BIOLOGY LETTERS

royalsocietypublishing.org/journal/rsbl

Research

Cite this article: Salloum PM, Jorge F, Dheilly NM, Poulin R. 2023 Adoption of alternative life cycles in a parasitic trematode is linked to microbiome differences. *Biol. Lett.* **19**: 20230091.
https://doi.org/10.1098/rsbl.2023.0091

Received: 22 February 2023
Accepted: 23 May 2023

Subject Areas:
evolution, ecology

Keywords:
bacteria, *Coitocaecum parvum*, complex life cycle, metacercariae, progenesis

Author for correspondence:
Robert Poulin
e-mail: robert.poulin@otago.ac.nz

†Joint first authors.

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6673606.

THE ROYAL SOCIETY PUBLISHING

Evolutionary biology

Adoption of alternative life cycles in a parasitic trematode is linked to microbiome differences

Priscila M. Salloum[1,†], Fátima Jorge[2,†], Nolwenn M. Dheilly[3,4] and Robert Poulin[1]

[1]Department of Zoology, and [2]Otago Micro and Nano Imaging, Electron Microscopy Unit, University of Otago, Dunedin 9016, New Zealand
[3]School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794, USA
[4]ANSES, Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail - Laboratoire de Ploufragan-Plouzané, Unité Génétique Virale de Biosécurité, 22440, Ploufragan, France

FJ, 0000-0002-3138-1729; RP, 0000-0003-1390-1206

For parasites with complex multi-host life cycles, the facultative truncation of the cycle represents an adaptation to challenging conditions for transmission. However, why certain individuals are capable of abbreviating their life cycle while other conspecifics are not remains poorly understood. Here, we test whether conspecific trematodes that either follow the normal three-host life cycle or skip their final host by reproducing precociously (via progenesis) in an intermediate host differ in the composition of their microbiomes. Characterization of bacterial communities based on sequencing of the V4 hypervariable region of the 16S SSU rRNA gene revealed that the same bacterial taxa occur in both normal and progenetic individuals, independent of host identity and temporal variation. However, all bacterial phyla recorded in our study, and two-thirds of bacterial families, differed in abundance between the two morphs, with some achieving higher abundance in the normal morph and others in the progenetic morph. Although the evidence is purely correlative, our results reveal a weak association between microbiome differences and intraspecific plasticity in life cycle pathways. Advances in functional genomics and experimental microbiome manipulation will allow future tests of the significance of these findings.

1. Introduction

The evolution of complex life cycles, requiring passage through two or more hosts of different species in a particular order, has been a convergent trend among multiple lineages of protozoan and metazoan parasites [1]. Although their evolution has been driven by natural selection for the advantages they offer [2–5], complex life cycles nevertheless pose challenges, by increasing the number of separate transmission events required per generation. Among the many adaptations to a complex cycle displayed by parasites, the ability to facultatively abbreviate, and thus simplify, the life cycle is an extreme case. For instance, low or unpredictable abundance of the next host species or unstable environmental conditions impeding transmission to the next host can favour individual trematodes capable of skipping that host and still completing their life cycle [6–8].

In the trematode *Coitocaecum parvum* (Opecoelidae), many individuals follow a three-host life cycle: after asexually multiplying in the snail first intermediate host and exiting the snail, they penetrate an amphipod second intermediate host, and encyst individually as 'normal' metacercariae to await ingestion by the fish definitive host, where they mature and reproduce sexually

[9]. However, some individuals truncate the cycle to just two hosts, developing within the amphipod as 'progenetic' metacercariae that achieve precocious sexual maturity and reproduce through self-fertilization, producing eggs without needing to reach a fish [9]. These eggs are released into water upon the amphipod's death. The alternative life cycles are not genetically determined, as genetically identical infective stages entering the same amphipod can develop into either normal or progenetic metacercariae [10]. Instead, external factors drive this developmental plasticity. Progenesis is more likely to occur when amphipod hosts are reared in water devoid of fish odours [11,12], when the metacercaria shares the amphipod with conspecific or heterospecific parasites [13], or when it has been in the amphipod for many weeks without reaching a fish [14]. However, these factors combined fall short from explaining all the unpredictability of life cycle pathways adopted by different individuals, particularly when they share the same host.

Increasingly, microbiomes (microbes dwelling inside animal bodies and all molecules they produce) are thought to contribute to the phenotype of animals [15–17], and the same applies to the phenotype of parasites and their interactions with hosts [18–20]. Many parasites, such as *C. parvum* [21], possess a core microbiome, distinct from that of their hosts and from the environmental microbiome. This core microbiome is maintained throughout the parasite's life cycle, implying vertical transmission and thus linking microbial transmission with successful parasite transmission [21]. Either through shared interests with the parasite or as a side-effect, these microbes and their gene products may exert influence on the parasite they inhabit. Indeed, microbes of parasites can impact both parasite development [22] and infectivity [23]. Yet there remains much to be understood about the implications of microbes for parasite biology.

Here, we test the hypothesis that the microbiome of *C. parvum* metacercariae is associated with the life cycle pathway they adopt. We predict that normal and progenetic metacercariae will harbour either distinct bacterial communities, or at least bacterial taxa unique to only one type of metacercaria or achieving different abundance in the two types. Our findings shed further light on the factors underpinning the plasticity of transmission routes followed by parasites, revealing influences extending beyond a simple host–parasite two-player interaction.

2. Methods

(a) Sample collection and sequencing

Some samples included here were collected during the 2019 austral summer and used previously [21]. Additional sampling of infected amphipods, *Paracalliope fluviatilis* (family Paracalliopiidae), was conducted at the same location in the 2021 summer (Lake Waihola, South Island, New Zealand; latitude −46.01499, longitude 170.10084). In both years, environmental samples (water and sediment from the field site) and samples of the laboratory environment (water in which amphipod hosts were maintained) were collected. Sample collection, processing, library preparation and microbiome sequencing protocols for both years are described in [21]. In some instances, more than one metacercariae was isolated from the same amphipod host (electronic supplementary material, table S1). Sequencing was carried out separately for each batch (2019 and 2021), targeting the V4 hypervariable region of the bacterial 16S SSU rRNA gene, using an Illumina MiSeq platform and v2 reagent cartridge (250 bp, paired-end), at the Otago Genomics & Bioinformatics Facility (New Zealand).

(b) Bioinformatics

De-multiplexed sequences were filtered separately for each batch, to remove potential sequencing-specific artefacts. Sequencing quality was checked with a FastQC report [24], following which adaptors, primers and overrepresented sequences were removed with the *cutadapt* plugin [25] implemented in QIIME 2 [26]. Sequences were trimmed and denoised using the *dada2* plugin in QIIME 2 [27]. To assign taxonomy, we trained the SILVA database version 138.1 targeting the region SSURef_NR09 [28] on our dataset using the Naive Bayes classifier on QIIME 2. This version of the SILVA database was updated in 2019, based on the DSMZ 'Bacterial Nomenclature up to date (PNU)'. Data quality was evaluated in QIIME 2 by comparing the observed composition of ZymoBIOMICS microbial community standards (MCS) against their expected composition. Feature tables were filtered to remove contamination. Depth filters were defined based on rarefaction curves (electronic supplementary material, figure S2), and two datasets were generated: one with lower depth but larger sample size (2 615 401 reads, $N = 81$), and another with greater depth but lower sample size (2 541 894 reads, $N = 61$). Results from both datasets were largely similar, thus results from the dataset with larger sample size are reported below; those for the other dataset are in the electronic supplementary material.

The resulting filtered 2019 and 2021 datasets were analysed separately and merged, and taxonomy was assigned based on the trained SILVA database. Amplicon sequence variants (ASVs) were aligned, rooted and unrooted phylogenetic trees were built, and QIIME 2 resulting output files were analysed in R v.4.1.3 [29]. Phyloseq v.1.38.0 [30] was used to group ASVs into higher taxonomic levels (family and phylum). All analyses were undertaken in the R package microeco v. 0.11.0 [31], unless otherwise specified.

(c) Statistical analyses of bacterial diversity, composition and abundance

Significance was based on a p-value ≤ 0.05 (accounting for multiple testing based on the false discovery rate). α-Diversity (observed diversity, Shannon diversity and Faith's PD) and β-diversity (Bray–Curtis, Jaccard distance, weighted and unweighted Unifrac) were used to assess differences in bacterial richness and composition at phylum and family levels between morphs, the environment and the laboratory environment, between years (2019 and 2021), and between *C. parvum* occurring alone in an amphipod and when co-occurring with another trematode (*Maritrema poulini*). Statistical significance was based on analyses of variance (ANOVAs) for α-diversity and permutational multivariate ANOVAs (perMANOVAs) for β-diversity. To account for amphipod host identity and year of collection on metacercariae microbiomes, generalized linear mixed models based on kernel matrices calculated from β-diversity distances were fitted using GLMMMiRKAT v1.2 [32]. Taxon abundance was calculated at phylum and family levels, with a Venn diagram at ASV level summarizing the number of unique and shared features between sample types. Tests of differential abundance were undertaken to look for taxa driving microbial community differences between morphs, using three approaches: metastat, which compares individual ASVs counts between two groups using Fisher's exact test to handle sparse samples (many zeroes), identifying statistical significance employing the false discovery rate [33]; LEfSe, which performs a class comparison that estimates the sizes of the significant variations [34]; and the corncob v.0.3.0 R package, which uses a β-binomial model to assess both differential abundance and differential variability of microbial taxon abundances across groups [35]. Differential abundance

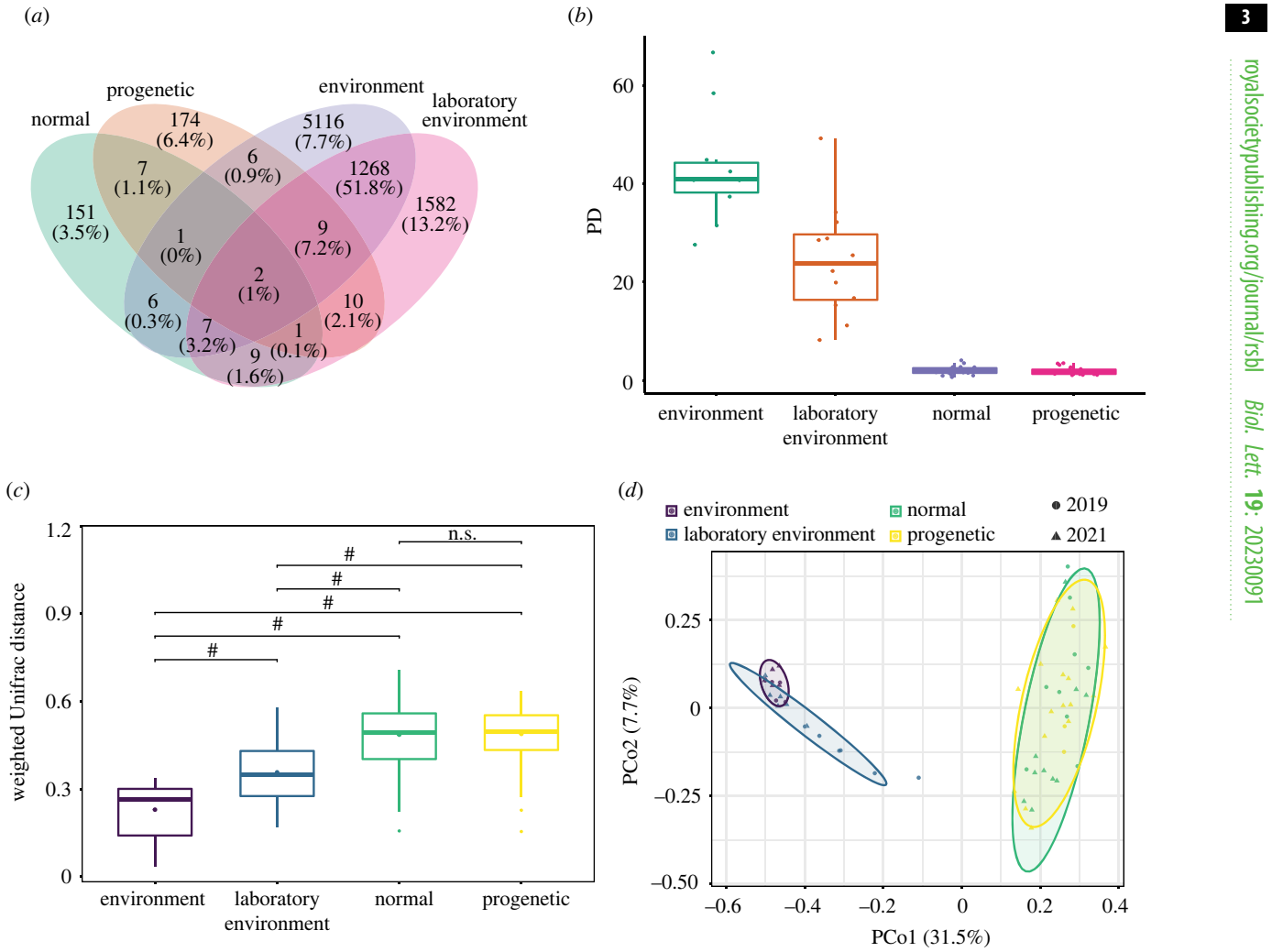

Figure 1. Microbiome richness and composition of normal and progenetic metacercariae, the environment and the laboratory environment. (*a*) Venn diagram at the ASV level. Numbers represent unique or shared ASVs among groups, while percentages represent the ratio sequences:total sequences. (*b*) α-Diversity at the family level, based on Faith's PD. (*c*) β-Diversity at the family level, based on weighted Unifrac ($^{\#}$ significant result; n.s.: non-significant). (*d*) PCoA of unweighted Unifrac distances at family level (2019 and 2021: year of sample collection).

methods do not necessarily return the same results, given specific features of the methods and the data, thus we employed three approaches for a more thorough evaluation of the data. For details, see electronic supplementary material.

3. Results

In the lower depth dataset, filtering removed 1097 and 1046 ASVs and 69 and 13 samples (including blanks) from the 2019 and 2021 datasets, respectively. The final merged dataset contains 8349 ASVs and 60 samples, including 38 metacercariae (19 of each morph, 383 ASVs) and 22 environmental samples (8017 ASVs). Final depth of the complete table ranged from 757 to 307 931; depth of metacercariae ranged from 757 to 52 389, and that of environmental samples from 1615 to 307 931. Results at lower taxonomic ranks (family) are presented along with results at higher ranks (phyla), since MCS analyses show taxonomic classification is less accurate at lower ranks (electronic supplementary material, figure S1; Results).

Metacercarial microbiomes clearly differed from the environment and the laboratory environment (figure 1), independent of year of collection (electronic supplementary material, figure S5 and tables S11–S14). The environment and the laboratory environment share many ASVs, but few ASVs are common between normal and progenetic morphs, and between each morph and the environment (figure 1*a*). However, the proportion of higher taxa (phylum and family) shared by normal and progenetic metacercariae is large (figure 2*a–c*).

The taxonomic composition at the phylum level is mostly similar between normal and progenetic morphs (figure 2*a,b*; electronic supplementary material, figure S6), with high relative abundance of Proteobacteria, Actinobacteriota, Firmicutes and Bacteroidota. Yet, the relative abundance of Firmicutes is higher in progenetic metacercariae, while Bacteroidota is more abundant in normal metacercariae. At the family level, Burkholderiaceae, Hymenobacteraceae, Beijerinckiaceae, Corynebacteriaceae, Nitrococcaceae, Microbacteriaceae and Caulobacteraceae are more abundant in normal morphs, whereas Moraxellaceae, Geodermatophilaceae, Bacillaceae, Pseudomonadaceae and Oxalobacteraceae are more abundant in progenetic morphs (figure 2*a,b*; electronic supplementary material, figure S7). Some taxa were only detected in a single sample (e.g. Catellicoccaceae and Alcaligenaceae), or with high abundance in one sample and very low abundance in another (figure 2*c*). In these cases, sample size was potentially insufficient to detect lower frequency taxa.

LEfSe and corncob tests of differential abundance between morphs were not significant at the taxonomic

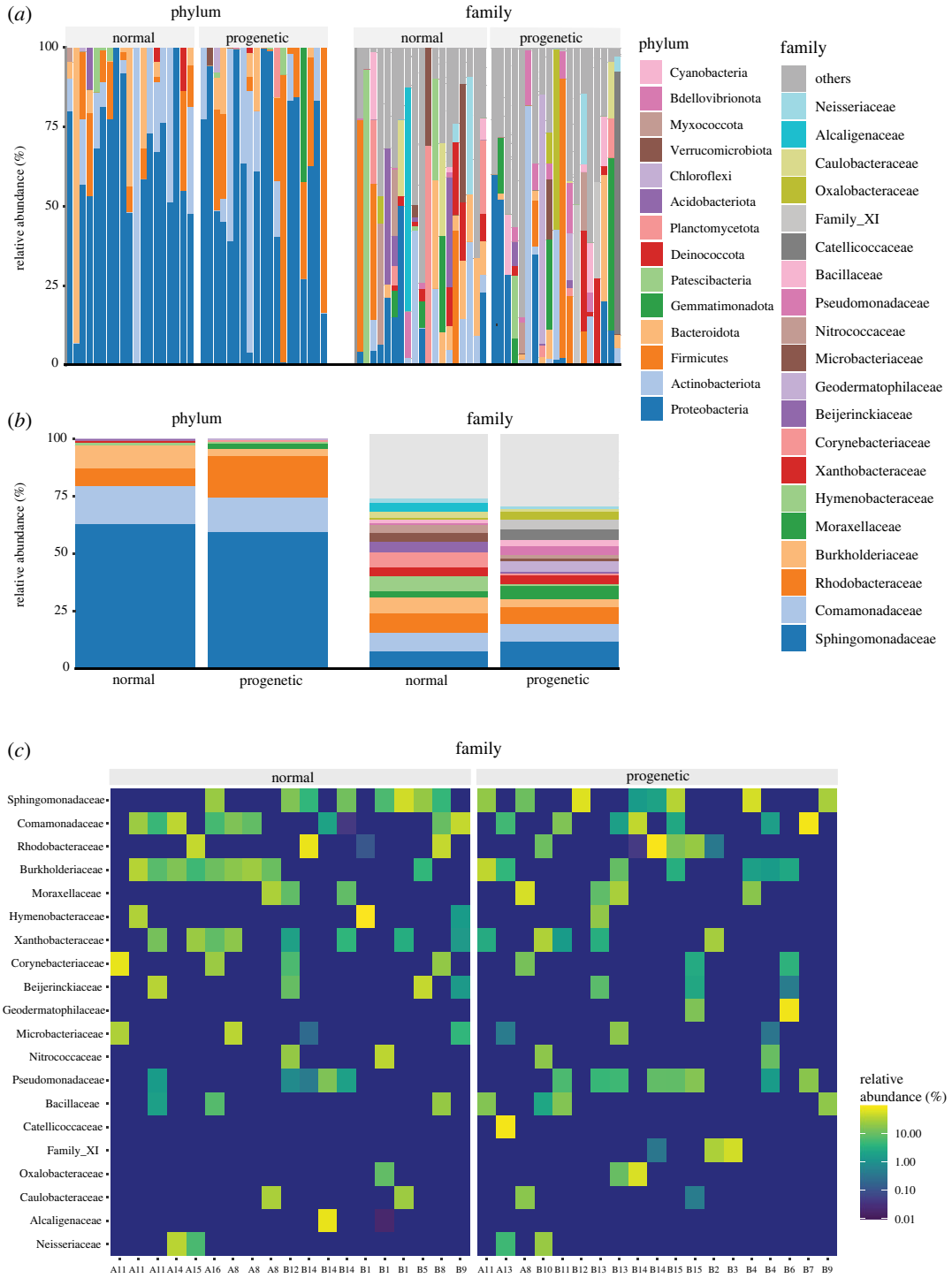

Figure 2. Taxonomic composition of the microbiome of normal and progenetic metacercariae. (*a*) Bar plots of taxonomic composition including all phyla and the 20 most abundant families, for each individual metacercaria. (*b*) Pooled taxonomic composition at phylum and family level. (*c*) Heat map showing relative abundance of the 20 most prevalent bacterial families in normal and progenetic morphs, matched by host identity (*x*-axis; each unique code is a different host individual).

levels tested, except corncob for the Cryomorphaceae family in 2021 (electronic supplementary material, figure S8 and table S2). However, metastat results showed that all 14 phyla occurring in metacercariae differed significantly in abundance between morphs (electronic supplementary material, table S5, results per year in electronic supplementary material, tables S3, S6 and S7). At the family level, 67 out of 92 taxa present in metacercariae differed significantly in abundance between normal and progenetic morphs (electronic supplementary material, table S8, results per year in the electronic supplementary material, tables S4, S9 and S10).

Microbial community richness (α-diversity) was similar between normal and progenetic morphs, even when considering different years or co-infection with *M. poulini* (figure 1*b*; electronic supplementary material, figure S5, and tables S11 and S12). There are α-diversity differences between metacercariae and environmental samples (electronic supplementary material, tables S11 and S12), and differences in environmental samples between years (electronic supplementary material, table S11).

Microbial diversity comparisons at phylum and family level (β-diversity) detected no significant difference between metacercariae morphs (electronic supplementary material,

tables S13 and S14), regardless of host identity and year of collection (figure 2*c*; electronic supplementary material, figures S9 and S10, and table S15); however, both are significantly different from the environment and laboratory environment (figure 1*c*,*d*, electronic supplementary material, tables S13 and S14). Temporal differences are stronger in environmental samples (electronic supplementary material, table S13). Finally, co-occurrence of *M. poulini* with *C. parvum* did not affect the microbial diversity of *C. parvum* metacercariae (electronic supplementary material, table S13).

4. Discussion

The ability of many helminths to facultatively truncate and simplify their life cycle is one of the most striking examples of phenotypic plasticity among parasites [6–8]. Although this phenomenon can be triggered by adverse conditions for transmission, its unpredictable adoption by different conspecifics exposed to the same conditions suggests that other determining factors play a role. Here, we show that individuals of the trematode *Coitocaecum parvum* following the 'normal' three-host life cycle ending in a fish host harbour a microbiome with subtle differences from that of conspecifics adopting a shorter two-host cycle with progenesis in the second intermediate host. Following earlier evidence that an individual's microbiome can shape its phenotype [15–17,36], our findings raise the question of whether symbiotic bacteria could influence the transmission route followed by parasites.

Our results indicate that *C. parvum* harbours a microbial community distinct from that of the external environment, a finding that supports an earlier study demonstrating that this trematode has a core microbiome different from that of its hosts or the environment [21]. The minor differences in microbial community composition between individuals sampled 2 years apart suggest the microbiome is resilient to major temporal changes in climatic and environmental conditions. There were nonetheless differences in the composition of microbial communities among individual parasites, even within the same morph (normal or progenetic). This can be explained by the imperfect vertical transmission of bacteria to the infective stages (cercariae) during the asexual proliferation stage within snail hosts, resulting in some cercariae inheriting certain bacterial taxa and others missing out [37,38], or by inter-individual variation in bacteria acquisition from the environment.

There were subtle differences between normal and progenetic metacercariae, however, with respect to the relative abundance of particular bacterial taxa, whether the metacercariae shared the same individual host or not. These differences likely arose prior to entry into the amphipod host, or shortly after. Indeed, within days of infecting their second intermediate host, metacercariae produce a cyst surrounding their body [39]. This consists of a thin and flexible envelope in species like *C. parvum*, or a thicker, double-walled cyst as in the frequently co-infecting *Maritrema poulini*. Regardless of the nature of the cyst, it is likely impermeable to bacteria. This means that bacterial communities develop in isolation within the encysted parasite, also explaining why the co-occurrence of *M. poulini* had no effect on *C. parvum*'s microbial community: exchanges of bacteria from host to parasite, or among parasites, are not possible following encystment.

Differences in the abundance of certain bacterial taxa between normal and progenetic metacercariae may be explained by the larger body size (roughly 5–10-fold) of the latter, as body size can modulate bacterial diversity and abundance [40]. Metastat analyses revealed that all 14 bacterial phyla detected in the metacercariae, and two-thirds of bacterial families, differed in relative abundance between the two morphs. However, body size does not explain everything: abundance was sometimes higher in progenetic metacercariae, and sometimes in normal ones, depending on the bacterial taxon (electronic supplementary material, tables S1 and S2). The real question is whether or not these differences in bacterial communities determine whether a metacercaria follows the normal three-host pathway, or achieves early maturity via progenesis and truncates its life cycle to two hosts. Natural selection might favour symbiotic microbes capable of influencing the trematode's development if their own transmission and fitness depend on what the parasite does. For instance, opportunities for horizontal transmission of bacteria (to fish) are only possible with the normal three-host cycle. However, too little is known about the functional genomics of these bacteria and their influence on developmental processes to speculate on their effects on trematode biology. Furthermore, the evidence we provide here is correlative. Demonstrating a causal relationship would require the experimental manipulation of microbiomes followed by monitoring of metacercarial development. Antibiotics can be used to alter microbial communities within trematodes [41]; however, this can only be achieved by targeting broad bacterial types, e.g. Gram-negative or Gram-positive taxa. Precisely targeting specific taxa only, such as those identified here, remains logistically impossible.

In summary, our findings reveal that alternative life cycle pathways involve more than differences in the timing of maturation: the two developmental morphs possess slightly different microbiomes. Although the evidence is correlative and the association with developmental pathways is weak, subtle differences in symbiotic bacteria may be associated with slightly different arrays of gene products that might influence other aspects of the parasite's biology and its impact on hosts. These findings reinforce earlier calls [18,19] for the inclusion of microbes into an integrated framework for the study of host–parasite interactions.

Data accessibility. Data and scripts used for the analyses are available as part of the electronic supplementary material [42].

Authors' contributions. P.M.S.: data curation, formal analysis, investigation, methodology, visualization and writing—original draft; F.J.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources and writing—review and editing; N.M.D.: conceptualization, funding acquisition, methodology and writing—review and editing; R.P.: conceptualization, funding acquisition, project administration, supervision and writing—original draft.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare having no conflict of interest.

Funding. This research was funded by grants UOO1718 to R.P. and N.M.D., and UOO2113 to R.P. and F.J., from the Marsden Fund (Royal Society of New Zealand).

Acknowledgements. We acknowledge the use of the New Zealand eScience Infrastructure (NeSI) high-performance computing facilities (https://www.nesi.org.nz). This study is part of the Parasite Microbiome Project.

References

1. Poulin R. 2011 The many roads to parasitism: a tale of convergence. *Adv. Parasitol.* **74**, 1–40. (doi:10.1016/B978-0-12-385897-9.00001-X)
2. Brown SP, Renaud F, Guégan JF, Thomas F. 2001 Evolution of trophic transmission in parasites: the need to reach a mating place? *J. Evol. Biol.* **14**, 815–820. (doi:10.1046/j.1420-9101.2001.00318.x)
3. Choisy M, Brown SP, Lafferty KD, Thomas F. 2003 Evolution of trophic transmission in parasites: why add intermediate hosts? *Am. Nat.* **162**, 172–181. (doi:10.1086/375681)
4. Parker GA, Chubb JC, Ball MA, Roberts GN. 2003 Evolution of complex life cycles in helminth parasites. *Nature* **425**, 480–484. (doi:10.1038/nature02012)
5. Benesh DP, Parker G, Chubb JC. 2021 Life-cycle complexity in helminths: what are the benefits? *Evolution* **75**, 1936–1952. (doi:10.1111/evo.14299)
6. Poulin R, Cribb TH. 2002 Trematode life cycles: short is sweet? *Trends Parasitol.* **18**, 176–183. (doi:10.1016/S1471-4922(02)02262-6)
7. Lefebvre F, Poulin R. 2005 Progenesis in digenean trematodes: a taxonomic and synthetic overview of species reproducing in their second intermediate host. *Parasitology* **130**, 587–605. (doi:10.1017/S0031182004007103)
8. Kasl EL, Font WF, Criscione CD. 2018 Resolving evolutionary changes in parasite life cycle complexity: molecular phylogeny of the trematode genus *Alloglossidium* indicates more than one origin of precociousness. *Mol. Phyl. Evol.* **126**, 371–381. (doi:10.1016/j.ympev.2018.04.027)
9. Holton AL. 1984 Progenesis as a means of abbreviating life histories in two New Zealand trematodes, *Coitocaecum parvum* Crowcroft, 1945 and *Stegodexamene anguillae* Macfarlane, 1951. *Mauri Ora* **11**, 63–70.
10. Lagrue C, Poulin R, Keeney DB. 2009 Effects of clonality in multiple infections on the life-history strategy of the trematode *Coitocaecum parvum* in its amphipod intermediate host. *Evolution* **63**, 1417–1426. (doi:10.1111/j.1558-5646.2009.00619.x)
11. Poulin R. 2003 Information about transmission opportunities triggers a life history switch in a parasite. *Evolution* **57**, 2899–2903.
12. Lagrue C, Poulin R. 2007 Life cycle abbreviation in the trematode *Coitocaecum parvum*: can parasites adjust to variable conditions? *J. Evol. Biol.* **20**, 1189–1195. (doi:10.1111/j.1420-9101.2006.01277.x)
13. Lagrue C, Poulin R. 2008 Intra- and interspecific competition among helminth parasites: effects on *Coitocaecum parvum* life-history strategy, size and fecundity. *Int. J. Parasitol.* **38**, 1435–1444. (doi:10.1016/j.ijpara.2008.04.006)
14. Lagrue C, Poulin R. 2009 Life cycle abbreviation in trematode parasites and the developmental time hypothesis: is the clock ticking? *J. Evol. Biol.* **22**, 1727–1738. (doi:10.1111/j.1420-9101.2009.01787.x)
15. Feldhaar H. 2011 Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecol. Entomol.* **36**, 533–543. (doi:10.1111/j.1365-2311.2011.01318.x)
16. Leclair M, Pons I, Mahéo F, Morlière S, Simon JC, Outreman Y. 2016 Diversity in symbiont consortia in the pea aphid complex is associated with large phenotypic variation in the insect host. *Evol. Ecol.* **30**, 925–941. (doi:10.1007/s10682-016-9856-1)
17. Takacs-Vesbach C, King K, Van Horn D, Larkin K, Neiman M. 2016 Distinct bacterial microbiomes in sexual and asexual *Potamopyrgus antipodarum*, a New Zealand freshwater snail. *PLoS ONE* **11**, e0161050. (doi:10.1371/journal.pone.0161050)
18. Dheilly NM, Poulin R, Thomas F. 2015 Biological warfare: microorganisms as drivers of host–parasite interactions. *Infect. Genet. Evol.* **34**, 251–259. (doi:10.1016/j.meegid.2015.05.027)
19. Dheilly NM *et al.* 2019 Parasite Microbiome Project: grand challenges. *PLoS Path.* **15**, e1008028. (doi:10.1371/journal.ppat.1008028)
20. Hahn MA, Piecyk A, Jorge F, Cerrato R, Kalbe M, Dheilly NM. 2022 Host phenotype and microbiome vary with infection status, parasite genotype, and parasite microbiome composition. *Mol. Ecol.* **31**, 1577–1594. (doi:10.1111/mec.16344)
21. Jorge F, Dheilly NM, Poulin R. 2020 Persistence of a core microbiome through the ontogeny of a multi-host parasite. *Front. Microbiol.* **11**, 954. (doi:10.3389/fmicb.2020.00954)
22. Jorge F, Dheilly NM, Froissard C, Poulin R. 2022 Association between parasite microbiomes and caste development and colony structure in a social trematode. *Mol. Ecol.* **31**, 5608–5617. (doi:10.1111/mec.16671)
23. Martinson VG, Gawryluk RMR, Gowen BE, Curtis CI, Jaenike J, Perlman SJ. 2020 Multiple origins of obligate nematode and insect symbionts by a clade of bacteria closely related to plant pathogens. *Proc. Natl Acad. Sci. USA* **117**, 31 979–31 986. (doi:10.1073/pnas.2000860117)
24. Andrew S. 2010 FastQC: a quality control tool for high throughput sequence data. See https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
25. Martin M. 2011 Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* **17**, 10–12. (doi:10.14806/ej.17.1.200)
26. Bolyen E *et al.* 2019 Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857. (doi:10.1038/s41587-019-0209-9)
27. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. 2016 DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583. (doi:10.1038/nmeth.3869)
28. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO. 2013 The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596. (doi:10.1093/nar/gks1219)
29. R Core Team. 2022 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
30. McMurdie PJ, Holmes S. 2013 phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**, e61217. (doi:10.1371/journal.pone.0061217)
31. Liu C, Cui Y, Li X, Yao M. 2021 microeco: An R package for data mining in microbial community ecology. *FEMS Microbiol. Ecol.* **97**, fiaa255. (doi:10.1093/femsec/fiaa255)
32. Koh H, Zhan LX, Chen J, Zhao N. 2019 A distance-based kernel association test based on the generalized linear mixed model for correlated microbiome studies. *Front. Genet.* **10**, 458. (doi:10.3389/fgene.2019.00458)
33. White JR, Nagarajan N, Pop M. 2009 Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLoS Comput. Biol.* **5**, e1000352. (doi:10.1371/journal.pcbi.1000352)
34. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011 Metagenomic biomarker discovery and explanation. *Genome Biol.* **12**, R60. (doi:10.1186/gb-2011-12-6-r60)
35. Martin BD, Witten D, Willis AD. 2022 corncob: Count Regression for Correlated Observations with the Beta-Binomial. See https://CRAN.R-project.org/package=corncob.
36. Strunov A, Lerch S, Blanckenhorn WU, Miller WJ, Kapun M. 2022 Complex effects of environment and *Wolbachia* infections on the life history of *Drosophila melanogaster* hosts. *J. Evol. Biol.* **35**, 788–802. (doi:10.1111/jeb.14016)
37. Greiman SE, Tkach VV, Vaughan JA. 2013 Transmission rates of the bacterial endosymbiont, *Neorickettsia risticii*, during the asexual reproduction phase of its digenean host, *Plagiorchis elegans*, within naturally infected lymnaeid snails. *Parasit. Vect.* **6**, 303. (doi:10.1186/1756-3305-6-303)
38. Greiman SE, Tkach VV. 2016 The numbers game: quantitative analysis of *Neorickettsia* sp. propagation through the complex life cycle of its digenean host using realtime qPCR. *Parasitol. Res.* **115**, 2779–2788. (doi:10.1007/s00436-016-5027-0)
39. Galaktionov KV, Dobrovolskij AA. 2003 *The biology and evolution of trematodes*. Dordrecht, The Netherlands: Kluwer Academic Publishers.
40. Godon JJ, Arulazhagan P, Steyer JP, Hamelin J. 2016 Vertebrate bacterial gut diversity: size also matters. *BMC Ecol.* **16**, 12. (doi:10.1186/s12898-016-0071-2)
41. Jorge F, Froissard C, Dheilly NM, Poulin R. 2022 Bacterial community dynamics following antibiotic exposure in a trematode parasite. *Int. J. Parasitol.* **52**, 265–274. (doi:10.1016/j.ijpara.2021.11.006)
42. Salloum PM, Jorge F, Dheilly NM, Poulin R. 2023 Adoption of alternative life cycles in a parasitic trematode is linked to microbiome differences. Figshare. (doi:10.6084/m9.figshare.c.6673606)