Hydrobiologia
https://doi.org/10.1007/s10750-022-04931-8

AQUATIC MICROBIOMES

Stenostomum leucops (Catenulida, Platyhelminthes) has a flexible microbiome in time and space

Marcos T. Rosa · Elgion L. S. Loreto

Received: 1 January 2022 / Revised: 16 June 2022 / Accepted: 20 June 2022

Abstract Multicellular organisms and their microbiomes can have a restricted and enduring relationship, sometimes reflected in their phylogenies, called phylosymbiosis. However, in some organisms, such as freshwater zooplankton, these relationships appear to be more flexible and more easily influenced by the environment. Here, we analysed the microbiome of a freshwater flatworm, *Stenostomum leucops* and sequenced the 16S RNA gene of the microbiota of a strain that was maintained in the laboratory for 12 years. This strain underwent four different cultivation conditions over the past 6 years, and the microbiome was characterised for each of these conditions. In addition, the microbiome of a wild *S. leucops* population was analysed. The microbiomes were highly variable between populations (Shannon index ranging from 0.26 to 2.06). It was not possible to determine a core microbiome, although *Bacillus, Pseudomonas* and *Ralstonia* were the predominant bacteria in populations under stable conditions. Under culture conditions where the water was contaminated with iron, *Rhodoferax ferrireducens,* a bacterium involved in iron reduction, was the predominant species. Our results are consistent with other studies on freshwater zooplankton. The microbiomes were very flexible and were influenced by the environment.

Keywords Phylosymbiosis · Microbiome · Iron contamination · Bacterial succession

Guest Editors: Isa Schön, Diego Fontaneto & Elena L. Peredo / Aquatic Microbiomes

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10750-022-04931-8.

M. T. Rosa
Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Santa Maria, Av. Roraima, 1000, Santa Maria, RS CEP 97105-900, Brazil
e-mail: marcostrindadebio@gmail.com

E. L. S. Loreto (✉)
Department of Biochemistry and Molecular Biology, Universidade Federal de Santa Maria, Av. Roraima, 1000, Santa Maria, RS CEP 97105-900, Brazil
e-mail: elgion@base.ufsm.br

Introduction

Multicellular eukaryotes always live in an environment full of microbes and interact with them. Often these interactions are very strong, and the organisms involved are completely interdependent. In extreme cases, for example, the bacteria become intracellular components of the host, as in some annelids, molluscs, and platyhelminths, where the bacteria are held in a specialised cell organelle, the trophosome, and provide energy to the host (Dubilier et al., 2008). In other cases, the microbe–host association involves the exchange of various materials such as nutrients, probiotics, and secondary metabolites (Phillips et al., 2012; Moeller et al., 2014). Indeed, at least for some

Published online: 12 July 2022

organisms, these associations are relevant to their fitness and evolution, once they are maintained throughout evolution and can be demonstrated through phylosymbiosis as defined by Lin and Bordenstein (2020) as "microbial community relationships that recapitulate the phylogeny of their host."

Phylosymbiosis is observed in several animal taxa. For example, in scleractinian corals (Pollock et al., 2018; Gault et al., 2021), sponges (Easson & Thacker, 2014), molluscs (Huot et al., 2020), and various insects such as bees (Figueroa et al., 2021), ants (Sanders et al., 2014), wasps, and *Drosophila* (Brooks et al., 2016). In addition, it has also been described in mammals such as mice, bats, and primates (Phillips et al., 2012; Moeller et al., 2014; Sanders et al., 2014). However, no phylosymbiosis signal has been found for several organisms such as birds (Hird et al., 2015; Kropáčková et al., 2017), Rotifera and Crustacea (Eckert et al., 2020, 2021). This last study showed that freshwater zooplankton shows no evidence of phylosymbiosis; in contrast, the microbiome of these organisms is very flexible and can be influenced by the external environment. Therefore, the extent of functional and evolutionary constraints affecting the microbiome and its relationships with its hosts is still an open question, and at this stage, further research on a wide variety of organisms is needed to answer this question.

The Catenulida is a basal group of Platyhelminthes, represented by around 95 described species of small flatworms, mostly freshwater, ranging in length from 0.5 to 5 mm (Noreña et al., 2005; Balsamo et al., 2020). They reproduce asexually by a process called paratomy, in which the development of structures typical of the anterior region in posterior parts of the body occurs, and after complete development, fission produces two or more individuals (Rieger, 1986; Noreña et al., 2005). Among Catenulida, representatives of the marine genus *Paracatenula* have a restricted relationship with some bacteria. Chemoautotrophic bacteria of the genus candidatus *Riegeria* are an obligate symbiont in the trophosomes of Paracatenula, accounting for half of the biomass of these worms and providing the primary energy source (Jäckle et al., 2019). Another Catenulida, *Stenostomum leucops*, also has a particular interaction with its microbiome that is thought to modulate phenotypic plasticity (Rosa et al., 2015). In addition, these worms can express genes that are maintained by their microbiome. At the very least, the worms express a green fluorescent protein (GFP) when they harbour bacteria containing plasmids with the GFP gene (Rosa & Loreto, 2019).

In this study, we examined the microbiome associated with *S. leucops* using a descriptive approach in which the microbiome was assessed over time by examining a laboratory strain over a six-year period in that the strain underwent four different cultivation procedures. In addition, the microbiome of the laboratory population was compared to a population recently collected in the wild. Furthermore, the microbiome associated with the worms was compared to that found in the environment where the worms live.

Materials and methods

Animals used and their maintenance

Two different sources of *Stenostomum leucops* were used for this study: (i) a wild population freshly collected from a pond used for fish farming, at the Federal University of Santa Maria, Santa Maria, Brazil (53° 17′ W; 29° 28′ S). One litre of water was taken from the edge of the pond, where the aquatic plants were squeezed to increase the probability of collecting *Stenostomum* that were on the plants, and (ii) a clonal isolation that was maintained in the laboratory for more than 12 years at 28 °C in reconstituted water and fed with milk powder. The morphological, DNA barcoding, and reproductive characteristics of these strains, as well as the maintenance procedures, were described in Rosa et al. (2015) and Rosa and Loreto (2021). The microbiome of the laboratory strain was established over time at four different time points (Fig. 1). The environmental microbial community (background) of extant populations was also evaluated.

Some problems were encountered during maintenance in the laboratory. For the first 8 years, the worms were maintained with reconstituted water, made with distilled water, and the animals were easy to keep (Fig. 1). During this time, a selection was performed, transferring only worms to a new culture to obtain cultures with only *S. leucops* and its microbiome. In 2017, the strain suddenly passed through a bottleneck and became more difficult to

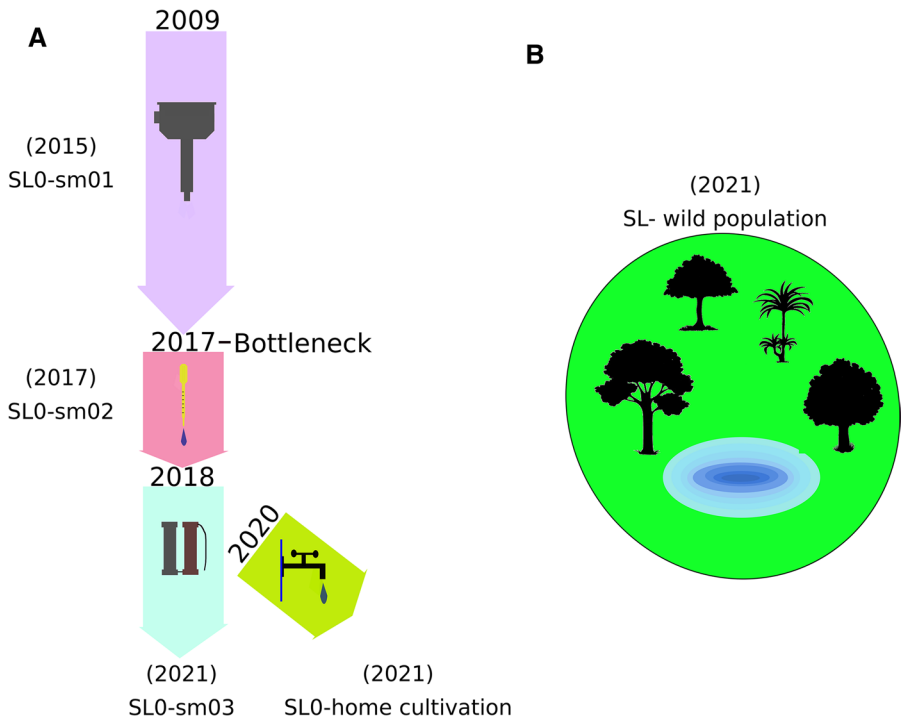

Fig. 1 **A** A laboratory *Stenostomum leucops* isoline and its maintenance phases: 2009–2017, with distilled water under selection, in which only the worms were transferred for new cultures for a cleaner culture of other zooplankton (SLO-sm01); in 2017, a bottleneck occurred and "new" bacterial sources were added to save the vitality of the culture (SLO-sm02); in 2018, iron contamination was detected in the water and the culture became to be maintained with deionized water (SLO-sm03). In 2020, a subpopulation was maintained at home with tap water (SLO-hc). Microbiomes were sequenced in 2015 (SLO-sm01), 2017 (SLO-sm02) and 2021 (SLO-sm03 and SLO-home cultivation). **B** A wild population was collected in September 2021 in a pond at the Federal University of Santa Maria, Santa Maria, Brazil (53° 17′ W; 29° 28′ S). These specimens were identified according to Noreña et al. (2005) and by DNA barcoding according to Rosa et al. (2015). Fresh animals were used for DNA isolation and used for barcoding and sequencing of the microbiome (2021)

maintain. To restore the vitality of the strain, we supplemented the culture with water from a dam (the same dam where the strain was collected). First, the water was filtered with a qualitative cellulose filter (Whatman 1004). Then, the water was centrifuged at 150 RCF for 5 min, and the supernatant was added to the culture aiming to improve the microbiota of the worms. The purpose of centrifugation was to prevent other worms from contaminating the culture. Iron contamination was found to be the cause of the bottleneck, and in 2018, deionized water was started to maintain the culture to produce the reconstituted water. The vitality of the strain increased. In 2020, due to the Sars-COV2 pandemic, we decided to maintain a subculture at home using tap water.

Microbiome analyses

For microbiome analyses, total DNA was isolated following the chymosin + silica particle protocol described by Oliveira et al. (2009). For each of the strains, a pool of 100 individuals freshly collected in culture was used. Worms were collected in the culture plates using a micropipette and first transferred to a drop of reconstituted water to minimise contamination with environmental microorganisms. They were then transferred to a microtube containing extraction buffer for DNA isolation. For environmental microbial community analysis, 25 ml of the culture water was filtered with Whatman 41 filter paper to the exclusion of worms and the bacteria were centrifuged at 1008 RCF for 5 min. The pellet

was resuspended in 2 ml of lysis buffer and used for DNA isolation.

Bacteria were identified by high-performance sequencing of the V3/V4 regions of the 16S rRNA gene. Amplification was performed using primers for the V3-V4 region of the 16S rRNA gene, 341F (CCTACGGGRSGCAGCAG; Wang & Qian, 2009) and 806R (GGACTACHVGGGTWTCTAAT; Caporaso et al., 2012). Libraries were sequenced using MiSeq Sequencing System instruments (Illumina Inc., USA). A MiSeq Reagent Kit v3 (600 cycles) was used for sequencing to generate paired-end reads (Neoprospecta Microbiome Technologies, Brazil).

The microbiome sequencing reads are available under the following NCBI accession numbers: SAMN12561444 (*Stenostomum leucops* Sl0-sm01); SAMN12561445 (*S. leucops* Sl0-sm02); SAMN24102714 (*S. leucops* SL0-sm03); SAMN24102715 (*S. leucops* SL0-hc); and SAMN24102716 (*S. leucops* SL0-wp).

For bioinformatics analysis, reads were first processed in Fastqc (Andrews 2010), using only reads with quality greater than 30. The first 5 nucleotides and the last 10 to 25 were removed using Trimgalore 0.6.7 (https://github.com/FelixKrueger/TrimGalore/releases/tag/0.6.7).

For microbiome classification based on 16S sequences, DADA2 (Callahan et al., 2016) was used and performed in the Galaxy platform (https://usegalaxy.org/). Mergepairs were performed using 12 as the overlap number. To assign Taxonomy and add Species assignment, bootstrap 50 was used as the minimum value, with complementary reverse option for comparison with the 16S sequences from the Silva database (2020 nr99_v138) (Quast et al., 2013). To characterise the predominant microbiome in populations, only bacterial species that had more than 100 reads in the sequencing analysis were analysed.

Determination of the iron content in water

For the determination of iron, an internal calibration curve with a linear calibration range of 1 to 100 µg/L was established using the SCP Science Kit. The instrument used was an inductively coupled plasma optical emission spectrometer (model Optima 4300 DV, Perkin Elmer, USA). The wavelengths monitored were 238.204 nm and 239.562 nm.

Results

Microbiome analyses

The number and size of reads used for microbiome estimation for each population can be found in Supplementary Table 1. The smaller dataset for SLO-sm01 contained 16,905 reads, and the larger dataset for SLwild_population contained 87,698 reads. Microbiome diversity analysis (Table 1) showed that SLO_sm01 had a reduced diversity (Shannon index = 0.26), whereas the other populations had a higher diversity (1.06 to 2.06). The populations showing higher diversity had distinct bacterial communities (β diversity index = 5.73). However, the Evenness index (J'), which ranged from 0.06 to 0.46, showed that there were a few highly dominant species and a large number of rare species.

The microbiomes found in *Stenostomum leucops* in the current laboratory population as well in the home cultivation and wild populations differed from those of the background environmental microbial community (Figs. 2A–C, 3), although the worms share some bacterial species with the environment. The microbial community in the *S. leucops* laboratory populations (SL0_sm01, SL0_sm02 and SL0_sm03) also differed from each other (Figs. 2D, 3). The current laboratory population, the home cultivation population and

Table 1 Shannon diversity index (H'); evenness index (J') and β diversity index for *Stenostomum leucops* populations analysed

	SLO_sm01	SLO_sm02	SLO_sm03	SLO_wp	SLO_hc	SLO_sm03_ background	SLO_wp_ background	LO_hc_ background
H'	0,26	2,06	1,06	1,79	1,58	1,78	1,87	1,91
J'	0,06	0,46	0,23	0,40	0,35	0,40	0,42	0,43
β'	5,73							

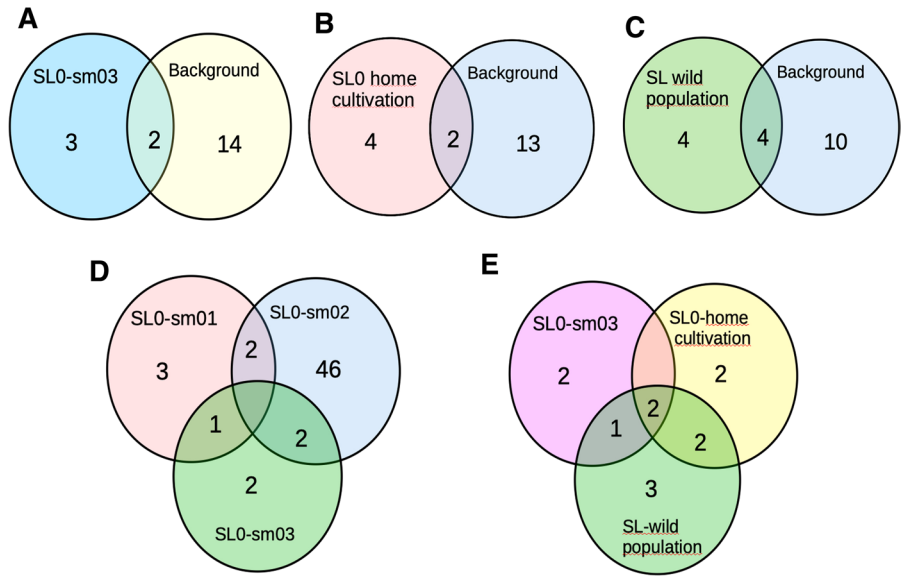

Fig. 2 Venn diagram comparing bacteria in worm populations. **A** number of bacterial amplicon sequence variants (**ASVs**) in the laboratory population (SL0_sm03) and its environmental microbial community (background); **B** bacteria in home cultivation and background; **C** bacteria in the wild and background; **D** number of bacterial OTUs in the laboratory populations (SL0_sm01, SL0_sm02, and SL0_sm03); **E** number of bacterial OTUs in extant populations (SL0_sm03, SL0_wp, and SL0_hc)

the sample obtained in the wild also showed distinct microbiomes (Figs. 2E, 3). A complete list of species (OTUs), their abundance, and frequency for each population can be found in the Supplementary spreadsheet. The laboratory population (SLO_sm01) had seven species when analysed after 6 years of laboratory maintenance (Fig. 2). After a bottleneck, we were forced to add new bacteria to avoid losing the worm strain (SLO_sm02); the number of bacterial species increased to 50, two of which were shared with the strain before the bottleneck. After 4 years of laboratory maintenance (SLO_sm03), this number decreased to 5, of which 2 were shared with SLO_sm02. Maintenance for 2 years using tap water (home cultivation) changed the diversity of microbiome, increasing to 6 bacterial OTUs. The comparison of microbiomes of extant populations, the laboratory population (SLO_sm03), the home cultivation (SLO_hc) population, and the wild population (SLO_wp), can be found in Fig. 2 E. A total of 2 bacterial OTU were shared in three populations. The wild population had 8 species, and 2 were shared with SLO_hc and 1 with SLO_sm03.

The predominant microbiome in the populations, i.e. the OTUs with abundance greater than 100, is shown in Table 2. In general, these species accounted for 95.3 to 100% of the reads obtained. None of the predominant species was represented in all populations. However, *Bacillus* was the predominant bacterial species in the first laboratory population (SLO_sm01) and was absent among the predominant species during the bottleneck (SLO_sm02). In addition, this bacterial species was also present in the wild population (SLO_wp) and in the home cultivation population (SLO_hc). Other predominant bacteria present in the home cultivation (SLO_hc), wild population, and extant laboratory populations (SLO_sm03) were *Pseudomonas* and *Ralstonia*. The bacterial species overrepresented during the bottleneck was *Rhodoferax ferrireducens* (42.7% of reads). Although this species is present in the current population (SLO_sm03), it accounts for only 0.1% of the reads.

Determination of iron content in water

During the maintenance of the laboratory, we had difficulty growing the worms, which we related to the iron contamination of the pipeline water. In addition, during this period, the most common bacterium in the worm microbiome was Rhodoferax ferrireducens, which is involved in iron reduction; therefore, we determined the iron content in some water sources (Table 3). In the tap water of the house and in the deionized water of the laboratory, the iron content was less than 5 µg L^{-1}; the water from the laboratory tap and the water from the dam contained more than 800 µg L^{-1}

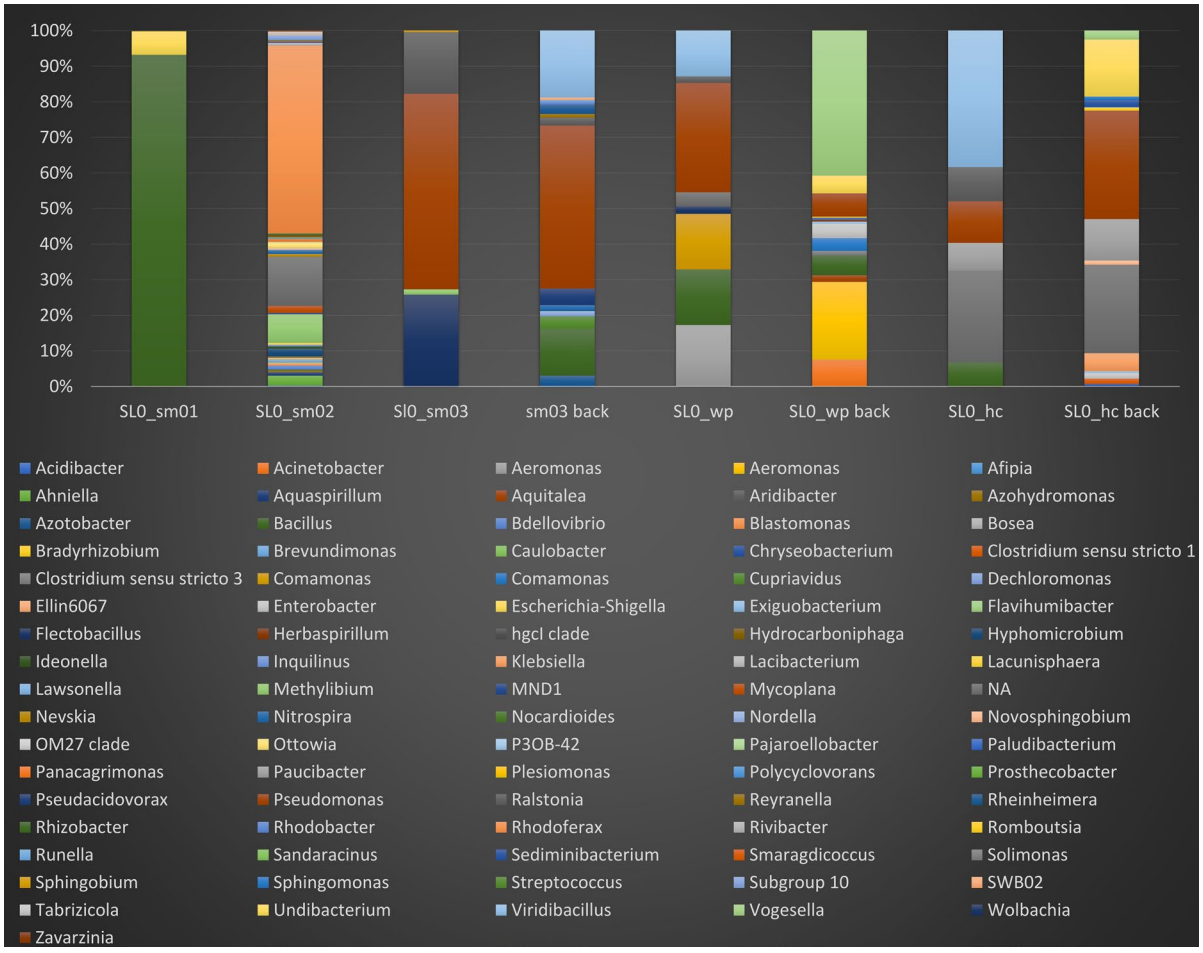

Fig. 3 Bacterial abundance in each population studied and in the environmental microbial community (background). The colours represent the different bacterial OTUs, and the height on the y-axis represents the respective abundance. Laboratory populations = SL0_sm01, SL0_sm02, and SL0_sm03; home cultivation = SL0_hc; wild population = SL0_wp. Background = sm03 back, SL0_hc back, SL0_wp back

Discussion

It is now clear that the microbiome plays an important role in the biology of its host (Bordenstein & Theis, 2015), although our current knowledge suggests that different organisms respond differently to this host–microbiome association in terms of physiology, stress tolerance, fitness, and evolvability. Organisms, such as corals, are closely linked to their microbiome, but also show some flexibility in this link to rapidly adapt to environmental changes (Voolstra & Ziegler 2020). Few studies have focused on freshwater zooplankton and have examined Crustacea (Cladocera and Copepoda) and Rotifera, which have very flexible microbiomes (Eckert et al., 2020, 2021; Akbar et al., 2022). The freshwater flatworm *S. leucops* exhibited a flexible microbiome, similar to the other freshwater zooplankton species studied to date.

The microbiome of the *Stenostomum leucops* laboratory strain followed over time or in different cultures was very flexible, even compared to a wild population. Although some bacteria are shared between the worms and the environment, the microbiome of the animals showed marked differences from the environmental bacterial community. When the most abundant bacteria were analysed, no core microbiome was found, although in the "stable" laboratory populations, which were easy to maintain and proliferative

Table 2 Predominant microbiome found in different *Stenostomum leucops* populations

SL0_sm01			SL0_sm02			SL0_sm03			SL0_hc (home)			SL0_wp (wild)		
Genus	Abundance	%	Genus	Abundance	%	Genus	Abundance	%	Genus	Abundance	%	Genus	Abundance	%
Bacillus	13983	93,2	*Rhodoferax*	13766	52,2	*Pseudomonas*	20420	55,0	*Viridibacillus*	16742	38,3	*Pseudomonas*	12373	30,7
Escherichia-Shigella	986	6,57	NA	3593	13,6	*Flectobacillus*	9615	25,9	NA	11259	25,8	*Aeromonas*	6974	17,3
			Methylibium	2106	8,0	*Ralstonia*	6434	17,3	*Pseudomonas*	5092	11,6	*Comamonas*	6312	15,7
			Ahniella	723	2,7	*Methylibium*	550	1,5	*Ralstonia*	4213	9,6	*Bacillus*	6295	15,6
			Hyphomicrobium	582	2,2	*Sphingobium*	130	0,3	*Paucibacter*	3425	7,8	*Viridibacillus*	5174	12,8
			Mycoplana	516	2,0				*Bacillus*	2986	6,8	*NA*	1616	4,0
			Ottowia	390	1,5							*Flectobacillus*	834	2,1
			Subgroup 10	352	1,3							*Ralstonia*	751	1,8
			Ottowia	303	1,1									
			Bdellovibrio	297	1,1									
			Nitrospira	249	0,9									
			Tabrizicola	219	0,8									
			Panacagrimonas	217	0,8									
			Aquaspirillum	211	0,8									
			Nevskia	206	0,8									
			Brevundimonas	201	0,8									
			Rivibacter	201	0,8									
			Ideonella	156	0,6									
			Rhizobacter	146	0,6									
			Blastomonas	132	0,5									
			Inquilinus	116	0,4									
			Lacunisphaera	116	0,4									
			Azohydromonas	114	0,4									
			Ellin6067	106	0,4									
			Pseudomonas	101	0,4									
Total	**15003**	**!""**		**26359**	**95.30**		**37149**	**100,0**		**43717**	**100,0**		**40329**	**100,0**

Table 3 Determination of iron content in water

Sample	Iron concentration—µg L^{-1}
Laboratory (pipeline water)	813
Laboratory deionized water	< 5
House (tap water)	< 5
Dam water (where was collected the wild population)	987

(SLO_sm01 and SLO_sm03), and in the wild population (SLO_wp), the most abundant bacterial species were *Bacillus* and *Pseudomonas*. However, in the home-kept population (SLO_hc), which also proliferated rapidly, the most abundant bacteria were *Pseudomonas.* In the current laboratory population (SLO_sm03), the home cultivation population, and the wild population, *Pseudomonas* and *Ralstonia* were also abundant.

In population SLO_sm02, which suffered a severe bottleneck due to iron contamination in the tap water of the laboratory building, the predominant bacterial species was *Rhodoferax ferrireducens.* This bacterial species can support growth by reducing Fe (III) (Finneran et al., 2003). However, during the period when this bacterial species was prevalent, it was difficult to maintain the *Stenostomum* population; although it had greater species diversity in the microbiome, the bacteria that were abundant in the "stable" laboratory populations were absent in SLO_sm02. Changes in the microbiota due to various chemicals can negatively affect host fitness and physiology (Brüssow, 2015; Jin et al., 2017; Pennycook & Scanlan, 2021). The water in the pond where the wild population (SLO_wp) was collected also had high iron concentrations, three times the limit for dissolved iron in drinking water (World Health Organization, 2008). However, *Rhodoferax ferrireducens* was not present in the SLO_wp sample. Thus, these results suggest that iron alone was not the stressor for *Stenostomum,* but that the microbiome affected by this metal promotes difficulties in laboratory husbandry of this species.

In conclusion, the *S. leucops* microbiome was variable over time in both a laboratory strain and a wild population. However, we can register here as a case report that the wild population and the laboratory population, in moments that were “easy” to maintain, shared several bacteria, such as *Bacillus*, *Pseudomonas*, *Flectobacillus*, and *Ralstonia*. These last results indicate that *S. leucops* is a promissory organism to perform experiments with microbiome shift.

Acknowledgements We thank Neoprospecta Microbiome Technologies for assistance in the sequencing. This study was

supported by research grants and fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq (421255/2018-8 and 305613/2020-0), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Declarations

Conflict of interest No potential conflict of interest was reported by the authors.

Funding Funding was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (305613/2020-0), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

References

Akbar, S., L. Gu, Y. Sun, L. Zhang, K. Lyu, Y. Huang & Z. Yang, 2022. Understanding host-microbiome-environment interactions: insights from Daphnia as a model organism. Science of the Total Environment 808: 152093.

Andrews. S., 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Balsamo, M., T. Artois, J. P. S. Smith, et al., 2020. The curious and neglected soft-bodied meiofauna: Rouphozoa (Gastrotricha and Platyhelminthes). Hydrobiologia 847: 2613–2644.

Bordenstein, S. R. & K. R. Theis, 2015. Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS Biology 13: e1002226.

Brooks, A. W., K. D. Kohl, R. M. Brucker, E. J. van Opstal & S. R. Bordenstein, 2016. Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. PLoS Biology 14: e2000225.

Brüssow, H., 2015. Growth promotion and gut microbiota: insights from antibiotic use. Environmental Microbiology 17: 2216–2227.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson & S. P. Holmes, 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods 13: 581–583.

Caporaso, J., C. Lauber, Walters, et al., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6: 1621–1624.

Dubilier, N., C. Bergin & C. Lott, 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nature Review Microbiology 6: 725–740.

Easson, C. G. & R. W. Thacker, 2014. Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges. Frontiers in Microbiology 5: 532.

Eckert, E., S. Amalfitano, A. Di Cesare, C. Manzari, G. Corno & D. Fontaneto, 2020. Different substrates within a lake harbour connected but specialised microbial communities. Hydrobiologia 847: 1689–1704.

Eckert, E. M., N. Anicic & D. Fontaneto, 2021. Freshwater zooplankton microbiome composition is highly flexible and strongly influenced by the environment. Molecular Ecology 30: 1545–1558.

Figueroa, L. L., J. J. Maccaro, E. Krichilsky, D. Yanega & Q. S. McFrederickc, 2021. Why did the bee eat the chicken? Symbiont gain, loss, and retention in the vulture bee microbiome. Mbio 12: e02317-e2321.

Finneran, K. T., C. V. Johnsen & D. R. Lovley, 2003. Rhodoferax ferrireducens sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). International Journal of Systematic and Evolutionary Microbiology. 53: 669–673.

Gault, J. A., B. Bentlage, D. Huang & A. M. Kerr, 2021. Lineage-specific variation in the evolutionary stability of coral photosymbiosis. Science Advandces 7: eabh4243.

Hird, S. M., C. Sánchez, B. C. Carstens & R. T. Brumfield, 2015. Comparative gut microbiota of 59 neotropical bird species. Frontiers in Microbiology 6: 1403.

Huot, C., C. Clerissi, B. Gourbal, R. Galinier, D. Duval & E. Toulza, 2020. Schistosomiasis vector snails and their microbiota display a phylosymbiosis pattern. Frontiers in Microbiology 10: 3092.

Jäckle, O., B. K. B. Seah, M. Tietjen, N. Leisch, M. Liebeke, M. Kleiner, J. S. Berg & H. R. Gruber-Vodicka, 2019. Chemosynthetic symbiont with a drastically reduced genome serves as primary energy storage in the marine flatworm Paracatenula. Proceeding of National Academy of Science USA 116: 8505–8514.

Jin, Y., S. Wu, Z. Zeng & Z. Fu, 2017. Effects of environmental pollutants on gut microbiota. Environmental Pollution 222: 1–9.

Kropáčková, L., M. Těšický, T. Albrecht, J. Kubovčiak, D. Čížková, O. Tomášek, J. F. Martin, L. Bobek, T. Králová, P. Procházka & J. Kreisinger, 2017. Codiversification of gastrointestinal microbiota and phylogeny in passerines is not explained by ecological divergence. Molecular Ecology 26: 5292–5304.

Lim, S. J. & S. R. Bordenstein, 2020. An introduction to phylosymbiosis. Proceeding of Royal Society B 287: 20192900.

Moeller, A. H., Y. Li, E. M. Ngole, S. Ahuka-Mundeke, E. V. Lonsdorf, A. E. Pusey, M. Peeters, B. H. Hahn & H. Ochman, 2014. Rapid changes in the gut microbiome during human evolution. Proceeding of National Academy of Science USA 111(431–16): 435.

Noreña, C., C. Damborenea & F. Brusa, 2005. A taxonomic revision of South American species of the genus Stenostomum O. Schmidt (Platyhelminthes: Catenulida) based on morphological characters. Zoological Journal of the Linnean Society 144: 37–58.

Oliveira, L. F. V., G. L. Wallau & E. L. S. Loreto, 2009. Isolation of high quality DNA: a protocol combining rennet and glass milk. Electronic Journal of Biotechnology 12: 1–6.

Pennycook, J. H. & P. D. Scanlan, 2021. Ecological and evolutionary responses to antibiotic treatment in the human gut microbiota. FEMS Microbiology Review 45: fuab018.

Phillips, C. D., G. Phelan, S. E. Dowd, M. M. McDonough, A. W. Ferguson, J. Delton Hanson, L. Siles, N. Ordóñez-Garza, M. San Francisco & R. J. Baker, 2012. Microbiome analysis among bats describes influences of host

phylogeny, life history, physiology and geography. Molecular Ecology 21: 2617–2627.
Pollock, F. J., R. McMinds, S. Smith, D. G. Bourne, B. L. Willis, M. Medina, R. V. Thurber & J. R. Zaneveld, 2018. Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. Nature Communications 9: 4921.
Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies & F. O. Glöckner, 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41: D1.
Rieger, R. M., 1986. Asexual reproduction and the turbellarian archetype. Hydrobiologia. 132: 35–45.
Rosa, M. T. & E. L. S. Loreto, 2019. The Catenulida flatworm can express genes from its microbiome or from the DNA it ingests. Scientific Report 9: 19045.
Rosa, M. T. & E. L. S. Loreto, 2021. Revisiting the regeneration of Stenostomum leucops (Catenulida, Platyhelminthes). Invertebrate Reproduction & Development. https://doi.org/10.1080/07924259.2021.1994475.
Rosa, M. T., C. M. Pereira, G. T. Ragagnin & E. L. S. Loreto, 2015. Stenostomum leucops Duges, 1828 (Platyhelminthes, Catenulida): a putative species complex with phenotypic plasticity. Papeis Avulsos De Zoologia 55: 375–383.
Sanders, J. G., S. Powell, D. J. Kronauer, H. L. Vasconcelos, M. E. Frederickson & N. E. Pierce, 2014. Stability and phylogenetic correlation in gut microbiota: lessons from ants and apes. Molecular Ecology 23: 1268–1283.
Voolstra, C. R. & M. Ziegler, 2020. Adapting with microbial help: microbiome flexibility facilitates rapid responses to environmental change. Bioessays 42: e2000004.
Wang, Y. & P.-Y. Qian, 2009. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS ONE 4: e7401.
World Health Organization. *Iron in drinking-water*. Background document for preparation of WHO Guidelines for drinking-water quality, 2008. Geneva, World Health Organization (WHO/SDE/WSH/03.04/8) 390. http://www.who.int/water_sanitation_health/dwq/chemicals/iron.pdf