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ORIGINAL PAPER

The Antarctic mite, *Alaskozetes antarcticus*, shares bacterial microbiome community membership but not abundance between adults and tritonymphs

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Abstract

The Antarctic mite (*Alaskozetes antarcticus*) is widely distributed on sub-Antarctic islands and throughout the Antarctic Peninsula, making it one of the most abundant terrestrial arthropods in the region. Despite the impressive ability of *A. antarcticus* to thrive in harsh Antarctic conditions, little is known about the biology of this species. In this study, we performed 16S rRNA gene sequencing to examine the microbiome of the final immature instar (tritonymph) and both male and female adults. The microbiome included a limited number of microbial classes and genera, with few differences in community membership noted among the different stages. However, the abundances of taxa that composed the microbial community differed between adults and tritonymphs. Five classes—Actinobacteria, Flavobacteriia, Sphingobacteriia, Gammaproteobacteria, and Betaproteobacteria—comprised ~ 82.0% of the microbial composition, and five (identified) genera—*Dermacoccus*, *Pedobacter*, *Chryseobacterium, Pseudomonas,* and *Flavobacterium*—accounted for ~ 68.0% of the total composition. The core microbiome present in all surveyed *A. antarcticus* was dominated by the families Flavobacteriaceae, Comamonadaceae, Sphingobacteriaceae, Chitinophagaceae and Cytophagaceae, but the majority of the core consisted of operational taxonomic units of low abundance. This comprehensive analysis reveals a diverse microbiome among individuals of different stages, with overlap likely due to their shared habitat and common feeding preferences as herbivores and detritivores. The microbiome of the Antarctic mite shows considerably more diversity than observed in mite species from lower latitudes.

Keywords Microbiome · Oribatid mite · 16S rRNA · Antarctica · Arthropod · Polar

Introduction

With long winters characterized by cold and dehydrating conditions and summers that rarely reach above freezing, the continent of Antarctica is one of the most extreme environments an organism can inhabit. This environment is home to a number of poorly understood organisms, such as the Antarctic mite, *Alaskozetes antarcticus* (Oribatida; Block and Convey 1995). This mite maintains abundant populations in terrestrial Antarctica by tolerating extreme temperatures, numerous freeze–thaw cycles, desiccating conditions, and limited periods for growth and reproduction (Young and Block 1980; Shimada et al. 1992; Convey 1994a, b; Block and Convey 1995; Benoit et al. 2008; Everatt et al. 2013). Desiccation and cold stresses are mitigated by seeking reprieve in sheltered microhabitats, which provide stabilized locations for mite development and enhance survival among all stages, and freeze–thaw

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cycles are survived via glycerol-enhanced supercooling (Young and Block 1980; Block and Convey 1995). *A. antarcticus*, an herbivore and detritivore, is adapted to survive the sub-optimal conditions of Antarctica and completes an unusually long life cycle of five to six years characterized by low feeding and growth rates as well as a low reproductive output (Block and Convey 1995). This prolonged lifecycle includes the management of many biological characteristics, which involves molt synchronization in early summer, followed by an inactive pre-molt phase, and regulation of gut content to reduce the chance of inoculative freezing (Block and Convey 1995).

Despite our understanding that microbes often have critical roles in the development and physiology of invertebrates, there is a distinctive gap in such information for Antarctic species and the few studies that have been completed are based on aquatic invertebrates (De Meillon and Golberg 1946; Webster et al. 2004; Herrera et al. 2017). No studies have directly examined microbial communities associated with the moss mite, *A. antarcticus*. This study characterizes the microbiome of *A. antarcticus* to determine the microbial community composition and to make comparisons between adult females (F), adult males (M), and tritonymphs (T). Our comparison specifically focuses on whole-body microbiomes and the overlapping (core) bacterial community found in the two sexes and the tritonymph. By identifying both conserved and differentiated communities among the mite groups, our goal was to gain a greater understanding of the microbial communities for *A. antarcticus* with respect to mite sex and stage of life in the extreme Antarctic environment and to compare these results with observations on mites from lower latitudes.

Methods

Field site description and sample collection

Mites were collected from Cormorant Island, near Palmer Station, Antarctica (− 64.793455S, − 63.966892W; Fig. 1) in the summer (*i.e.,* January) of 2016. To ensure standardization, mites were held for two weeks at 4 °C under long daylength (20-h light:4-h dark) conditions typical of summer at Palmer Station. Mites were provided access to algae (*Prasiola crispa*), local mosses, and other organic debris from the site of collection. The organic substrate for these mites contained Antarctic midges, *Belgica antarctica*, and collembolans. Females, males, and tritonymphs were separated based on described morphological characteristics (Block and Convey 1995). Mites were surface sterilized by rinsing 1 min in each of the following solutions: 70% ethanol, 2% sodium hypochlorite, and 70% ethanol. This was followed by four rinses in sterile phosphate-buffered saline (PBS; 81 mM Na_2HPO_4, 19 mM NaH_2PO_4, 150 mM NaCl, pH 7.4. Samples were frozen at − 70 °C until used.

DNA extraction and quantification

For each sample, four mites were manually homogenized in liquid nitrogen and DNA was extracted using a DNeasy PowerSoil Kit (Qiagen, Carlsbad, CA, USA). Female, male, and tritonymph groups each consisted of eight samples (32 mites total, per group). DNA was sent to the Center for Bioinformatics & Functional Genomics at Miami University (Oxford, OH, USA) or the University of Minnesota Genomics Center (Minneapolis, MN, USA) for 16S rRNA gene sequencing. DNA concentration was determined using a

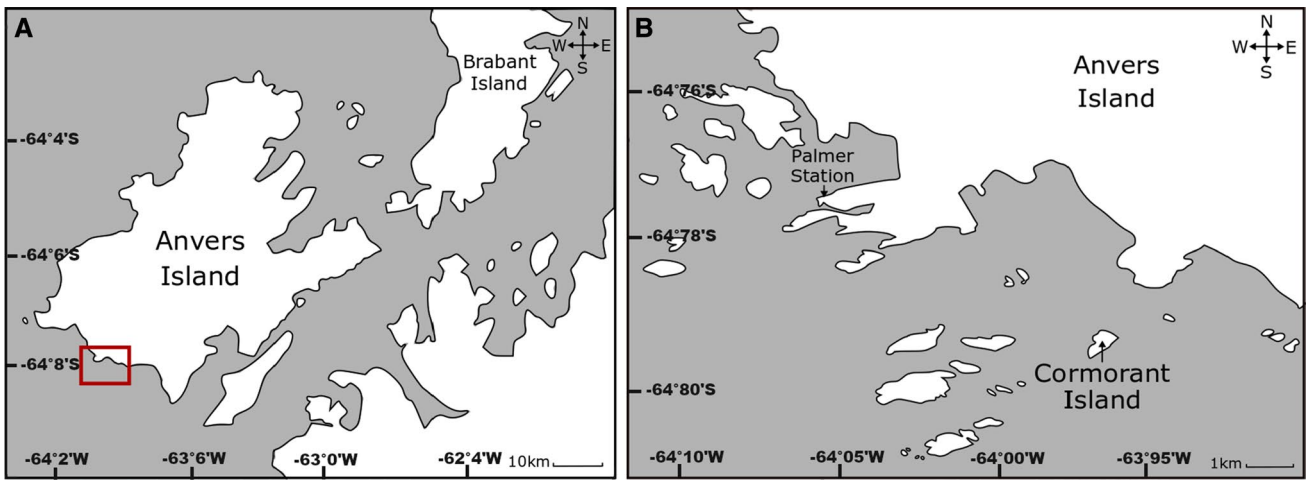

Fig. 1 Location of Cormorant Island, Antarctica (− 64.793455S, − 63.966892W), **a** relative to Anvers and Brabant Islands in the Palmer Archipelago and **b** relative to Palmer Station. The box inside **a** indicates the geographic border used for **b**

Qubit dsDNA HS Assay kit and a Qubit 3.0 Fluorometer (Invitrogen, Burlington, ON, Canada). Earth Microbiome Project primers 515f and 806r were used to isolate the V4 region of 16S rRNA gene sequences (Caporaso et al. 2011; Apprill et al. 2015); an extraction blank served as a negative control.

Sequence analysis

Sequence analysis was conducted similarly to previous studies (Schuler et al. 2017). MiSeq Illumina 2 × 300 bp chemistry was used to sequence amplicons. Sequence reads were processed using mothur (v.1.39.3; Schloss et al. 2009), following the MiSeq SOP (Kozich et al. 2013), and with QIIME (v.1.9.1; Caporaso et al. 2011), through the Nephele pipeline (v.1.7), referencing SILVA taxonomy database versions 99 and 123, respectively (sequence reads and taxonomies from mothur are included in Online Resource 1). Chimeras were removed with UCHIME (Edgar et al. 2011), reads classified as archaea and eukarya were removed, singletons were removed, and a 3% dissimilarity was allowed (i.e., a distance of 0.03) in the analysis, corresponding to a 97% OTU identification rate, which is sufficient for species representation in the sample (Fuka et al. 2013). Remaining unique sequences were classified into operational taxonomic units (OTUs), and relative abundances were normalized to the total number of reads (Online Resource 2). α-diversity was assessed through Shannon and inverse Simpson diversity indices, as well as through the number of observed OTUs for an OTU definition (sobs). β-diversity was calculated using the Jaccard index and the Yue & Clayton measure of dissimilarity and compared in mothur through a homogeneity of molecular variance (HOMOVA) analysis. Community composition abundances were visualized for hierarchical viewing as krona charts (Ondov et al. 2011; Online Resources 3–7). Between-group comparisons were conducted by analysis of molecular variance (AMOVA) and Linear discriminant analysis Effect Size (LEfSe; Online Resources 8–10). Krona charts and LEfSe were generated in the Galaxy web platform (Afgan et al. 2018). Rarefaction (Online Resource 11) and non-metric multidimensional scaling (NMDS) analyses using sequence recovery data were both conducted in mothur. Core OTUs were isolated in mothur by selecting shared sequences between female, male, and tritonymph groups (Online Resources 12–15). Data management, statistical testing, and graphical representation were conducted in Microsoft Excel (v.14.6.5) and R using reshape2 (Wickham 2007), ggplot2 (Wickham 2009), VennDiagram (Chen 2018), dplyr (Wickham et al. 2017), Rmisc (Hope 2013), car (Fox and Weisberg 2011), lsmeans (Lenth 2016), multcompView (Graves et al. 2015), and exported via the xlsx package (Dragulescu 2014). ANOVA-based analyses were completed using these packages according to methods previously described (Mangiafico 2015). Results from mothur and the Nephele implementation of QIIME were compared via linear regression in R for all groups at the bacterial class level (Fig. 2). Sequence data have been added to the NCBI Sequence Read Archive (SRA) database (BioProject number PRJNA514361) and project descriptions were included as Online Resource 16.

Results

Relative abundance, α- and β-diversity, community composition, and number of sequences from both mothur (364,394) and QIIME (372,362) were similar. Although variation existed in calculations for sobs between mothur and QIIME, the results were corroborative. A dual-pipeline comparison of class relative abundance between mothur and QIIME was used for verification purposes, and no difference existed between the two methods of analyses ($y = 0.9882x + 0.0011$, $R^2 = 0.9842$; Fig. 2). This study targeted the V4 region of the 16S rRNA gene for high specificity and qualitative comparisons to other studies (Yang et al. 2016), and although PCR validation was not performed, sole utilization of high-throughput sequencing is highly effective (Yang et al. 2015). Downstream analyses were also consistent, and although mothur generates a larger number of OTUs than QIIME (López-García et al. 2018), calculations from both pipelines indicated that α-diversity was similar between groups (Fig. 3a–d). Rarefaction (Online Resource 11) and summary statistics

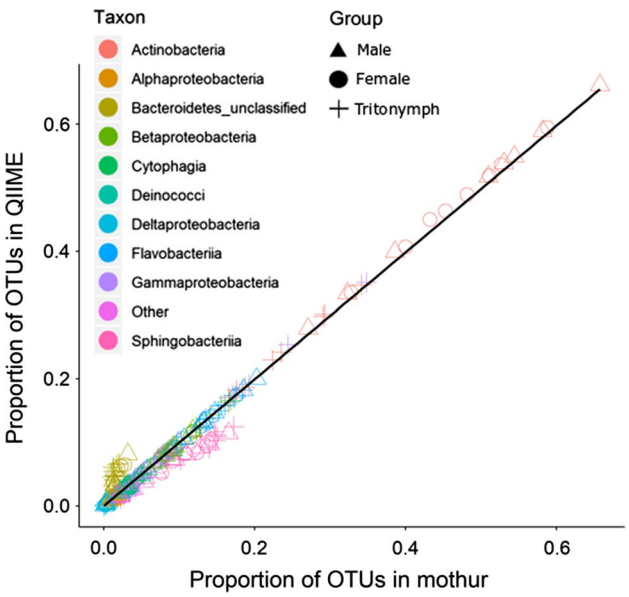

Fig. 2 Between-pipeline regression of the ten most abundant classes and the remaining 'other' classes. Shape indicates group, compared classes are listed, $y = 0.0011 + 0.9882x$, $r^2 = 0.9842$

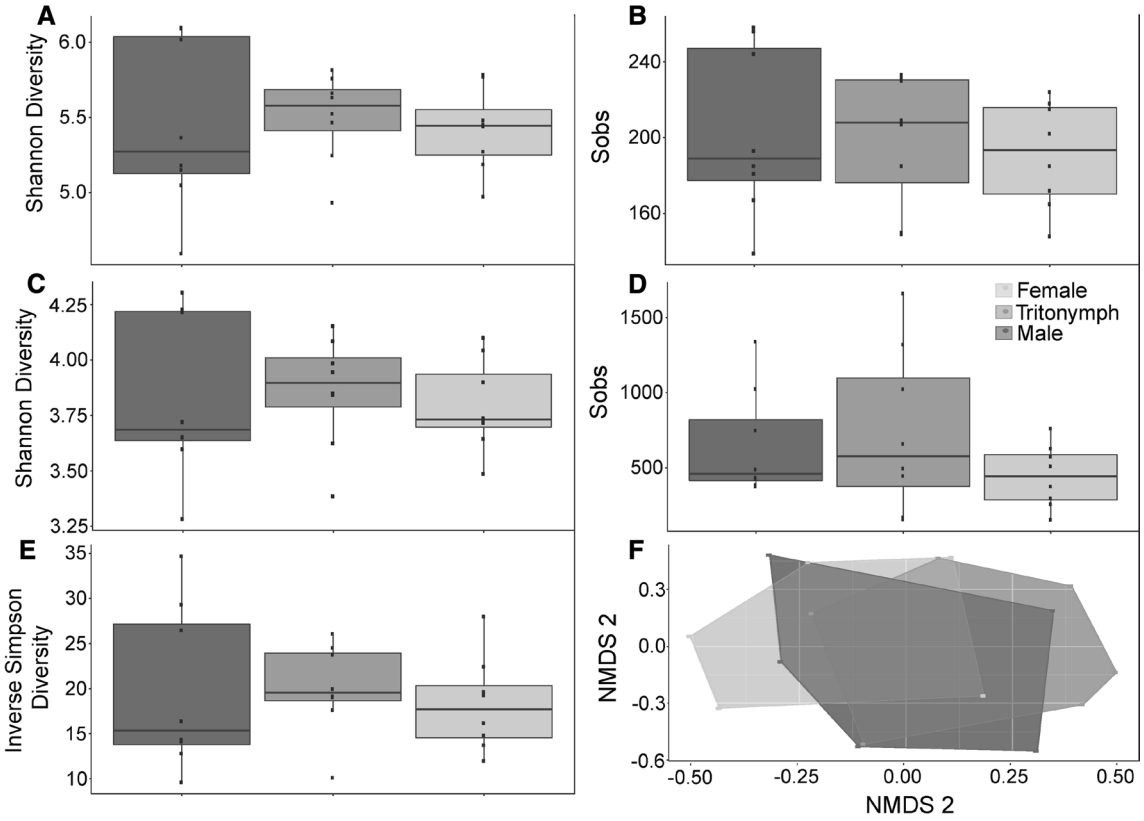

Fig. 3 Measures of α-diversity and sample similarities, **a** Nephele output of Shannon diversity, **b** Nephele output of sobs, **c** mothur output of Shannon diversity, **d** mothur output of sobs, **e** mothur output of inverse Simpson diversity, and **f** NMDS generated from thetayc calculations in mothur

Table 1 Summary statistics and α-diversity descriptions for group, total, and core microbial community composition

Group	Total number of sequences	Good's coverage (%)	Total sobs	Inverted Simpson diversity average	Shannon diversity average
Female	72,431	97.33	3554	18.251	3.794
Male	132,570	97.74	5225	19.712	3.832
Tritonymph	159,393	97.64	5930	20.034	3.859
Total	364,394	97.57	14,709	19.332	3.828
Core	9947	78.86	753	–	–

(Table 1) showed adequate sampling depth and coverage. Calculation of sobs, inverse Simpson, and Shannon calculations showed no overall differences in richness or α-diversity (Fig. 3a–e), and dissimilarity measures showed no differences in β-diversity or homogeneity among females, males, and tritonymphs (Fig. 3f; Table 2). Microbial community membership was the same in *A. antarcticus* regardless of sex or life-stage, with a microbial core composition reliably representing all samples (resolved to the family level; Fig. 4), but microbial abundances were different between adult and tritonymph *A. antarcticus* (Table 3, Online Resources 8 and 10).

Table 2 Between-community HOMOVA for β-diversity using the Jaccard index (jclass) and the Yue and Clayton (thetayc) measure of dissimilarity within mothur

HOMOVA	*b*-value	Mean square	*p* value
Jclass	0.005	0.244	0.250
Thetayc	4.976	0.043	0.117

Sobs were compared in mothur for females (3554), males (5225), and tritonymphs (5930) to establish a core microbiome (Fig. 4; Table 1). In characterizing the core

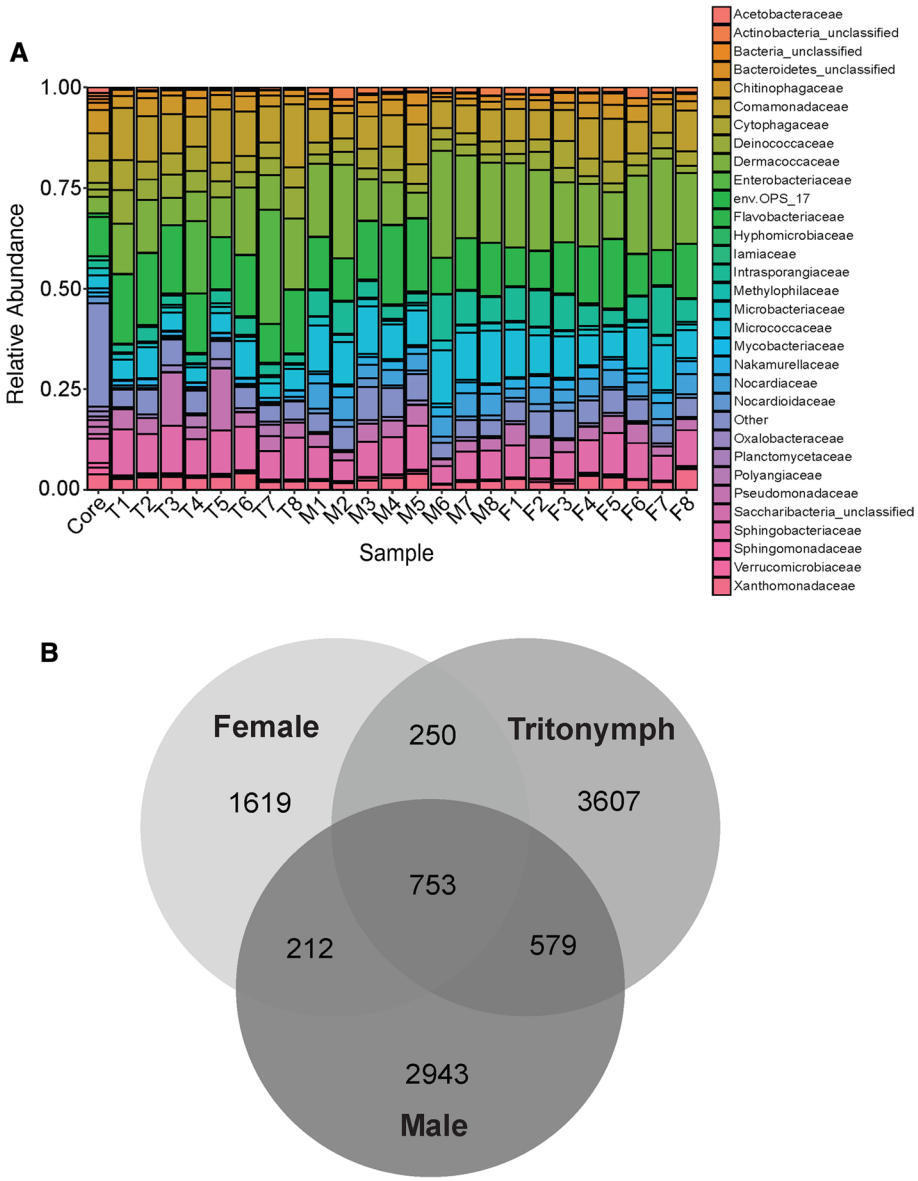

Fig. 4 **a** Mothur-derived relative microbial abundance of bacterial families in *A. antarcticus* with the core microbial composition displayed on the left. **b** Venn diagram representing the number of OTUs recovered for each group. An extracted core microbial composition consisted of 753 OTUs shared between tritonymphs, males, and females. Tritonymph samples, T1–T8; males, M1–M8; females, F1–F8. Taxa are stacked to correspond with the legend

Table 3 Between-group AMOVA using the Jaccard index and the Yue and Clayton measure of dissimilarity within mothur

AMOVA	Comparison	Fs	*p* value
Jclass	Female–tritonymph–male	1.137	< 0.001
	Female–tritonymph	1.196	0.001
	Female–male	1.026	0.207
	Tritonymph–male	1.193	0.001
Thetayc	Female–tritonymph–male	3.669	0.009
	Female–tritonymph	4.831	0.006
	Female–male	0.485	0.487
	Tritonymph–male	4.121	0.020

microbiome, we identified 753 OTUs that were present in female, male, and tritonymph groups. Despite representing only 5.12% of the sobs, Good's coverage of the core microbiome was calculated at 78.9%, indicating that this core community constitutes the bulk of the *A. antarcticus* microbiome (Table 1). The core microbiome was dominated by the families Flavobacteriaceae (10.5%), Comamonadaceae (7.3%), Sphingobacteriaceae (6.5%), Chitinophagaceae (6.1%), and Cytophagaceae (5.8%; Fig. 4a). Further analysis of relative abundance showed that many members of the shared core microbiome exist in relatively low abundance, with the 20 most abundant families representing only 69.1% of the community. Investigating the core at the class and genus levels corroborate the finding that the core fairly represents microbial composition but is limited by the group with the lowest abundance. For example, Actinobacteria abundance found

in the core could not, and did not, exceed 18% of the total relative abundance at the class level due to the relatively low abundance found in the tritonymph group.

The total bacterial microbiome was resolved at the class level for 364,394 OTUs in mothur, with five classes comprising ~ 82.0% of the microbial composition: Actinobacteria (33.6%), Flavobacteriia (15.3%), Sphingobacteriia (11.5%), Gammaproteobacteria (11.3%), and Betaproteobacteria (10.7%; Fig. 5). Driving overall differences in class-level abundances between adult and tritonymph *A. antarcticus* were the classes Actinobacteria and Gammaproteobacteria (Fig. 5b). Genus-level identity was resolved for 220,898 sequences (59.3% of total) with QIIME, a value similar in resolution to previous research (Fagen et al. 2012). Five genera explained ~ 68.0% of the microbial composition: *Dermacoccus* (21.5%), *Pedobacter* (15.7%), *Chryseobacterium* (13.7%), *Pseudomonas* (9.4%), and *Flavobacterium* (8.4%; Fig. 6). Only four genera differed in relative abundance and were associated with the differences in Actinobacteria and Gammaproteobacteria at the class level. The stage-specific difference in Actinobacteria was driven by genera dissimilarities in *Dermacoccus* ($p < 0.001$), *Pseudomonas* ($p < 0.001$), and *Rhodococcus* ($p < 0.01$), while the difference in Gammaproteobacteria was driven by the stage-specific dissimilarities in *Deinococcus* ($p < 0.05$; Fig. 6b).

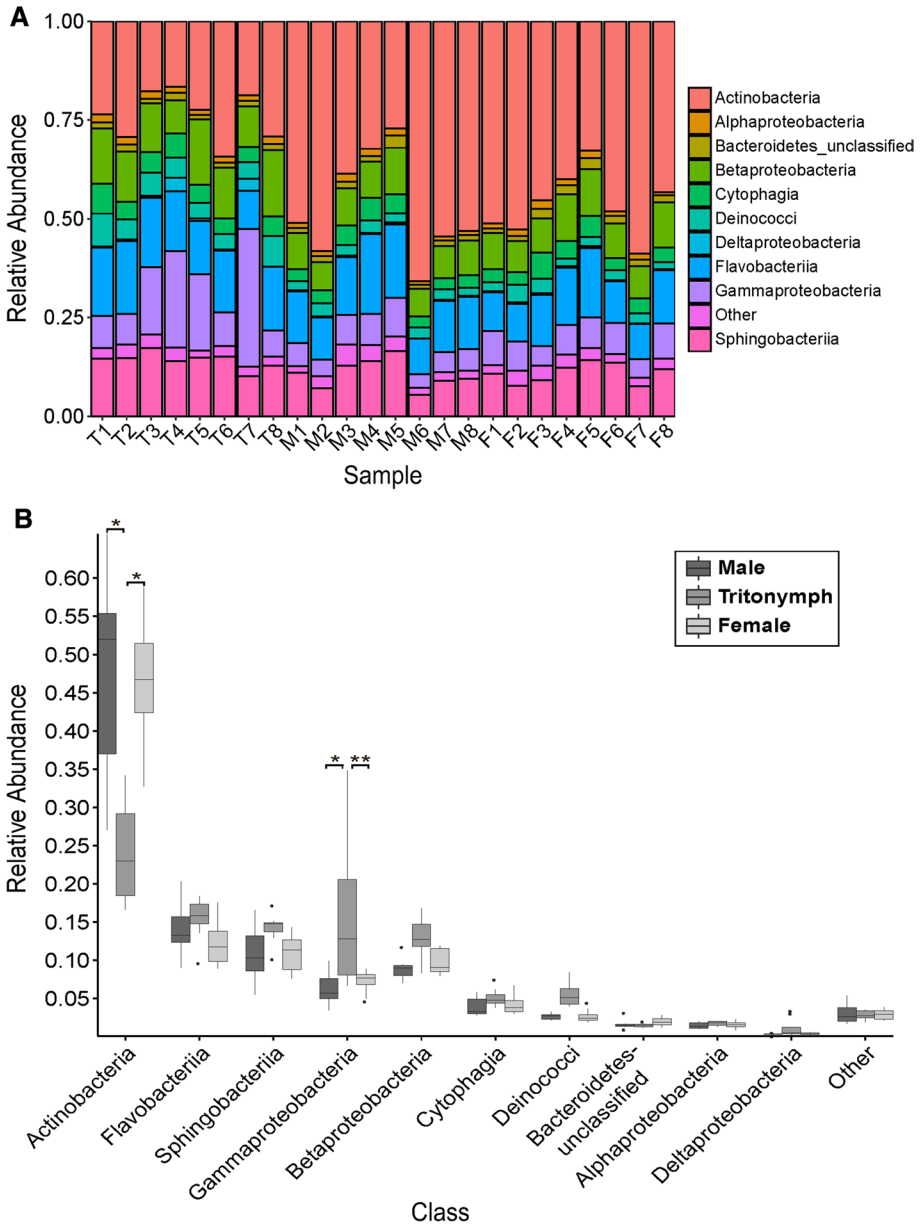

Fig. 5 **a** Relative microbial abundance of bacterial classes in *A. antarcticus*. **b** Relative abundance comparisons between microbial classes. Tritonymph samples, T1–T8; males, M1–M8; females, F1–F8. Asterisks indicate significance; *$p < 0.001$; **$p < 0.005$. Taxa are stacked to correspond with the legend

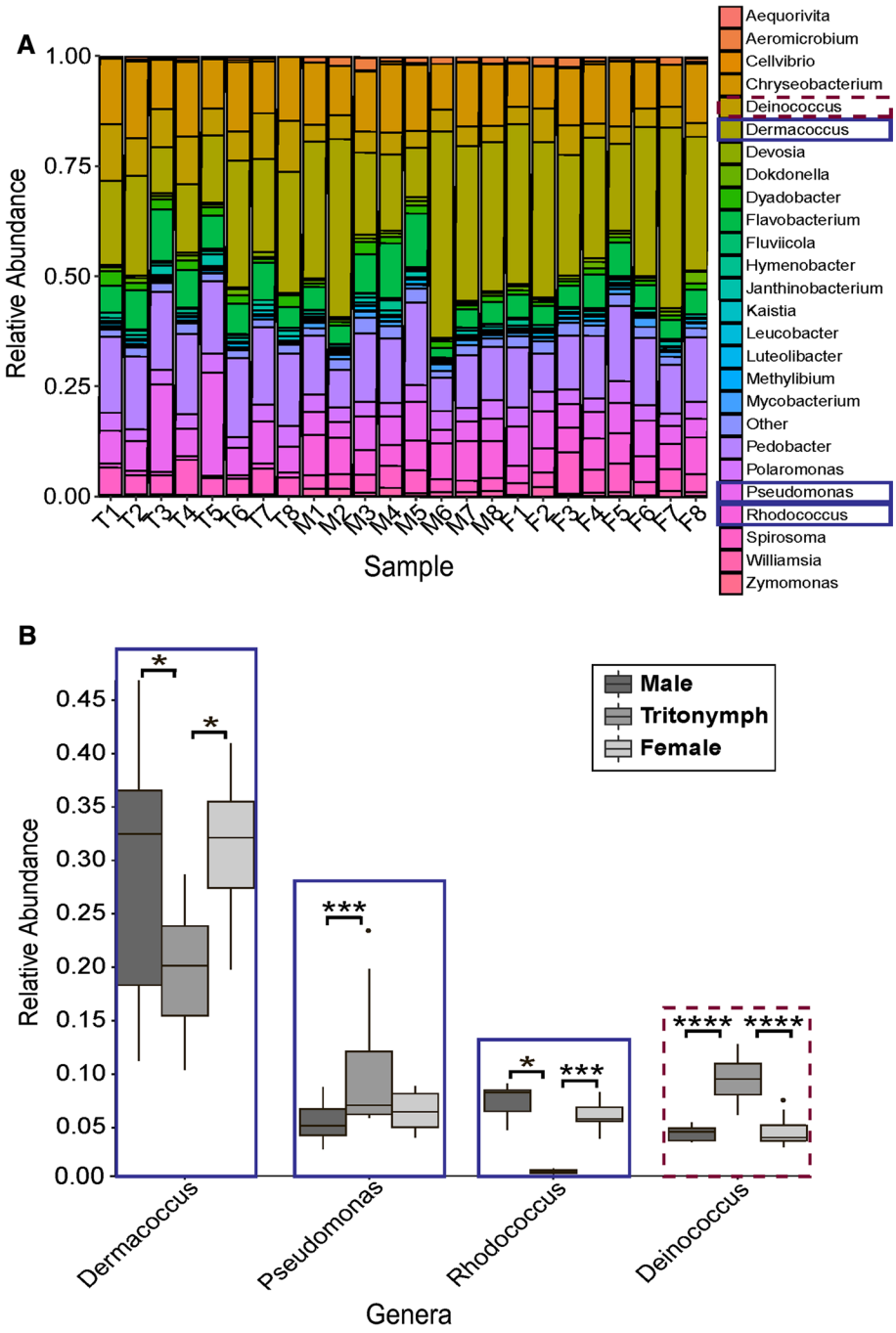

Fig. 6 **a** Relative microbial abundance of bacterial genera in *A. antarcticus*. **b** Relative abundance comparisons between microbial genera. Tritonymph samples, T1–T8; males, M1–M8; females, F1–F8. Solid boxes denote the Actinobacteria class; dashed boxes denote Gammaproteobacteria; asterisks indicate significance; $*p < 0.001$; $***p < 0.01$; $****p < 0.05$. Taxa are stacked to correspond with the legend

Discussion

We provide a survey of both adult sexes and the final nymphal stage of *A. antarcticus* and found that microbial community membership was similar for adult females, males, and tritonymphs but that microbial abundances differed between stages. However, overall community membership was not dominated by any single taxon, with the top five most abundant taxa accounting for ~ 82% of the bacterial composition at the class level and ~ 68% at the genus level. The consistence in taxa across the three groups further underscores the conserved nature of the *A. antarcticus* microbiome, with only two classes and four genera differing in representation. However, differences in abundance between those identified taxa were significant enough to drive overall differences between adults and tritonymphs. Similarities in microbial community composition were not surprising because both sexes and all life stages of this mite are herbivores and detritivores and occupy the same microhabitat. Interestingly, when microbiomes

from thirteen species of mites from various lower latitude habitats were described, three of the 13 mite species were dominated by a single bacterial genus (≥ 99% of sequences), eight of 13 were dominated by two or fewer genera (≥ 90%), and 11 of the 13 species were dominated by three or fewer genera (≥ 95%; Hubert et al. 2016). In the Antarctic mite, however, 17 bacterial genera accounted for ≥ 95% of the sequences, far more than in any of the 13 previous species of mites previously examined (Hubert et al. 2016, 2017). Distribution of microbial community members in *A. antarcticus* also appears to be more general than in the dust mite, *Dermatophagoides farinae*, the Asian citrus psyllid, *Diaphorina citri*, and two populations of an abundant soil mite, *Tyrophagus putrescentiae* (Fagen et al. 2012; Chan et al. 2015; Hubert et al. 2017). The distribution of bacterial taxa in *A. antarcticus* is likely affected by soil composition (Nielsen et al. 2012) as well as by harshness of the Antarctic environment, the contributions of which have yet to be addressed.

Soils in extreme environments, such as those of the *A. antarcticus* microhabitat, often share limitations in nutrient and water availability, and are accompanied by increased abundance of bacterial specialists such as Actinobacteria (Greer et al. 2010). Actinobacteria were significantly less abundant in tritonymphs of *A. antarcticus* than in adults, with three genera driving this difference, *Dermacoccus, Pseudomonas,* and *Rhodococcus*. The most abundant, *Dermacoccus*, was lower in tritonymphs than in both adult groups and is typically found in bacterial communities of multiple organisms, soil, seawater, and in extreme environments such as hot springs and the Marianas Trench (Pathom-Aree et al. 2006; Haeder et al. 2009; Ruckmani et al. 2011; Stackebrandt and Schumann 2014). *Pseudomonas* was less abundant in tritonymphs than in males and was previously found in soils occupied by *A. antarcticus*; it may contribute to reduced supercooling ability in mites (Shimada et al. 1992). *Pseudomonas* is also a common component of the microbiome in terrestrial invertebrates, including ticks and other mites (Narasimhan and Fikrig 2015; Hubert et al. 2016, 2017; Pakwan et al. 2017), where it possibly contributes to digestive processes, based on its prevalence in digestive systems of other arthropods (Narasimhan and Fikrig 2015; Pakwan et al. 2017). Lastly, *Rhodococcus* was less abundant in tritonymphs than in adults. This genus is commonly isolated from extreme environments, specifically cold areas (Bej et al. 2000). Similar to *Pseudomonas*, *Rhodococcus* has been isolated from other arthropods and is an obligate symbiont in some triatomine bugs (Díaz et al. 2016). However, the specific role of *Rhodococcus* in the *A. antarcticus* microbiome is unclear and warrants future studies. Due to the close proximity of mites at varying developmental stages, transmission of *Rhodococcus* may occur through an extracellular process (*i.e.*, coprophagy) during juvenile development, similar to the bacterial acquisition of the soil mite, *Tyrophagus putrescentiae* (Hubert et al. 2018).

Gammaproteobacteria was the only other class that differed in relative abundance between adults and tritonymphs, with one associated genus driving this difference, *Deinococcus*. Similar to Actinobacteria, *Deinococcus* is prevalent in dry soils and possibly exhibits desiccation tolerance (Aislabie et al. 2008). Such a characteristic may have prompted the evolution of other phenotypes for survival in harsh environments, such as ionizing-radiation resistance (Rainey et al. 2005). Interestingly, the microbes that differ in abundance between tritonymphs and adults of *A. antarcticus* have similar functionality and are likely associated with adaptation to the stressors found in extreme environments. These differences may be associated with dietary or size differences between the two stages, which may promote shifts in the microbiome. With major microbial community abundances being composed of taxa specialized for extreme environments, it appears that the arid and cold environment of Antarctica has likewise restricted the microbial composition found in *A. antarcticus*.

Despite differences in Actinobacteria and Gammaproteobacteria, other differences in microbiome community composition between adults and tritonymphs of *A. antarcticus* were slight. The core bacterial community in all surveyed stages of *A. antarcticus* was represented by a large amount of diversity, with approximately 125 identified families in 753 observed OTUs. Flavobacteriaceae represented the single most abundant family within the core; this taxon is found in many environments and appears to be psychrophilic (Bernardet et al. 1996). The diversity that exists in the microbial core composition of females, males, and tritonymphs of *A. antarcticus* likely reflects the diversity of bacteria associated with the habitat occupied by these mites, where many factors likely drive the proposed association between mites and habitat. One potential influence on mite-habitat dynamics is low nutrient availability in the habitat, which may prompt limitations in the microbial community (Radwan 2008). Such limitations may influence the bacteria associated with dietary intake in mites and may contribute to alterations in factors such as chitin digestion—considering that oribatid mite gut microbiomes may have coevolved alongside mite physiology (Gong et al. 2018).

Varying nutrition is a major contributor to the microbiome associated with the stored product mite, *Tyrophagus putrescentiae* (Erban et al. 2017), and this feature potentially contributes to the microbiome associated with *A. antarcticus*. The Antarctic mite microbiome is likely sculpted by cohabitation with seabirds and other marine animals, where the soil around seabird colonies contains high levels of nitrogen as well as other specific nutritional building blocks (Bokhorst and Convey 2016). Breakdown of nitrogen by-products such as uric acid (the

main nitrogenous component of bird guano) necessitates the presence of bacterial components in the soil or within the mites for nitrogen metabolism. Influences of nitrogen on growth, survival, and reproduction in the grasshopper, *Ageneotettix deorum* (Van Borm et al. 2002) further support the involvement of nitrogen in soil-mite dynamics. High nitrogen concentration has also been found in the dominant algal species, *Prasiola crispa* (Bokhorst and Convey 2016), a main dietary component of *A. antarcticus* (Worland and Lukešová 2000). Similar to some *Tetraponera* ants (Joern and Behmer 1997), and in accordance with the elevated relative abundance of *Pseudomonas* in the samples, *A. antarcticus* may undergo nitrogen recycling by *Pseudomonas*.

Microbiome analyses among mites have been limited, with most studies focusing on medically and agriculturally important mites and ticks (Zindel et al. 2013; Chan et al. 2015; Zolnik et al. 2016; Hubert et al. 2017; Pekas et al. 2017; Swei and Kwan 2017). Only two studies have examined microbiomes associated with oribatid mites (Moquin et al. 2012; Gong et al. 2018). In one study, tydeid, neonanorchestid, and oribatid mites showed relatively high bacterial diversity but minimal OTU overlap between mites and the bryophytic soil crusts in New Mexico where they reside (Moquin et al. 2012). High diversity is characteristic of detritivores and invertebrates that reside in soil (Bahrndorff et al. 2018), a feature that appears to be exaggerated in *A. antarcticus*. Additionally, approximately 66% of arthropod species are infected with *Wolbachia* (Hilgenboecker et al. 2008), but no sequences affiliated with *Wolbachia* were recovered from the Antarctic mite, suggesting that *Wolbachia* has not reached the mite's isolated habitat in Antarctica. *Wolbachia* was also not noted in the Antarctic midge collected from the same habitat (unpublished results). Considering the potentially multifaceted influences of mite microbiome, island habitat, soil nitrogen, and distance from bird colonies, further research is necessary to establish the dynamics of the microbiome–mite–environment interaction in *A. antarcticus*.

Contributions of the microbiome to mite physiology are likely important and substantial, considering the uniformity in the microbial community membership in *A. antarcticus* regardless of sex or life-stage. Furthermore, the bacterial taxa associated with differences in taxa abundances between adults and tritonymphs have known associations with nutritional limitations in the soil as well as desiccation- and cold-tolerance, all of which are likely related to developmental processes and may influence mite survival. Future studies on the effect of site nutrition and soil composition in relation to the microbiome of *A. antarcticus* will be critical for identifying how this mite has become prevalent both locally and throughout large sections of the Antarctic Peninsula.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Čech M, Chilton M, Clements D, Coraor N, Grüning B, Guerler A, Hillman-Jackson J, Jalili V, Rasche H, Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D (2018) The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 46:W537–W544

Aislabie J, Jordan S, Barker G (2008) Relation between soil classification and bacterial diversity in soils of the Ross Sea region, Antarctica. Geoderma 144:9–20

Apprill A, McNally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137

Bahrndorff S, de Jonge N, Hansen JK, Lauritzen JMS, Spanggaard LH, Sørensen MH, Yde M, Nielsen JL (2018) Diversity and metabolic potential of the microbiota associated with a soil arthropod. Sci Rep 8:2491

Bej AK, Saul D, Aislabie J (2000) Cold-tolerant alkane-degrading *Rhodococcus* species from Antarctica. Polar Biol 23:100–105

Benoit JB, Yoder JA, Lopez-Martinez G, Elnitsky MA, Lee RE Jr, Denlinger DL (2008) Adaptations for the maintenance of water balance by three species of Antarctic mites. Polar Biol 31:539–547

Bernardet JF, Segers P, Vancanneyt M, Berthe F, Kersters K, Vandamme P (1996) Cutting a Gordian knot: emended classification and description of the genus Flavobacterium, emended description of the family Flavobacteriaceae, and proposal of Flavobacterium hydatis nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). Int J Syst Evolut Microbiol 46:128–148

Block W, Convey P (1995) The biology, life cycle and ecophysiology of the Antarctic mite *Alaskozetes antarcticus*. J Zool 236:431–449

Bokhorst S, Convey P (2016) Impact of marine vertebrates on Antarctic terrestrial micro-arthropods. Antarct Sci 28:175–186

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci 108:4516–4522

Chan TF, Ji KM, Yim AKY, Liu XY, Zhou JW, Li RQ, Yang KY, Li J, Li M, Law PTW (2015) The draft genome, transcriptome, and microbiome of *Dermatophagoides farinae* reveal a broad spectrum of dust mite allergens. J Allergy Clin Immunol 135:539–548

Chen B (2018) VennDiagram: generate high-resolution Venn and Euler plots. R package version 1.6.20

Convey P (1994a) Growth and survival strategy of the Antarctic mite *Alaskozetes antarcticus*. Ecography 17:97–107

Convey P (1994b) Sex ratio, oviposition and early development of the Antarctic oribatid mite *Alaskozetes antarcticus* (Acari: Cryptostigmata) with observations on other oribatids. Pedobiologia 2:161–168

De Meillon B, Golberg L (1946) Preliminary studies on the nutritional requirements of the bed bug (*Cimex lectularius* L.) and the tick *Ornithodorus moubata* Murray. J Exp Biol 24:41–63

Díaz S, Villavicencio B, Correia N, Costa J, Haag KL (2016) Triatomine bugs, their microbiota and *Trypanosoma cruzi*: asymmetric responses of bacteria to an infected blood meal. Parasites Vectors 9:636

Dragulescu A (2014) xlsx: read, write, formal Excel 2007 and Excel 97/2000/xp/2003 files. R package version 04 2

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200

Erban T, Ledvinka O, Nesvorna M, Hubert J (2017) Experimental manipulation shows a greater influence of population than dietary perturbation on the microbiome of *Tyrophagus putrescentiae*. Appl Environ Microbiol 83:e00128–e217

Everatt M, Convey P, Worland M, Bale J, Hayward S (2013) Heat tolerance and physiological plasticity in the *Antarctic collembolan*, *Cryptopygus antarcticus*, and mite, *Alaskozetes antarcticus*. J Therm Biol 38:264–271

Fagen JR, Giongo A, Brown CT, Davis-Richardson AG, Gano KA, Triplett EW (2012) Characterization of the relative abundance of the citrus pathogen Ca. Liberibacter asiaticus in the microbiome of its insect vector, *Diaphorina citri*, using high throughput 16S rRNA sequencing. Open Microbiol J 6:29

Fox J, Weisberg S (2011) Car: companion to applied regression. https://www.CRAN. R-project org/package= car

Fuka MM, Wallisch S, Engel M, Welzl G, Havranek J et al (2013) Dynamics of bacterial communities during the ripening process of different croatian cheese types derived from raw Ewe's milk cheeses. PLoS ONE 8:e80734

Gong X, Chen TW, Zieger SL, Bluhm C, Heidemann K, Schaefer I, Maraun M, Liu M, Scheu S (2018) Phylogenetic and trophic determinants of gut microbiota in soil oribatid mites. Soil Biol Biochem 123:155–164

Graves S, Piepho HP, Selzer, ML (2015) Package 'multcompView'

Greer C, Whyte L, Niederberger T (2010) Microbial communities in hydrocarbon-contaminated temperate, tropical, alpine, and polar soils. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 2313–2328

Haeder S, Wirth R, Herz H, Spiteller D (2009) Candicidin-producing Streptomyces support leaf-cutting ants to protect their fungus garden against the pathogenic fungus Escovopsis. Proc Natl Acad Sci 106:4742–4746

Herrera LM, García-Laviña CX, Marizcurrena JJ, Volonterio O, de León RP, Castro-Sowinski S (2017) Hydrolytic enzyme-producing microbes in the *Antarctic oligochaete* Grania sp. (Annelida). Polar Biol 40:947–953

Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH (2008) How many species are infected with Wolbachia?—a statistical analysis of current data. FEMS Microbiol Lett 281:215–220

Hope R (2013) Rmisc: Ryan miscellaneous. R package version 1.

Hubert J, Kopecky J, Sagova-Mareckova M, Nesvorna M, Zurek L, Erban T (2016) Assessment of bacterial communities in thirteen species of laboratory-cultured domestic mites (Acari: Acaridida). J Econ Entomol 109:1887–1896

Hubert J, Erban T, Kopecky J, Sopko B, Nesvorna M, Lichovnikova M, Schicht S, Strube C, Sparagano O (2017) Comparison of microbiomes between red poultry mite populations (*Dermanyssus gallinae*): predominance of Bartonella-like bacteria. Microb Ecol 74:947–960

Hubert J, Nesvorna M, Sopko B, Smrz J, Klimov P, Erban T (2018) Two populations of mites (*Tyrophagus putrescentiae*) differ in response to feeding on feces-containing diets. Front Microbiol 9:2590

Joern A, Behmer ST (1997) Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). Oecologia 112:201–208

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79:5112–5120

Lenth RV (2016) Least-squares means: the R package lsmeans. J Stat Softw 69:1–33

López-García A, Pineda-Quiroga C, Atxaerandio R, Pérez A, Hernández I, García-Rodríguez A, González-Recio O (2018) Comparison of mothur and QIIME for the analysis of rumen microbiota composition based on 16S rRNA amplicon sequences. Front Microbiol 9:1–11

Mangiafico S (2015) An R companion for the handbook of biological statistics, version 1.09 i.

Moquin S, Garcia J, Brantley S, Takacs-Vesbach C, Shepherd U (2012) Bacterial diversity of bryophyte-dominant biological soil crusts and associated mites. J Arid Environ 87:110–117

Narasimhan S, Fikrig E (2015) Tick microbiome: the force within. Trends Parasitol 31:315–323

Nielsen UN, Osler GH, Campbell CD, Burslem DF, van der Wal R (2012) Predictors of fine-scale spatial variation in soil mite and microbe community composition differ between biotic groups and habitats. Pedobiologia 55:83–91

Ondov BD, Berqman NH, Phillippy AM (2011) Interactive metagenomic visualization in a Web browser. BMC Bioinform 12:385

Pakwan C, Kaltenpoth M, Weiss B, Chantawannakul P, Jun G, Disayathanoowat T (2017) Bacterial communities associated with the ectoparasitic mites Varroa destructor and *Tropilaelaps mercedesae* of the honey bee (*Apis mellifera*). FEMS Microbiol Ecol 93:160

Pathom-Aree W, Stach JE, Ward AC, Horikoshi K, Bull AT, Goodfellow M (2006) Diversity of actinomycetes isolated from challenger deep sediment (10,898 m) from the Mariana Trench. Extremophiles 10:181–189

Pekas A, Palevsky E, Sumner JC, Perotti MA, Nesvorna M, Hubert J (2017) Comparison of bacterial microbiota of the predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae) and its factitious prey *Tyrophagus putrescentiae* (Acari: Acaridae). Sci Rep 7:2

Radwan S (2008) Microbiology of oil-contaminated desert soils and coastal areas in the Arabian Gulf region. In: Dion P, Nautiyal CS (eds) Microbiology of extreme soils. Springer, Berlin, pp 275–298

Rainey FA, Ray K, Ferreira M, Gatz BZ, Nobre MF, Bagaley D, Rash BA, Park MJ, Earl AM, Shank NC (2005) Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran Desert soil and description of nine new species of the genus Deinococcus obtained from a single soil sample. Appl Environ Microbiol 71:5225–5235

Ruckmani A, Kaur I, Schumann P, Klenk HP, Mayilraj S (2011) *Calidifontibacter indicus* gen. nov., sp. nov., a member of the family Dermacoccaceae isolated from a hot spring, and emended description of the family Dermacoccaceae. Int J Syst Evol Microbiol 61:2419–2424

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Schuler CG, Havig JR, Hamilton TL (2017) Hot spring microbial community composition, morphology, and carbon fixation: implications for interpreting the ancient rock record. Front Earth Sci 5:97

Shimada K, Pan C, Ohyama Y (1992) Variation in summer cold-hardiness of the Antarctic oribatid mite *Alaskozetes antarcticus* from contrasting habitats on King George Island. Polar Biol 12:701–706

Stackebrandt E, Schumann P (2014) The family Dermacoccaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes. Springer, Berlin, pp 301–315

Swei A, Kwan JY (2017) Tick microbiome and pathogen acquisition altered by host blood meal. ISME J 11:813

Van Borm S, Buschinger A, Boomsma JJ, Billen J (2002) Tetraponera ants have gut symbionts related to nitrogen–fixing root–nodule bacteria. Proc R Soc Lond B 269:2023–2027

Webster NS, Negri AP, Munro MM, Battershill CN (2004) Diverse microbial communities inhabit Antarctic sponges. Environ Microbiol 6:288–300

Wickham H (2007) Reshaping data with the reshape Package. J Stat Softw 21:1–20

Wickham H (2009) ggplot2: elegant graphics for data analysis

Wickham H, Francois R, Henry L, Müller K (2017) dplyr: a grammar of data manipulation. R package version 0.7.4

Worland MR, Lukešová A (2000) The effect of feeding on specific soil algae on the cold-hardiness of two Antarctic micro-arthropods (*Alaskozetes antarcticus* and *Cryptopygus antarcticus*). Polar Biol 23:766–774

Yang YW et al (2015) Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in mouse feces. Appl Environ Microbiol 81:6749–6756

Yang B, Wang Y, Qian PY (2016) Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. BMC Bioinform 17:135

Young S, Block W (1980) Experimental studies on the cold tolerance of *Alaskozetes antarcticus*. J Insect Physiol 26:189–200

Zindel R, Ofek M, Minz D, Palevsky E, Zchori-Fein E, Aebi A (2013) The role of the bacterial community in the nutritional ecology of the bulb mite *Rhizoglyphus robini* (Acari: Astigmata: Acaridae). FASEB J 27:1488–1497

Zolnik CP, Prill RJ, Falco RC, Daniels TJ, Kolokotronis SO (2016) Microbiome changes through ontogeny of a tick pathogen vector. Mol Ecol 25:4963–4977