EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **116**: 432–441, 2019
doi: 10.14411/eje.2019.044
ORIGINAL ARTICLE

High-diversity microbiomes in the guts of bryophagous beetles (Coleoptera: Byrrhidae)

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Key words. Coleoptera, Byrrhidae, bryophagy, gut microbiota, Illumina MiSeq, richness

Abstract. The diversity and role of the gut microbiota of insects is a rapidly growing field of entomology, primarily fueled by new metagenomic techniques. Whereas endosymbionts in the guts of xylophagous or herbivorous insects are well studied, the microbiomes in moss-eating (bryophagous) insects remain uncharacterized. Using the Illumina MiSeq platform, we determined the composition of microbiomes in the gut, abdomen and on the body surface of two bryophagous species: *Simplocaria semistriata* (Fabricius, 1794) and *Curimopsis paleata* (Erichson, 1846) (Coleoptera: Byrrhidae). Gut microbiomes differed substantially from abdominal microbiomes in the same individuals, which indicates the need to separate them during dissection. Microbiomes in the gut and abdomen differed markedly from surface microbial assemblages. Gut microbiomes in bryophages had the highest MOTU richness, diversity and relative rarity. The eudominant bacteria in the guts and abdomens of bryophages were *Novosphingobium*, *Bradyrhizobium*, *Ralstonia* and *Caulobacter*, which are responsible for the detoxification of secondary metabolites or nitrogen fixation. These are less common in the surface samples and, therefore, likely to be associated with the specific ability of bryophages to feed on mosses.

INTRODUCTION

Many insects establish symbiotic interactions with microorganisms in their gut, body cavities, or cells (Dillon & Dillon, 2004). Protista have been identified in the digestive tract of lower termites and wood roaches (Hongoh, 2010); whereas fungi and methanogenic archaea are frequent in the gut of xylophagous and detritivorous beetles and termites (Egert et al., 2005; Brune, 2010). Bacteria are found in the gut community of most insects, including herbivores. Most gut microbes are commensals or parasites; however, some are known to provide beneficial services to their hosts (Engel & Moran, 2013). They can affect resistance against pathogens or parasites (Hedges et al., 2008; Oliver et al., 2010), intestinal cell renewal and systemic growth (Buchon et al., 2009), production of pheromones (Dillon et al., 2002) and kairomones (Leroy et al., 2011), or mating attractiveness (Sharon et al., 2010). Their beneficial role in diet processing through degradation of complex food components (Warnecke et al., 2007) and toxins (Kikuchi et al., 2012), nitrogen fixation (Nikoh et al., 2011), ammonia recycling (Hongoh et al., 2008), and nutrient supplementation via the synthesis of vitamins (Akman et al., 2002) and essential amino acids (Douglas, 1998) is also crucial.

Whereas the microbiota of xylophagous and herbivorous insects has been extensively studied, the microbiome of bryophagous (moss-feeding) insects has remained poorly characterized, except for an obligate endosymbiotic taxon in the bacteriomes of moss bugs (Hemiptera: Peloridiidae; Kuechler et al., 2013). At present, nothing is known about microbial assemblages in the guts or abdomens of

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bryophagous insects, even though the utilization of mosses as a source of food is a unique phenomenon due to the specific properties of mosses. Bryophytes contain several compounds that reinforce the cell wall and hinder its digestion by inhibiting potential symbiotic organisms (Vanderpoorten & Goffinet, 2009). Moreover, mosses have evolved effective defenses against herbivores by producing antimycotics (Frahm, 2004) and antibiotics (McCleary et al., 1960), organically soluble fractions (Parker et al., 2007) and high levels of water-soluble phenolic compounds (Davidson et al., 1989; Glime, 2006). It is assumed that all these mechanisms strongly affect the gut microbiota of their occasional consumers, and, as a result, deter the majority of herbivores from feeding on mosses (Gerson, 1969).

The aim of the present study was to determine the molecular operational taxonomic units (MOTUs) of bacteria in the gut and/or abdominal microbiomes in two bryophagous species *Curimopsis paleata* (Erichson, 1846) and *Simplocaria semistriata* (Fabricius, 1794) (Coleoptera: Byrrhidae). Adults of *S. semistriata* are recorded feeding, mating and ovipositing on mats of the moss *Dicranella heteromalla* (Hedw.) Schimp. and also feeding on the moss *Mnium hornum* Hedw. (Johnson, 1990). In the genus *Curimopsis*, all species are thought to be strictly bryophagous based on collecting, rearing or dissections. The dissections revealed fragments of undetermined moss in the alimentary tract of *C. paleata* (Johnson, 1986). Interestingly, *Curimopsis nordensis* Tshernyshev, 2013, a new species that occurs in Russian alpine tundra and steppes, also feeds on carrion, so at least some adults of the genus *Curimopsis* can feed also on non-vegetable matter (Tshernyshev, 2013).

We aim to determine, whether the gut microbiomes in both species, which are in permanent contact with a moss diet, are less diverse and distinctly different in their composition from that of their surface microbiota, which are in permanent contact with bacteria-rich soil. We compare the bacterial assemblages associated with both species and subsequently in different parts of their bodies in terms of their composition, species richness and diversity, overlap and relative rarity. Finally, we aimed to determine the MOTUs significantly associated with gut and/or abdominal microbiomes and discuss their potential role in moss digestion in both of the bryophagous species studied. In addition to the principal analysis, we compare the gut and abdominal microbiomes of the same bryophagous individuals to determine whether it is necessary to separate body parts before metagenomic analyses. This aspect is often neglected in studies on small insects because it is difficult, but its omission could confound the results.

MATERIALS AND METHODS

Sampling and dissection of beetles

Beetles were collected from November to December 2013 in deciduous forests near Ostrava, Czech Republic (49°52′04″N, 18°14′17″E). We focused on two species of the family Byrrhidae, a unique group of moss-feeding beetles: *C. paleata* and *S. semistriata*. Both are widespread Palearctic species with minute bodies and perennial activity. The beetles were captured individually on the surface and inside cushions of the moss *Dicranella heteromalla*. Individuals were placed in separate plastic boxes together with moss using sterilized tweezers to avoid contamination. The boxes were stored in a refrigerator until dissection (for a maximum of three days after collection to avoid considerable changes in the composition of bacterial assemblages). In order to obtain surface samples, each beetle was washed by vortexing in a 1.5-mL micro centrifuge tube with 1 mL sterile solution of 1% Tween 80 (Sigma-Aldrich, Saint Louis, USA) and phosphate-buffered saline (PBS) for 30 s at 2100 rpm. The procedure was repeated so that the beetle was completely clean and the second wash was discarded (Bateman et al., 2016). Gut contents and abdominal tissues were separated on paraffin wax, which was previously sterilized by pouring ethanol on it and igniting it. For further metagenomic analysis, surface washes, gut contents and abdominal tissues of five *C. paleata* and five *S. semistriata* individuals were obtained. In addition, we verified the bryophagy of both species by analyzing the gut contents of five individuals of *C. paleata* and five of *S. semistriata* that were dissected under a binocular microscope. This revealed that fragments of phylloids and rhizoids formed >95% of the gut contents and the remaining material probably consisted of soil particles.

DNA isolation, PCR amplification and library preparation

Microbial DNA from the surface wash, gut contents and abdominal tissues of each beetle was isolated using the PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, CA) following the manufacturer's standard protocol and then subjected to sequencing library preparation. To ensure the recovery of broad spectra of bacterial and archaeal diversity, we used the universal F515/R806 bacterial ribosomal primers for the MiSeq platform to amplify the V4 region of 16S rDNA (Caporaso et al., 2010b). A two-step PCR was used during library preparation. First-step amplifications were done in quintuple reactions using only gene-specific primers to avoid PCR artifacts caused by long primers with attached sequencing adapters and identifiers, as well as the stochasticity in the PCR amplification (Berry et al., 2011; Schmidt et al., 2013). The first-step PCR was performed according to Caporaso et al. (2010b) with minor modifications consisting of initial denaturation at 94°C for 3 min; 25 cycles at 94°C for 45 s, 50°C for 60 s, 72°C for 90 s; and a final extension at 72°C for 10 min. The number of cycles was kept low to prevent potential depletion of specific primers from the degenerate mixture. Quintuple PCR reactions were pooled, purified (UltraClean® PCR Clean-Up Kit; MoBio) and subjected to a second-step PCR. The latter included 15 cycles of PCR amplification (following the same cycling profile as in the first-step PCR) with fused Illumina primers containing sequencing adapters and sample-unique multiplex identifiers necessary for demultiplexing the reads from each sample. Each PCR reaction volume (25 µL) contained 14.25 µL of molecular biology-grade water, 2.5 µL ExTaq 10× buffer, 0.2 mM dNTPs, 0.8 µM of each primer, 1.25 U ExTaq polymerase (all Clonetech, Mountain View, CA) and 20 ng extracted DNA or 2 µL cleaned PCR product in the second-step PCR. All PCR products were purified and checked using an agarose gel and quantified with the Quant-iT kit (Life Technologies, Carlsbad, CA). Subsequently, equimolar proportions of all samples were pooled to create a final sequencing library at 7.5 ng/µL and submitted for sequencing on the MiSeq platform (Illumina Technologies, San Diego, CA) at the Interdisciplinary Center for Biotechnology Research of the University of Florida, USA. Raw demultiplexed sequencing data with sample annotations are available at the Short Read Archive database (http://www.ncbi.nlm.nih.gov/Traces/sra/) under accession number SRP055203 and further details can be found under the BioProject accession number PRJNA275854.

Processing of sequencing data and statistical analysis

Sequencing data were processed using QIIME 1.8.0 (Caporaso et al., 2010a), including quality checking, demultiplexing, read clustering and taxonomic assignments. Forward and reverse reads were joined to create contigs. Afterward, reads were demultiplexed in a parallel way with quality filtering that included a maximum unacceptable Phred quality score of 20 and a maximum number of consecutive poor quality base calls of 12 due to lower-quality overlaps of paired-end reads. Resulting reads were clustered into MOTUs using UCLUST (Edgar, 2010) with 97% similarity threshold against the bacterial 16S rRNA reference database Greengenes gg_13_8 release (DeSantis et al., 2006). Finally, we compiled information on read counts for all MOTU clusters from all samples together with taxonomic information into a MOTU table, which was used for comparing and describing the diversity of the samples. To enable comparison of beta diversity at the same sequencing depth rarefaction of resample datasets from all samples was carried out to the lowest observed read count. Resampling was done at a depth of 13,400 sequence reads to allow the inclusion of all samples. Singletons (clusters with only one read in individual samples) were discarded before producing the final dataset.

We analyzed data in R 3.4.3 (R Development Core Team, 2017) and Canoco 5.03 (Ter Braak & Smilauer, 2012). We used the "Rarity" library (Leroy, 2016) to calculate rarity indices for assemblages of bacterial MOTUs in individual samples. First, we calculated rarity weights for each MOTU using the weighting function "W" (Leroy et al., 2012) and the improvements proposed by Leroy et al. (2013). Rarity cut-off points were counted using the Leroy method on a set of actual species assemblages. The obtained rarity weights were used to calculate the index of relative rarity. Extrapolated MOTU richness (estimation of unobserved MOTUs) was based on abundances in subsamples using the abundance-based coverage estimator (ACE) in the "vegan" library (Oksanen et al., 2017). The diversity of assemblages was calculated using Fisher's alpha diversity (Fisher et al., 1943).

We analyzed differences in microbial composition in the gut and abdomen of bryophages using PERMANOVA with strata defined by individuals and expressed them using the Jaccard (J) and Renkonen (P) similarity indices (Renkonen, 1938). We used the generalized linear mixed model in the "lme4" library (Bates et al., 2014) with normal distribution of residuals and random effect of individuals to determine the relationship between the species of bryophage and origin of samples as explanatory variables and (a) Fisher's alpha diversity, (b) ACE (abundance-based coverage estimation) of species richness and (c) index of relative rarity as dependent variable. The generalized linear model with a negative binomial distribution ("MASS" library) and likelihood ratio test were used to compare the MOTU richness recorded in bryophages in our dataset with the MOTU richness of various herbivorous beetles [Chrysomelidae: *Paridea angulicollis* (Motschulsky, 1854), *Smaragdina semiaurantiaca* (Fairmaire, 1888), *Cryptocephalus koltzei* Weise, 1887, *Lema fortunei* Baly, 1859; Scarabaeidae (Rutelinae): *Adoretus tenuimaculatus* Waterhouse, 1875, *Adoretus* sp. Dejean, 1833, *Exomala orientalis* (Waterhouse, 1875), *Anomala luculenta* Erichson, 1848; Tenebrionidae: *Opatrum subaratum* Faldermann, 1835] recorded by Yun et al. (2014). Rarefaction curves were plotted using the "vegan" library, line plots were created using the "sciplot" library (Morales, 2017) and Venn diagrams were constructed using the "gplots" library (Warnes et al., 2016). We analyzed differences in microbiota in relation to the species of beetles and their particular body part (gut-, abdomen- and surface-associated) using RDA and principal component analysis (PCoA) in Canoco 5.03 and PERMANOVA in R with strata defined by individuals. For RDA, the number of depicted MOTUs were reduced to 25 based on best fit. We determined the particular bacterial MOTUs significantly associated with the gut and/or abdomen of bryophagous beetles using the pairwise Wilcoxon rank sum test for multiple testing and false discovery rate method for correcting p-values using a paired test.

RESULTS

Comparison of the bacterial assemblages of the species of bryophagous beetle studied

We classified the sequences into 402 MOTUs belonging to 22 phyla, 55 classes, 91 orders and 182 families of bacteria, and one MOTU belonging to Archaea. On average, we recorded 78.5 MOTUs per sample. Gammaproteobacteria were the most abundant class on the surfaces of both *S. semistriata* (68%) and *C. paleata* (49%), whereas Alphaproteobacteria were the most abundant class in the gut and abdomen of both, *S. semistriata* (65%, 39% respectively) and *C. paleata* (62%, 55% respectively). We recorded great differences in the composition of bacterial microbiomes based on species (PERMANOVA: df = 24; F = 1.68; $P < 0.001$; $R^2 = 0.040$) and greater differences for particular body parts (df = 24; F = 8.02; $P < 0.001$; $R^2 = 0.385$).

At the order level, the most abundant MOTUs on the surface of beetles were the Enterobacteriales for *S. semistriata* (34%) and Pseudomonadales for *C. paleata* (28%). The surface assemblages of bacteria on both species of beetles were similar with the most dominant MOTUs *Pseudomonas* (24%), *Burkholderiaceae* (16%) or *Pedobacter* (12.5%). In the abdomen, the most abundant order was Sphingomonadales in both of the species of beetles (26.5%, 21% respectively). The bacterial assemblages in the abdomens of these beetles were also similar in composition and dominated by the MOTUs *Novosphingobium* (24%), *Bradyrhizobium* (20%), *Ralstonia* (14%) or *Caulobacter* (12.5%). In contrast, the bacterial assemblages in the guts of the two species differed. That in the gut of *S. semistriata* was dominated by Enterobacteriales (34.5%) and Rickettsiales, namely *Rickettsia* (14.5%), while that in *C. paleata* was dominated by another Rickettsiales, *Wolbachia* (17%) and by Entomoplasmatales (23%). A detailed composition at the order level is provided in Fig. 1 and the genera most strongly associated with both species of beetles are depicted in Fig. 2. The bacterial assemblages associated with *Curimopsis paleata* and *Simplocaria semistriata* did not differ either in terms of Fisher's alpha diversity (df = 22, F = 0.36, $P = 0.519$), species richness (df = 22, F = 0.42, $P = 0.481$) or relative rarity (df = 22, F = 0.20, $P = 0.623$).

Diversity of bacterial assemblages in guts and abdomens of bryophages

As indicated by the first two axes of the PCoA, accounting for 58% of the variability in the data, assemblages on the body surfaces differed strongly from those in their abdomens and guts, which occur in a separate and distant cluster. The microbiota in the gut and abdomen was quite

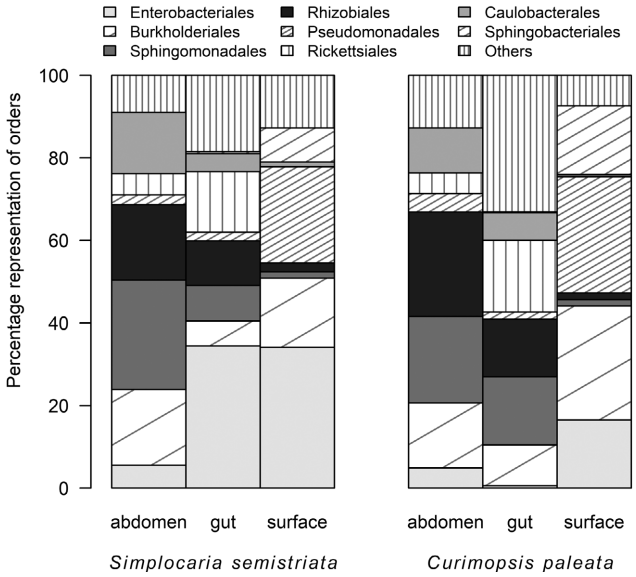

Fig. 1. The composition at the order level of the bacterial assemblages associated with the abdomens, guts and surfaces of the bryophagous beetles *Curimopsis paleata* and *Simplocaria semistriata*.

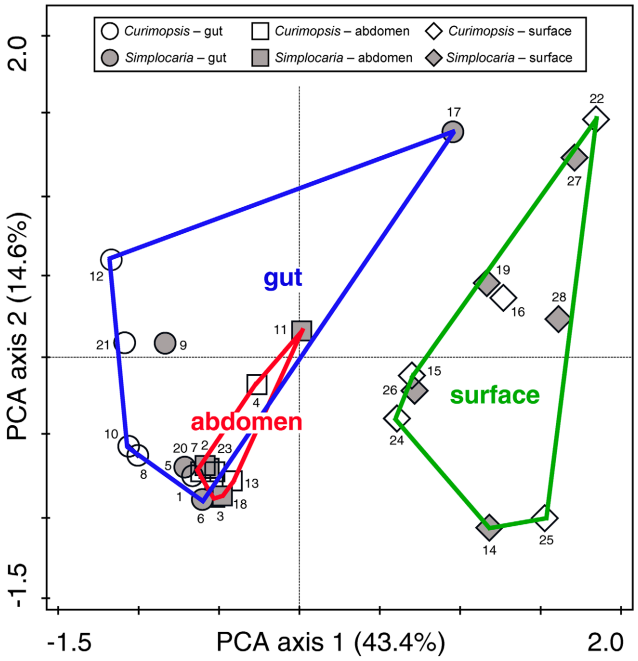

Fig. 3. Results of the PCoA of data for the different body parts of the bryophagous beetles, *Curimopsis paleata* and *Simplocaria semistriata*.

similar in both species (Fig. 3) generally, but there was a substantial difference between the assemblages in the guts and abdomens at the level of individuals (df = 16; F = 5.63; P < 0.001), with J = 21.8% average MOTU overlap and mean $P = 0.437$ (Fig. 4). Fisher's alpha diversity index was significantly dependent on the origin of the sample (df = 23; F = 10.98; $P < 0.001$). The highest overall (gamma) diversity was recorded in the gut microbiome (n = 241 MOTUs for *S. semistriata*, n = 215 MOTUs for *C. paleata*). As shown in Fig. 5a, the gut microbiome also had the highest average richness (96.22 MOTUs per sample) and the abdomen the lowest (63.89 MOTUs per sample). Fisher's alpha diversity also reflects this pattern (Fig. 5b). Rarefaction curves showed that the species richness recorded in gut samples was higher (i.e., in terms of the number of MOTUs) than recorded for other samples. Estimated species richness differed significantly also based on the origin of the sample (df = 23; F = 6.82; $P = 0.002$). The species richness of the gut microbiota of beetles was higher than that recorded for the surface and abdomen microbiota (Fig. 6a). In addition, the species richness recorded in the gut of bryophagous beetles was significantly higher than that recorded for herbivorous beetles (df = 16; LR = 12.96; $P < 0.001$; Fig. 6b).

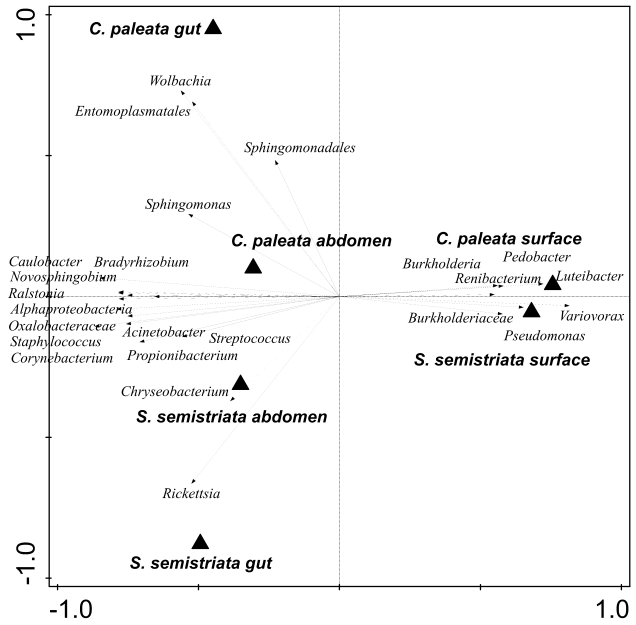

Fig. 2. Results of the RDA analysis showing the MOTUs associated with different body parts for each of the two species of bryophagous beetles studied.

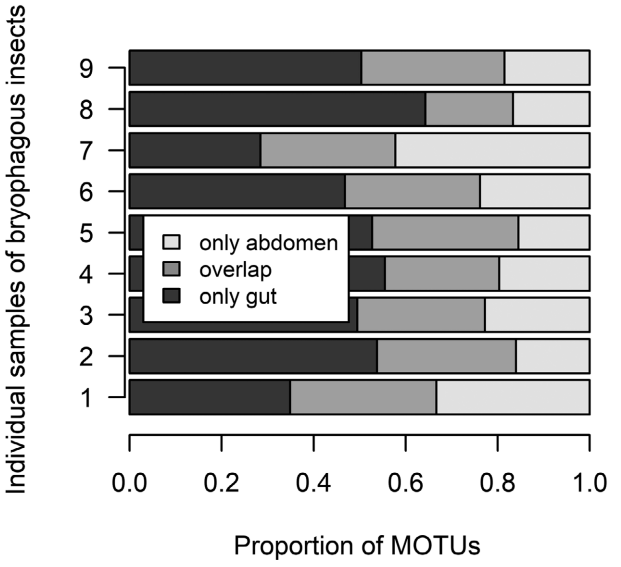

Fig. 4. Bar plots showing the proportional overlap in bacterial MOTUs assemblages recorded in the gut and abdomen of individual bryophagous beetles.

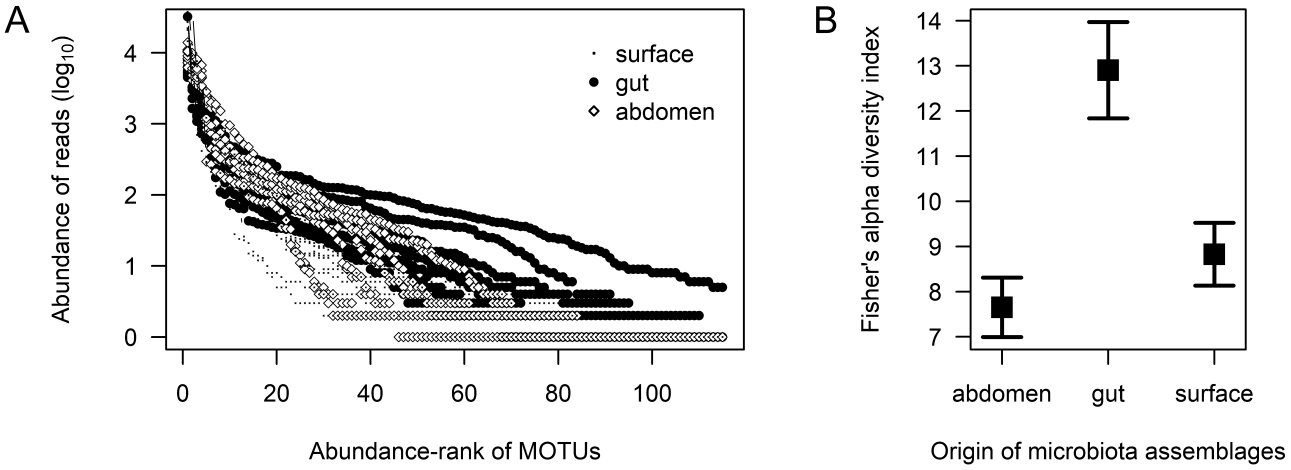

Fig. 5. A – Abundance-rank plot (Whittaker, 1965) of the microbiota recorded in the abdomen, gut and on the surface of the bryophagous beetles; curve length reflects MOTU richness, whereas a gentle slope reflects MOTU evenness. B – Differences in Fisher's alpha diversity of microbial assemblages recorded for the different body parts.

The highest proportion of origin-specific MOTUs was recorded in the gut (20.10%), followed by on the surface (13.76%) and the lowest in the abdomen (10.32%) (Fig. 7a). Consequently, index of relative rarity of assemblages differed significantly (df = 23; F = 9.88; $P < 0.001$), with the highest index values (many MOTUs with low frequency in the dataset) recorded for gut microbial assemblages (Fig. 7b). The eudominant taxa, *Bradyrhizobium, Caulobacter, Novosphingobium* and *Ralstonia*, were not only significantly more abundant in the abdomen than the gut, but also significantly more abundant in the gut than on the surface (for all: $U_{abd/gut} = 36$, $P_{abd/gut} = 0.012$; $U_{gut/sur} = 36$, $P_{gut/sur} = 0.012$).

DISCUSSION

High richness and low similarity of gut bacterial assemblages

Comparable metagenomic studies report relatively few microbial species in most insect guts (Engel & Moran, 2013) and many previous identifications based on 16S rRNA gene sequences reveal fewer than 20–30 bacterial MOTUs per insect taxon (Dillon & Dillon, 2004; Robinson et al., 2010; Wong et al., 2011). We recorded great overall richness in bacterial MOTUs, particularly in the guts of bryophages. The overall high number of MOTUs detected may reflect the greater accuracy of the metagenomic approach used. For studies comparing bacterial richness, culture-independent DNA metabarcoding is more appropriate than conventional cultivation methods with subsequent taxonomic and/or molecular identifications (Broderick et al., 2004; Vaz-Moreira et al., 2011). A comparison with the results reported for herbivorous beetles (Yun et al., 2014) revealed that in bryophagous beetles there were more MOTUs per individual. For this comparison, we used only those MOTUs recorded in the gut of bryophages. A comparable metagenomic study of herbivorous beetles by Kelley & Dobler (2011) indicate that the gut of *Cryptocephalus* spp. (Chrysomelidae) harbours only 15–30 bacterial MOTUs per species. In contrast, Montagna et al. (2014) report that *Cryptocephalus* sp. averaged 86.3 MOTUs per individual, which is consistent with our results. A higher MOTU richness is reported for some non-herbivorous

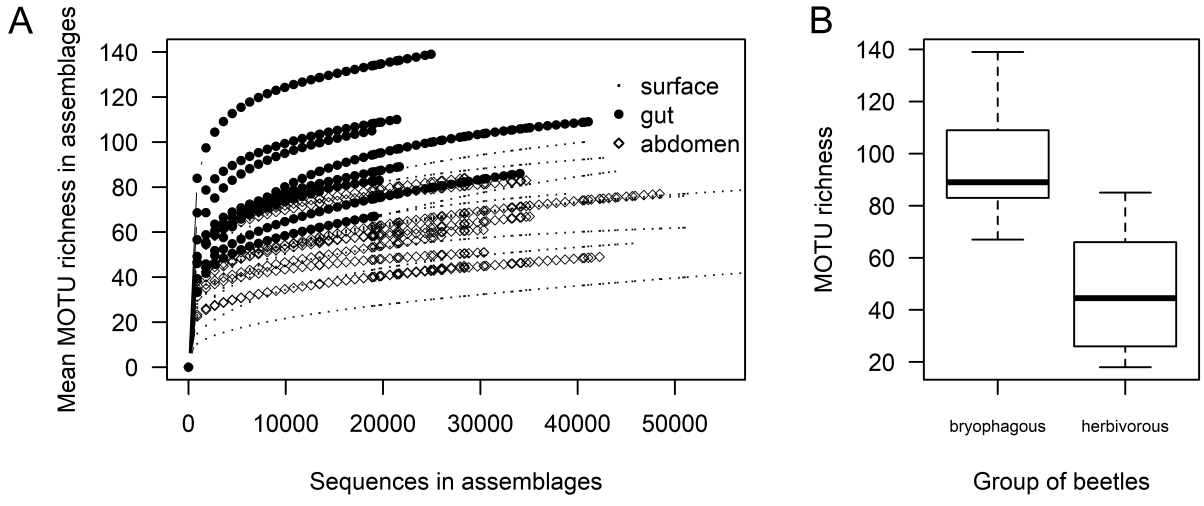

Fig. 6. A – Rarefaction curves showing the richness of bacterial MOTUs recorded in the gut, abdomen and on the surface of bryophagous beetles. B – Comparison of the MOTU richness of bryophagous beetles and that of herbivorous beetles (Yun et al., 2014).

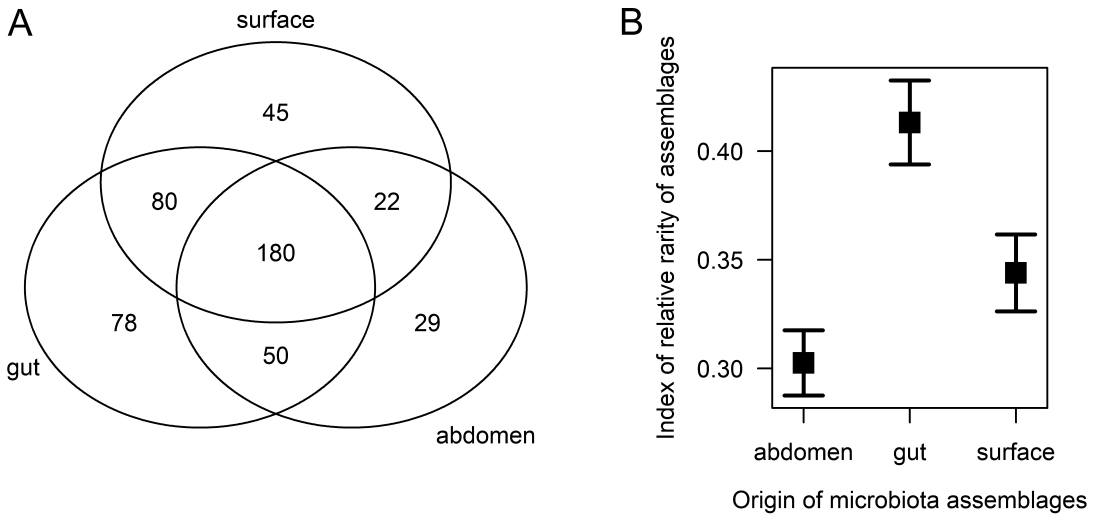

Fig. 7. A – Venn diagram showing the overlap in the bacterial microbiomes recorded for different body parts of bryophagous beetles. B – Index of the relative rarity of microbiomes recorded for the different body parts.

beetles: 164 MOTUs for *Melolontha melolontha* (Scarabaeidae; Egert et al., 2005), 140 MOTUs for *Megetra cancellata* and 177 MOTUs for *Epicauta longicollis* (both Meloidae; Colman et al., 2012). The high MOTU richness recorded here in the gut of bryophagous beetles could be associated with the very specific diet and ecology of these insects.

Both seasonality and the substrate from which the beetles were sampled may substantially influence richness and diversity of bacterial assemblages. We collected our specimens at the beginning of winter. Although the effect of timing remains to be determined, bacterial diversity in the phyllosphere increases in response to drought and heat (Peñuelas et al., 2012). Similarly, insect gut microbiota changes with the season (Behar et al., 2008). We collected samples from the moss *D. heteromalla*, which forms low cushions barely a few millimeters above the soil. Soil bacterial assemblages are very complex (Borneman et al., 1996) and determine the composition and structure of gut microbial assemblages in insects living in direct contact with the soil (Huang & Zhang, 2013). Consistently, high bacterial richness of up to 695 MOTUs, are reported in the detritivorous beetles *Onthophagus* sp. (Yun et al., 2014).

The bacterial microbiome in the guts of beetles has the greatest proportion of specific MOTUs and the highest index of relative rarity compared to that recorded in other body parts, indicating that many bacterial MOTUs in the guts of bryophagous beetles differed among individuals. In some insects, gut bacterial communities vary among individuals within a species and consist mainly of bacteria without specific adaptation(s) to life in the gut of their host species (Cariveau et al., 2014), which indicates that the diet of the host might have some influence (Broderick et al., 2004). Nevertheless, a relatively static community is also documented (Tang et al., 2012). Physicochemical conditions in gut compartments, such as pH, redox potential, or availability of particular substrates, may select for particular species. Thus, even when acquired independently during each generation, gut communities are not expected to be random assemblages of bacteria derived from the food or local environment (Engel & Moran, 2013). Instead, the high abundance and ubiquitous presence of soil bacteria, such as *Variovorax*, *Pedobacter*, *Bacillus*, *Pseudomonas* and *Erwinia* on the surface of the bodies of *S. semistriata* and *C. paleata* could be explained by the proximity to the soil of mosses such as *D. heteromalla*. Indeed, the bacteria that are predominant on the surface of the beetles are also widespread in the rhizosphere of plants (Mahaffee & Kloepper, 1997) and, in the case of *Pseudomonas* and *Erwinia* are also endophytic bacteria of many species of plants (Cankar et al., 2005).

Little overlap in the microbiomes in the gut and abdomen

The bacterial assemblages in the guts and abdomens of individual bryophagous beetles differed substantially. This indicates the possibility of confounding two distinct microbial niches if gut and abdomen are not separated during dissection, as is the case of most studies on such small insects. Despite the careful dissection of the guts and abdomens of beetles, we cannot completely exclude the possibility of cross-contamination. Thus, the recorded dissimilarity of the assemblages in the gut and abdomen could be even more significant, further emphasizing the importance of their segregation during dissection.

Ecology and function of potential symbionts

Although symbiotic bacteria are often acquired via the diet or soil, they can be involved in digestion and other processes (Kelley & Dobler, 2011). Proteobacteria and Firmicutes are often the predominant bacterial phyla in the majority of insect guts (Vasanthakumar et al., 2008; Douglas, 2011; Colman et al., 2012), which is consistent with our findings. The eudominant bacterial genera significantly more associated with the gut and abdomen of both byrrhids were *Bradyrhizobium, Caulobacter, Novosphingobium* and *Ralstonia*. These bacteria were not only more abundant in the gut than on the surface of their body, but also more abundant in the abdomen than in the gut, indi-

cating a more intimate association with the organisms and possible involvement in the metabolic processing of bryophytes. ***Bradyrhizobium***, the commonest soil bacterium fixing nitrogen in legumes (Klimaszewski et al., 2013), has been repeatedly associated with *Sphagnum* moss (Bragina et al., 2012) and reported in the guts of Cerambycidae (Grünwald et al., 2010), Staphylinidae (Klimaszewski et al., 2013) and Tortricidae fed on artificial diets (Landry et al., 2015). Many other MOTUs recorded in the gut and abdomen belong to Bradyrhizobiaceae, which are typical rhizosphere bacteria fixing nitrogen (De Jesús-Laboy et al., 2011). They have been also isolated from bryophytic mites (Moquin et al., 2012) and *Sphagnum* mosses (Bragina et al., 2012). ***Caulobacter*** are predominant in the mycangia of *Xyleborus glabratus* (Curculionidae; Hulcr et al., 2012), the gut of *Acromyrmex* ants (Van Borm et al., 2002), in Cicadellidae (Rogers & Backus, 2014), Noctuidae (Snyman et al., 2016) and the midgut of *Pyrrhocoris* bugs and in their diet, lime seeds (Sudakaran et al., 2012), and in symbiotic interactions with *Sphagnum* mosses (Bragina et al., 2012). ***Novosphingobium*** is reported associated with the gut of *Cryptocephalus* spp. (Chrysomelidae; Montagna et al., 2014), *Anoplophora glabripennis* (Cerambycidae; Geib et al., 2009), oral secretions of Curculionidae (Cardoza et al., 2009), herbivorous Cicadellidae (Rogers, 2016), gall midges (Ojha et al., 2017) and also mosses (Bragina et al., 2012; Graham et al., 2017). ***Ralstonia*** is a plant-associated genus abundant in the microbiota of Cicadellidae (Rogers & Backus, 2014), *Cryptocephalus* spp. (Chrysomelidae; Montagna et al., 2014) and associated with ciliates in the rumen (Irbis & Ushida, 2004).

Some of these microorganisms can provide their host with nitrogen, which is typically deficient in plant materials (Benemann, 1973). Rapid passage through the midgut may reduce the ability to extract nitrogenous compounds from food. Therefore, an association with nitrogen-fixing bacteria may be especially beneficial. Some genera present in the microbiota of bryophages, such as *Bradyrhizobium*, fix nitrogen. Closely related to the nutritional role of symbiotic microorganisms is their ability to detoxify (Engel & Moran, 2013). Bryophytes contain a broad spectrum of secondary metabolites that protect them from being eaten (Gerson, 1982). For example, symbionts of the bryophagous Peloridiidae are thought to play a role in excretion or detoxification of ingested moss (Kuechler et al., 2013). Similarly, the microorganisms found in the bryophagous beetles studied, such as *Novosphingobium* and *Ralstonia* can degrade phenols and aromatics.

CONCLUSION

The recorded MOTU diversity in the microbiomes in the gastrointestinal tract of bryophagous beetles was high. Substantial differences in the composition of the assemblages recorded in the gut and abdomen indicate the importance of separating body parts before any metagenomic analysis. High bacterial diversity may stem from the particular diet of bryophagous beetles, as the most abundant MOTUs recorded are involved in metabolic processes associated with digestion; alternatively, they may reflect the greater possibilities of metagenomics. Further studies are required to distinguish bacteria, which are either incorporated only transiently through the diet or are ingested accidentally with soil particles, from those involved in bryophyte digestion. This vast bacterial assemblage could potentially serve as a source of enzymes degrading a variety of chemical compounds present in mosses. Moreover, further research is likely to help us understand the unique ability of bryophagous beetles to digest such a specific and generally deleterious source of food, moss.

ACKNOWLEDGEMENTS. This research was funded by the Ministry of Education, Youth and Sports of the Czech Republic (LO1208), the Institute of Environmental Technologies (CZ.1.05/2.1.00/03.0100), NextGenProject (CZ.1.07/2.3./20.0303), Extension and Appreciation of Instruments IET (CZ.1.05/2.1.00/19.0388), University of Ostrava (SGS28/PrF/2014, SGS20/PrF/2015, SGS23/PrF/2016), European Regional Development Fund BIOCEV (CZ.1.05/1.1.00/02.0109) and Czech Science Foundation (GA18-08803S). We would like to thank Editage (www.editage.com) for English language editing.

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Received January 21, 2019; revised and accepted August 22, 2019
Published online November 26, 2019