknown. This warrants further research as these
5 are the only genera of nematode to evolve to kill slugs and snails out of the entire
6 Nematoda phylum (consisting of an estimated 1 million species).
7 Our aim was to discover whether *P. hermaphrodita* affects the gut microbiota
8 of two gastropod host species of differing susceptibility to the nematode. The highly
9 susceptible slug *Deroceras reticulatum* (Fig 1 C), a common pest species with
10 worldwide importance [27], is killed by *P. hermaphrodita* in 4-21 days [18, 20] while
11 *Ambigolimax valentianus* (Fig 1 D) is resistant to being killed by *P. hermaphrodita*
12 [28, 29]. How and why these slug species differ so dramatically in their susceptibility
13 to *P. hermaphrodita* is unknown. To investigate the potential role of the gut
14 microbiome we used 16S SSU ribosomal DNA metagenomic profiling to track
15 changes in the gut microbiome of each species before and after infection with *P.*
16 *hermaphrodita.*
17
18
19 **Materials and methods**
20
21 **Source of invertebrates**
22
23 *P. hermaphrodita* (strain DMG0001) (Nemaslug®) (Fig 1 A, B) was purchased
24 from BASF Agricultural Specialities and stored at 4°C for one week before the
25 experiment.
26 *D. reticulatum* (Fig 1 C) and *A. valentianus* (Fig 1 D) were collected from
27 Maghull, Liverpool (OS grid reference SD373027) and kept in non-airtight plastic
28 boxes (35 x 23 x 22 cm) lined with moist tissue paper at 15°C for 7 days and checked
29 daily for any signs of infection by *Phasmarhabditis* nematodes e.g. swollen mantle,
30 lesions. The slugs were immediately fed a diet of iceberg lettuce and carrots using
31 protocols by McDonald-Howard et al. (2022) [30].
32
33 **Infection of gastropod hosts with *P. hermaphrodita***
34

We used a standard bioassay to infect *D. reticulatum* and *A. valentianus* with *P. hermaphrodita* (see Cutler and Rae, 2020 and Sheehy et al. 2022)[23, 24]. Briefly, infective stage *P. hermaphrodita* DMG0001 were added in doses of either 500 or 1000 nematodes in 2 ml of water to cotton bungs at the bottom of separate 20 ml universal tubes. Two adult slugs (either *D. reticulatum* or *A. valentianus*) were added to each tube, a cotton wool plug was placed on top, and the lid loosely closed. The slugs were exposed for 7 days at 10°C in the dark after which faeces was collected using a pipette tip to transfer to a 2 ml Eppendorf tube for DNA extraction.

Profiling the gut microbiota from faeces of *D. reticulatum* and *A. valentianus*

Individuals were grouped as follows. Group 1 (five *D. reticulatum* fed a lab diet for seven days); Group 2 (five *D. reticulatum* fed a lab diet for 14 days); Group 3 (five *D. reticulatum* fed a lab diet and infected on day seven with *P. hermaphrodita* with faeces collected seven days post infection – 14 days in total); Group 4 (three *A. valentianus* fed a lab diet for seven days); Group 5 (three *A. valentianus* fed a lab diet for 14 days); Group 6 (three *A. valentianus* infected with *P. hermaphrodita* with faeces collected seven days post infection – 14 days in total). Faeces was collected from each individual slug for DNA extraction.

DNA was extracted from faeces using DNeasy PowerSoil Pro Kit (QIAGEN) following the manufacturer's instructions. Presence of bacterial DNA was checked after extractions using PCR amplification of the hypervariable regions of the 16S rRNA gene. This was carried out using the primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3') [31] with the following thermocycler conditions: 3 min at 95°C followed by 35 cycles of 15 seconds at 95°C, 30 seconds at 55°C, 1.5 min at 72°C and a final step of 8 mins at 72°C. Amplicons were visualised using agarose gel electrophoresis to confirm that PCRs had worked; in all cases bands of the correct size were present and no amplification of bacterial DNA could be seen in the extraction negative control or the PCR negative control.

DNA samples were sent for 16S rRNA metagenomic sequencing (Novogene). The V4 hypervariable region of the 16S rRNA gene was amplified using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). All PCR reactions were carried out with

Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Sequencing libraries were generated with NEBNext® Ultra^{TM} DNA Library Prep Kit for Illumina and quantified via Qubit and Q-PCR. Libraries were sequenced on an Illumina NovaSeq 6000 platform to generate 2x250 bp paired end reads.

Analysis of the raw reads occurred at Novogene using the following method. Paired-end reads were merged using FLASH (V1.2.7) [32]. Quality filtering on the raw tags were performed under specific filtering conditions to obtain high-quality clean tags according to the QIIME (V1.7.0) [33]. The tags were compared with the reference database (SILVA database) using UCHIME algorithm [34] to detect chimera sequences. Detected chimera sequences were then removed to obtain Effective Tags. All Effective Tags were processed by UPARSE software (v7.0.1090) [35]. Sequences with ≥97% similarity were assigned to the same Operational Taxonomic Units (OTUs).

For each OTU, QIIME (Version 1.7.0) in Mothur method was performed against the SSU rRNA database of SILVA Database for species annotation at each taxonomic rank (Threshold:0.8~1) [36]. MUSCLE (Version 3.8.31) [37] was used to obtain the phylogenetic relationship of all OTUs.

OTUs abundance information was normalised using a standard of sequence number corresponding to the sample with the least sequences. OTUs were analysed for Alpha diversity (Wilcoxon test function) and Beta diversity (AMOVA - Analysis of Molecular Variance) to obtain richness and evenness information in samples. AMOVA was also used to compare the taxonomic compositions of infected and non-infected slugs in weighted PCoA. Analysis of Alpha and Beta diversity were all performed on the normalized data and calculated with QIIME (Version 1.7.0). Significant intra-group variation is detected via MetaStats based on their abundance.

Results

The microbiomes of *D. reticulatum* and *A. valentianus*

After quality filtering all groups produced high quality data with an average of over 99,000 tags with 90% of these tags resulting in taxa annotation (Good's coverage of >0.97 for both *D. reticulatum* and *A. valentianus*). The average number of OTUs

per sample for each group was similar within species; Group 1: 746, Group 2: 979, and Group 3: 849 for *D. reticulatum* and for *A. valentianus*; Group 4: 452, Group 5: 403, and Group 6: 463. There is a richer diversity of bacteria associating with *D. reticulatum* compared *A. valentianus* when considering number of OTUs

A core microbiome was identified for *D. reticulatum* with 774 OTUs present (Fig 2 A), consisting of bacteria from the phylum Pseudomonadota (39%), Bacteroidota (21%) Bacillota (16%) and Actinomycetota (12%) the remaining 12% spread across 16 phyla (Verrucomicrobiota, Acidobacteriota, Bdellovibrionota, Gemmatimonadota, Chloroflexota, Desulfobacterota, Cyanophyta, Abditibacteriota, Campilobacterota, Methylomirabilota, Patescibacteria, Planctomycetota, Deferribacterota, Nitrospirota, RCP2-54 and Spirochaetota).

The core microbiome for *A. valentianus* is represented by 384 OTUs across 8 phyla (Fig 2 B). Bacteria from the phylum Pseudomonadota (54%), Bacterioidota (23%) and Actinomycetota (12%) were the most abundant with the remaining 11% spread across 5 phyla (Bacillota, Verrucomicrobiota, Bdellovibrionota, Chloroflexota and Cyanophyta). Whilst several phyla are represented in both core microbiomes the abundance of these phyla differs substantially. The core microbiome of *D. reticulatum* shows a much richer diversity than the core microbiome of *A. valentianus.*

The core microbiomes for *D. reticulatum* and *A. valentianus* indicate several shared bacterial associations at the phylum level. *D. reticulatum* and *A. valentianus* associate with a wide range of bacteria from several phyla including Pseudomonadota and Bacteroidota (Fig 3A & 3B).

No effect of sustained laboratory-based diet on the microbiome of *D. reticulatum* and *A. valentianus*

The sustained laboratory-based diet does not lead to a significant difference in the microbiome of *D. reticulatum* or *A. valentianus.* Group 1 (*D. reticulatum*) and Group 4 (*A. valentianus*) were fed a diet of lettuce and carrot for seven days before faeces collection. Whilst Group 2 (*D. reticulatum*) and Group 5 (*A. valentianus*) were fed a diet of lettuce and carrot for 14 days before faeces collection. Neither richness (alpha diversity analysis with Wilcoxon test function $P > 0.5$) or microbiome structure (beta diversity analysis by AMOVA $P > 0.1$) showed a significant change due to the sustained laboratory diet. Shown in Figure 3 A & B the relative abundance at the

phylum level is similar between Group 1 and Group 2 for *D. reticulatum* and between Group 4 and Group 5 for *A. valentianus*. Therefore, the laboratory-based diet does not affect microbiome diversity in either slug species.

Malacopathogenic nematode infection results in a microbiome shift in susceptible gastropod host but not in resistant host species

Susceptible *D. reticulatum* hosts infected with *P. hermaphrodita* exhibit a shift in their microbiome (Fig 4 A). Beta diversity analysis using weighted Unifrac Wilcoxon test indicates a significant change ($P < 0.05$) in the microbiome structure of *D. reticulatum* hosts infected with *P. hermaphrodita.* However, resistant *A. valentianus* hosts infected with *P. hermaphrodita* do not exhibit a shift in their microbiome (Figure 4 B). Beta diversity analysis using weighted Unifrac Wilcoxon test indicated no significant change ($P > 0.05$) in the microbiome structure of *A. valentianus* hosts infected with *P. hermaphrodita.*

Infection with *P. hermaphrodita* results in a change in the composition of the microbiome of susceptible slug host *D. reticulatum*. A Principal Coordinates Analysis (PCoA) based on weighted Unifrac distance indicates the taxonomic composition of the communities in each sample (Fig. 5). Group 3 shows a significant difference in the composition of bacterial communities compared to Group 1 ($P<0.01$) and Group 2 ($P<0.05$) (Fig 5 A). This indicates that infection with *P. hermaphrodita* is affecting the microbiome of the host (Fig 5a). PCoA analysis showed there was no significant difference in the composition of the microbiome of *A. valentianus* when infected by *P. hermaphrodita* (Group 6) compared to uninfected *A. valentianus* in Groups 4 and 5 (Fig 5 B) ($P>0.05$).

Bacteria with significant intra-group variation were detected via MetaStats based on their abundance, through this analysis a significant decrease in the abundance of Pseudomonadota in *D. reticulatum* hosts was seen after infection with *P. hermaphrodita* between Group 1 and Group 3 ($P < 0.0005$) and Group 2 and Group 3 ($P < 0.002$) (Figure 6 A). This decrease specifically affected bacteria from the class of Gammaproteobacteria. Additionally, there are significant decreases ($P < 0.0005$) in the abundance of bacteria in *D. reticulatum* (Group 3) after infection with *P. hermaphrodita* from the following phyla: Thermodesulfobacteriota, Campilobacterota and Deferribacterota. Conversely, there is a significant increase in the abundance of

bacteria from the phylum Bacteroidota in *D. reticulatum* hosts (Group 3) after infection with *P. hermaphrodita* (Figure 6 B). Indicating dysbiosis is associated with *P. hermaphrodita* infection.

Discussion

In this study we have determined that bacteria from the phyla Pseudomonadota, Bacteroidota and Actinomycetota were abundantly present in the core microbiomes of *D. reticulatum* and *A. valentianus*. Jackson (2021) also found Pseudomonadota, Bacteroidota and Actinomycetota to be the most abundant taxa in *A. valentianus* collected in Californica [12]. These three phyla are commonly found in gastropod microbiomes. For example, Joynson et al. examined the microbiome of the common black slug, *Arion ater* and found the most abundant genera to be *Enterobacter, Citrobacter, Pseudomonas, Escherichia, Acinetobacter* and *Sphingobacteriaceae* [38]. Reich et al. (2018) found the most abundant phyla in the microbiome of the protected Kerry Spotted Slug *Geomaculus maculosus* to be Pseudomonadota, Bacteroidota, Planctomycetota, Acidobacteriota, Verrucomicrobiota, Bacillota and Actinomycetota [39]. Both species showed a large abundance of Pseudomonadota before infection, this abundance of Pseudomonadota was also seen in the work from Cardoso et al (2012) on the microbiome of the giant land snail *Lissachatina fulica* [10]. Though Cardoso also showed that when the land snail was fed a diet rich in sugarcane there was a greater abundance of Bacteroidota and Bacillota [10], demonstrating the malleability of the microbiome in response to external forces, in this instance diet.

We also investigated whether and how infection with *P. hermaphrodita* affected the gut microbiota of two host gastropod species. Whilst susceptible host species *D. reticulatum* showed a significant shift in the microbiome, in particular the abundance of Pseudomonadota and Bacteroidota, the resistant species *A. valentianus* showed no such effect.

Further investigation is still required to understand whether the parasitic nematode directly alters the microbiome of the slug or if the microbiome is shifting as a secondary effect of ill health of the slug host during a nematode infection. There is evidence in other host/parasite systems to show parasites can directly affect their host's microbiome [40-42]. Walk (2015) showed that chronic *Heligmosomoides*

polygyrus infection in the duodenum of mice caused in an increase in the abundance of *Lactobacillaceae* and *Enterobacteriaceae* [40]. Gaulke et al (2019) demonstrated that *Pseudocapillaria tomentosa* infection disrupts zebrafish gut microbiome, notable increase in abundance of the genus *Mycoplasma* and *Pelomonas* whilst Fusobacterium and Plesiomonas decreased in their abundance. Through this work Gaulke et al identified interactions between the gut microbiota leading to parasite success [41].

Furthermore, infection with whipworm (*Trichuris muris*) reduces microbiome alpha-diversity in mice [43], but can promote growth of *Lactobacillus* [44]. Houlden et al (2015) also showed that the presence of the parasite is required to maintain the shift in the microbiota as once the parasite is removed the microbiota transitions back to that of an uninfected animal [43], suggesting that the parasite is directly changing the microbiome of the host rather than a microbiome shift being an indirect response. In addition to the parasites changing the microbiota of the host, White et al. (2018) found the parasitic nematode *T. muris* acquired microbiota from its mouse host, which was needed for its fitness [45]. Interestingly infection by the nematode was only successful if microbiota was present (compared to germ free mice). Furthermore, in a comprehensive analysis by Hahn et al. (2022) they showed the microbiome of sticklebacks' changes with not just infection of the cestodes parasite *Schistocephalus solidus* but this is also dependant on the genotype of the parasite [46].

It is not only parasitic helminths that can alter the microbiome of their hosts. Koch and Schmid-Hempel (2011) showed the adaptive value of the microbiota of social bees, which can protect against parasites such as the trypanosomatid gut parasite *Crithidia bombi* [47]. Furthermore, Yun et al. (2022) found strains of honeybees (*Apis cerana*) resistant to sacbrood virus (SBV) had a unique microbiome containing the acetic acid bacterium *Bombella intestine*, the lactic acid bacteria *Lactobacillus spp.* (as well as others) and bees infected with SBV lost crucial bacterial species [48]. The authors suggest a selection of bacteria that could be used as probiotics to keep honeybees healthy. Understanding the multidirectional interactions between parasites, microbiome and the host's immune system during infections could open new avenues of treatments and prevention [42].

Our results show a clear holobiont dysbiosis associated with a *P. hermaphrodita* infection in the susceptible species *D. reticulatum,* but not in the resistant slug species *A. valentianus*, this dysbiosis is consistent with previous studies looking at host/parasite systems in vertebrates [8, 40, 49].

Figure Legends

Figure 1 The infective juvenile stage of *P. hermaphrodita* (A) develops into self-fertilising adults (B) that reproduce on a slug cadaver. *P. hermaphrodita* can infect and kill the susceptible slug species *D. reticulatum* (C) but not the resistant slug species *A. valentianus* (D).

Figure 2 Venn diagrams showing the unique and shared bacterial taxa among groups. (A) *D. reticulatum.* Group 1 fed lab diet faeces collected at day 7; Group 2, fed lab diet faeces collected at day 14; Group 3, infected with *P. hermaphrodita* with faeces collected 7 days after infection. A large core microbiome of 774 OTUs with 20 phyla represented but dominated by bacteria from the phylum Pseudomonadota. (B) *A. valentianus.* Group 4 fed lab diet faeces collected at day 7; Group 5, fed lab diet faeces collected at day 14; Group 6, infected with *P. hermaphrodita* with faeces collected 7 days after infection. A core microbiome of 384 OTUs with just 8 phyla represented but in similarity to *D. reticulatum* the core microbiome is dominated by bacteria from the phylum Psuedomonadota.

Figure 3 A *D. reticulatum* associates with a wide range of bacteria from several phyla Psuedomonadota, Bacillota, Actinomycetota and Bacteroidota. Whilst the laboratory diet (Group 2) has minimal effect on the relative abundance of these phyla, infection with the malacopathogenic nematode *P. hermaphrodita* affects the relative abundance of Pseudomonadota and Bacillota. **Figure 3 B** *A. valentianus* associates with a wide range of bacteria from several phyla Psuedomonadota, Bacillota and Actinomycetota. Neither the sustained laboratory diet nor the infection with *P. hermaphrodita* significantly affect the relative abundance of the bacteria associated with *A. valentianus.*

Figure 4 Beta diversity analysis using weighted Unifrac. (A) Wilcoxon test indicates a significant change ($P < 0.05$) in the microbiome structure of the *D. reticulatum* hosts when infected with the malacopathogenic nematode *P. hermaphrodita.* (B) Wilcoxon test does not indicate a significant change in the microbiome structure of the *A. valentianus* hosts when infected with the malacopathogenic nematode *P. hermaphrodita*.

Figure 5A Infection with *P. hermaphrodita* results in a change in the composition of the microbiome of susceptible slug host *D. reticulatum*. A Principal Coordinates Analysis (PCoA) based on weighted Unifrac distance indicates the taxonomic composition of the communities in each sample. Group 3 shows a significant difference in the composition of bacterial communities than Group 1 or Group 2, this indicates that infection with *P. hermaphrodita* is affecting the microbiome of the host. **Figure 5B** A Principal Coordinates Analysis (PCoA) based on weighted Unifrac distance indicates the taxonomic composition of the communities in each sample. Group 6 (infection with *P. hermaphrodita*) results in a slight change in the composition of the microbiome of susceptible slug host *A. valentianus*, though this change is not significant.

Figure 6 Bacteria with significant intra-group variation detected via MetaStats based on their abundance. (A) A significant decrease (** $P < 0.0005$, * $P < 0.002$) in the abundance of Pseudomonadota in *D. reticulatum* hosts (Group 3) after infection with *P. hermaphrodita* specifically affected bacteria from the class of Gammaproteobacteria. (B) A significant increase (** $P > 0.005$) in the abundance of Bacteroidota in *D. reticulatum* hosts (Group 3) after infection with *P. hermaphrodita*.

Author contributions

Laura Sheehy
Conceptualization-Equal, Data curation-Equal, Formal analysis-Equal, Investigation-Equal, Writing – original draft-Equal, Writing – review & editing-Equal

Kerry McDonald-Howard
Formal analysis-Equal, Investigation-Equal, Project administration-Equal, Writing – review & editing-Equal

Hayley Jones
Supervision-Equal

Chris Williams

Supervision-Equal, Writing – review & editing-Equal

Gareth Weedall
Formal analysis-Equal, Supervision-Equal, Writing – review & editing-Equal

Robbie Rae
Conceptualization-Equal, Supervision-Equal, Writing – original draft-Equal, Writing – review & editing-Equal

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Ethics statement
None required

Conflict of interest
None declared

Data Availability statement

Data has been submitted to the European Nucleotide Archive (ENA) under the ID PRJEB56262 (ERP141185).

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