

Assessment of Bacterial Communities in Thirteen Species of Laboratory-Cultured Domestic Mites (Acari: Acaridida)

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Abstract

House dust mites (HDMs) and stored-product mites (SPMs) of various species inhabit human homes and stored agricultural products. These mites are carriers and hosts of microorganisms that enable their survival. The bacteriome from 13 species of SPMs and HDMs was analyzed and compared by 454 pyrosequencing of partial 16S rRNA gene amplicons. Altogether 128,052 sequences were obtained and assigned to 71 operational taxonomic units (OTUs) at the 97% identity level. The number of sequences in the OTUs between species of mites ranged from 6 to 31 in the individual mite species. We did not find any significant effect of diet or evolutionary origin of mites or their interaction on the composition of the mite bacteriome. In mite species with low bacterial diversity, the bacterial communities were dominated by potential symbiotic or parasitic bacteria, i.e., *Cardinium* in *Dermatophagoides farinae* (Hughes, 1961) and *Aeroglyphus robustus* (Banks 1906) and the enteric bacteria *Erwinia* in *Blomia tropicalis* Van Bronswijk, de Cock & Oshima, 1974 and *Xenorhabdus* in *Tyroborus lini* (Oudemans, 1924). Among the bacterial species identified, *Staphylococcus*, *Bacillus*, *Kocuria*, *Brevibacterium*, *Corynebacterium*, and *Brachybacterium* likely serve as food sources for the mites. The domestic acaridid mites carried high numbers of various bacteria that are potential threats to human health. These results contribute to the general understanding of the ecology of mite adaptation to human-made habitats.

Key words: bacteria, symbiont, feeding, parasite, allergen

Synanthropic acaridid mites of medical and economic importance include the house dust mites (HDMs) and the stored-product mites (SPMs; Colloff 2009). Collectively, these groups are called “domestic mites” because many storage mites from the families Acaridae, Glycyphagidae, and Chortoglyphidae are also found in house dust (Colloff and Spieksma 1992). The evolutionary origin of domestic mites lies in different habitats (OConnor 1979, 1982, 1984; Klimov and OConnor 2013). OConnor (1979) categorized these mites according to habitat occurrence into four groups: 1) mites associated with specific resources (*Sancassania*, *Carpoglyphus*, and *Aeroglyphus*); 2) mites associated with field resources (*Tyrophagus*, *Tyroborus*, and *Glycyphagus*); 3) mites associated with the nests of mammals (*Acarus*, *Aleuroglyphus*, *Lepidoglyphus*, *Chortoglyphus*, and *Blomia*); and 4) mites associated with the nests of birds (*Dermatophagoides*). The mite species associated with specific resources comprise the mites from heterogeneous habitats, e.g., *Carpoglyphus lactis* (L., 1758), which infest dried fruits (Hubert et al. 2011, 2015); *Sancassania berlesii* (Michael, 1903), which inhabit dung, rotting vegetation, stables, and barns (OConnor 1979), and also a poultry farm (Principato et al. 2005); and

Aeroglyphus robustus (Banks 1906), which colonizes the nests of social insects (OConnor 1979).

Domestic mites produce a variety of allergenic agents (Musken et al. 2000, Arlian 2002, Colloff 2009, Thomas et al. 2010), the most significant of which are feces-defecated allergens—primarily digestive enzymes (Tovey et al. 1981, Erban and Hubert 2015, Erban et al. 2016). Mite–microbial interactions, whether bacterial or fungal, are proposed to be essential for the effective use of nutrients from the derivatives of skin in the dust environment (Erban and Hubert 2010). The mites also feed on the microorganisms in grain stores (Sinha 1966). In addition, the feces of mites contain bacterial enzymes and toxins that are important components of mite allergens (Erban et al. 2016).

The food sources of mites colonized by microorganisms are habitats where mite–microbial associations and interactions occur (Horak 1987; Hay et al. 1992a,b; Erban and Hubert 2010, 2012; Erban et al. 2016). As demonstrated by the increase in the growth of mite populations fed a *Micrococcus lysodeikticus*-supplemented diet (Erban and Hubert 2008), mites feed facultatively on bacteria. The SPMs are able to acquire bacteria from the environment as an

adaptation to changes in the diet (Smrz and Catska 2010, Hubert et al. 2012b, Zindel et al. 2013). Gram-positive bacteria (e.g., *Bacillus* and *Staphylococcus*) that have been identified in both HDMs and SPMs potentially supply the mites with nutrients (Oh et al. 1986, Hubert et al. 2012a). Mites are also common hosts of the gram-negative bacterium *Cardinium* (Hubert et al. 2012a, Kopecky et al. 2013), which is a putative nutritive symbiont; however, this bacterium may also be a parasite (Penz et al. 2012). *Dermatophagoides* spp., *Acarus siro* (L. 1758), and *Tyrophagus putrescentiae* (Schrank, 1781) are inhabited by a *Bartonella*-like bacteria that may also have a symbiotic function (Valerio et al. 2005, Kopecky et al. 2014a).

A few studies indicate that some bacteria are harmful to the mites. The bacteria isolated from dead specimens of *Tyrophagus similis* Volgin were found to be pathogenic to mites in biotests (Kikuta and Takagi 2001). A suspension of the spores of *Bacillus* spp. prolonged the developmental time for tritonymphs of *Dermatophagoides pteronyssinus* (Trouessart, 1897) (Saleh et al. 1991). *Bacillus thuringiensis* var. *tenebrionis*, a producer of Cry 3A toxin, when added to mite diet at relatively high concentrations suppressed the growth of *Dermatophagoides farinae* (Hughes, 1961), *T. putrescentiae*, *A. siro*, and *Lepidoglyphus destructor* (Schrank, 1871) (Erban et al. 2009) populations. *Bacillus cereus* isolated from *T. putrescentiae* reared on dry dog food decreased the population growth of mites after being added to two nutritionally distinct diets (Erban et al. 2016).

In this study, the bacteriomes of two species of HDMs and 11 species of SPMs were analyzed by pyrosequencing the 16S rRNA gene amplicons. The goals of the study were as follows: 1) to describe the bacteriome of 13 species of domestic mites; 2) to compare the relationship between the evolutionary origins of mites and their bacteriomes; and 3) to analyze the effects of rearing diet on the mite-associated bacteriome.

Materials and Methods

Mites

The 11 species of SPMs were *A. siro*, *Ae. robustus*, *Aleuroglyphus ovatus* (Troupeau 1878), *Blomia tropicalis* Van Bronswijk, de Cock & Oshima, 1974, *Carpoglyphus lactis* (L., 1758), *Chortoglyphus arcuatus* (Troupeau, 1879), *Glycyphagus domesticus* (De Geer, 1778), *Lepidoglyphus destructor*, *S. berlesii*, *T. putrescentiae*, and *Tyrobobus lini* (Oudemans, 1924). The two species of HDMs were *D. farinae* and *D. pteronyssinus* (Trouessart, 1897). The mites originated from laboratory cultures maintained at the Crop Research Institute, Prague, Czechia (see Table 1). The mites were mass-reared in IWAKI 70-ml capacity tissue culture flasks with 25-cm² surface areas (IWAKI flasks, Catalog number 3100-025; Sterilin, Newport, United Kingdom). The flasks were maintained in Secador desiccator cabinets (Bel-Art Products, Pequannock, NJ) with a saturated KCl or NaCl solution to provide 85 or 75% relative humidity, respectively, in an air-conditioned room at 25 ± 1°C. Two different rearing diets were used. The stored-product mite diet (SPMd) was a mixture of oat flakes, wheat germ, and dried yeast extract (ratio 10:10:1 wt; Pangamin, Rapeto, Bezdrzuzice, Czechia). The house dust mite diet (HDMd) was a mixture of dog food (Ontario-pet, Placek, Podebrady, Czechia), wheat germ, dried fish food (Lon-Bio, Aquatropic, Praha, Czechia), Pangamin, and gelatin (ratio 10:10:3:1 wt; SERVA Electrophoresis, Heidelberg, Germany). Both diets were powdered and sieved and then heated in a thermostat (70°C) for 0.5 h to suppress microbial growth (Erban and Hubert 2008). Approximately 1.5 g of diet was placed into each flask.

Table 1. The list of sampled species and their origin and the rearing conditions of the mites in the experiment

Family	Name	Sampling time	Sampled/obtained Site	Year	Collector/ obtained from	Rearing cond.		Evol. origin
						RH (%)	Diet	
Acaridae	<i>Acarus siro</i> (L. 1758)	10 Sept	Grain, Buštěhrad, Czechia	1996 ^a	E Zdarkova	85	SPMd	3
	<i>Aleuroglyphus ovatus</i> (Troupeau 1878)	10 June	Obtained from Fera, UK	1998	K Wildey	85	SPMd	3
	<i>Sancassania berlesii</i> (Michael, 1903)	10 Sept	Obtained from Fera, UK	1998	K Wildey	85	HDMd	1
	<i>Tyrophagus putrescentiae</i> (Schrank, 1781)	10 June	Grain, Buštěhrad, Czechia	1996 ^a	E Zdarkova	85	SPMd	2
	<i>Tyrobobus lini</i> (Oudemans, 1924)	10 Sept	Food processing fact., Prague, Czechia,	1965 ^a	E Zdarkova	85	HDMd	2
Echinypodidae	<i>Blomia tropicalis</i> Van Bronswijk, de Cock & Oshima, 1974	10 June	Obtained from University of Cartagena, Colombia	2008	J Zakzuk	85	SPMd	3
	<i>Carpoglyphus lactis</i> (L., 1758)	10 June	Food processing fact., figs, Prague, Czechia	1965 ^a	E Zdarkova	85	HDMd	1
	<i>Chortoglyphus arcuatus</i> (Troupeau, 1879)	10 Sept	Food processing fact., Prague, Czechia,	1965 ^a	E Zdarkova	85	HDMd	3
	<i>Dermatophagoides farinae</i> (Hughes, 1961)	10 June	Obtained from Medical University of Silesia, Poland	2005	K Solarz	75	HDMd	4
	<i>Dermatophagoides pteronyssinus</i> (Trouessart, 1897)	10 June	Obtained from Fera, UK	1998	K Wildey	75	HDMd	4
Glycyphagidae	<i>Glycyphagus domesticus</i> (De Geer, 1778)	10 Sept	Food processing fact., Czechia	1965 ^a	E Zdarkova	85	HDMd	2
	<i>Lepidoglyphus destructor</i> (Schrank, 1871)	10 June	Food processing fact., Czechia	1965 ^a	E Zdarkova	85	HDMd	3
Aeroglyphidae	<i>Aeroglyphus robustus</i> Banks	10 June	Apiary, Czechia	2005	S. Tuckova	85	HDMd	1

The two environmental factors are included, i.e. diet and evolutionary origin.

^a The common practices before the year 2000 was to mix the laboratory strain with field strain to protect genetic diversity of the strain. SPMd—stored-product mite diet, HDMd—house dust mite diet; Evolutionary origin (according to OConnor 1979): (1) mites associated with field resources; (2) mites associated with the nests of mammals; and (4) mites associated with the nests of birds.

The mites were collected after one month of cultivation. To avoid cross contamination between the cultures of mites, each species was collected during one day and after ethanol cleaning of the workplace. Unsexed mite adults and juveniles were collected from the rearing chambers using a sterile paintbrush and a Stemi 2000-C dissecting stereomicroscope (Carl Zeiss, Jena, Germany). Then, the mites were weighed in Eppendorf tubes using a Mettler AE 240 microbalance (Mettler-Toledo, Columbus, OH) with an accuracy of 10 µg. Each tube was filled with 1 ml of sodium hypochlorite (5%) for 1 min, and the sample was centrifuged at 3,000g for 1 min. Then, the sodium hypochlorite was replaced by 1 ml of absolute ethanol (Lach-Ner, Neratovice, Czechia) for 1 min, and the sample was centrifuged at 3,000 × g for 1 min. Next, the ethanol was replaced by PBST composed of phosphate buffered saline (3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 1.3 mM KCl, and 135 mM NaCl) with 0.05% detergent Tween 20 (Catalog number P9416; Sigma-Aldrich, Saint Louis, MO). The sample was cleaned three times with PBST using centrifugation (Kopecky et al. 2014b). Finally, the mites were homogenized in 100 µl of PBST in a 2-ml glass Potter-Elvehjem homogenizer with a Teflon pestle (Art. No. 6302; Kartell Labware Division, Noviglio, Italy). The DNA was extracted using a DNeasy Tissue kit (Qiagen, Valencia, CA) and was cleaned with a GeneClean Turbo kit (MP Biomedicals, Solon, OH). The quality of DNA was checked by amplification of the 16S rRNA gene using universal eubacterial primers (Lane 1991).

Assessment of the Bacteriome by 454 Pyrosequencing

The bacterial, tag-encoded, FLX 16S rDNA amplicon parallel pyrosequencing and postsequencing processing were conducted at the Medical Biofilm Research Institute (Lubbock, TX) as described by Dowd et al. (2008), Middelbos et al. (2010), and Ghosh et al. (2011). The sequenced region of the 16S rRNA gene corresponded to the positions 28–349 (numbered based on *Escherichia coli*).

Bioinformatics

The sequences were analyzed by combining MOTHUR v. 1.36.1 (Schloss et al. 2009) and UPARSE (Edgar 2013). At first, sequences were demultiplexed and trimmed in MOTHUR according to the description of Schloss et al. (2011) and web pages (http://www.mothur.org/wiki/454_SOP, accessed in February 2016). The trimmed sequences were aligned to SILVA-based bacterial reference alignment database (Pruesse et al. 2007, Quast et al. 2013), and chimeras were removed using UCHIME (Edgar et al. 2011). Operational taxonomic units (OTUs) were constructed in UPARSE at a 97% identity level and classified according to the Ribosomal Database Project training set v. 15 (<http://rdp.cme.msu.edu>; Cole et al. 2014). OTUs similar to chloroplasts and Archaea were removed. The obtained representative sequences were processed using the BLASTN program in the NCBI platform (<https://blast.ncbi.nlm.nih.gov/>; Altschul et al. 1997). The best search hits were chosen based on the highest bit score. The symbiotic bacteria *Cardinium* and *Solitalea*-like and *Bartonella*-like bacteria were identified by comparing the sequences with those previously identified in mites by Sanger sequencing (Hubert et al. 2012a, Kopecky et al. 2014a). The comparison was performed in CodonCode 5.1.5 (CodonCode Corporation, Centerville, MA). The taxonomic composition of the samples was visualized with KRONA projection (Ondov et al. 2011).

Statistical Analyses

Abundance data were transformed to a shared file and processed in MOTHUR according to the procedure described in Kozich et al.

(2013) and web pages (http://www.mothur.org/wiki/MiSeq_SOP). The data were subsampled to obtain 3741 reads per sample, and α -diversity indices were calculated. The subsampled dataset was exported to the PAST 3.06 (Hammer et al. 2001). The diets (HDMd and SPMd) and evolutionary origin (Table 1) of mites were set as environmental variables. The criterion for evolutionary origin was based on the classification of O'Connor (1979). The effect of both variables (diets and evolutionary origin of mites) to bacteriome composition was tested by two-way Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson et al. 2013) using Bray–Curtis dissimilarities and 10,000 permutations. The position of the species was visualized using principal coordinate analyses (PCoA) calculated in MOTHUR on the Bray–Curtis dissimilarity matrix. The results were visualized using XLSTAT (<http://www.xlstat.com/en/>, Addinsoft, New York, NY).

Results

Comparison of the Bacterial Communities in the Mite Species

The dataset was composed of 128,052 16S rRNA partial sequences, which were assigned to 71 OTUs (Table 2). The number of samples from individual mite species ranged from 3,741 to 14,080 sequence reads and from 6 to 31 OTUs (Fig. 1). The composition of the bacteriome for different species was visualized by Krona projection (Fig. 2). Bacteriomes of *A. robustus*, *B. tropicalis*, and *D. farinae* were characterized by the presence of one dominant taxon, *Cardinium* or *Erwinia*, covering 99% of sequences of the genera. Also the bacteriome of *A. siro* was formed by symbiotic or parasitic *Solitalea*-like and *Bartonella*-like bacteria. The bacterial communities in these species of mites had the lowest numbers of OTUs (sOTUs) and inverse Simpson diversity indices (Fig. 1). The bacterial community of *G. domesticus* was characterized by an inverse Simpson diversity index six times higher than that of *D. farinae*.

The two-way PERMANOVA model showed no significant effect of diet or the evolutionary origin of mites or their interaction on the bacteriome compositions using the Bray–Curtis matrix ($F=0.18$, $P=0.81$; $F=0.28$, $P=0.41$; $F=-0.93$, $P=0.57$). The PCoA visualization showed that *T. putrescentiae* with *A. ovatus* and *T. lini* mites had different bacteriomes than the other species (Fig. 3).

The Bacterial Taxa Identified in the Mite Communities

More than 50% of the sequences in the bacterial communities of *D. pteronyssinus*, *C. lactis*, *C. arcuatus*, and *S. berlesei* belonged to *Actinobacteria* (Fig. 2). The most numerous were the sequences related to *Kocuria*, *Brevibacterium*, *Corynebacterium*, *Brachybacterium*, and sequences of low similarity to *Arthrobacter* (Table 2). All the mite species contained sequences from the phylum *Firmicutes*; the most numerous sequences belonged to *Staphylococcus*, *Bacillus*, *Filobacillus*, and unclassified *Bacillaceae_2*. *Firmicutes* represented >50% of the sequences in the bacterial communities of *G. domesticus*, *L. destructor*, *A. ovatus*, and *S. berlesei* (Fig. 2). The sequences of *Alphaproteobacteria* were found in *A. siro* and *T. putrescentiae*, where they represented 75 and 33% of sequences, respectively (Fig. 3). In *L. destructor*, *Betaproteobacteria* formed 6% of the bacterial community (Fig. 2). The sequences clustering inside *Gammaproteobacteria* formed 99, 74, and 29% of the sequences in the bacterial communities of *B. tropicalis*, *T. lini*, and *C. arcuatus*, respectively. The gammaproteobacterial sequences were related to *Erwinia* in *B. tropicalis* and to *Xenorhabdus* in the other two species (Table 2).

Table 2. The distribution of OTUs97 among sample species of mites and characterization of OTU consensus according to RDP and GenBank

OTUs	RDP identification taxa	id.	GenBank identification taxon (similarity)	Accs. No.	aOTU	Species of mites												
						Aer rob	Gly dom	Tyr put	Der pte	San ber	Car lac	Cho arc	Lep des	Tyr lin	Aca sir	Ale ova	Blo tro	Der far
OTU1	Kocuria	0.9902	<i>Kocuria korensis</i> (99)	NR_116745	21010	0	7	2,234	2,784	6,930	5,783	42	127	1,491	13	1,578	20	1
OTU2	Bartonella-like	nd	<i>Bartonella henselae</i> (93)	NR_074335	14349	0	0	3,849	3	1	0	0	1	1	10,480	3	5	6
OTU4	Staphylococcus	0.9839	<i>Staphylococcus arlettae</i> (98)	NR_024664	14189	60	1,933	5,573	83	148	2,384	361	1,560	8	0	2,067	0	12
OTU6	Cardinium	nd	can. <i>Amoebophilus asiaticus</i> (89)	NR_074574	11620	4,773	2	0	4	1	0	1	5	0	1	0	1	6,832
OTU3	Erwinia	0.2276	<i>Erwinia uzbekensis</i> (96)	NR_113061	11151	0	0	0	0	0	0	0	0	0	1	0	11,150	0
OTU5	Xenorhabdus	0.2785	<i>Xenorhabdus innexi</i> (93)	NR_042325	10125	0	0	0	0	3,437	0	0	0	6,680	0	7	0	1
OTU7	Bacillus	0.9954	<i>Bacillus cereus</i> (100)	NR_074540	8619	1	44	249	1,050	1	0	0	7,129	0	140	0	5	0
OTU8	Micrococcaceae	nd	<i>Arthrobacter tecti</i> (90)	NR_042251	5769	0	0	0	5,768	1	0	0	0	0	0	0	0	0
OTU9	Staphylococcus	0.9944	<i>Staphylococcus saprophyticus</i> (100)	NR_074999	4271	18	8	0	0	2	54	4,165	19	0	1	4	0	0
OTU11	Solitalea-like	nd	<i>Bizionia halysiensis</i> (80)	NR_109525	3383	0	1	0	1	0	1	0	2	0	3,370	3	5	0
OTU66	Enterobacteriaceae	0.0897	<i>Serratia rubidaea</i> (92)	NR_114232	3180	0	0	0	0	273	0	0	0	2,904	0	0	3	0
OTU10	Corynebacterium	0.7119	<i>Corynebacterium nuruki</i> (98)	NR_117816	2879	0	0	1	0	0	0	2,863	15	0	0	0	0	0
OTU12	Bacillaceae_2	nd	<i>Virgibacillus campisalis</i> (89)	NR_108840	2588	1	2,582	0	0	4	1	0	0	0	0	0	0	0
OTU13	Brachylobacterium	0.9923	<i>Brachylobacterium alimentarium</i> (99)	NR_026269	1841	0	0	0	0	0	1,571	270	0	0	0	0	0	0
OTU18	Brevibacterium	0.9132	<i>Brevibacterium avium</i> (96)	NR_026485	1567	0	1,142	0	0	0	425	0	0	0	0	0	0	0
OTU14	Bacillaceae_2	nd	<i>Salinibacillus xinjiangensis</i> (89)	NR_125634	1431	0	1,431	0	0	0	0	0	0	0	0	0	0	0
OTU27	Bacillaceae_2	nd	<i>Lentibacillus salinarum</i> (89)	NR_044305	1,369	0	1,368	0	0	1	0	0	0	0	0	0	0	0
OTU15	Brevibacterium	0.8461	<i>Brevibacterium siliginense</i> (97)	NR_115086	1,321	0	0	0	0	0	0	1,305	16	0	0	0	0	0
OTU16	Staphylococcus	0.9954	<i>Staphylococcus lentus</i> (100)	NR_043418	1,289	0	0	0	0	0	88	1,200	1	0	0	0	0	0
OTU17	Brevibacterium	0.4823	<i>Brevibacterium siliginense</i> (96)	NR_115086	1,070	0	145	0	0	1	920	0	4	0	0	0	0	0
OTU21	Staphylococcus	0.9902	<i>Staphylococcus kloostii</i> (99)	NR_024667	838	2	5	28	0	0	570	160	20	0	0	53	0	0
OTU19	Brevibacterium	0.8461	<i>Brevibacterium iodinum</i> (97)	NR_026241	690	0	0	0	0	0	0	684	6	0	0	0	0	0
OTU20	Massilia	0.9818	<i>Massilia aurea</i> (100)	NR_042502	569	0	0	0	0	0	0	0	568	0	0	0	1	0
OTU24	Bacillaceae_2	nd	<i>Virgibacillus halotolerans</i> (88)	NR_108860	564	0	564	0	0	0	0	0	0	0	0	0	0	0
OTU28	Bacillaceae_2	nd	<i>Oceanobacillus indicitreducens</i> (90)	NR_113330	354	0	354	0	0	0	0	0	0	0	0	0	0	0
OTU59	Bacillaceae_2	nd	<i>Virgibacillus campisalis</i> (87)	NR_108840	293	0	293	0	0	0	0	0	0	0	0	0	0	0
OTU22	Brevibacterium	0.779	<i>Brevibacterium oceanii</i> (99)	NR_042458	216	0	0	0	0	0	0	216	0	0	0	0	0	0
OTU23	Brevibacterium	0.9132	<i>Brevibacterium oceanii</i> (97)	NR_042458	210	0	0	0	0	0	0	207	3	0	0	0	0	0
OTU51	Brevibacterium	0.8126	<i>Brevibacterium siliginense</i> (98)	NR_115086	182	0	0	0	0	0	0	182	0	0	0	0	0	0
OTU29	Bacillaceae_2	nd	<i>Virgibacillus albus</i> (90)	NR_109613	142	0	128	0	0	0	0	0	0	0	0	14	0	0
OTU25	Methylobacterium	0.9818	<i>Methylobacterium rhodesianum</i> (99)	NR_041028	138	0	138	0	0	0	0	0	0	0	0	0	0	0
OTU26	Brevibacterium	0.9132	<i>Brevibacterium antiquum</i> (95)	NR_029079	118	0	0	0	0	0	118	0	0	0	0	0	0	0
OTU30	Salirhabdus	nd	<i>Paraliobacillus ryukyensis</i> (88)	NR_113871	110	0	103	7	0	0	0	0	0	0	0	0	0	0
OTU32	Bacillaceae_2	nd	<i>Virgibacillus byunensis</i> (87)	NR_116615	103	0	10	93	0	0	0	0	0	0	0	0	0	0
OTU48	Staphylococcus	0.9693	<i>Staphylococcus arlettae</i> (96)	NR_024664	58	0	39	8	2	0	0	2	5	0	0	1	0	1
OTU33	Bacillaceae_2	nd	<i>Oceanobacillus polygوني</i> (89)	NR_114348	48	0	27	21	0	0	0	0	0	0	0	0	0	0
OTU31	Brevibacterium	0.779	<i>Brevibacterium siliginense</i> (94)	NR_115086	47	0	0	0	0	0	47	0	0	0	0	0	0	0
OTU36	Acinetobacter	0.9693	<i>Acinetobacter lwoffii</i> (99)	NR_113346	36	0	1	0	12	0	0	0	19	0	0	0	4	0
OTU57	Bacillaceae_2	nd	<i>Virgibacillus campisalis</i> (89)	NR_108840	36	0	36	0	0	0	0	0	0	0	0	0	0	0
OTU43	Brevibacterium	0.7454	<i>Brevibacterium siliginense</i> (96)	NR_115086	35	0	0	0	0	0	0	35	0	0	0	0	0	0

(continued)

(continued)

Table 2. Continued

OTUs	RDP identification taxa	id.	GenBank identification taxon (similarity)	Accs. No.	aOTU	Species of mites													
						Aer rob	Gly dom	Tyr put	Der pte	San ber	Car lac	Cho arc	Lep des	Tyr lin	Aca sir	Ale ova	Blo tro	Der far	
OTU69	Brevibacterium	0.6448	<i>Brevibacterium avium</i> (97)	NR_026485	25	0	9	0	0	0	16	0	0	0	0	0	0	0	
OTU34	Brevibacterium	0.1766	<i>Brevibacterium luteolum</i> (94)	NR_114872	23	0	0	0	0	0	0	23	0	0	0	0	0	0	
OTU39	Enterobacteriaceae	nd	<i>Serratia liquefaciens</i> (99)	NR_121703	23	0	23	0	0	0	0	0	0	0	0	0	0	0	
OTU42	Gracilbaccillus	nd	<i>Paradiobacillus ryukyuensis</i> (88)	NR_113871	18	0	18	0	0	0	0	0	0	0	0	0	0	0	
OTU62	Virgibacillus	0.2276	<i>Virgibacillus soli</i> (92)	NR_116243	18	0	6	12	0	0	0	0	0	0	0	0	0	0	
OTU41	Oceanobacillus	0.986	<i>Oceanobacillus neutriphilus</i> (98)	NR_116424	16	0	0	1	0	0	15	0	0	0	0	0	0	0	
OTU74	Brevibacterium	0.2785	<i>Brevibacterium siliguriense</i> (95)	NR_115086	13	0	0	0	0	0	13	0	0	0	0	0	0	0	
OTU35	Bartonella-like	nd	<i>Bartonella henselae</i> (92)	NR_074335	10	0	0	0	0	0	0	0	0	0	0	0	10	0	
OTU37	Brevundimonas	0.9735	<i>Brevundimonas vesicularis</i> (99)	NR_113586	10	0	0	0	1	0	0	0	9	0	0	0	0	0	
OTU45	Staphylococcus	0.9839	<i>Staphylococcus aureus</i> (99)	NR_075000	9	0	0	0	0	0	0	0	4	0	0	0	5	0	
OTU46	Corynebacterium	0.986	<i>Corynebacterium variabile</i> (100)	NR_102874	9	0	0	0	0	0	0	0	9	0	0	0	0	0	
OTU44	Massilia	0.9881	<i>Massilia timonae</i> (100)	NR_026014	8	0	0	0	5	0	0	0	0	2	0	1	0	0	
OTU56	Corynebacterium	0.5332	<i>Corynebacterium nuruki</i> (97)	NR_117816	8	0	0	0	0	0	0	0	8	0	0	0	0	0	
OTU52	Streptococcus	1	<i>Streptococcus intermedius</i> (100)	NR_102797	7	0	0	0	0	0	0	0	0	0	0	0	0	7	
OTU65	Propionibacterium	0.996	<i>Propionibacterium acnes</i> (100)	NR_074675	6	0	0	0	0	0	0	0	0	3	0	0	0	2	
OTU58	Bacillaceae_2	nd	<i>Oceanobacillus limi</i> (95)	NR_109124	4	0	4	0	0	0	0	0	0	0	0	0	0	0	
OTU64	Acinetobacter	0.9839	<i>Acinetobacter calcoaceticus</i> (99)	NR_102826	4	0	0	0	2	0	0	0	2	0	0	0	0	0	
OTU47	Staphylococcus	0.9954	<i>Staphylococcus epidermidis</i> (100)	NR_113957	3	0	0	0	0	0	0	0	0	0	0	0	3	0	
OTU49	Solirubrobacter	nd	<i>Staphylococcus epidermidis</i> (100)	NR_108192	3	0	3	0	0	0	0	0	0	0	0	0	0	0	
OTU50	Lachnospiracea	0.779	<i>Solirubrobacter ginsenosidimutans</i> (88)	NR_108192	3	0	0	0	0	0	0	0	0	0	0	0	0	0	
OTU68	Variovorax	0.9818	<i>Blautia hydrogenotrophica</i> (95)	NR_746054	3	0	0	0	3	0	0	0	0	0	0	0	0	0	
OTU76	Bacillaceae_2	nd	<i>Variovorax paradoxus</i> (100)	NR_074654	3	0	0	0	0	0	0	0	1	0	0	0	2	0	
OTU55	Friedmanniella	0.1766	<i>Virgibacillus campisalis</i> (88)	NR_108840	3	0	3	0	0	0	0	0	0	0	0	0	0	0	
OTU60	Pseudonocardiaceae	nd	<i>Tessaracoccus oleagris</i> (97)	NR_108681	2	0	0	0	0	0	0	0	0	0	0	0	2	0	
OTU61	Geodermatophilaceae	0.0571	<i>Amrycolatopsis ruanii</i> (90)	NR_109305	2	2	0	0	0	0	0	0	0	0	0	0	0	0	
OTU63	Frigoribacterium	0.6783	<i>Geodermatophilus brasiliensis</i> (96)	NR_126197	2	0	2	0	0	0	0	0	0	0	0	0	0	0	
OTU67	Prevotellaceae	0.0354	<i>Frigoribacterium endophyticum</i> (97)	NR_134732	2	0	0	0	0	0	0	0	2	0	0	0	0	0	
OTU70	Chryseobacterium	0.986	<i>Prevotella stercorea</i> (85)	NR_041364	2	0	0	0	0	0	0	0	0	0	0	0	2	0	
OTU71	Novosphingobium	0.9714	<i>Chryseobacterium indoltheticum</i> (100)	NR_042926	2	0	0	0	0	0	0	0	0	0	0	0	2	0	
OTU72	Brachyobacterium	0.0571	<i>Novosphingobium lentum</i> (99)	NR_042056	2	0	0	0	0	0	0	0	0	0	0	2	0	0	
OTU75	Massilia	0.9714	<i>Brachyobacterium faecium</i> (90)	NR_074655	2	0	0	0	0	0	0	0	2	0	0	0	0	0	
			<i>Massilia brevitalea</i> (99)	NR_044274	2	2	0	0	0	0	0	0	0	0	0	0	0	0	

Aca-sir, *Acarus siro*; Aer-ova, *Aeroglyphus robustus*; Ale-ova, *Aleuroglyphus ovatus*; Blo-tro, *Blomia tropicalis*; Car-lac, *Carpoglyphus lactis*; Cho-arc, *Chortoglyphus arcuatus*; Der-far, *Dermatophagoides farinae*; Der-pte, *Dermatophagoides pteronyssinus*; Gly-dom, *Glycophagus domesticus*; Lep-des, *Lepidoglyphus destructor*; San-ber, *Sancassania berlesii*; Tyr-put, *Tyrophagus putrescentiae*; and Tyr-lin, *Tyrobionus lini*; id. - confidence threshold; nd - not determined—for RDP match.

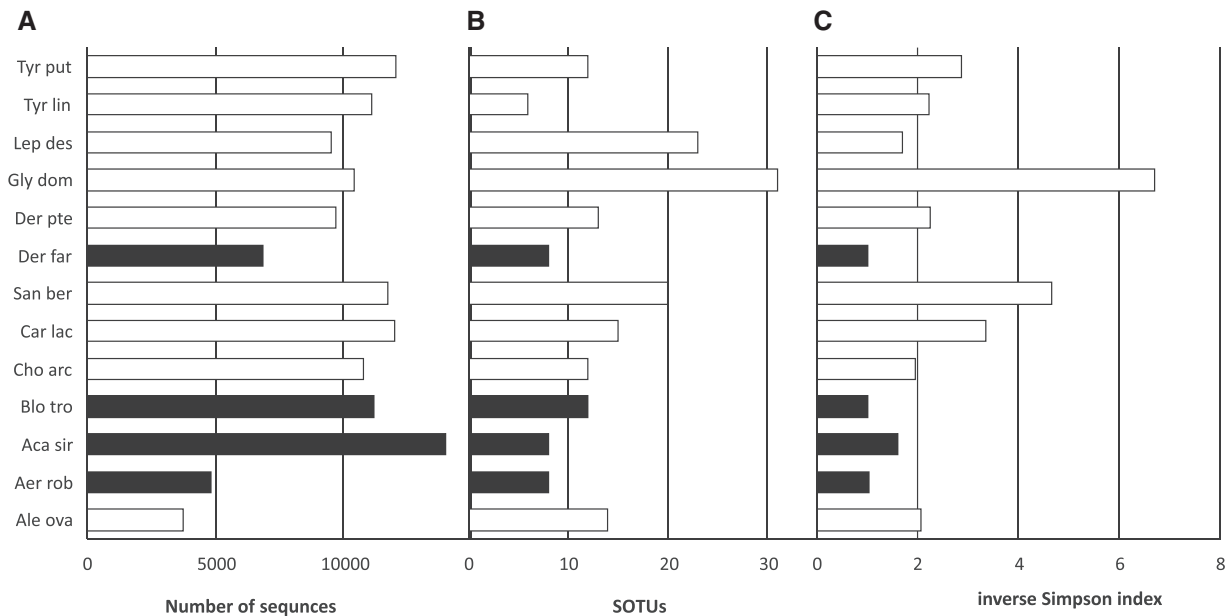


Fig. 1. Composition and diversity of bacteriome in house dust and stored-product mites assessed by 454 pyrosequences using OTUs97, the black columns indicate species with symbiotic bacteria. (A) Number of sequences. (B) Number of OTUs97 (sOTUs). (C) Inverse Simpson index. Aca-sir, *Acarus siro*; Aer-ova, *Aeroglyphus robustus*; Ale-ova, *Aleuroglyphus ovatus*; Blo-tro, *Blomia tropicalis*; Car-lac, *Carpoglyphus lactis*; Cho-arc, *Chortoglyphus arcuatus*; Der-far, *Dermatophagoides farinae*; Der-pte, *Dermatophagoides pteronyssinus*; Gly-dom, *Glycyphagus domesticus*; Lep-des, *Lepidoglyphus destructor*; San-ber, *Sancassania berlesei*; Tyr-put, *Tyrophagus putrescentiae*; and Tyr-lin, *Tyroborus lini*.

Discussion

Diversity of Bacteria

This study analyzed and compared the bacterial communities of 13 species of SPMs and HDMs for the first time. In general, low diversity in bacterial communities was correlated with the dominance of symbiotic bacteria. The proposed nutritive endosymbionts were *Cardinium* in *D. farinae* and *A. robustus* and the enteric bacterium *Erwinia*, which forms ~99% of bacteriome in *B. tropicalis*. In contrast, the highest diversity was found in the species without detected symbionts, *G. domesticus* and *S. berlesei*. The presence or absence of symbionts is suggested as the reason for the nonsignificant effect of the evolutionary origin of mites. The mites obtain and change symbionts frequently under various habitats and diets and symbionts cause variability among populations of the same species (Smrz and Catska 2010). In this study, the bacteriome was described in laboratory populations of 13 mite species; however, a different composition of symbionts can be expected in field conditions. The bacteriome in the field populations of mites (e.g., *Rhizoglyphus robini* (Zindel et al. 2013), *T. putrescentiae* (Hubert et al. 2012b), and *C. lactis* (Hubert et al. 2015) showed differences among populations of the same species based on diet.

A Possible Association of Mites and Bacteria

The mite–bacterial associations were divided into the following groups: 1) ingested bacteria; 2) gut symbionts; 3) parasites or symbionts in the fat body and reproductive organs; and 4) pathogenic bacteria (cf. Hubert et al. 2012b). Although we did not observe any significant effect of diet on bacteriome composition, the mites can obtain bacteria with their ingested diets (Smrz and Catska 2010). The ingested bacteria enter the gut with diets, and although both the diets HDMd and SPMd used in this study were pasteurized, the mites disseminate bacteria from old cultures to new ones on their bodies and in feces, just as they also disseminate fungi (Hubert et al.

2004). The food for SPMs and HDMs includes *Actinobacteria* and *Bacillales*; among these, *Kocuria* was apparently the most common, based on the previous study of *A. siro*, *L. destructor*, and *T. putrescentiae* (using SPMd and HDMd) (Hubert et al. 2012a), even when the specimens were treated with antibiotics (Kopecky et al. 2014b). *Kocuria* were also isolated from a field population of *D. pteronyssinus* (Tang et al. 2013). Moreover, *Micrococcus*, which is taxonomically closely related to *Kocuria*, accelerated the population growth of SPMs and HDMs compared with the controls (Erban and Hubert 2008). Members of *Bacillales* including *B. cereus*, *B. licheniformis*, *Staphylococcus aureus*, *S. epidermidis*, and *S. capitis* were identified in a field population of *D. pteronyssinus* (Tang et al. 2013). The addition of *B. cereus* caused suppression of *T. putrescentiae* growth. The suppressive effect was higher in the high-fat, high-protein diet than in the low-fat, low-protein food (Erban et al. 2016) though the high-fat, high-protein food is more favorable for *T. putrescentiae* population growth (Erban et al. 2015, 2016; Rybanska et al. 2016). This result indicated the need for balance between the mite and its nutritive symbiont (Erban et al. 2016). Moreover, the addition of antibiotics to the diet did not eliminate *Bacillales* bacteria in SPMs (Kopecky et al. 2014b); thus, the *Firmicutes* taxa may belong to other ingested bacteria (Hubert et al. 2012a).

Microorganisms produce exoenzymes that modify the environment in which they are growing, e.g., decaying plant debris in the soil (Luxton 1972, Maraun et al. 2003). Similarly, an effective use of difficult to digest nutrients (e.g., from skin, hair, nails, and feathers) by mites is possible because of the symbiotic interactions of mites with bacteria and microscopic fungi, which then serve as a food source for mites (Erban and Hubert 2010). In particular, neutral and alkaline proteases and exo-chitinases of *B. cereus* associated with *T. putrescentiae* grown on dry dog food were found in the feces extract (Erban et al. 2016). The *B. cereus* proteases are capable of degrading keratin, and thus, the symbiosis is also applicable to the dust environment where the bacterial proteases can degrade keratin

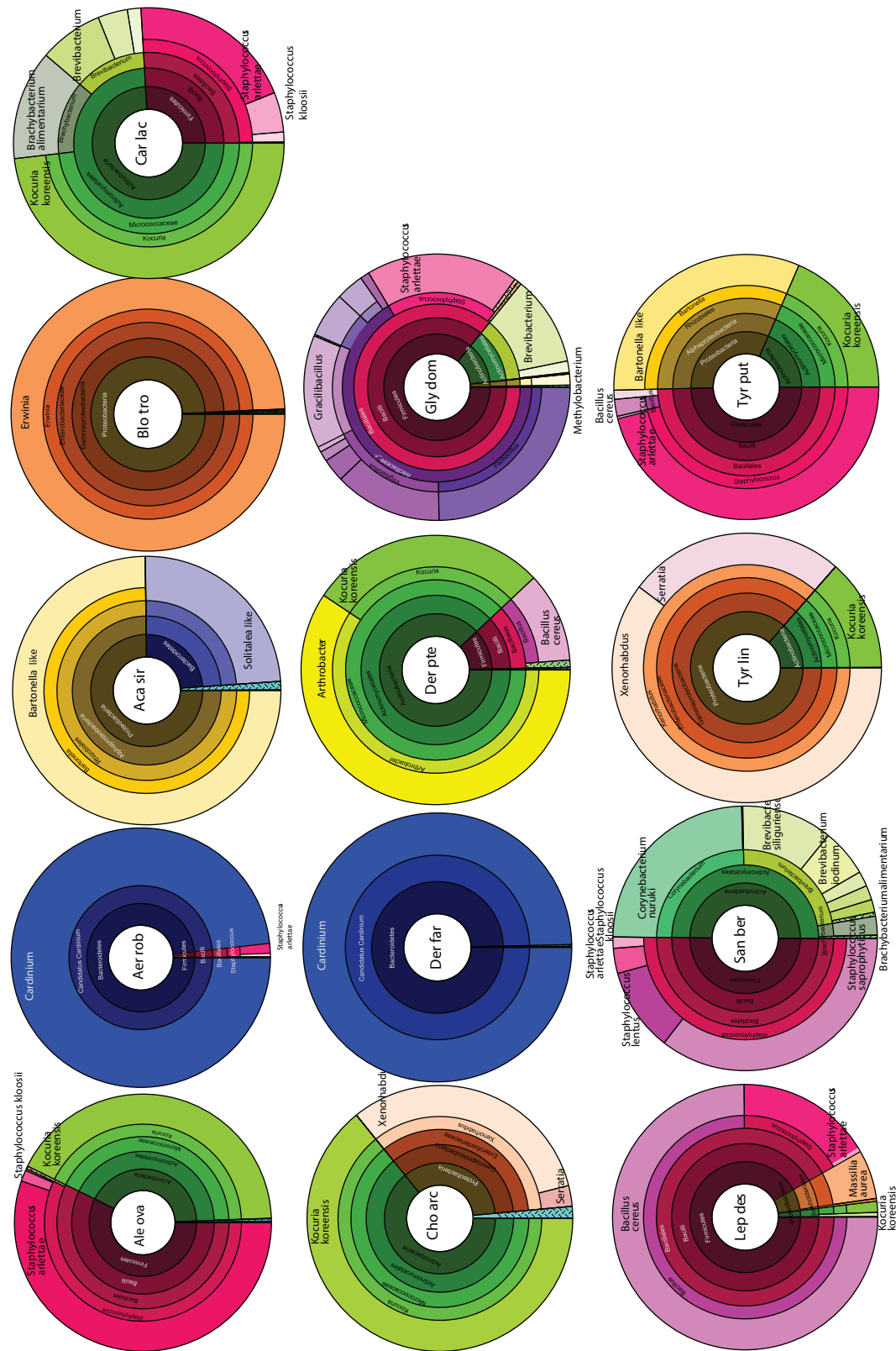


Fig. 2. The Krona visualization of bacteriome in house dust and stored-product mites assessed by 454 pyrosequences using OTUs97. Aca-sir, *Acarus sir*; Aer-ova, *Aeroglyphus robustus*; Ale-ova, *Aleurroglyphus ovatus*; Blo-tro, *Blomia tropicalis*; Car-lac, *Carpoglyphus lactis*; Cho-arc, *Chortoglyphus arcuatus*; Der-far, *Dermatophagoides farinae*; Der-pte, *Dermatophagoides pteronyssinus*; Gly-dom, *Glyciphagus domesticus*; Lep-des, *Lepidoglyphus destructor*; San-ber, *Sancassania berlessei*; Tyr-put, *Tyrophagus putrescentiae*; and Tyr-lin, *Tyroborus lini*.

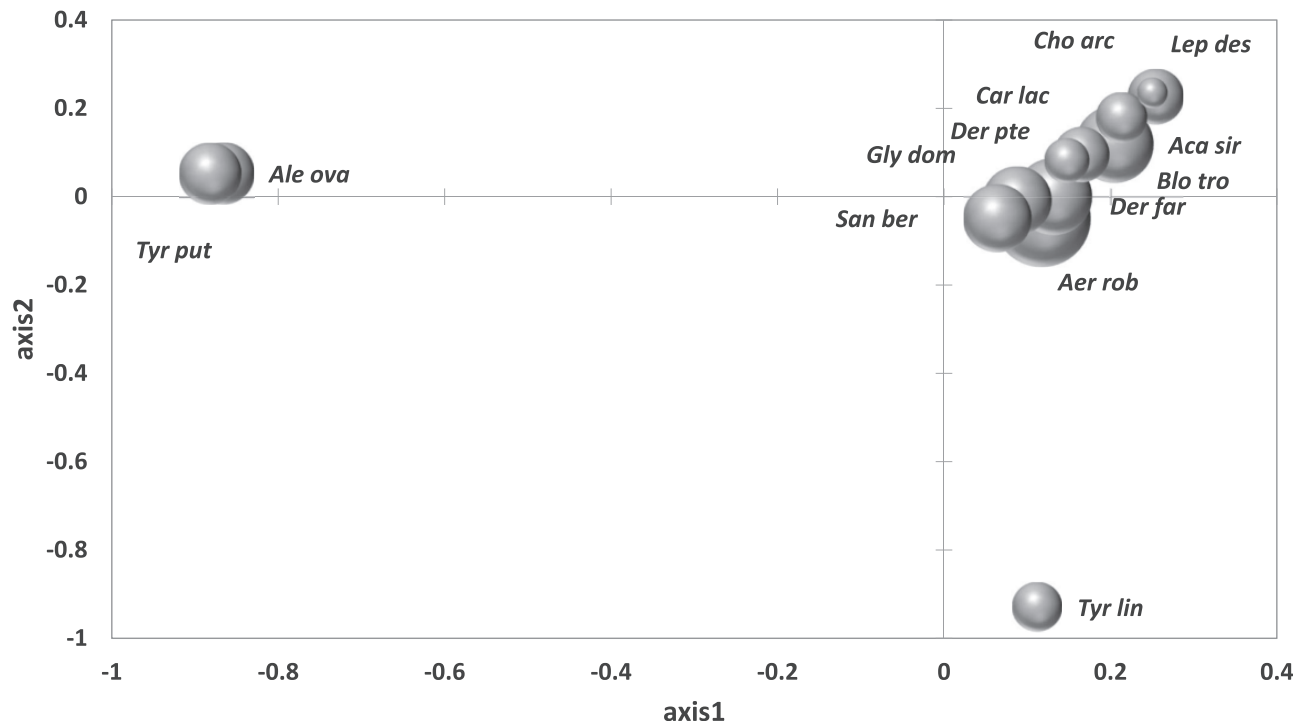


Fig. 3. PCoA of bacteriome in house dust and stored-product mites analyzed in Bray–Curtis dissimilarity matrix, $Rsq = 0.45$; first axes explained 32%, the second 18%, and the third 13% of variability in dataset. The third dimension is represented by the size of the point according to XLSTAT visualization.

Aca-sir, *Acarus siro*; Aer-ova, *Aeroglyphus robustus*; Ale-ova, *Aleuroglyphus ovatus*; Blo-tro, *Blomia tropicalis*; Car-lac, *Carpoglyphus lactis*; Cho-arc, *Chortoglyphus arcuatus*; Der-far, *Dermatophagoides farinae*; Der-pte, *Dermatophagoides pteronyssinus*; Gly-dom, *Glycyphagus domesticus*; Lep-des, *Lepidoglyphus destructor*; San-ber, *Sancassania berlesei*; Tyr-put, *Tyrophagus putrescentiae*; and Tyr-lin, *Tyroborus lini*.

substrates (Erban et al. 2016). This “external-rumen” mode of feeding has been proposed for soil mites and springtails. The microbial enzymes prehydrolyze structural polysaccharides to oligosaccharides, which are then more easily digested by the arthropods (Maraun et al. 2003). The microbial predigestion of mite food increases both the attractiveness and the digestibility of food. The food boli with a mixture of bacteria and the debris of plant cells observed in *C. lactis* are an indication of this type of feeding (Hubert et al. 2015). *Actinobacteria* may also indicate external rumen feeding. For example, *Micrococcus roseus* is a symbiotic organism of termites that produces an extracellular cellulase that participates in the digestion of plant tissues (Sarkar and Upadhyay 1994). High numbers of another related species, *Kocuria marina*, which possesses cellulolytic activity, were found in the gut of *Dendroctonus rhizophagus* (Curculionidae: Scolytinae) (Morales-Jimenez et al. 2012). We propose that during feeding on grain debris, the SPMs prefer partially hydrolyzed plant cells overgrown with actinobacteria, including those that we found in the microbiomes of the mites (i.e., *Kocuria*, *Brevibacterium*, *Corynebacterium*, *Brachybacterium*, and an unclassified *Micrococcaceae*). Hence, these bacteria were most likely ingested with the diets.

The microbes identified as gut symbionts were the *Erwinia*-like bacteria in *B. tropicalis* and *Bartonella*-like bacteria in *A. siro* and *T. putrescentiae*. *Erwinia* was likely not from the diets because *B. tropicalis* shared the diet with other mite species where *Erwinia* were absent. *Erwinia*-related bacteria also inhabit the guts of insects, e.g., the bark beetle (*Ips typographus* L.) (Skrodenyte-Arbaciauskiene et al. 2012), and *Candidatus E. dadicola* affects the mating success of *Bactrocera oleae* (Diptera: Tephritidae) (Estes et al. 2014). In flies, the bacteria contribute essential amino acids

and metabolize urea as an available nitrogen source (Ben-Yosef et al. 2014). To confirm the type of relationship, further study is required to localize *Erwinia* in *B. tropicalis*.

The *Bartonella*-like bacteria may colonize the mesodeum of *A. siro* and are found in the postcolonial diverticula (Sobotnik et al. 2008, Hubert et al. 2012b, Kopecky et al. 2014a). In another study, sequences of high similarity to *Bartonella* were also found in *Dermatophagoides* spp. mites (Valerio et al. 2005). In this study, the species of HDMs lacked *Bartonella*, which was consistent with our previous results (Hubert et al. 2012b, Kopecky et al. 2014a). Whether the occurrence of the *Bartonella*-like bacteria is variable among populations or in time remains an open question.

In this study, a high number of *Bacteroidetes*-related sequences were found in three mite species and low numbers of sequences were found in seven other mite species. *Cardinium*- and *Solitalea*-like bacteria (phylum Bacteroidetes) are among the bacteria that have symbiotic or parasitic relationships with mites (Hubert et al. 2012a). Previously, using PCR with species-specific primers, we found *Cardinium* in all mite species that were tested (Kopecky et al. 2013).

Xenorhabdus and *Photorhabdus* are well-recognized entomopathogenic bacteria (Nielsen-LeRoux et al. 2012); however, it is not known whether these bacteria have acaropathogenic effects on SPMs (Hubert et al. 2012b). Previously, sequences similar to the enteric *Gammaproteobacteria* *Providencia* and *Xenorhabdus* were observed in *D. farinae* and *L. destructor* (Hubert et al. 2012b). In this study, *Xenorhabdus* sequences were observed in *T. lini* and *C. arcuatus*.

Betaproteobacteria formed 6% of the bacterial community in *L. destructor*. The sequences were similar to those of *Massilia*.

These bacteria are found primarily in soils, but *Massilia*-like bacteria are also found in cochineal insects of the genus *Dactylopius* (Hemiptera: Dactylopiidae) (Ramirez-Puebla et al. 2010). Moreover, similar bacteria are also found in laboratory cultures of *C. lactis* (Hubert et al. 2015). Therefore, this genus might represent another group that is symbiotic with mites.

Medical Aspects of Mite–Bacterial Interactions

The differences in the microbiomes of mites may help to explain the results from studies, in which mite extracts differed in their effects on the gene expression and cytokine secretion of human dermal fibroblasts (Rockwood et al. 2013). Laboratory cultures of HDMs reared on a diet similar to the HDMd used in this study (Erban and Hubert (2008)) were used for preparation of a vaccine containing mite proteins (Batard et al. 2006). The microbial proteins from these populations of mites contributed to the total proteins in the vaccines (Finkelman et al. 2006, Trivedi et al. 2003). *Bartonella* and other G^- species are likely to be the sources of the endotoxin found in the mite allergenic extracts (Valerio et al. 2005). The endotoxin (LPS) contents of standardized allergen vaccines are higher in *D. farinae* than in *D. pteronyssinus* (Trivedi et al. 2003). Here we found strong difference in the bacteriome of *D. farinae* containing G^- *Cardinium*, whereas G^+ actinobacteria prevailed in *D. pteronyssinus*.

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