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Bacterial diversity associated with the brown stone centipede, *Lithobius forficatus* (Chilopoda, Lithobiomorpha)

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Many associations with microbial species significantly affect the biology, ecology and evolution of the host. Yet, our understanding of the species composition of the gut microbiota remains limited for many host species. Here, we provide a new step towards filling this gap, and characterize the bacterial microbiota of 60 specimens of *Lithobius forficatus*, a brown stone centipede commonly found in Finland. Many specimens analysed in this study were found to have a very species-rich bacterial community, while others hosted communities clearly dominated by one bacterial species. The most abundant phylotypes included some potential pathogens such as *Borrelia* and *Pseudomonas*, a honeybee gut symbiont *Gilliamella* and some maternally inherited symbiotic bacteria, including *Wolbachia* and Rickettsiaceae. While females and males were found to carry similar bacterial communities, population had a significant effect on the bacterial community composition. Bacterial species richness did not differ between sexes or between populations in *Lithobious forficatus*.

Introduction

Studies on the microbiota in the intestinal tract of arthropods have revealed a rich microbial species diversity (Ladygina *et al*. 2009, Thakuria *et al*. 2010, Agamennone *et al*. 2015, Bahrndorff *et al*. 2016, 2018, Tyagi *et al*. 2021), with diverse and important roles in their host's ecology and evolution (Kennedy *et al*. 2020). For example, the microbiota may not only contribute to digestion and detoxification of food (Brune & Ohkuma 2010, Boone *et al*. 2013) but can also benefit the host by supplying essential nutrients, mediating defence against pathogens, or influencing the social interaction and behaviours of their host (Engel & Moran 2013). According to some studies, functional microbiota is usually stable, predictable, and independent of dietary shifts (Tinker & Ottesen 2016). In contrast, other studies have shown that some hosts lack resident microbial species, but are rather colonized by transient gut communities with no obvious beneficial function for their hosts (Hammer *et al*. 2017, Duplouy *et al*. 2020), and often more representative of the

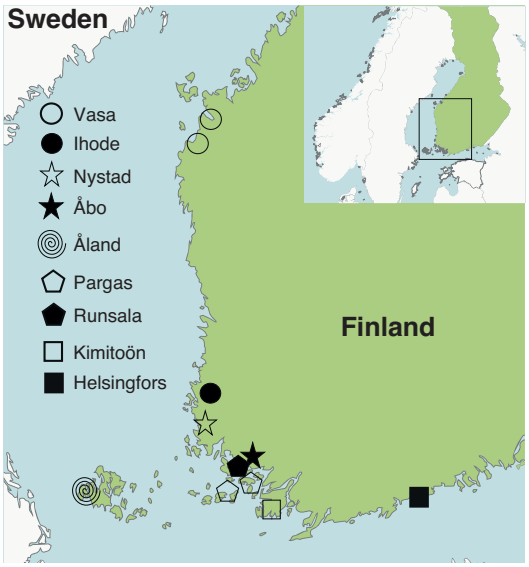

Fig. 1. Sampling localities in Finland.

microbiota associated with the host environment including their diet (Shukla *et al*. 2016, Xiang *et al*. 2019, Kennedy *et al*. 2020). For example, in predatory species, the prey species consumed drastically reshape the transient and environmentally derived gut microbiota for several weeks post-feeding (Kennedy *et al*. 2020).

Nonetheless, although the intimate association between hosts and microbes has been emphasized by many studies, little is known about the gut microbiome of many arthropod species (Engel & Moran 2013). This is because studies of the arthropod microbiota mostly focus on a few taxa within this highly species-rich phylum. To our knowledge, despite their high diversity and function as key species in terrestrial arthropod communities globally, no research has provided a comprehensive characterization of the microbiota of centipedes.

Lithobiomorphs, or stone centipedes, are terrestrial chilopods that have been roaming our planet since the Devonian Period (Edgecombe 2011). They are most diverse in the northern hemisphere where they are surface-active nocturnal top predators of local arthropod communities, hunting in the leaf litter and upper soil-layers (Voigtländer 2011). Lithobiomorphs feed mainly on small arthropods but there is also some evidence that they may occasionally consume plant matter (Lewis 1981). Although a narrow spectrum of bacteria was previously isolated from the centipede *Scolopendra subspinipes* (Soopramanien *et al*. 2019, Akbar *et al*. 2020, Soopramanien *et al*. 2021) including *Kocuria* sp., *Micrococcus* sp., *Staphylococcus* and *Bacillus* bacteria, we are not aware of any comprehensive analysis of the bacterial microbiota for any chilopod species.

Our aim was to present the first characterization of the bacterial diversity associated with the brown centipede *Lithobius forficatus*, one of the largest and the most common species of the order Lithobiomorpha found in Finland (as in many European countries), and test whether this microbial diversity differed between populations and sexes.

Material and methods

Samples

A total of 62 specimens, hand-collected from various habitats were included in this study (Fig. 1 and Appendix 1). Most of the specimens were collected during the summer 2023, but three were collected in September 2020, and one in June 2015. Additionally, we screened the ENA database (December 2022) for any centipede microbiota 16S rRNA depositions and found no other projects on microbiota from centipede hosts publicly available.

Molecular studies

All specimens were individually preserved in 96% ethanol and stored at –20 °C in a freezer on the same day until further handling. The samples were individually washed in two baths of 1xPBS in sterile conditions. We dissected a central section from each specimen before extracting the DNA from tissues in sterile conditions using Qiagen DNeasy Blood and Tissue kit (Qiagen, Germany) following the optimized protocol described by Duplouy *et al*. (2018). Three sterile samples of water were similarly handled as controls for contamination across the whole procedure. The hypervariable V5–V6 region of

the *16S ribosomal RNA* (*rrs*) gene was amplified using the primers 784F (5′-AGGATTAGATACCCTGGTA) and 1061R (5′-CRRCACGAGCTGACGAC; Toft & Andersson, 2010). This hypervariable region enables for discrimination of the bacterial taxa without the amplification of the mitochondrial 16S rRNA from the host. The barcoding sequencing was performed by the Institute for Molecular Medicine Finland (FIMM, Finland) using a MiSeq ver. 3. sequencing platform (Illumina, USA) with both reverse and forward primers. Libraries were cleaned and analysed using Mothur ver. 1.44.0 (Schloss *et al*. 2009) and the rrs SILVA.nr_v138 database reference files (Yilmaz *et al*. 2014). We selected all 250–350 bp long sequences, with no more than eight homopolymers, no ambiguous position, no chimera, and which aligned to the rrs SILVA.nr_v138 database. Any phylotype showing a 5× higher proportion in the negative control than in any sample was considered as contaminant and removed from the sample using an in-house R script (Minard *et al*. 2019). Two specimens (22A & 24D) were discarded as their microbiota was similar to that of the sterile water controls.

To test whether our sampling was sufficient to detect all microbial phylotype associated with this host species, we built a species accumulation curve in R. The number of samples was on the *x*-axis while the number of microbial phylotypes detected on the *y*-axis. The early sharp rise of the curve indicates that with the increase of sampling quantity, a large number of new microbial species is discovered. The curve should flatten out as the microbial phylotypes do not anymore increase significantly with sample size. Sampling is sufficient when the curve reaches a plateau.

Statistical analyses

We performed all statistical analyses in R 4.4.0 GUI 1.80 for MacOS (R Core Team 2022), using the *vegan* package (http://CRAN.R-project.org/package=vegan). To analyse bacterial composition (β-diversity) variations among samples, we first computed a Bray-Curtis dissimilarity matrix using nonmetric multidimensional scaling (NMDS) (Anderson & Willis 2003), calculated homogeneity of variance (ie. distance to centroids) of the treatment groups (sex or population) using the command *betadisper*, and used permutational analyses of variance (PERMANOVA with n = 9999 permutations) using the command *Adonis2* (Anderson 2001) with the population or sex (female, male, or unknown in case of juveniles) as explanatory variables. To estimate the α-diversity (diversity of the microbiota within each sample) of the microbiota, we calculated the Shannon diversity index (H') for each specimen. We then log-transformed it and tested the effect of population of origin and sex of the specimens on this index using a linear model. We tested whether particular species were significantly more often found associated with certain treatment groups (sex or population), and thus represented indicator species for those treatment groups, using the *multipatt* command from the R library *indicspecies* (De Cáceres & Legendre 2009).

Real-time qPCR

We conducted a small pilot study to test the protocols prior to this study, including only the four specimens collected in 2015 and 2020. As a result, we detected a presence of *Borrelia* in two of the samples (446 and 447). We wanted then to identify whether these individuals had fed on *Ixodes ricinus* or *I. persulcatus* ticks just prior to being collected in the field. The samples were screened for the tick's DNA using real-time quantitative PCR (qPCR) using species-specific duplex qPCR assays with primers targeting the ITS2 region of *Ixodes ricinus* and *I. persulcatus* following the protocol of Sormunen *et al*. (2016). The assay was carried out in 10 µl reaction volume, including 5 µl SensiFAST™ Hi-Rox 2X (Bioline), 0.4 µl of mixed forward and reverse primers (IXO-I2-F4 and IXO-I2-R4), 0.15 µl Iri-I2-P4 probe, 0.15 µl Ipe-I2-P4 probe, 2.3 µl RNase free H20, and 2 µl DNA. Primers are listed in Table 1. The thermal cycling profile used was 3 min at 95 °C, then 50 cycles at 95 °C for 3 s, and 40 s at 60 °C. As positive controls we used *I. ricinus* and *I. persulcatus* DNA extracts available from the lab.

As the analysis of the composition of the microbiota revealed the presence of the bacte-

rium *Borrelia burgdorferi sensu lato*, a common infection in ticks, we decided to further screen our DNA samples for it. We did this by using primers targeting 23S rRNA of *B. burgdorferi sensu lato* (Bb23Sf and Bb23Sr) and a dual-labelled probe, Bb23Sp (Table 1). The assay (protocol by Sormunen *et al*. 2016) consisted of a single qPCR run in 10 µl reaction volume, including 5 µl SensiFAST™ Hi-Rox 2X (Bioline), 0.4 µl of mixed forward and reverse primer (Bb23Sf + Bb23Sr), 0.1 µl Bb23S probe, 2.3 µl RNase free H20, and 2 µl DNA. To screen the presence of *Borrelia* the profile was kept at 95 °C for 5 min, followed by 50 cycles at 95 °C for 3 s, and 30 s at 60 °C. As a positive control we used Borrelia AFZELII DNA Control (Amplirun®, Vircell).

Both the *Ixodes* and *Borrelia* assays described above were carried out using Bio-Rad CFX96™ Real-Time System and Thermal Cycler. All samples were analysed in two replicates. Two blank water samples were used as negative controls in each assay. All qPCR results were analysed using Bio-Rad Manager. Samples were considered positive when successful amplification (cutoff threshold set at 10^1 RFU, Ct value < 40) was detected in both replicate reactions.

Results

Microbial communities

We identified 845 unique bacterial phylotypes (taxonomy-based OTUs) from 60 Finnish centipede specimens, which is unlikely to represent the full diversity of microbial diversity associated with this host, as suggested by the species accumulation curve not reaching a plateau (Appendix 2).

Many specimens carry a very species-rich bacterial community, while others are mostly dominated by one bacterial species. The most abundant phylotypes included some potential pathogens, such as *Borrelia* (e.g. specimens 1B, 446 and 447) and *Pseudomonas* (e.g. 26), a honeybee gut symbiont *Gilliamella* (e.g. 449 and 12B), some maternally inherited symbiotic bacteria, including *Wolbachia* (e.g. 1A, 15B and 19) and *Rickettsiaceae* (e.g. 8C), and diverse other uncharacterized bacteria from different families (Fig. 2).

The analyses of the community composition (β-diversity) of the microbiota associated with the centipedes revealed no difference in the bacterial communities between females and males (PerMANOVA: $F_{2,57} = 0.931$, $p = 0.642$), with each sex group showing the same distance to centroids (Fig. 3A and B). In contrast, population had a significant effect on the bacterial composition of the microbiota of *L. forficatus* (PerMANOVA: $F_{8,45} = 1.416$, $p = 1e\text{-}4$), with the distance to centroids differing between populations (Fig. 3C and D). However, three populations are only represented by a very small sample size (i.e. three populations include one or three specimens; Helsingfors ($n = 3$), Nystad ($n = 3$) and Ihode ($n = 1$); (Appendix 1).

Species richness (α-diversity) did not differ between sexes, as no difference was found in the bacterial species the males and females carried. Both the Shannon diversity index and the species richness values did not differ among sexes and populations (ANOVA: $F_{2,57} > 0{,}46$, $p > 0.05$ (Fig. 4A and Appendix 3A); and $F_{8,45} > 1.5$, $p > 0.05$ (Fig. 4B and Appendix 3B); respectively).

Table 1. Primers used in the screening for *Ixodes* tick and *Borrelia burgdorferi* bacteria DNA in the centipede DNA extracts.

Primer	Primer/probe target	5′→3′	Source
IXO-I2-F4	*Ixodes* spp./ITS2	TCTCGTGGCGTTGATTTGC	Sormunen *et al.* 2016
IXO-I2-R4	*Ixodes* spp./ITS2	CTGACGGAAGGCTACGACG	Sormunen *et al.* 2016
Ipe-I2-P4	*I. persulcatus*/ITS2	[FAM]-TGCGTGGAAAGAAAACGAG-[BHQ1]	Sormunen *et al.* 2016
Iri-I2-P4	*I. ricinus*/ITS2	[HEX]-TGCTCGAAGGAGAGAACGA-[BHQ1]	Sormunen *et al.* 2016
Bb23Sf	*B. burgdorferi*/23S RNA	CGAGTCTTAAAAGGGCGATTTAGT	Courtney *et al.* 2004
Bb23Sr	*B. burgdorferi*/23S RNA	GCTTCAGCCTGGCCATAAATAG	Courtney *et al.* 2004
Bb23Sp	*B. burgdorferi*/23S RNA	[FAM]-AGATGTGGTAGACCCGAAGCCGAGTG-[BHQ1]	Courtney *et al.* 2004

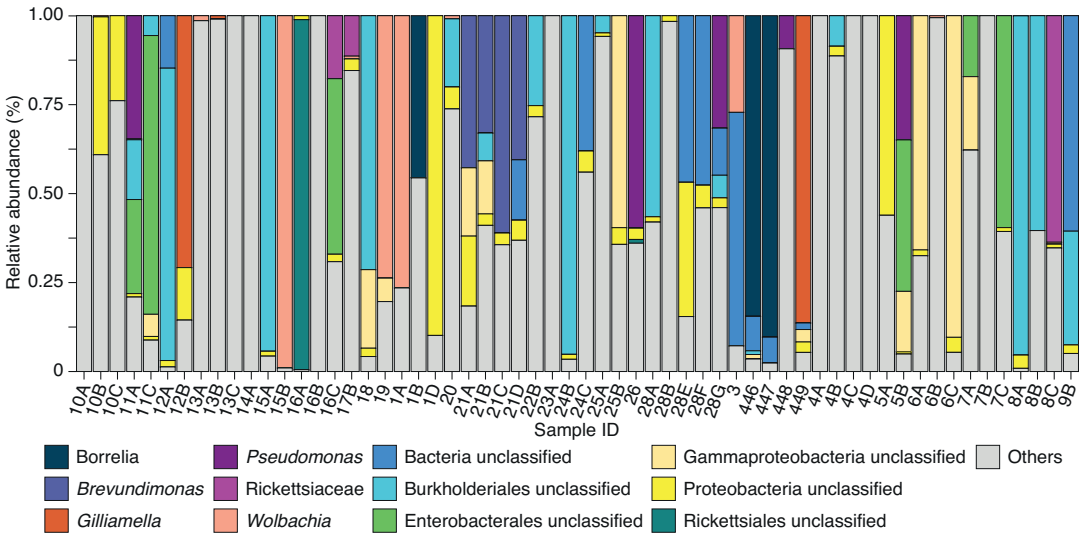

Fig. 2. Relative abundances of the 12 dominant bacterial phylotypes in 60 *Lithobius forficatus* specimens from mainland Finland and the Åland Islands.

Real-time qPCR

The qPCR assays showed that *Borrelia burgdorferi sensu lato* was present in samples 446 and 447, and also in smaller amounts in samples 448 and 449. The qPCR screening however revealed no traces of *Ixodes ricinus* or *I. persulcatus* in the centipedes.

Discussion

We found that the studied 60 *L. forficatus* specimens carried diverse bacterial communities, represented by 845 bacterial phylotypes, that did not differ between sexes, and among populations. This diversity included bacterial species such as the pathogenic bacterium *Borrelia* or the symbiotic bacterium *Wolbachia*, for which ecology and evolution in other arthropod hosts have been the focus of many studies. However, the role of many other bacterial members of the microbiota of *L. forficatus* remains unclear.

Microbial community composition can vary depending upon many factors in the host environment, including diet (Ng *et al*. 2018, Ebert *et al*. 2021). Because the studied *Lithobius* specimens were not starved before being killed and stored in ethanol, the highly variable microbiota characterized from the specimens could still be a composite of the centipedes' own microbiota and that of their prey. In Finland, the primary vectors of *Borrelia* are the ticks *Ixodes ricinus* and *I. persulcatus*, which facilitate the dispersal and transmission of *Borrelia* to its final hosts (i.e. mammals including humans), where it may cause serious illness (e.g. de Taeye *et al*. 2013). The presence of *Borrelia* was confirmed also by the qPCR analysis, which is often more sensitive than PCR metabarcoding sequencing methods (Fig. 2). As generalist predators, chilopods can easily alternate between prey types (Lewis 1981), and although there is no record of lithobiid species feeding on *Ixodes* ticks, previous studies reported diverse mites as part of the diet of centipedes. For example, mesostigmate mites are part of the diet of *L. validus* Meinert (Bonato *et al*. 2021), while oribatid and parasitiform mites are included in the diet of *L. lapidicola* Meinert (Roberts 1956). The detection of *Gilliamella* bacteria in one of the specimens, may further support the idea of contamination of the centipede-associated microbiota with bacterial species from their prey. Indeed, *Gilliamella* are best known as bacterial symbionts of bees, for which they break down sugars and other carbohydrates to improve the host diet and health (Zheng *et al*. 2016). Although there is no evi-

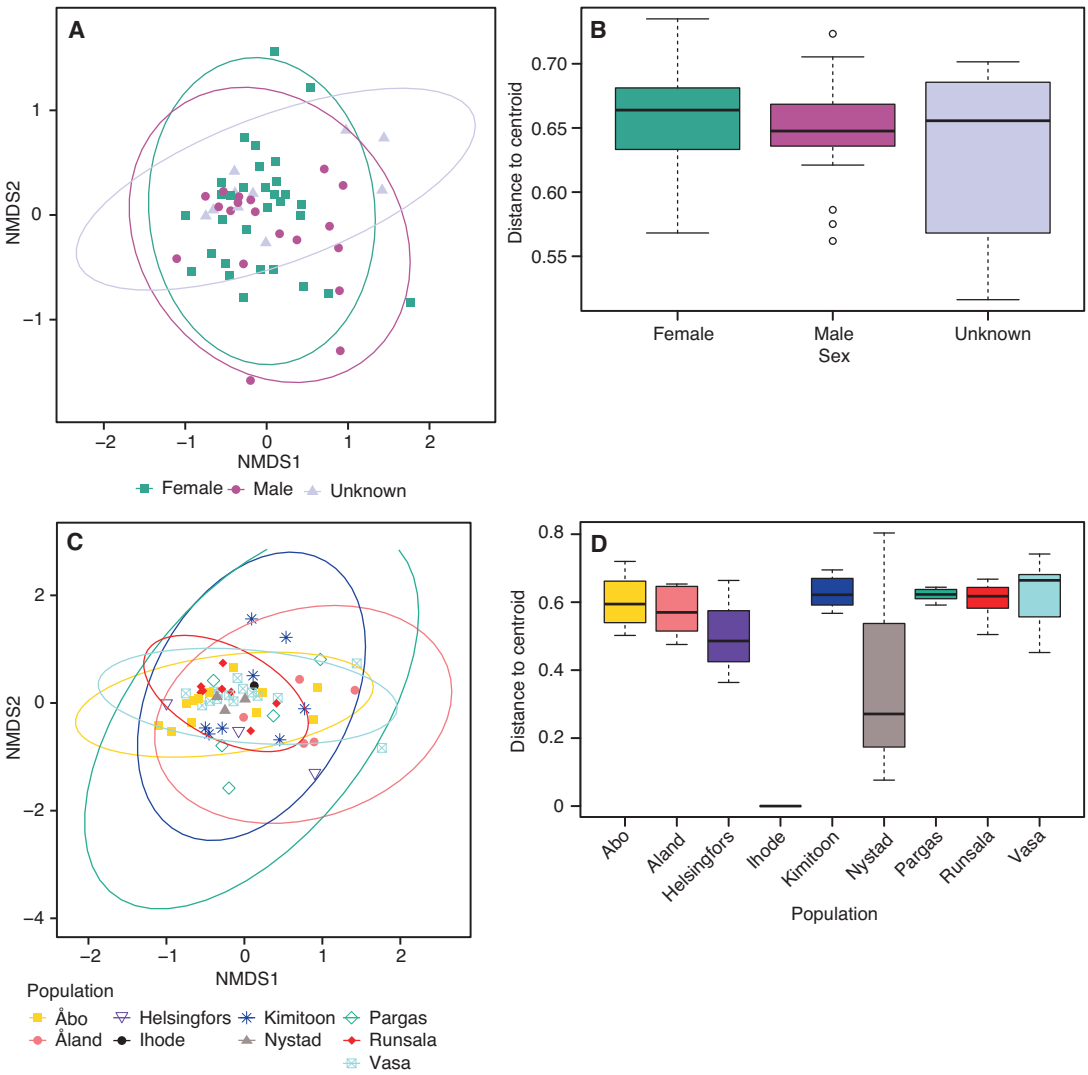

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plots based on Bray-Curtis distances for the bacterial communities associated with *Lithobius forficatus*. — **A** and **B**: sex. — **C** and **D**: population of origin. Ellipses in **A** and **C** are 95% confidence limits for groups with enough data points; in **B** and **D** horizontal lines are medians, boxes upper and lower 25% confidence limits, error bars 95% confidence limits, and circles outliers for groups with enough data points.

dence that chilopods would feed on bees, it is possible that other pollen-consuming prey species also bear *Gilliamella*.

Another possibility is that both the *Borrelia* and *Gilliamella* bacteria are naturally hosted by centipede species, and they could have another function in *Lithobius* than what they have in ticks or bees, a function that is yet to be characterized in centipedes. Indeed, the *L. forficatus* specimens that tested positive for *Borrelia* were not found positive for the presence of *Ixodes ricinus* and *I. persulcatus* ticks DNA. Additionally, *Borrelia* bacteria have also been found in the microbiota of mosquitoes (Melaun *et al.* 2016), suggesting the bacteria are not restricted to tick hosts, and the centipedes might themselves naturally carry the bacteria, or at least host them for many days after a meal on *Ixodes* ticks. Nonetheless, these uncertainties around the origin of the microbial associations may be avoided in future studies by either further starving the specimens before killing, manipulating

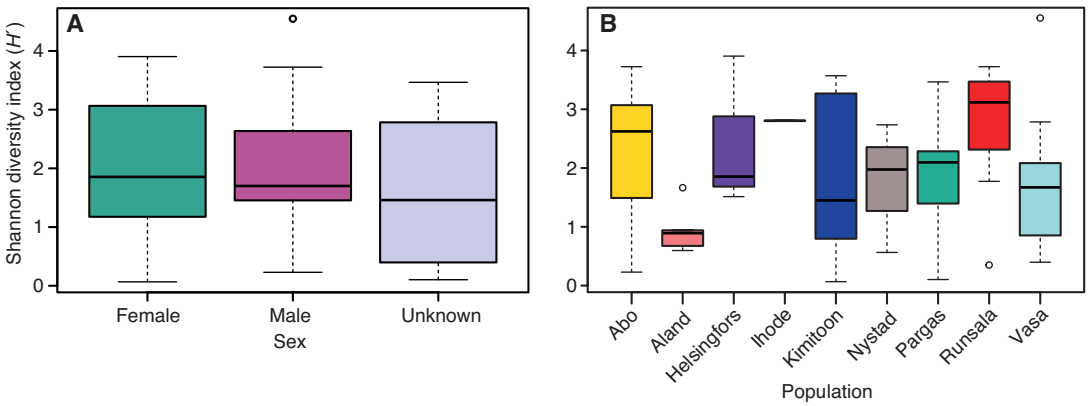

Fig. 4. Variation in Shannon's diversity index (*H'*) among (**A**) sexes, and (**B**) populations. Horizontal lines are medians, boxes upper and lower 25% confidence limits, error bars 95% confidence limits, and circles minima and maxima. For comparisons of species richness among groups *see* Appendix 3.

the microbiota through different diet or antibiotic treatments, and/or by characterizing the microbiota of the prey independently to that found in the predatory centipedes.

We also identified diverse species of bacterial symbionts such as *Wolbachia* (14/60 samples, 23%), *Spiroplasma* (3/60, 5%), or other *Rickettsia* (16/60, 27%), many strains of which are maternally inherited and can affect their host life history and reproduction (Moran 2006). For example, in diverse insect species *Wolbachia* and *Spiroplasma* can manipulate the reproductive system of their hosts to benefit the reproductive output of infected females over uninfected ones (Montenegro *et al*. 2006, Jaenike *et al*. 2010, Correa & Ballard 2016, Duplouy & Hornett 2018). As we found these symbionts in both male and female specimens, it is unlikely that the bacteria kill the males of *L. forficatus*, as it was shown in many insect species (Duplouy *et al*. 2010, Harumoto *et al*. 2018). In spiders, *Rickettsia* symbionts can affect the dispersal of their host through the landscape (Goodacre *et al*. 2009) but *Rickettsia* bacteria were also characterized as parasites of *Ixodes* ticks (Li *et al*. 2019), and their detection in centipedes could again be just from an *Ixodes* meal in the gut of the predatory hosts. However, in our data, *Rickettsia* and *Borrelia* were not detected from the same host specimens, suggesting their presence is not linked, and *Rickettsia* may remain a symbiont in the centipede species. Laboratory rearing of this arthropod, and experimental testing of their effect, will in the future clarify the exact role of these bacteria in *L. forficatus*.

Few bacteria isolated from myriapods have been assigned potential medical properties, including anti-cancer properties for the bacteria *Kocuria varians* isolated from *Scolopendra subspinipes* (Soopramanien *et al*. 2019), or antibiotic properties for *Actinobacter bacteria* from the digestive tracts of *Nedyopus dauydoffiae* (Glukhova *et al*. 2018). We did not detect *Kocuria* bacteria from the microbiota associated with *L. forficatus*, but rather showed that the studied specimens carried a wide diversity of bacterial species, including bacteria important for human and bee health, and for which many aspects of their ecology remain unclear (Farrell *et al*. 1991, McNamara 1998). Broader sampling and experimental work with these top predators will be needed to further explain and confirm the structure and function of their associated microbiota.

Data availability

All metadata are provided together with the article. The raw microbiota data are accessible from the European Nucleotide Archive (http://www.ebi.ac.uk/ena, European Molecular Biology Library-European Bioinformatics Institute, EMBL-EBI) under the project ID PRJNA1091151.

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Appendix 1. Collection details. * M = male, F = female, ? = unknown. ** A = adult, J = juvenile.

Population	Location	Sex*	Stage**	Length (cm)	Lat. °N, Long. °E	Date	Habitat	Collector	Sample ID
Åbo	Svalas	F	A	2	60.43543, 22.35769	1.X.2020	compost heap	V. Weijola	10A
Åbo	Svalas	F	A	2.5	60.43543, 22.35769	1.X.2020	compost heap	V. Weijola	10B
Åbo	Svalas	M	A	2	60.43543, 22.35769	1.X.2020	compost heap	V. Weijola	10C
Åbo	Runsala, botanical garden	M	A	2	60.436504, 22.174501	15.V.2023	oak-dominated forest, decaying *Tilia* sp.	V. Vahtera	22A
Åbo	Runsala, botanical garden	M	A	2.5	60.436504, 22.174501	15.V.2023	oak-dominated forest, decaying birch	V. Vahtera	24D
Åbo	Svalas	?	J	0.5	60.43543, 22.35769	15.V.2023	suburban garden	V. Vahtera	28A
Åbo	Svalas	?	A	0.7	60.43543, 22.35769	15.V.2023	suburban garden	V. Vahtera	28B
Åbo	Svalas	F	A	2.5	60.43543, 22.35769	15.V.2023	suburban garden	V. Vahtera	28E
Åbo	Svalas	F	A	1.5	60.43543, 22.35769	15.V.2023	suburban garden	V. Vahtera	28F
Åbo	Svalas	M	A	1.5	60.43543, 22.35769	15.V.2023	suburban garden	V. Vahtera	28G
Åbo	Lundo, near SF Caravan	F	A	2.5	60.502862, 22.589132	20.VII.2022	pine forest	V. Vahtera	3
Åbo	Lauste	M	A	2	60.42967, 22.35449	2.VIII.2022	mixed forest, decaying conifer	V. Vahtera	8A
Åbo	Lauste	M	A	2	60.42967, 22.35449	2.VIII.2022	mixed forest, decaying conifer	V. Vahtera	8B
Åbo	Lauste	M	A	2	60.42967, 22.35449	2.VIII.2022	mixed forest, decaying conifer	V. Vahtera	8C
Åland	Eckerö, Skag	?	A	NA	60.222778, 19.560825	11.VI.2015	unknown	V. Vahtera	449
Åland	Hammarland, Strömma, Jumalön	F	A	2	60.304260, 19.751050	10.VI.2015	stony beach with lichens and loose stones	V. Vahtera	11A
Åland	Hammarland, Strömma, Jumalön	M	A	2	60.304260, 19.751050	10.VI.2015	stony beach with lichens and loose stones	V. Vahtera	11C
Åland	Eckerö, Skag	M	A	2	60.288650, 19.593490	11.VI.2015	unknown	V. Vahtera	12A
Åland	Eckerö, Skag	?	A	2	60.288650, 19.593490	11.VI.2015	unknown	V. Vahtera	12B
Helsingfors	Helsingfors, Kajsaniemiparken	M	A	3	60.17394, 24.94874	29.VII.2022	urban park, decaying oak	V. Vahtera	7A
Helsingfors	Helsingfors, Kajsaniemiparken	F	A	2.5	60.17394, 24.94874	29.VII.2022	urban park, decaying oak	V. Vahtera	7B
Helsingfors	Helsingfors, Kajsaniemiparken	F	A	2	60.17394, 24.94874	29.VII.2022	urban park, decaying oak	V. Vahtera	7C
Ihode	Highway E8 close to Ihode	F	A	2	60.997654, 21.572926	21.V.2023	pine forest, under moss	V. Vahtera	20
Kimitoön	Kimitoön, Sandskär	F	A	2	60.041268, 22.354245	14.VII–15.IX.2020	sandy beach meadow, few meters from the shoreline	A. Karhilahti, V. Rinne & A. Teräs	13A
Kimitoön	Kimitoön, Sandskär	F	A	2	60.041268, 22.354245	14.VII–15.IX.2020	sandy beach meadow, few meters from the shoreline	A. Karhilahti, V. Rinne & A. Teräs	13B
Kimitoön	Kimitoön, Sandskär	M	A	2	60.041268, 22.354245	14.VII–15.IX.2020	sandy beach meadow, few meters from the shoreline	A. Karhilahti, V. Rinne & A. Teräs	13C
Kimitoön	Kimito, Tappo, Dalis	F	A	2	60.088250, 22.666380	14.V.2023	pine forest, under lichens, growing on a rock	V. Vahtera	15A
Kimitoön	Kimito, Tappo, Dalis	F	A	1.5	60.088250, 22.666380	14.V.2023	pine forest, under lichens, growing on a rock	V. Vahtera	15B

continued

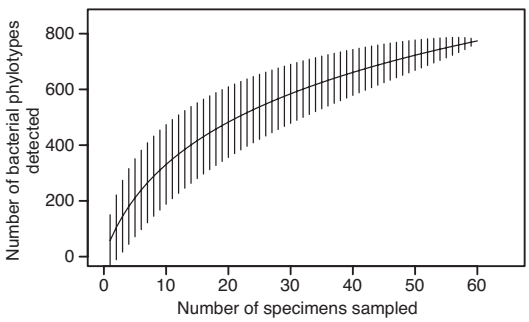
Appendix 1. Continued.

Population	Location	Sex*	Stage**	Length (cm)	Lat. °N, Long. °E	Date	Habitat	Collector	Sample ID
Kimitoön	Kimito, Tappo, Dalis	F	A	2	60.088250, 22.666380	14.V.2023	decaying pine stump	V. Vahtera	19
Kimitoön	Kimito, Tappo, Dalis	M	A	2.5	60.08782, 22.66356	30.7.2022	spruce-dominated forest, decaying conifer	V. Vahtera	5A
Kimitoön	Kimito, Tappo, Dalis	F	A	2.5	60.08782, 22.66356	30.7.2022	spruce-dominated forest, decaying conifer	V. Vahtera	5B
Nystad	Hiu	M	A	2	60.82657, 21.38077	13.8.2022	mixed forest, decaying conifer	V. Vahtera	6A
Nystad	Hiu	F	A	2	60.82657, 21.38077	13.8.2022	mixed forest, decaying conifer	V. Vahtera	6B
Nystad	Hiu	F	A	2	60.82657, 21.38077	13.8.2022	mixed forest, decaying conifer	V. Vahtera	6C
Pargas	Nago, Sandö	M	A	2	60.170861, 22.108544	5.VI–9.VII.2020	sandy beach meadow, few meters from the shoreline	A. Karhilahti, V. Rinne & A. Teräs	14A
Pargas	Ålön, Mustfinn	?	J	1	60.307844, 22.100543	29.V.2023	pine forest close to the shoreline, under a stone	V. Vahtera	16A
Pargas	Ålön, Mustfinn	?	J	1	60.307844, 22.100543	29.V.2023	pine forest close to the shoreline, under a stone	V. Vahtera	16B
Pargas	Ålön, Mustfinn	M	A	2	60.307844, 22.100543	29.V.2023	pine forest close to the shoreline, under a stone	V. Vahtera	16C
Pargas	Ålön, Mustfinn	F	A	2	60.308453, 22.099518	29.V.2023	stony beach, under a stone	V. Vahtera	17B
Runsala	Runsala, botanical garden	F	A	2	60.436504, 22.174501	15.V.2023	oak-dominated forest, decaying *Tilia* sp.	V. Vahtera	22B
Runsala	Runsala, botanical garden	F	A	2	60.436504, 22.174501	15.V.2023	oak-dominated forest, under dead oak leaves	V. Vahtera	23A
Runsala	Runsala, botanical garden	?	A	1	60.436504, 22.174501	15.V.2023	oak-dominated forest, decaying birch	V. Vahtera	24B
Runsala	Runsala, botanical garden	F	A	1.5	60.436504, 22.174501	15.V.2023	oak-dominated forest, decaying birch	V. Vahtera	24C
Runsala	Åbo, Runsala	M	A	2.5	60.43360, 22.17345	8.VIII.2022	oak-dominated forest, decaying deciduous tree	V. Vahtera	4A
Runsala	Åbo, Runsala	F	A	2.5	60.43360, 22.17345	8.VIII.2022	oak-dominated forest, decaying deciduous tree	V. Vahtera	4B
Runsala	Åbo, Runsala	F	A	2	60.43360, 22.17345	8.VIII.2022	oak-dominated forest, decaying deciduous tree	V. Vahtera	4C
Runsala	Åbo, Runsala	F	A	2	60.43360, 22.17345	8.VIII.2022	oak-dominated forest, decaying deciduous tree	V. Vahtera	4D
Vasa	Malax, Petalax	M	A	NA	62.783652,21.386926	19.IX.2020	unknown	V. Vahtera	446
Vasa	Malax, Petalax	F	A	NA	62.783652,21.386926	19.IX.2020	unknown	V. Vahtera	447
Vasa	Malax, Petalax	M	A	NA	62.783652,21.386926	19.IX.2020	unknown	V. Vahtera	448
Vasa	Vasa	F	A	2	63.114654, 21.642987	14.4.2023	conifer-dominated urban forest area, under a stone	V. Vahtera	18
Vasa	Malax, Petalax, Viitala	F	A	2	62.78386, 21.38687	16.IX.2022	pine-dominated mixed forest, under a stone	V. Vahtera	1A

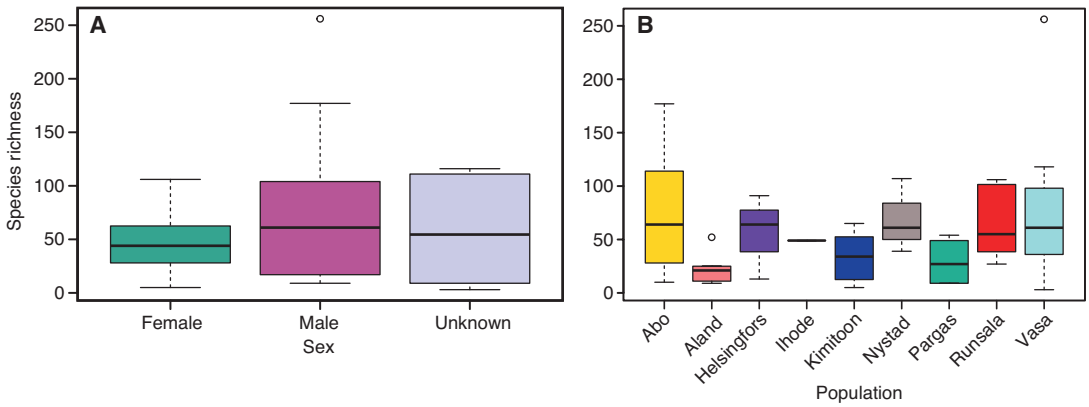
continued

Appendix 1. Continued.

Population	Location	Sex*	Stage**	Length (cm)	Lat. °N, Long. °E	Date	Habitat	Collector	Sample ID
Vasa	Malax, Petalax, Viitala	F	A	2	62.78386, 21.38687	16.IX.2022	pine-dominated mixed forest, under a stone	V. Vahtera	1B
Vasa	Malax, Petalax, Viitala	?	A	1	62.78386, 21.38687	16.IX.2022	pine-dominated mixed forest, under a stone	V. Vahtera	1D
Vasa	Malax, Bergö	F	A	2	62.962053, 21.213773	18.V.2023	spruce forest, decaying spruce stump	V. Vahtera	21A
Vasa	Malax, Bergö	?	J	2	62.962053, 21.213773	18.V.2023	spruce forest, decaying spruce stump	V. Vahtera	21B
Vasa	Malax, Bergö	?	J	0.5	62.962053, 21.213773	18.V.2023	spruce forest, decaying spruce stump	V. Vahtera	21C
Vasa	Malax, Bergö	M	A	1.5	62.962053, 21.213773	18.V.2023	spruce forest, decaying spruce stump	V. Vahtera	21D
Vasa	Malax, Petalax, nature trail	F	A	1.5	62.784149, 21.395508	20.V.2023	spruce-dominated mixed forest, decaying birch	V. Vahtera	25A
Vasa	Malax, Petalax, nature trail	M	A	1.5	62.784149, 21.395508	20.V.2023	spruce-dominated mixed forest, decaying birch	V. Vahtera	25B
Vasa	Malax, Petalax, nature trail	M	A	2	62.784149, 21.395508	20.V.2023	spruce-dominated mixed forest, fallen pine	V. Vahtera	26
Vasa	Malax, Petalax, Viitala	F	A	2	62.78386, 21.38687	17.IX.2022	clear-cutting area, spruce stump	V. Vahtera	9B



Appendix 2. Bacterial species accumulation curve for all 60 centipede specimens in this study.



Appendix 3. Variation in species richness among (**A**) sexes and (**B**) populations. Horizontal lines are medians, boxes upper and lower 25% confidence limits, error bars 95% confidence limits, and circles maxima