



Phylogenetic and trophic determinants of gut microbiota in soil oribatid mites



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ABSTRACT

Gut microbiota are determined by both the food ingested and physiological conditions of the host. In soil food webs, detritivore animals occupy various trophic niches, spanning from primary decomposers to predators. However, the relative contribution of food resources and species attributes of consumers to gut microbial communities in soil detritivores has not yet been explored. In this study, we investigated gut bacteria and fungi of oribatid mites (Oribatida, Acari), ubiquitous and diverse soil microarthropods feeding on a variety of food resources, to uncover the contribution of host phylogenetic relatedness and trophic niches to the assemblages of gut microbiota. Abundance and community composition of bacteria and fungi were characterized by qPCR and Illumina sequencing, respectively. Gut bacterial communities were more closely correlated with host phylogenetic affinity, whereas gut fungal communities were more closely correlated with the trophic niches of the host. Community phylogenetic analysis suggests that deterministic processes predominated in the assembly of both bacterial and fungal communities in most of the studied oribatid mite species. Integrating phylogenetic distance and trophic niche distance of hosts resulted in the highest correlation coefficients between host species and their gut microbial communities suggesting that both evolutionary history and current trophic niches shape gut microbial communities. Bacteria in the gut may comprise commensals or mutualists facilitating digestion which potentially coevolved with the host, while the fungal community in the gut reflects the trophic niches of the consumer likely suggesting that they form part of the diet and serve as food resources of soil detritivore microarthropods.

1. Introduction

Host – microbial associations are crucial to many plants and animals in their development and adaptation to the environment (Ley et al., 2008; Shapira, 2016). Microbiomes, including genes and genomes of microbiota, play an essential role for the fitness and adaptation of host species (Hacquard et al., 2015; Shapira, 2016), reflecting in the phrase “the second genome of the host” (Berendsen et al., 2012; Grice and Segre, 2012). In animals, microbiomes function as counterpart of the host and influence host metabolism and nutrient assimilation (Lee and Hase, 2014). Reciprocally, physiological conditions of the host impact community assembly of host associated microorganisms (Dethlefsen et al., 2006; Yun et al., 2014). Further, the diet of consumers contributes to the microbial community compositions in their guts (Knapp

et al., 2009), resulting in non-random patterns of gut microbial community composition.

Deterministic processes drive the composition of gut microbiota in termites, nematodes and primates (Sekelja et al., 2011; Otani et al., 2014; Brune and Dietrich, 2015; Berg et al., 2016), where gut microbial communities are more similar in phylogenetically and trophically closely related host species than in distantly related species. On the one hand, phylogenetically related species may share morphological, physiological and ecological traits, and therefore community composition of gut microbiota likely reflects host phylogenetic affinity. On the other hand, in particular in detritivores, microorganisms form part of the diet and function as food resource, thereby gut microbiota of detritivores may reflect the diet and trophic niches of the consumer. However, the relative contribution of consumer species attributes, represented by

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their phylogenetic affinities and food resources, to gut microbial community assembly has not yet been explored. In this study, we used oribatid mites (Oribatida, Acari), a ubiquitous and diverse soil microarthropod taxon that feeds on a variety of food resources, to investigate the driving factors of gut microbiota and to uncover the contribution of host phylogenetic relatedness and trophic niches to the assemblages of gut microbiota.

Oribatid mites are important soil decomposers that regulate soil microbial activity and contribute to litter decomposition and nutrient cycling in particular in forest ecosystems (Maraun and Scheu, 2000; Maraun et al., 2007). In the soil food web, different oribatid mite species occupy a variety of trophic niches as indicated by the stable isotopic signatures (Schneider et al., 2004; Maraun et al., 2011). The species include primary decomposers using detritus and root exudates as resources, secondary decomposers consuming microbes and/or microbial residues, and predators feeding on other soil animals. Decomposers at different trophic levels thus may differ in respect to their gut microbial communities. Further, earlier work suggested that oribatid mites predominantly feed on fungi but are unable to digest complex compounds such as chitin and cellulose (Siepel and de Ruiter-Dijkman, 1993; Smrž and Norton, 2004; Smrž and Čatská, 2010). Microorganisms in their gut, however, may contribute to the digestion of complex compounds, with the microorganisms benefitting from continuous resource input and dispersal due to vertical and horizontal movement of the animal hosts in soil facilitating colonization of new habitats (Behan and Hill, 1978; Renker et al., 2005; Alejandra Perotti and Braig, 2011).

Oribatid mite – gut microbiota interactions have been investigated in a number of studies (Siepel and de Ruiter-Dijkman, 1993; Renker et al., 2005). However, recent advances in using high-throughput sequencing techniques and community phylogenetic approaches allow investigating host – microbial associations in soil detritivore animals in unprecedented detail (Wolf and Rockett, 1984; Hubert et al., 2001; Smrž and Norton, 2004; Pompanon et al., 2012; Cadotte et al., 2013). In this study, we analyzed the relative contribution of oribatid mite phylogeny and trophic niches to their gut microbial assemblages. We hypothesized that (I) the composition of gut microbiota communities correlates with the trophic niches of oribatid species as indicated by stable isotope signatures, and (II) phylogenetic relationship reflecting similarity in physiological attributes of hosts drives gut microbiota communities. Further, we hypothesized that (III) microbial community assembly in the gut of oribatid mites is shaped by the host environment and this is reflected by coexisting gut microbiota being phylogenetically closely related.

2. Materials and methods

2.1. Study site

Oribatid mites were sampled in a beech forest of the Hainich-Dün in central Germany (51.3371°N, 10.3592°E). The site forms part of the “Biodiversity Exploratories”, a large scale long term functional biodiversity research project (<http://www.biodiversity-exploratories.de>; Fischer et al., 2010). The site is located at 432 m a.s.l., mean annual temperature is 7.5 °C and mean annual precipitation is 670 mm. The forest consists of old European beech trees (*Fagus sylvatica*) predominantly ranging between 70 and 110 years. The soil is characterized as Luvisol and the topsoil (0–10 cm) is rather acidic with a pH (H₂O) of 4.2–4.4.

2.2. Sampling and sample preparation

Litter and top soil samples were collected on 14th November 2016. Oribatid mites were extracted by heat (Kempson et al., 1963). To avoid degradation of microbial DNA in the gut of consumers, animals were sorted in 96% ethanol within 6 h after sampling and stored at –80 °C; prey DNA in the gut of consumers are detectable for at least 12 h after

ingestion (Sheppard and Harwood, 2005). Individuals were identified to species level using Weigmann and Miko. (2006) except for *Oribatella* which was determined to genus level only, as determination to the species needs bleaching of the individuals which detrimentally affects the integrity of DNA. However, according to the previous data *Oribatella calcarata* (Koch, 1835) dominates at the study site (C. Bluhm, unpubl. data). In total, seven oribatid mite species dominating at the study site and being generally abundant in temperate beech forests (Bluhm et al., 2015) were selected for this study: *Achipteria coleoptrata* (Linnaeus, 1758), *Eupelops hirtus* (Berlese, 1916), *Nothrus silvestris* (Nicolet, 1855), *Oribatella* spp., *Platynocheilus peltifer* (Koch, 1839), *Steganacarus magnus* (Nicolet, 1855) and *Tritegeus bisulcatus* (Grandjean, 1953).

2.3. Molecular gut content analysis

Prior to DNA extraction, oribatid mites were subjected to sterilization to remove contamination from surface microbial DNA using a customized washing protocol (K. Heidemann, unpubl. data). Animals were transferred to 1% Tween 20 and vortexed for 2 min, and then to 98% ethanol and vortexed for 5 min, followed by 1% Tween 20 and vortexed for 2 min. Thereafter, they were transferred to 5% bleach and vortexed for 5 min and finally vortexed twice in 1% Tween 20. All operations were conducted under sterile conditions.

According to the preliminary study on sufficient amount of DNA for gut microbiota analysis (Supplementary Fig. S1), pooling eight individuals yields compatible number of microbial OTUs as compared to that of 16 individuals. Therefore, eight individuals of each oribatid mite species were pooled for one DNA extraction using Tissue Genomic DNA Purification Mini Prep Kit (Genaxxon BioScience, Ulm, Germany). Five replicate oribatid mite samples (with eight individuals each) were extracted for each species except for *S. magnus* with only three replicates (due to limited numbers of individuals). V3-V4 region of bacterial 16S rRNA gene and fungal ITS2 region were amplified using primer pairs 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') (Klindworth et al., 2013), ITS3.KYO2 (5'-GATGAA-GAAGCYAGYRAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Toju et al., 2012), respectively. These primers were designed with overhang Miseq adapters at the 5' end (5'-TCGTCGGCAGCGTCAGATGTGATAAGAGACAG-3' for forward primers and 5'-GTCTCGTGGGCTCGGAGATGTGATAAGAGACAG-3' for reverse primers).

Each DNA extract was amplified in triplicate, including No Template Control (NTC), in 50 µl reaction volume containing 10 µl 5-fold Phusion GC Buffer, 50 ng of DNA, 0.2 µM each primer, 1 Unit Thermo Scientific® Phusion High-Fidelity DNA Polymerase, 5% DMSO and 0.2 mM of each of the four deoxynucleoside triphosphates. After initial denaturation at 98 °C for 1 min, the targeted DNA region was amplified by 25 cycles at 98 °C for 45 s, optimal annealing temperature (55 °C for bacteria and 48 °C for fungi) for 45 s and 72 °C for 30 s, followed by a final elongation step at 72 °C for 5 min using a Biometra TAdvanced thermal cycler (Biometra, Göttingen, Germany). The triple PCR products were combined for each sample and purified using PCR DNA Purification Mini Prep Kit (Genaxxon BioScience, Ulm, Germany). Amplicon concentration was measured using Qubit™ dsDNA HS Assay Kit and a Qubit fluorometer (Invitrogen GmbH, Karlsruhe, Germany). Purified PCR products were sent to Göttingen Genomics Laboratory for paired-end sequencing by 2 × 300 bp Illumina Miseq platform. Raw sequencing reads were deposited in NCBI SRA database under the accession number SRP116306.

2.4. Illumina Miseq sequence data processing

Paired-end sequence data were joined, demultiplexed and analyzed using QIIME pipeline (Caporaso et al., 2010). Sequences < 200 bp with an average quality score < 20 and ambiguous characters were discarded. After chimeras and singletons were detected and removed by usearch6.1, operational taxonomic units (OTUs) were clustered on the

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