Journal of Thermal Biology 82 (2019) 222–228

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Vanishing benefits - The loss of actinobacterial symbionts at elevated temperatures

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ARTICLE INFO

Keywords:
Ambient temperature
Antibiotic-producing *Actinobacteria*
Gut microbiome
Terrestrial isopod
Host growth
Holobiont

ABSTRACT

Only a few insect species are known to engage in symbiotic associations with antibiotic-producing *Actinobacteria* and profit from this kind of protection against pathogens. However, it still remains elusive how widespread the symbiotic interactions with *Actinobacteria* in other organisms are and how these partnerships benefit the hosts in terms of the growth and survival. We characterized a drastic temperature-induced change in the occurrence of *Actinobacteria* in the gut of the terrestrial isopod *Porcellio scaber* reared under two different temperature (15 °C and 22 °C) and oxygen conditions (10% and 22% O_2) using 16S rRNA gene sequencing. We show that the relative abundance of actinobacterial gut symbionts correlates with increased host growth at lower temperature. Actinobacterial symbionts were almost completely absent at 22 °C under both high and low oxygen conditions. In addition, we identified members of nearly half of the known actinobacterial families in the isopod microbiome, and most of these include members that are known to produce antibiotics. Our study suggests that hosting diverse actinobacterial symbionts may provide conditions favorable for host growth. These findings show how a temperature-driven decline in microbiome diversity may cause a loss of beneficial functions with negative effects on ectotherms.

1. Introduction

Ambient temperature is among the most important abiotic factors known to affect the performance of animals in general and ectotherms in particular (Angilletta, 2009). However, it is seldom realized that most studied organisms live in symbiosis with microorganisms. Already in 1965, nearly half of the insect species, which represent the largest animal group on earth, was estimated to harbour symbiotic microbes (Buchner, 1965) and only half a century later, we realize that virtually all organisms may harbour symbionts (Hurst, 2016). Several recent studies have demonstrated that the structural and functional composition of gut microbiota might be strongly shaped by environmental temperature (Bestion et al., 2017; Kikuchi et al., 2016; Kohl and Yahn, 2016). How the temperature-induced changes in gut microbiota may affect the host performance is poorly understood and rarely addressed in experimental studies (see an example for the effect of temperature on salamander's digestion, Fontaine et al., 2018).

The complex interaction between environmental temperature and organisms is further complicated by the fact that other abiotic and biotic factors associated with ambient temperature might affect organismal performance. Oxygen for example – which naturally correlates with environmental temperature – may play a key role in mediating temperature effects especially in habitats, in which oxygen availability is generally low (e.g., soil, digestive tract or water; Brune and Friedrich, 2000; Forster et al., 2012; Harrison et al., 2018). Our previous studies showed that not only ambient temperature, but also oxygen conditions are limiting factors for early growth and development of the terrestrial isopod *Porcellio scaber* (Horvathova et al., 2015a, 2017). Terrestrial isopods have also recently been proposed as an excellent model to investigate symbiotic associations along the spectrum from parasitism to mutualism (Bouchon et al., 2016). In general, isopods harbour a highly diverse microbiome varying between populations, which indicates important contributions of environmental microbes in the host-associated microbiota (Dittmer et al., 2016). According to the original definition by Anton De Bary (1879), symbiosis refers to any close association between two heterospecific organisms, independent of the effects on the organisms involved, whereby the symbiosis can be mutualistic, commensalistic, or parasitic. Arthropods may benefit from mutualistic associations by improved growth and survival, enhanced resistance to pathogens or obtaining essential nutrients (Douglas, 2009; Engel and Moran, 2013; Kaltenpoth and Engl, 2014). However, to what extent are temperature effects on arthropods mediated through changes in their

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https://doi.org/10.1016/j.jtherbio.2019.04.015
Received 17 October 2018; Received in revised form 15 April 2019; Accepted 20 April 2019
Available online 03 May 2019

symbiont community, or through interactions with other components of environment, remains to be tested.

The discovery of antibiotics has revolutionized modern medicine and greatly improved the quality of human life. Most of the available antibiotics originate from *Actinobacteria*, specifically from the genus *Streptomyces* (almost 40% of the total known microbial antibiotics (Berdy, 2012)). *Actinobacteria* are particularly common and widespread in the terrestrial environment, especially soils (Blay et al., 2017; Janssen, 2006), and are therefore regularly encountered in many organisms. Interestingly, the idea that antibiotics can be commonly "prescribed" not only for humans but also for other organisms emerged only recently (Kaltenpoth, 2009). Thus far, our knowledge on the role of *Actinobacteria* in protection of animals against pathogens is restricted to insects, namely, fungus-growing ants, wasps and beetles (Kaltenpoth, 2009; Kaltenpoth and Engl, 2014; Seipke et al., 2012). Leaf-cutting attine ants and pine beetles (*Dendroctonus frontalis*) use symbiotic *Actinobacteria* from their integument to defend their food sources against specialized fungal pathogens (Currie et al., 1999; Scott et al., 2008). Female beewolf digger wasps (*Philanthus triangulum*) cultivate *Streptomyces* bacteria in their antennal glands and apply them to the brood chamber to protect larvae against mould fungi (Kaltenpoth et al., 2005). Although a diverse range of *Actinobacteria* have been isolated from the guts of different invertebrates and vertebrates, "*the link to the* [metabolite] *producing microorganisms and the fitness consequences of the symbiosis for the host often remain enigmatic"* (Florez et al., 2015). *Actinobacteria* are known to be common members of isopod gut and faecal microbiome (Bouchon et al., 2016; Zimmer, 2002a), but the nature of the symbiotic interactions remains elusive. Additionally, symbiotic *Actinobacteria* have been revealed as one of the most dominant group in isopod gut microbiome of our studied population (Horvathova et al., 2016, Table S1). In the present study, we examined the effects of environmental temperature and oxygen conditions on the growth rate and gut microbiota of adults of the common woodlouse *P. scaber* reared under two different temperature (15 °C and 22 °C) and two oxygen conditions (10% and 22% O_2). We wanted to further test whether the relationship between temperature and oxygen conditions and host performance is mediated by changes in the community structure of symbiotic *Actinobacteria*.

2. Material and methods

2.1. Experimental animals

Individuals of terrestrial isopod *Porcellio scaber* were collected during the summer of 2014 in Kraków, Poland. Isopods were kept in separate plastic boxes (205 × 150 × 97 mm) for the period of almost one year in the laboratory under constant temperature (20 °C) and light (16 h:8hr light:dark) conditions and fed with decayed alder leaves. Forty adult males were randomly selected from the boxes (April 2015), weighed to the nearest 0.01 mg (Mettler Toledo XP26, Greifensee, Switzerland) and transferred individually to the experimental boxes (52 × 48 mm, 100 ml) containing wet sand and a piece of clay pot. The experimental boxes with individual males were equally distributed into four experimental groups controlling for the uniform distribution of the initial body mass between the experimental groups.

2.2. Experimental conditions

Boxes with individuals were maintained in two climatic chambers (15 °C and 22 °C, POL-EKO APARATURA, Sp.j., Poland), which contained two plexi-chambers (40 × 50 × 55 cm, YETI – Advertising Agency, Poland) with regulated normal (22%) and low oxygen (10%) levels (ROXY-4 four channel gas regulator Sable Systems Europe GmbH, Germany). This experimental set-up gave us four temperate and oxygen combinations: 15 °C and 22% oxygen, 15 °C and 10% oxygen, 22 °C and 22% oxygen, and 22 °C and 10% oxygen. Isopods were fed with decayed alder leaves that are preferred by terrestrial isopods as a food source due to their high microbial activity (Zimmer et al., 2003). Leaves from the nearby forest were mixed and left within a plastic bag for microbial conditioning. Conditioned leaves were kept in the freezer at −5 °C and were provided *ad libitum* once at the beginning of the experiment. The amounts of leaves were sufficient to last for the entire experimental period (leaves were still present in the boxes at the end of the experiment). Faeces were not removed from the boxes to allow for coprophagy (Hassall and Rushton, 1982). Forty individuals were weighed to the nearest 0.01 mg after four and eight weeks of growth. The period of eight weeks has been shown long enough for detection of the effect of symbionts on the isopod growth (Horvathova et al., 2015b, 2016). Growth rate is an important life-history trait, which is often used to investigate the effect of environmental conditions on isopod performance (Hassall et al., 2003; Horvathova et al., 2015a).

2.3. 16S rRNA gene sequencing and processing

After final weighing, individuals were maintained without food for three days prior to dissections. One male died during the experimental rearing period. Isopods were decapitated, and the hindgut of each individual was stored at −20 °C. Total DNA was extracted from the hindguts of 39 males (n = 10 per experimental group except for the group at 22 °C and with 10% O_2, where n = 9) using the Wizard genomic DNA purification kit (Promega). Amplification and Illumina sequencing of 16S rRNA was done following established protocols (Caporaso et al., 2011). The V4 region of bacterial and archeal 16S rRNA genes were PCR amplified in triplicates using primers 515f and 806r (Caporaso et al., 2011). The samples were indexed using a 12 bp barcode added to the 5′end of the 806r primer (Table S2). Two types of negative controls were included in each batch of PCR reactions: two extraction negative controls and two PCR negative controls. Amplicon libraries were pooled at equimolar ratios and sequenced on an Illumina MiSeq machine, producing 2 × 150 bp reads. Raw sequences were processed using the QIIME software package (Caporaso et al., 2010b). The reads were demultiplexed, quality controlled and trimmed, retaining only reads with at least 75bp of consecutive high-quality bases. To assign sequences to operational taxonomic units (OTUs), we clustered sequences that shared 97% or greater sequence identity and then aligned against the Greengenes database (version 13_8) using PyNAST (Caporaso et al., 2010a) with default parameters set by QIIME. The resulting BIOM table contained 3,993,769 reads with a mean of 102,404 ± 47,916 (SD) for 39 samples (Table S2).

2.4. Estimates of bacterial diversity

All data were tested for normality of distribution and homogeneity of variance. We evaluated the effect of temperature and oxygen on both α-diversity (microbial diversity within individual guts, i.e. OTU richness and Phylogenetic Diversity whole tree index, PD) and β-diversity (microbial diversity across individual guts, i.e. unweighted and weighted Unifrac metric distances). Weighted and unweighted UniFrac represent qualitative and quantitative phylogenetic measure of β-diversity respectively; unweighted UniFrac considers only the absence or presence of lineages (i.e., taxonomic composition), while weighted UniFrac directly accounts for differences in relative abundances of lineages within communities (Lozupone et al., 2011). Rarefaction curves of the OTU and PD estimates were constructed in QIIME at 10 subsamplings of every 1200 reads and were used to verify adequate depth of sampling. The total number of reads per sample was rarified to the average number of reads for a given sample. Further analyses were performed using exclusively this dataset.

2.5. Univariate and multivariate analyses of bacterial communities

Differences in isopod body mass increase and gut microbial α-

diversity measures (OTU and PD index) between experimental conditions were analyzed by two-factorial ANCOVA with temperature and oxygen as fixed factors and the initial body mass as a covariate, all non-significant interactions were stepwise removed. The interaction term between temperature and oxygen was always retained in the minimum model. We performed additional analyses to test whether bacterial gut diversity (in terms of overall gut diversity *vs* diversity of *Actinobacteria*) has an effect on isopod growth rate by including PD measures as a covariate. PD was chosen as a preferred measure of overall bacterial diversity because it considers the phylogenetic relatedness of bacteria within a sample. The difference in body mass increase between individuals harboring a low and high proportion of bacteria potentially capable of antibiotic production was analyzed by one-way ANCOVA with the initial body mass as a covariate. Body mass increase was calculated as the difference between the initial body mass and body mass after two months. ANCOVAs were calculated with the SAS 9.4 statistical software package (SAS Institute Inc., Cary, NC, USA). The phylogenetic topology tree of *Actinobacteria* was constructed using APE package in R. Two-factorial permutational MANOVA with temperature and oxygen as fixed factors, and the interaction term between the two factors were used to analyze differences in gut microbial β-diversity using both unweighted and weighted UniFrac distance metrices. Similarity percentage (SIMPER) analysis identified the taxa that were mainly responsible for the differences observed between the experimental conditions using Bray-Curtis matrix. The permutational MANOVA and SIMPER analyses were computed using the VEGAN package implemented in R. Principal coordinate analysis of bacterial communities was performed in Canoco 5.0 software for Windows using weighted UniFrac distance matrix. We report $p < 0.05$ as significant and means are quoted with ± SEM throughout unless stated otherwise. Other statistical parameters are reported in the main text and figure legends.

2.6. Characterization of actinobacterial symbionts

We searched for the literature sources that characterize antimicrobial activity (anti-bacterial, fungal or viral) of *Actinobacteria* that were identified to the genus level in our study (Table S3). Taxonomy-based functional profiling of bacterial communities was successfully employed in different microbiome studies (Curiel Yuste et al., 2014; Rothig et al., 2016; Yun et al., 2014). The abundance of antibiotic-producing *Actinobacteria* in isopod gut was calculated as the sum of relative abundances (percentage) of identified genera with the potential to produce antibiotics. We set the threshold of 8% abundance of antibiotic-producing *Actinobacteria* to characterize isopod microbiome with high *vs* low potential ability to produce antibiotics. Antibiotic-producing *Actinobacteria* were generally highly abundant in low temperature conditions (range between 0.1 and 27.3%) with an average of 8.3 and under-represented in warm temperature conditions (range between 0.5 and 7.2% with an average of 2.2%). The threshold value of 8% relative abundance thus represents the average value for low temperature and the sub-maximum value for high temperature. The diversity of *Actinobacteria* for individual isopods was calculated as the number of genera identified at ≥0.1% abundance. High abundance of *Actinobacteria* more likely reflects a natural condition rather than the effects of laboratory rearing (our unpublished study: 12% average abundance for individuals kept under 15 °C without previous rearing in the laboratory, Table S1).

3. Results

Our experimental manipulation of environmental conditions showed that ambient temperature shaped the gut bacterial community composition of the isopods, while oxygen concentration had no significant effect. The relative abundance of the most dominant phyla, *Proteobacteria*, *Actinobacteria*, *Tenericutes* and *Cyanobacteria*, differed between temperatures (Fig. 1A, see also Fig. S1 and Table S4) as confirmed by SIMPER analysis (Table S5). Principal coordinate analysis revealed that individual microbiomes clustered strongly by ambient temperature but not by oxygen level (Fig. 1B). We found that experimental temperature had a significant effect on the isopods' microbiome, explaining as much as 26 or 33% of the variance in the unweighted and weighted phylometric distances between the samples (PERMANOVA: $p = 0.001$ for both measures). Oxygen did not significantly shape isopods' microbiome (PERMANOVA: $p > 0.48$ for both measures). The interaction between temperature and oxygen was not significant (PERMANOVA: $p > 0.56$ for both measures). Overall, microbiomes were found to be more similar for individuals from 22 °C than from 15 °C (Fig. S2). This pattern was most likely driven by dominant *Proteobacteria* in 22 °C (i.e., members of *Enterobacteriaceae*, Fig. S1).

We examined the potential beneficial effect of gut symbionts on isopod host by correlating symbiont diversity in general and actinobacterial diversity specifically on isopod growth. Isopod body mass gain strongly correlated with higher overall bacterial gut diversity in the cold but not in the warm environment (ANCOVA with temperature × PD index: $p = 0.03$, Fig. 2A, Table S6). This effect was independent of isopod initial body mass ($p = 0.12$, Table S6). The positive correlation can be likely attributed to the diversity of *Actinobacteria* at the low temperature (Fig. 2B and C, Table S6, see also Fig. S3). For the subsequent analysis of how the potential production of antimicrobial compounds is associated with isopod growth, we selected those individuals harbouring high and low levels of *Actinobacteria*, which are known to produce antibiotics (the threshold value of 8% relative abundance, see Material and Methods) and tested for growth differences. Individual isopods with high levels of *Actinobacteria* present in their gut microbiome clearly outperformed their conspecifics with low levels of *Actinobacteria* at the low ambient temperature (Bonferroni-corrected $p = 0.02$, Fig. 2D, Table S6). *Actinobacteria* known to produce antibiotics were almost absent in the high temperature treatment (range between 0.5 and 7%, with an average of 2.2%) resulting in a single group with low abundance of *Actinobacteria*. The results did not qualitatively change after we adjusted the threshold value up (20% of relative abundance: Bonferroni-corrected $p = 0.01$) or down (4% of relative abundance: Bonferroni-corrected $p = 0.04$).

4. Discussion

Ectotherms are considered particularly vulnerable to climate warming because their basic physiological functions such as locomotion, growth, and reproduction are strongly influenced by environmental temperature (Angilletta, 2009; Huey and Kingsolver, 1989). Environmental temperature is naturally correlated with oxygen availability and oxygen may become a limiting factor for ectotherms especially under warm conditions, when demand for oxygen is high (Verberk et al., 2016; Walczynska et al., 2015). However, if we consider that most, if not all animals are associated with symbiotic microorganisms (Hurst, 2016), and many of those play a pivotal role in metabolism of the host (e.g., Hillyer et al., 2016; Lee et al., 2018), we may well expect that ectotherm response to changes in environmental conditions might be associated with changes in the symbiont community.

In this study, we showed that ambient temperature, but not oxygen concentration, shapes the gut microbial communities of the terrestrial arthropod, *Porcellio scaber*. Specifically, the relative abundance in major bacterial phyla differed between temperatures. Members of *Proteobacteria* were highly abundant under higher-temperature condition, which supports the evidence that *Proteobacteria* is the most dominant bacterial phylum in arthropod gut microbiome (Degli Esposti and Martinez Romero, 2017). Under lower-temperature condition, the average abundance of *Actinobacteria* exceeded 15% and represents the highest proportion recorded in arthropods thus far (previously reported range 3–10% (Bouchon et al., 2016; Jones et al., 2013; Yun et al., 2014)). The observed differences in gut microbiome composition between temperatures may reflect differences in cell-type structure and

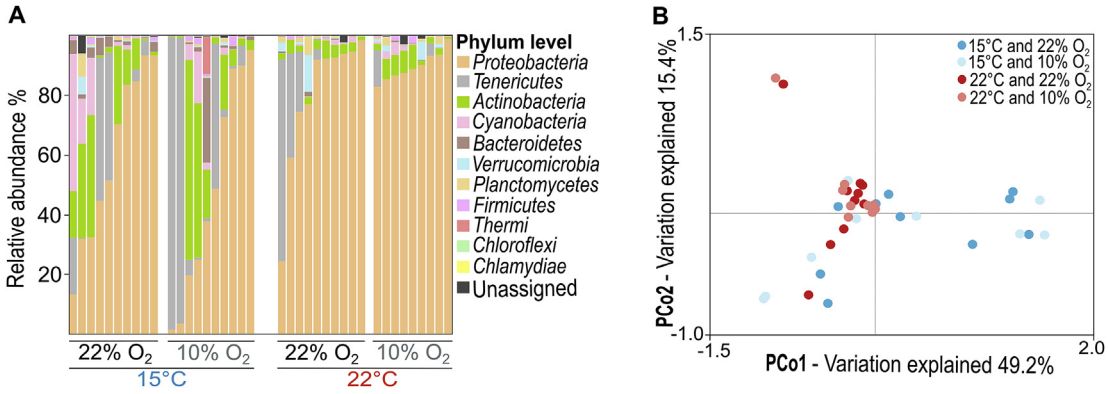

Fig. 1. The effect of environmental factors on the isopod gut microbiome. **(A)** Relative abundance of the bacterial phyla presented within the hindgut of the terrestrial isopod *Porcellio scaber* reared under two different temperatures (15 °C and 22 °C) and oxygen conditions (10% and 22% O_2). **(B)** Principal coordinate plot showing the degree of similarity between isopod gut bacterial communities (weighted UniFrac values). The graph demonstrates that the isopod microbiome is more similar for individuals from 22 °C than for individuals from 15 °C irrespective of oxygen level. The greater distribution spread of data for 15 °C (blue symbols) along the PC1 axis also shows that the isopod microbiome is overall more diverse under the low temperature condition. Each circle corresponds to an individual gut microbiome sample (n = 10 per experimental group except for the group at 22 °C and with 10% O_2, where n = 9). The percentage of the variation explained by the plotted principal coordinates is indicated on the axes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

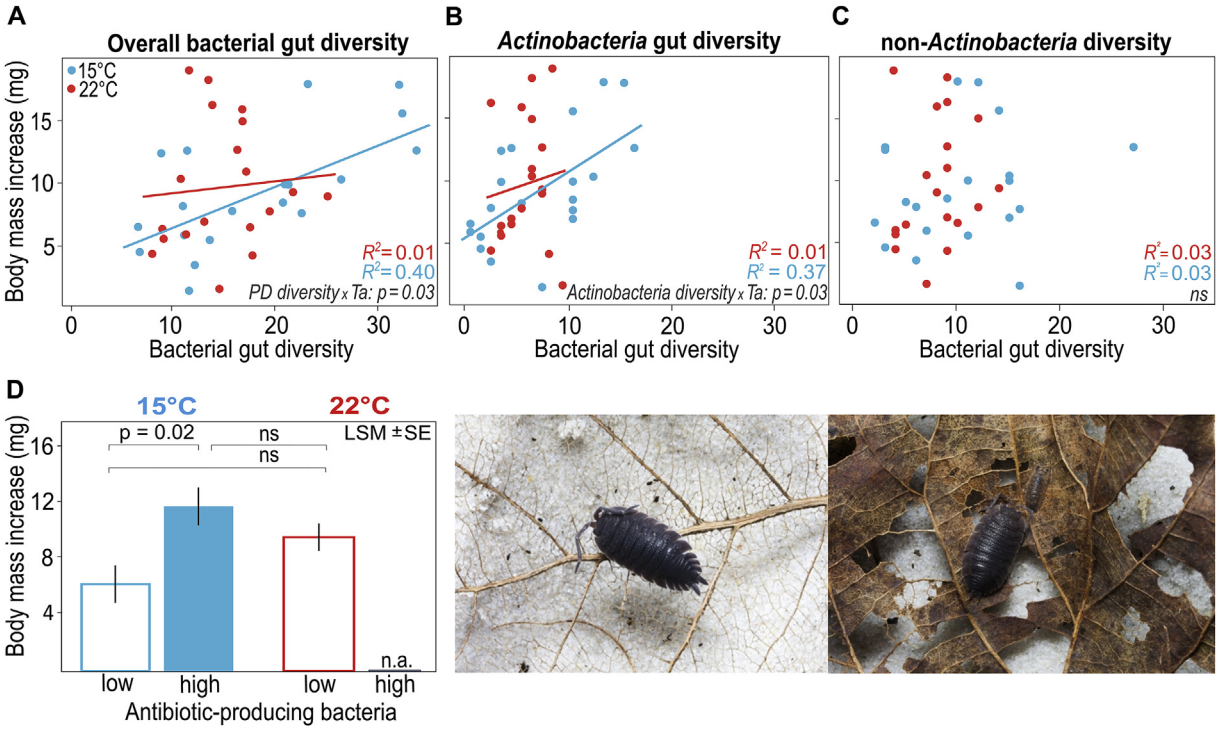

Fig. 2. The association between bacterial gut diversity and body mass increase for the isopod *P. scaber* under low and high temperature conditions (15 °C and 22 °C with oxygen groups combined due to a non-significant oxygen effect). **(A)** The significant interaction between temperature (T_a) and overall bacterial gut diversity (presented here as the phylogenetic diversity index, PD) shows that individuals with highly diverse microbiome grow faster under low temperature conditions. Note that no significant differences in overall bacterial gut diversity were observed between experimental conditions (see Fig. S4 and Table S6). **(B)** The significant interaction between temperature and diversity of *Actinobacteria* shows that individual isopods with higher diversity of gut *Actinobacteria* grow faster under the low temperature condition. The partial set of data on actinobacterial diversity was calculated as the number of genera with ≥0.1% relative abundance. **(C)** The lack of a significant association between the diversity of bacterial groups other than *Actinobacteria* and isopod mass increases. Bacterial diversity was calculated as the total number of genera within the phyla *Bacteroidetes*, *Firmicutes*, *Planctomycetes, Tenericutes*, *Verrucomicrobia* and *Thermi* with ≥0.1% relative abundance. **(D)** Individuals with high abundance of bacteria with a potential to produce antibiotics in the gut microbiome outperformed the conspecifics with low levels of such bacteria (n = 10 for 15 °C "high", n = 10 for 15 °C "low", n = 19 for 22 °C "low" group). Significance was tested using ANOVA with post-hoc Bonferroni correction. Note that antibiotic-producing Actinobacteria were highly underrepresented under warm temperature conditions. Data show least-square means ± SE. The pictures depict an adult male (left), an adult female (right) and a subadult (right) of terrestrial isopod *Porcellio scaber* feeding on favourite natural food source, alder leaf.

the turnover rates of the predominant taxa (Young, 2007). *Actinobacteria* with a thick peptidoglycan cell wall are gram-positive bacteria, which form complex fungi-like mycelia and are therefore generally considered slow-growing species (Prosser and Tough, 1991). At higher temperatures, *Actinobacteria* might be likely outcompeted by smaller, fast-growing gram-negative *Proteobacteria* (Sjostedt et al., 2012). *Proteobacteria* were dominated by the members of *Enterobacteriaceae* (Fig. S1), which inhabit the digestive tract of a wide range of animals

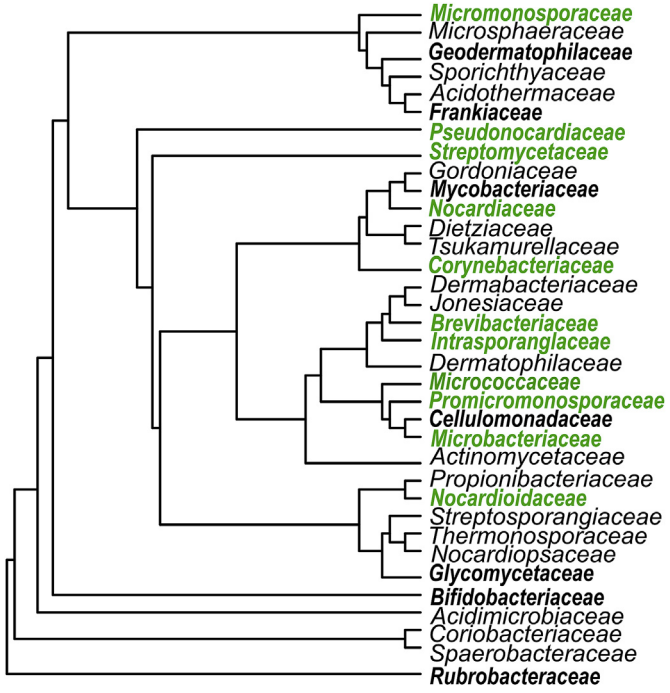

Fig. 3. Family-level phylogenetic topology tree of *Actinobacteria* with nearly 50% of the known families present in isopod gut microbiome (tree modified after (Ventura et al., 2007)). *Actinobacteria* in bold represent families that were identified in isopod guts; families with confirmed antibiotic-producing ability indicated with green (see Table S3).

(Gordon and FitzGibbon, 1999; Kohl et al., 2017; Whittaker et al., 2016) and therefore might prefer higher (more stable) temperatures compared to free-living *Actinobacteria* (Goodfellow and Williams, 1983). *Enterobacteriaceae* are among the most abundant organisms in the microbiome of arthropods (Degli Esposti and Martinez Romero, 2017), which further supports our previous findings (30% of the isopod gut bacterial community, Horvathova et al., 2016). The phylum *Tenericutes* was represented only by a single genus *Candidatus* Hepatoplasma, which is generally found at high frequencies in the midgut glands of a variety of isopod species (Fraune and Zimmer, 2008; Wang et al., 2007). The midgut glands might secrete digestive fluids with symbiotic bacteria into the hindgut, where *Hepatoplasma* may be involved in cellulose digestion (Bouchon et al., 2016; Wang et al., 2007). However, the recent molecular evidence questions the bacterial origin of cellulolytic enzymes (Bredon et al., 2018; Kostanjsek et al., 2010). High relative abundance of *Cyanobacteria* (i.e., the chloroplast DNA; Fig. S1) and *Tenericutes* could also reflect differential patterns in isopod feeding behaviour, owing to the slower digestion rates under lower temperature conditions (Zimmer, 2002a). Terrestrial isopods often encounter hypoxia in leaf-litter and soil habitats with high microbial activity (Schmitz and Harrison, 2004), and therefore limiting effects of environmental oxygen might be more common than previously expected in terrestrial habitats (Harrison et al., 2018). For example, insect gut bacterial communities showed to depend on the oxygen levels in the host habitat (the prevalence of anaerobes vs aerobes, Yun et al., 2014). In the present study, we did not find significant differences in the relative abundance of major bacterial groups depending on the environmental oxygen level, and most of the bacterial genera identified in isopod microbiome could be classified as aerobes (according to Yun et al., 2014). Although we cannot exclude the possibility that the observed changes in isopod gut microbiome were mediated exclusively through alteration of host physiology rather than through a direct effect of environmental temperature (Stevenson et al., 2014), our results represent a clear effect of ambient temperature on gut microbial communities of a terrestrial arthropod.

Actinobacteria have been hypothesized to engage in protective symbiosis and to possibly provide general healthcare for insects, but the underlying mechanisms remain unknown (Kaltenpoth, 2009). The effects of the microbiota on host physiology are largely driven by bacterial metabolites (Douglas, 2009; Kohl and Carey, 2016), including antibiotics produced by symbiotic *Actinobacteria*. We detected diverse members of *Actinobacteria* with high antibiotic-producing capabilities (e.g., *Streptomyces*, *Amycolatopsis* and *Pseudonocardia*; see also Table S3) and found evidence for a positive correlation of *Actinobacteria* and growth rate of an isopod host. Furthermore, this host benefit was temperature dependent and occurred only under our colder temperature regime. An increase in ambient temperature indicates the potential to deprive the host of beneficial symbionts and to eradicate the typically positive effect of temperature on growth rate in ectotherms (Fig. 2D). Our experimental temperatures do not represent extremes for the rough woodlouse but rather moderate temperatures occurring over the broad distribution of this species (Sfenthourakis and Taiti, 2015). The temperature of 15 °C may not be optimal for growth, but the presence of antibiotic-producing bacteria may compensate by enabling a high growth rate likely through offering protection against pathogens. Indeed, we found the higher presence of potentially pathogenic genus *Vibrio* (Le Roux et al., 2015) only under lower-temperature condition (see Fig. S5). Although we can only speculate about the adaptive advantages provided by symbionts, terrestrial isopods are generally slow-growing animals and mature only after reaching a threshold size, which can take 14 months or more under natural temperature conditions (Zimmer, 2002b). Growth rate is an important life-history trait, and faster growth may allow the animals to attain this minimum size for reproduction earlier, especially under unfavorable environmental conditions (Zimmer and Kautz, 1997). Our study provides a novel understanding of how the interaction between temperature and host-symbiotic associations may influence host life-history strategies.

With members of nearly 50% of the known *Actinobacteria* families present in the analyzed isopod gut microbiome (according to (Ventura et al., 2007)), our study reveals an astonishing diversity of *Actinobacteria* in this soil-dwelling arthropod. Most of these identified families include members that are known to produce antibiotics (Fig. 3, Table S3). The common rough woodlouse has previously been shown to prefer food sources inoculated with antibiotic-producing *Streptomyces* and *Pseudonocardia* (Ihnen and Zimmer, 2008), and this finding has more weighted importance in view of our present findings. This biofilmivore (Horvathova et al., 2016) likely also encounters harmful bacteria and fungi in its food, but the presence of antibiotic-producing *Actinobacteria* can potentially offset pathogen activity. The common rough woodlouse has a wide distribution and is one of the most successful crustacean colonizers of land habitats (Hornung, 2011; Slabber and Chown, 2002). Terrestrial isopods, the suborder *Oniscidea*, have invaded most areas of the world and occupy almost the whole range of terrestrial habitats including high mountains, caves, deserts and isolated islands (Sfenthourakis and Taiti, 2015; Taiti and Wynne, 2015). In addition, Oniscideans are not exclusively terrestrial, but also include amphibious and aquatic species that have secondarily returned to live in salt lakes or subterranean freshwaters (Taiti and Xue, 2012). All these features qualify isopods in general and the common rough woodlouse in particular as a potential model organism for cost-effective and large-scale screening of bioactive secondary metabolites from diverse habitats because it acts as a living vacuum cleaner for the collection of a wide range of environmental microbes including antibiotic-producing bacteria.

5. Conclusions

Recent evidence indicates that climate warming may impact diverse organisms not only directly but also indirectly through disruption of associations with parasitic/mutualistic partners (Kiers et al., 2010; Kikuchi et al., 2016; Wernegreen, 2012). The loss of algal symbionts in

bleaching corals exemplifies the extreme case of climate impacts on host-symbiont associations (Douglas, 2003). A temperature-driven loss of microbiome diversity may affect the essential functions with negative effects on host survival (Bestion et al., 2017), host tolerance to environmental stresses (Anbutsu et al., 2017; Burke et al., 2010; Engl et al., 2017), host digestion efficiency (Fontaine et al., 2018) or the host growth rate (this study). The absence of actinobacterial symbionts might thus represent just one of the likely examples for the effects of elevated temperature on host-symbiont interactions. Given the importance of the gut microbiota for host fitness, microbial symbionts may play key roles in facilitating or constraining host adaptation to changing environmental conditions. Our results from investigations on the interplay between the environment, host and symbionts thus provide a strong rationale for the "holobiont" concept (i.e., the host and its associated microbiome (Zilber-Rosenberg and Rosenberg, 2008)). Future studies should aim to investigate the host in concert with its symbionts to improve our understanding of organismal responses to a changing environment.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This project was supported by MAESTRO (2011/02/A/NZ8/00064) and PRELUDIUM grants (2014/13/N/NZ8/02469) of the National Science Centre Poland. TH was also supported by AVČR PPPLZ grant (L200961851). We thank Roey Angel, Manfred Bauchinger, Alica Chroňáková, Lucy Gillis and Martin Zimmer for critical comments. We thank Andrzej Antoł for help with the analysis in R, Mateusz Sobczyk for help with the analysis in CANOCO and Katarzyna Dudek for help with generating sequence data. Sequence data that support the findings of this study are deposited in NCBI's Sequence Read Archive with accession number SRP125004. We are grateful for the anonymously provided comments that helped to improve the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.04.015.

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