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INVERTEBRATE MICROBIOLOGY

Detection and Identification of Species-Specific Bacteria Associated with Synanthropic Mites

Jan Hubert · Jan Kopecký · M. Alejandra Perotti · Marta Nesvorná · Henk R. Braig · Markéta Ságová-Marečková · Lilia Macovei · Ludek Zurek

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Abstract Internal bacterial communities of synanthropic mites *Acarus siro*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae* (Acari: Astigmata) were analyzed by culturing and culture-independent approaches from specimens obtained from laboratory colonies. Homogenates of surface-sterilized mites were used for cultivation on non-selective agar and DNA extraction. Isolated bacteria were identified by sequencing of the 16S rRNA gene. PCR amplified 16S rRNA genes were analyzed by terminal restriction fragment length polymorphism analysis (T-RFLP) and cloning sequencing. Fluorescence *in situ* hybridization using universal bacterial probes was used for direct bacterial localization. T-RFLP analysis of 16S rRNA gene revealed distinct species-specific bacterial communities. The results were further confirmed by cloning and sequencing (284 clones). *L. destructor* and *D. farinae* showed more diverse communities then *A. siro* and *T. putrescentiae*. In the cultivated part of the community, the mean CFUs from four mite species ranged from 5.2×10^2 to 1.4×10^3 per mite. *D. farinae* had significantly higher CFUs than the other species. Bacteria were located in the digestive and reproductive tract, parenchymatical tissue, and in bacteriocytes. Among the clones, *Bartonella*-like bacteria occurring in *A. siro* and *T. putresecentiae* represented a distinct group related to *Bartonellaceae* and to *Bartonella*-like symbionts of ants. The clones of high similarity to *Xenorhabdus cabanillasii* were found in *L. destructor* and *D. farinae*, and one clone related to *Photorhabdus temperata* in *A. siro*. Members of *Sphingobacteriales* cloned from *D. farinae* and *A. siro* clustered with the sequences of "*Candidatus* Cardinium hertigii" and as a separate novel cluster.

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J. Hubert (✉) · J. Kopecký · M. Nesvorná · M. Ságová-Marečková
Crop Research Institute,
Drnovská 507,
Praha 6-Ruzyně 161 06, Czech Republic
e-mail: hubert@vurv.cz

J. Hubert
Medical Center Prague,
Mezi Vodami 205,
Praha 4 143 00, Czech Republic

M. A. Perotti
School of Biological Sciences, Environmental Biology,
University of Reading,
Whiteknights,
Reading, Berkshire RG6 6AS, UK

H. R. Braig
School of Biological Sciences, Bangor University,
Bangor LL57 2UW Wales, UK

L. Macovei
The Forsyth Institute,
Boston, MA 02142, USA

L. Zurek
Department of Entomology, Kansas State University,
Manhattan, KS 66506, USA

L. Zurek
Department of Diagnostic Medicine and Pathobiology,
Kansas State University,
Manhattan, KS 66506, USA

Introduction

Mites are among the most diverse and successful of all invertebrates. It was estimated that 48,200 species of mites exist [23]. They have exploited an incredible array of habitats, and because of their microscopic size they go largely unnoticed. Many mites live freely in the soil or water, but there is also a large number of species living as parasites on plants or animals. In urban habitats, synanthropic mites (Acari: Astigmata) are the most abundant and frequent pests.

Synanthropic mites represent a serious threat to human health due to the production of human allergens [8] and dissemination of pathogenic fungi [18, 29, 31, 52]. They are also important pests of stored food products, where the direct losses are caused by mite feeding on food or feed including grain, oilseeds, dried fruit, dried meat, and cheeses [33].

Only a few studies focused on bacteria and eukaryotes associated with mites although bacteria seem to be closely associated with their life strategies and feeding [28, 46, 47]. A diverse bacterial community was detected in the sheep scab mite *Psoroptes ovis* [26], while relatively poor (diversity and abundance) bacterial communities were found in *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* [43, 51, 62, 63, 66]. *Tyrophagus putrescentiae* interaction with bacteria was reported in contaminated Iberian ham causing damage of ham [19]. Different bacterial communities were determined in the gut and on surface of *T. putrescentiae* originating from different habitats based on the cultivation methods [53]. Smrž and Čatská [54] suggested that bacteria associated with *T. putrescentiae* including *Serratia*, *Pseudomonas*, *Brevudimonas*, and *Stenotrophomons* spp. were symbiotic and participated in chitin digestion and utilization. In *A. siro*, the transmission microscopy (TEM) observation of the gut showed that the postcolonic diverticula (sometimes called "Malphigian tubules [32]") harbored symbiotic bacteria [55]. In addition, TEM observations of the gut in this species revealed that bacteria in the gut are active and produce exoenzymes ("hydrolyzed halos" around bacteria) [30].

In this study, we focused on four species of medical importance *Acarus siro*, *D. farinae*, *Lepidoglyphus destructor*, and *T. putrescentiae*. *D. farinae* and *T. putrescentiae* inhabit house dust, while *A. siro*, *L. destructor*, and *T. putrescentiae* infested stored products, especially in areas with humid climate [2, 56]. We determined their internal bacterial communities by cultivation approach, terminal restriction fragment length polymorphism analysis (T-RLFP), and cloning/sequencing of the 16S rRNA gene as well as fluorescence *in situ* hybridization (FISH) to show differences among the species of mites.

Material and Methods

Sample Preparation

Four species of synantropic mites *A. siro* L., *T. putrescentiae* (Schrank) (Acari: Acaridiidae), *L. destructor* (Schrank) (Acari: Glycyphagidae), and *D. farinae* Hughes (Acari: Pyroglyphidae) originated from the laboratory cultures maintained at Crop Research Institute, Prague, Czech Republic (details in Erban and Hubert [12]) were used in this study.

The stored-product mite diet (SPMd) used for *A. siro* and *T. putrescentiae* was a mixture of oat flakes, wheat germs, and Pangamin—dried yeast extract (Rapeto, Bezdruzice, Czech Republic) (ratio 10:10:1 wt). The house-dust mite diet (HDMd) used for *L. destructor* and *D. farinae* was composed of dog food (Ontario-pet, Placek, Podebrady, Czech Republic), wheat germ, Aqua-tropic—dried fish food (Lon-Bio, Praha, Czech Republic), Pangamin, and gelatin (SERVA Electrophoresis GmbH, Heidelberg, Germany) (ratio 10:10:3:1 wt). Both diets were powdered and sieved and heated in thermostat (70°C) for 0.5 h to suppress the microbial growth [12]. About 1.5 g of the diet was placed into Iwaki tissue cell chambers (P-Lab, Praha, Czech Republic) and chambers were maintained in Secador desiccators (P-Lab) at 85% relative humidity and 25±3°C in darkness. From each culture, 200 mg of each mite species was transferred to Eppendorf tubes, surface sterilized (Zurek et al. 2000), and homogenized in 100 μl of phosphate buffered saline (PBS) (MP Biomedicals, Aurora, OH, USA) with the Potter–Elvehjem homogenizer (Kavalier Sázava, Czech Republic) and used for bacterial culturing and DNA extraction.

T-RLFP

Total DNA was extracted from mite homogenates using the DNAEASY Tissue kit (Qiagen, Valencia, CA, USA) and cleaned with GENECLEAN® TURBO kit (MP Biomedicals, Solon, OH). The bacterial 16S rRNA gene fragments were PCR amplified with 6-carboxyfluorescein (FAM) labeled forward primer 27f (5′-AGAGTTTGATCMTGGCKCAG [49]) and reverse primer pH (5′-AAGGAGGTGATC CAGCCGCA [5]). PCR protocol consisted of initial denaturation at 94°C at 2 min, followed by 35 cycles of 45 s at 94°C, 45 s at 57°C, 1 min 30 s at 72°C, and a final extension step at 72°C for 5 min. The amplicon was purified and cleaved by *Alu*I. After inactivation of the restriction enzyme and purification with Sigma Spin™ Post-Reaction Clean-Up Columns (Sigma-Aldrich, St. Louis, MO, USA), fragment analysis was performed at Genomac International, s.r.o. (Prague, Czech Republic) with a 96-capillary sequencer (Applied Biosystems, Foster City, CA, USA). T-RFLP analyses were performed in one run for all samples.

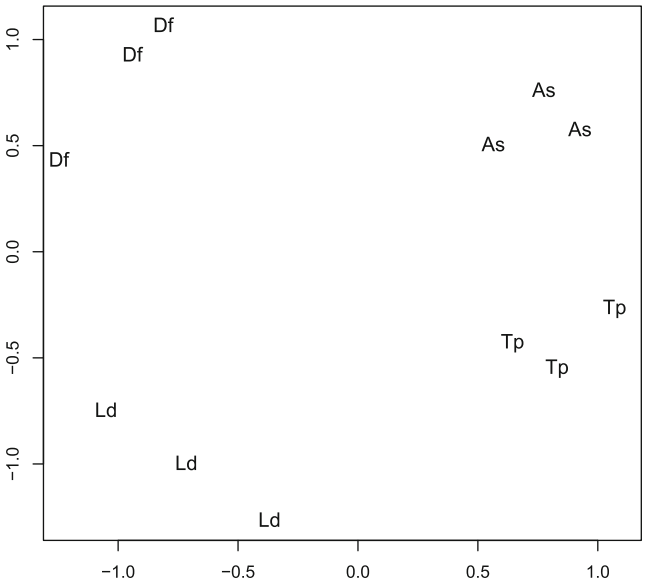


Figure 1 Bacterial communities associated with *Lepidoglyphus destructor* (*Ld*), *Tyrophagus putrescentiae* (*Tp*), *Dermatophagoides farinae* (*Df*), and *Acarus siro* (*As*). T-RFLP profiles of bacterial diversity expressed by Sammon's method of multidimensional scaling. Three replicates were sampled and processed independently

Bacterial Isolation and Identification

The mite homogenates were serially diluted to 10^{-6}, plated on TSB (tryptone soy broth) agar (HiMedia, Mumbai, India), and cultivated for 48 h at 37°C. Ten homogenates per species were used. The CFUs were counted and individual colony morphotypes were identified using PCR amplification and sequencing of the 16S rRNA gene. Specifically, the genomic DNA was prepared by boiling the cells with subsequent centrifugation, and the supernatant was used as a template. The primers and PCR conditions are described below. The CFUs were recalculated per one mite and data were processed by analysis of variance (ANOVA) with Tukey's post hoc comparison using STATISTIX (Analytical Software, Tallahassee, FL, USA). As control, both rearing diets (10 mg) were homogenized, diluted, and spread on the plates by the same protocol as the mite homogenates.

Table 1 The comparison of colony forming units (CFU) of cultivated part of bacterial community among tested species of mites

Mite species	*N*	Bacterial CFU
A. siro	12	637±525 a
D. farinae	10	1395±999 b
L. destructor	10	537±188 a
T. putrescentiae	10	521±360 a

CFU was recalculated per one mite and the means±standard deviations are presented. The letters indicate Tukey's differences

N numbers of replicates

16S rRNA Gene Cloning and Sequencing

PCR amplification of 16S rRNA gene was performed with universal bacterial primers—UF: 5′-AGA GTT TGA TYM TGGC 3′ (position 8–23) and UR: 5′-GYT ACC TTG TTA CGA CTT (position 1496–1514) [3]. The amplification was done for DNA extracted from both mite homogenates and bacterial isolates. Amplification was done on a Peltier Thermal Cycler (MJ Research, Waltham, MA, USA). A total volume of 25 μl PCR reaction mixture contained a final concentration of 200 μM dNTPs, 3 mM $MgCl_2$, forward and reverse primers (100 nM each), 0.5 U *Taq* polymerase (all Promega, Madison, WI, USA), and 300 ng template DNA. The amplification conditions were as follows: 2 min at 94°C followed by 30 cycles of 90 s at 94°C, 90 s at 50°C, and 60 s at 72°C, followed by final extension for 10 min at 72°C and 4°C hold [3]. Resulting PCR products were purified with GFX PCR DNA and Gel Band Purification Kit (Amersham Bioscience, UK). For identification of isolated bacteria, sequences were determined on an ABI 3700 DNA Analyzer at the K-State DNA Sequencing Facility using the same bacterial universal primers and analyzed as described above. For culture-independent approach, the PCR amplicons originating from the mite homogenates were cloned using pGEM®-T Easy Vector (Promega). Selected clones (ca. 70 per one species) were sequenced in the UC-Riverside Core Instrumentation facility from the T6 and SP6 primers on the ABI 3730xl sequencer. Nearly full-length sequences were assembled with CodonCode Aligner, version 1.5.2 (CodonCode Corporation, Dedham, MA, USA) and assigned to bacterial taxonomy using Ribosomal Database Project naïve Bayesian rRNA classifier [64].

Phylogenetic Analysis

An initial alignment of partial 16S rRNA gene sequences was performed using Muscle 3.6 [11]. Phylogeny was inferred by Bayesian analysis using Markov chain Monte Carlo sampler in Phase 2.0 software [34]. Neighbor-joining method was based on the Jukes–Cantor distance matrix (1,000 bootstrap resamplings) in Phylip 3.67 package [16], and maximum-likelihood method with Jukes–Cantor substitution model (1,000 bootstrap resamplings) in Phyml software [22]. The resulting phylograms were finalized using MEGA version 4 [61].

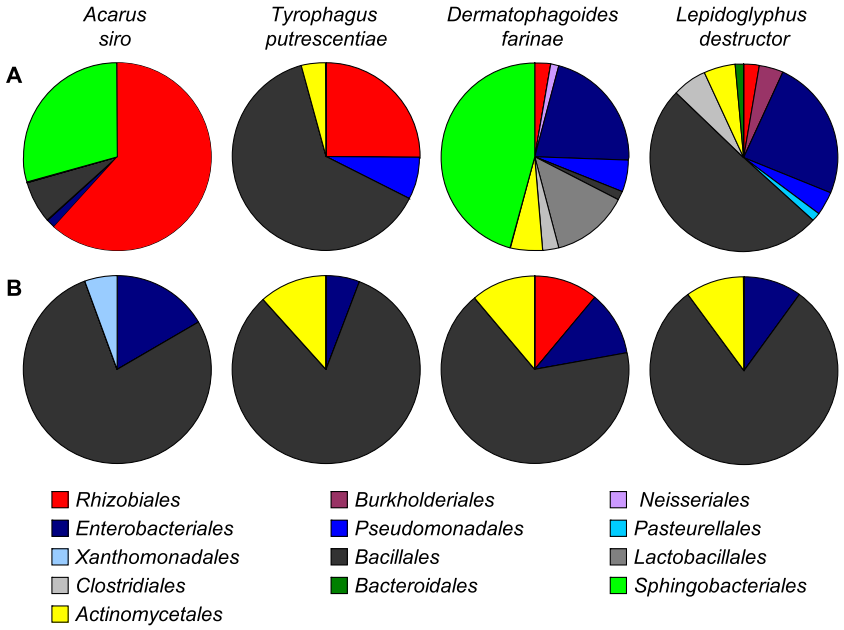


Figure 2 Phylogenetic assignment of bacterial 16S rRNA gene sequences cloned from DNA extracted from mite whole body homogenates (**a**) and amplified from the isolates representing isolated bacterial community members (**b**)

FISH

Fluorescence *in situ* hybridization was performed using universal bacterial probes [45]. Mite specimens were fixed in 4% formaldehyde and preparation of whole-mite specimens for the hybridizations followed the FISH protocol described in Perrotti et al. [45]. For the hybridizations, samples were incubated at 45°C in darkness for a maximum of 20 h, then washed for 1 h with the same hybridization buffer followed by washing in PBTA (phosphate buffer with Triton X-100 plus sodium azide) at room temperature. Samples were mounted in PBS/glycerol and examined under the microscope. The bacterial probes EUB-338 [1] and EUB-338 II and III [9] used in this experiment were equimolar mixed in the hybridization buffer (following remarks of ProBase). No-probe and competition suppression controls using excess unlabeled probes were performed. The hybridized specimens were visualized using Confocal Zeiss LSM510 microscope with Coherent Multiphoton laser. Length measurements were performed by the Zeiss LSM Image Examiner.

Results

T-RFLP

Based on T-RFLP analysis of 16S rRNA gene, distinct bacterial communities were identified among the tested mite species. Specifically, the bacterial communities were separated by the type of diet along the first axis in Sammon's multidimensional scaling (Fig. 1).

Bacterial Isolation

In the isolated bacteria from homogenized and surface-sterilized mites, the mean bacterial CFU numbers ranged from 5.2×10^2 to 1.4×10^3 per mite. Significant differences in CFU numbers were observed among mite species ($F_{(3,38)}$= 4.96, P=0.005); Tukey's comparison showed that *D. farinae* carried significantly higher CFU number than the other mite species (Table 1).

The 16S rRNA genes were sequenced from 54 colonies representing the culturable part of bacterial communities associated with *L. destructor* (10 sequences), *D. farinae* (9), *A. siro* (18), and *T. putrescentiae* (17), and from 30 colonies isolated from uninoculated rearing diets. The sequences were deposited in GenBank under accession nos. JN392772–JN392855 (Supplementary Table S1). There were no remarkable differences among the types of the diet (Table S1).

Cloning

The 16S rRNA genes were cloned and 280 sequences representing the bacterial communities associated with *L. destructor* (67 clones), *D. farinae* (74), *A. siro* (68), and *T. putrescentiae* (71) were deposited in GenBank under accession nos. JN236220–JN236499 (Supplementary Table S1). The phylogenetic assignment of cloned bacteria showed that the communities differed among the species (Fig. 2).

Rhizobiales were represented by 60 cloned sequences of unidentified bacteria related to *Bartonellaceae* (Fig. 3). The sequences showed separated species-specific clusters originating from *A. siro* and *T. putrescentiae*, respectively. In

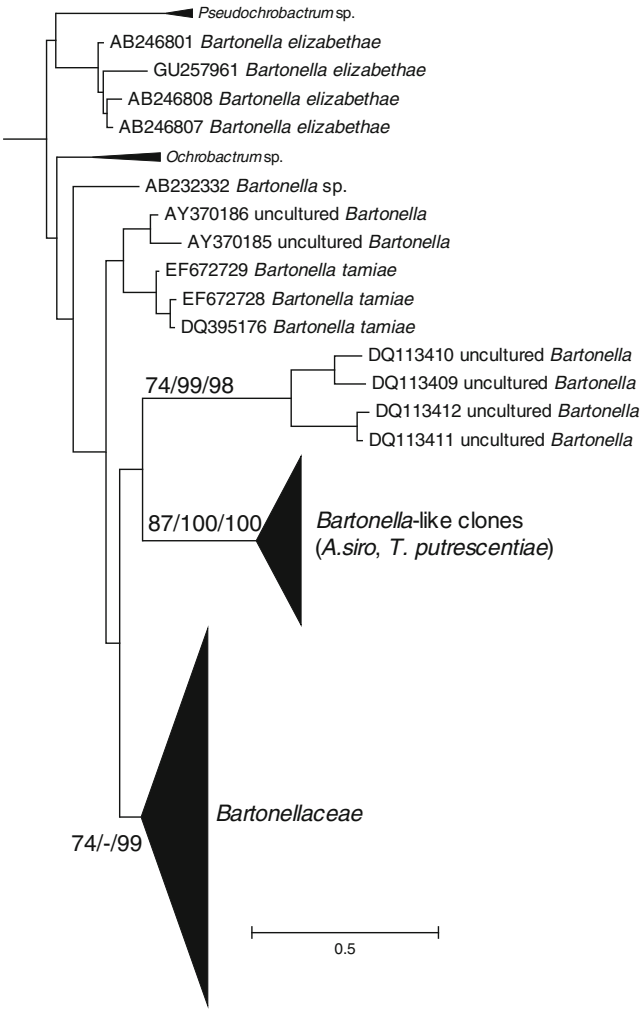


Figure 3 Phylogenetic analysis of clones related to *Bartonella* together with their closest neighbors available in public databases. Phylogram was constructed by Bayesian analysis of 60 cloned sequences of the gene for 16S rRNA gene in this study and 425 full-length sequences from the order *Rhizobiales*. The *figure* shows a subtree including all *Bartonellaceae* and related clusters. Branch lengths correspond to mean posterior estimates of evolutionary distances (*scale bar*, 0.5). Branch labels indicate the Bayesian posterior probability and bootstrap values from maximum-likelihood and neighbor-joining analyses supporting the respective branch

comparison, among the cultured bacteria, one isolate from *D. farinae* was identified as *Rhizobium* sp.

Bacteroidales were presented as one sequence of high similarity to *Prevotella* in the cloned sequences of *L. destructor*. *Sphingobacteriales* found in *D. farinae* and *A. siro* were represented by 54 cloned sequences (Supplementary Table S1). The sequences clustered in two separated species-specific clusters including clones originating from *A. siro* (20 clones) and *D. farinae* (34 clones), respectively (Fig. 4).

Enterobacteriales were found among the clones and isolates of all mite species. The majority of *Enterobacteriales* cloned sequences from *D. farinae* and *L. destructor* belonged to the genera *Providencia* and *Xenorhabdus*. The *Xenorhabdus* sequences from both species, i.e., three sequences from *D. farinae* and 12 sequences from *L. destructor*, clustered together with *Xenorhabdus cabanillasii*. Two sequences from *L. destructor* showed similarity to *Providencia rustigianii*, *Providencia alcalifaciens*, and *Providencia heimbachae*. Other sequences from *D. farinae* and *L. destructor* showed similarity to *Providencia rettgeri*, *Providencia vermicola*, and *Providencia stuartii* (Supplementary Fig. S1).

Bacillales were found among the clones and isolates from all tested mite species. The sequences from both clones and isolates showed high similarities to the genera *Bacillus* or *Staphylococcus*. *Lactobacillales* were found in *D. farinae* only; six sequences belonged to *Enterococcus* and four to *Streptococcus*. The majority of *Bacillus* sp. clones and isolates clustered with *Bacillus cereus*. Clones and isolates related to *Staphylococcus* from all species formed two clusters, the first one with *Staphylococcus kloosii* and the second with *Staphylococcus nepalensis* and *Staphylococcus cohnii* (Supplementary Fig. S2).

The sequences of *Actinomycetales* were found among the clones and isolates from *D. farinae*, *L. destructor*, and *T. putrescentiae*. The sequences of *Kocuria* sp. were found among the clones and isolates from *L. destructor* and *T. putrescentiae* as well as from isolates of *D. farinae*.

FISH

Bacteria were located in the digestive tract (Fig. 5a, b, d), parenchymal tissues (Fig. 5e), reproductive tissues (Fig. 5a, b, d), and bacteriocytes (Fig. 5c). In the digestive tract, the bacteria were localized as ingested food inside food boli. In *A. siro*, the bacteria were observed in postcolonal diverticula (Fig. 5a).

Discussion

Bacterial communities of synanthropic mites are of particular interest since they might contain pathogenic microorganisms and therefore represent the source for allergies and infections in humans [37]. In the present study, several previously described bacterial taxa [43, 63] were confirmed and a number of bacterial species was extended for *D. farinae*, while the bacterial communities were newly described for *A. siro*, *L. destructor*, and *T. putrescentiae*. The bacterial community composition varied between mite species; the communities in *D. farinae* and *L. destructor* were more diverse than those in *A. siro* and *T. putrescentiae*. There is a possibility that dried fish in rearing diet added to the differences in the bacterial community composition. However, no remarkable differences of bacteria associated to the rearing diets (SPMd and HDMd) were found by the cultivation approach.

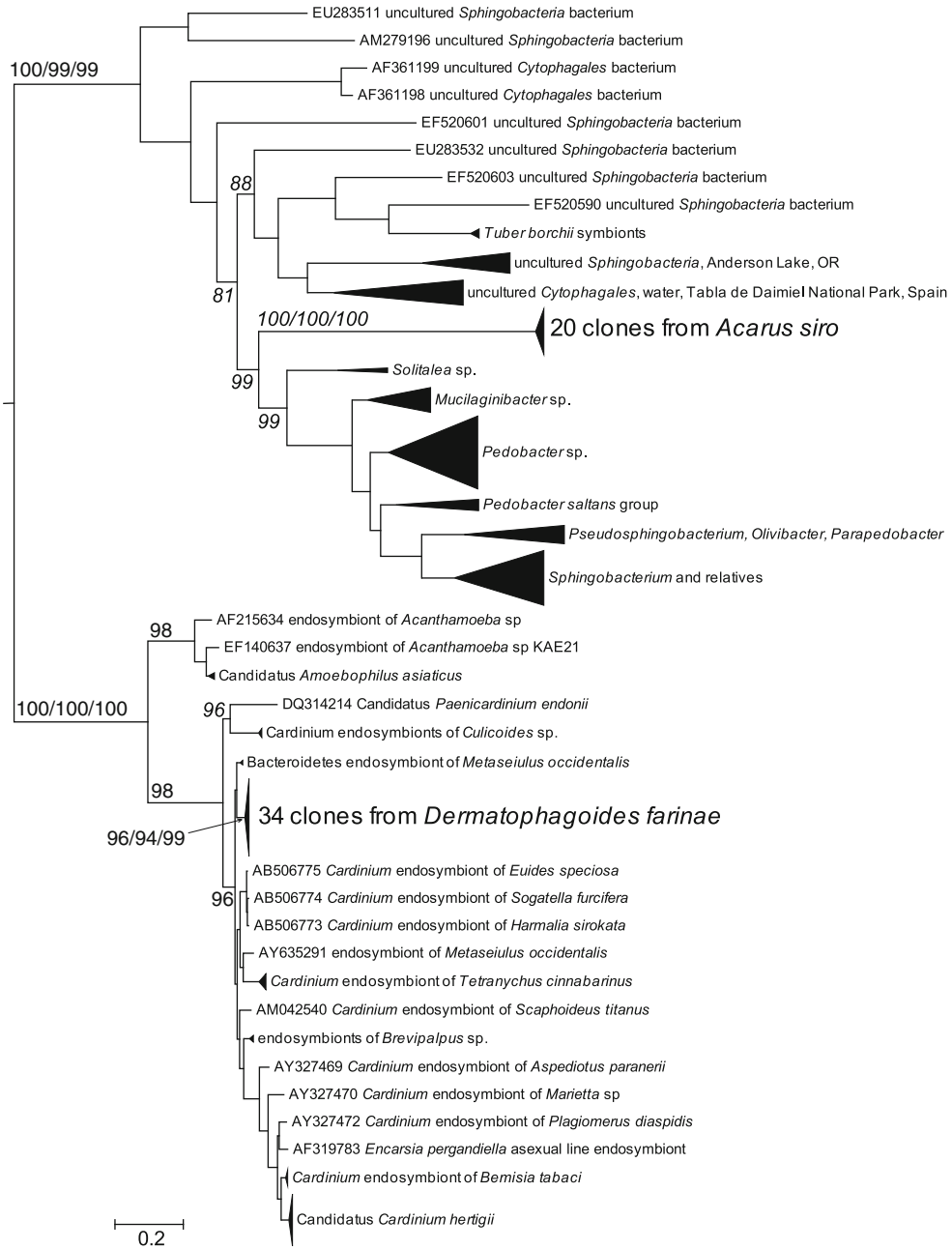


Figure 4 Phylogenetic analysis of clones belonging to *Bacteroidetes* together with their closest neighbors available in public databases. Phylogeny was inferred by Bayesian analysis of 54 cloned sequences of the gene for 16S rRNA in this study work and 175 full-length sequences of their closest relatives from the phylum *Bacteroidetes*. Branch lengths correspond to mean posterior estimates of evolutionary distances (*scale bar*, 0.2). Branch labels indicate the Bayesian posterior probability and for selected branches also supporting bootstrap values from maximum-likelihood and neighbor-joining analyses. The phylograms were outgrouped using the *E. coli* sequence U00096

Among the identified microbes, we commonly detected the genera *Bacillus* and *Staphylococcus*. Those two genera were previously isolated from *T. putrescentiae* naturally occurring in medical laboratory [15, 20]. Some staphylococci sequences clustered with *S. cohnii*, putative human pathogen occurring in the hospital environment, on skin and mucous membranes of the patients [48]. In the bed dust, the occurrence of *Bacillus* and *Staphylococcus* altogether with *Dermatophagoides* spp. mites has been reported [27].

Previously, enzymatic studies showed that mites are equipped with lysozyme that can hydrolyze the β(1→4) glycosidic bonds in peptidoglycan. *Dermatophagoides* spp. whole mites extracts and spent growth media showed hydrolytic activity to members of *Bacillales* and *Micrococcus lysodekticus*, while the lysis of *Escherichia coli*, but also *Lactobacillales* bacteria, was about 15-fold lower [39]. The presence of digestive lysozyme [7, 12, 57, 58] together with digestive cysteine and serine proteases [13, 14, 25, 44, 50] indicated that mites may be able to hydrolyze some bacteria and utilize them as a source of nutrients. Consequently, some of the identified bacterial taxa *Bacillus* and *Staphylococcus* were also found in both mite diets suggesting that they are ingested with food and may be used as a nutritional source. In stored grain habitats, mites feed on carbohydrate-rich

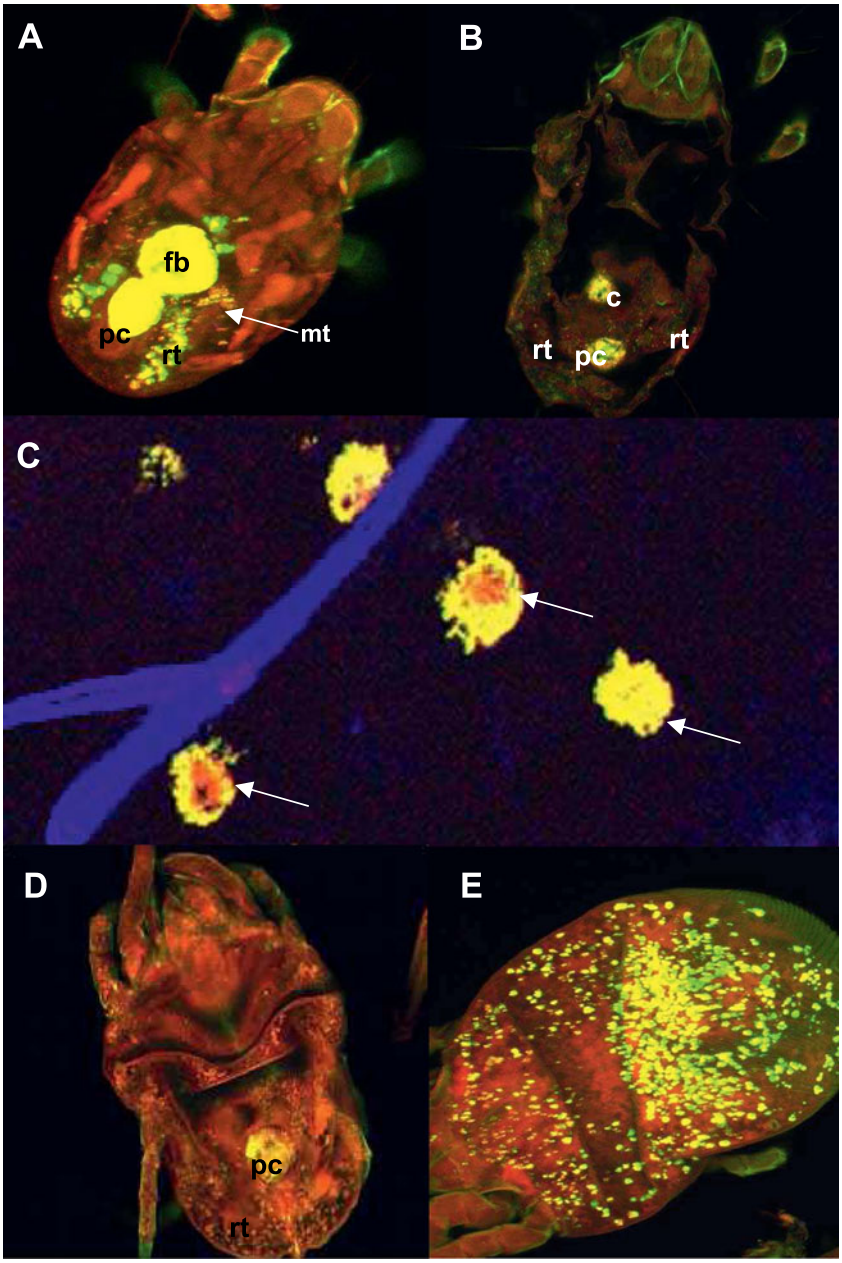


Figure 5 Visualization of associated bacteria to mites based on fluorescent in situ hybridization (Cy5 labeled universal bacterial probes); **a** total view on *Acarus siro*; **b** total view on *Tyrophagus putrescentiae*; **c** detail of bacteriocyte (pointed by *arrows*) in *Acarus siro*; **d** total view on *Dermatophagoides farinae*; **e** *Dermatophagoides farinae*, bacteria associated to parenchymal tissues. Legends: *c* colon (digestive tract), *fb* food bolus, *mt* postcolonal diverticula (digestive tract), *pc* postcolon (digestive tract), *rt* reproductive tract, *s* sensilus

sources and feeding on microorganisms could provide supplemental nitrogen.

The detection of *Enterobacter*, *Escherichia*/*Shigella*, and unclassified *Enterobacteriaceae* in the communities of tested mite species confirmed the occurrence of enteric bacteria in synanthropic mites as reported previously in *Dermatophagoides* spp. [63]. The genera *Cronobacter* (*Enterobacter*) and *Enterococcus* have been reported from the gut of stored product beetles [6, 38, 67] and the gut of the stable fly, *Stomoxys calcitrans* [24]. Some insects were suggested to participate in bacterial contamination of stored products and other substrates including food [6, 38, 40, 41, 67, 69].

We found 16 clones of high similarity to *Xenorhabdus cabanillasii* in *L. destructor* and *D. farinae*, and one clone of high similarity to *Photorhabdus temperata* in *A. siro*. Both *Xenorhabdus* and *Photorhabdus* are Gram-negative proteobacteria that form entomopathogenic symbioses with nematodes [17] and are carried in the digestive tract of nematodes of the families *Steinernematidae* and *Heterorhabditidae*. *X. cabanillasii* was also described in a symbiotic relationship in *Steinerma diaprepesi* [60]. Since *Xenorhabdus* and *Photorhabdus* are known to kill insect by their toxins [10], we hypothesize that they may have a similar pathogenic character in mites.

Bartonella species are Gram-negative bacteria that are human pathogens, and their potential vectors are arthropods [4]. *Bartonella* spp. are common symbiotic bacteria in ticks [35] and recently have been described from *D. farinae* and *D. pteronyssinus* [63] but were not found in *Psoroptes ovis* [26]. Valerio et al. [63] suggested *Bartonella* sp. as a dominant symbiont in house dust mites. The *Bartonella*-like bacteria were detected in *A. siro* and *T. putrescentiae* in this study. Our clones were related to *Bartonellaceae*, but formed a separate cluster close to *Bartonella*-like symbionts of ants of the genus *Tetraponera*. In ants, the *Bartonella*-like symbionts were present in the intestine [59] while in *A. siro*, an abundant bacterial community resided in postcolonal diverticula (this study and Sobotnik et al. [55]). The high incidence (63%) of *Bartonella*-like bacteria among the obtained sequences suggested that postcolonal diverticula was the main colonization site for *Bartonella*-like bacteria in *A. siro*. However, *Bartonella*-like bacteria were also found in *T. putrescentiae*, which is probably lacking postcolonal diverticula [53]. The localization of *Bartonella*-like bacteria in mite body is still open.

Some bacterial species in the phylum *Bacteroidetes* are known as manipulators of arthropod reproduction that includes cytoplasmic incompatibility [31]. Members of the clade "*Candidatus* Cardinium hertigii" were found in various arthropods and nematodes [21], and they are also common in spider mites [42]. Cytophaga-like organisms were observed in the related oribatid mite *Oppiella nova* [65]. The previous study showed the absence of *Cardinium* in *Rhizoglyphus robini* and *T. putrescentiae* [68], while the evidence of symbiotic proteobacteria closely related to endosymbionts from ticks and spider mites was reported in *Dermatophagoides* mites [63]. In this study, the cloned sequences of *Sphingobacteriales* formed two separate clusters. In *D. farinae*, the clones clustered to the sequences of "*Ca*. Cardinium hertigii". In *A. siro*, the cloned sequences of *Sphingobacteriales* formed a separate cluster distinct from "*Ca*. *Cardinium hertigii*". The position of the *Sphingobacteriales* sequences from *A. siro* indicated that they may constitute a new taxon of endosymbiotic bacteria.

Finally, bacteria were present not only in the digestive tract of mites but also in the reproductive tract, parenchymal tissue, and in bacteriocytes in the examined mite species as shown in the results of FISH with the universal eubacterial primers. Similar distribution of bacteria was found in *Brevipalpus* spp. mites in which *Cardinium* cells were detected in every body part including legs and palps using electron microscopy [36]. The presence of bacteria in bacteriocytes is probably novel for mites.

Mite species used in this study belong to pests of human-made habitats. It was documented that they act as vectors transporting fungi of medical importance [18, 24, 31]. In this study, we found that some *Enterobacteriaceae* and *Bacillales* related to pathogenic species (e.g., *S. cohnii*) occur in house-dust and stored-product mites. Consequently, mites can distribute them through human habitats by the same way as they spread fungi [52].

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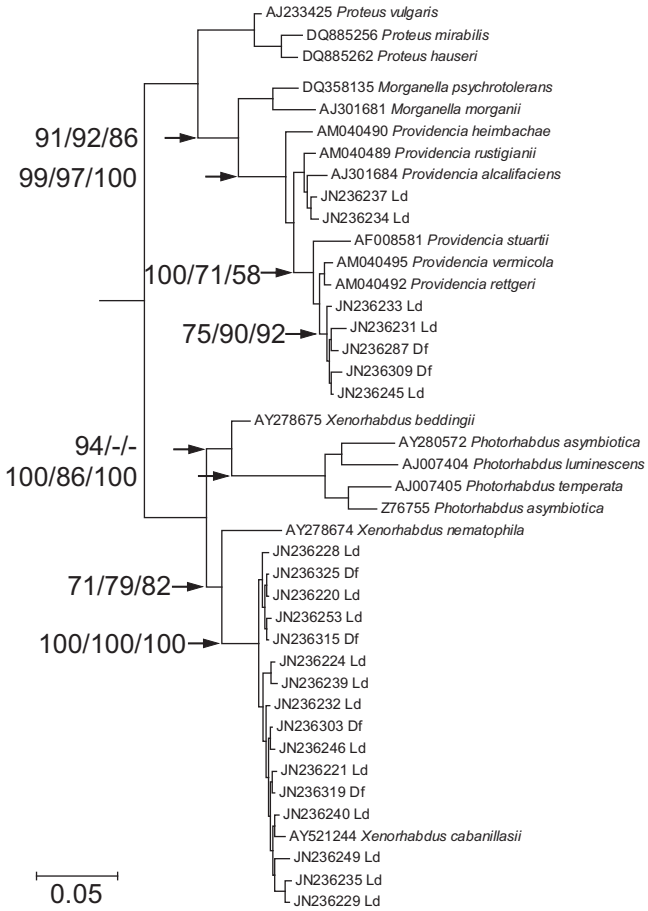
Supplementary Table S1

Taxonomical assessment and the numbers of bacterial clones and isolates obtained from the tested species of mites: *Acarus siro* (As); *Dermatophagoides farinae* (Df); *Lepidoglyphus destructor* (Ld) and *Tyrophagus putrescentiae* (Tp). The sequences obtained from isolates are indicated as isol. The classification reliability is given by confidence threshold (ct) value (RDP Classifier, Wang et al. 2007).

Order	Family	Genus	ct	Diet		As		Df		Ld		Tp	
			%	SPMd	HDMd	cl.	isol.	cl.	isol.	cl.	isol.	cl.	isol.
Actinomycetales	*Micrococcaceae*	*Kocuria*	96-100						1	2	1	3	2
		Micrococcus	100	2	3								
	Propionibacteriaceae	*Propionibacterium*	100					4		1			
Bacteroidales	*Prevotellaceae*	*Prevotella*	100							1			
Sphingobacteriales	*Sphingobacteriaceae*	uncl. *Sphingobacteriaceae*				20							
	uncl. *Sphingobacteriales*							34					
Bacillales	*Bacillaceae*	*Bacillus*	99-100	3	1		8	1	3	33	2	35	7
	Staphylococcaceae	*Staphylococcus*	100	1	1	5	6		3	3	6	10	7
Lactobacillales	*Enterococcaceae*	*Enterococcus*	100					6					
	Streptococcaceae	*Streptococcus*	100					4					
Clostridiales	*Incertae Sedis XI*	*Finegoldia*	100					1		1			
	Veillonellaceae	*Selenomonas*	97-92							2			
	Lachnospiraceae	*Coprococcus*	100					1					
		uncl. *Lachnospiraceae*								1			
Rhizobiales	uncl. *Rhizobiales*					42						18	
	Rhizobiaceae	*Rhizobium*	100						1				
	Methylobacteriaceae	*Methylobacterium*	100					2		2			
Neisseriales	*Neisseriaceae*	*Neisseria*	100					1					
Enterobacteriales	*Enterobacteriaceae*	*Enterobacter*	94-100					6					
		Escherichia/Shigella	100				1	1					
		Providencia	100					2		5			
		Xenorhabdus	100					4		12			
		uncl. *Enterobacteriaceae*		1		1	2	3	1		1		1
		Pantoea	100	5	7								
Pseudomonadales	*Moraxellaceae*	*Enhydrobacter*	100							1			
		Acinetobacter	100	1	3								
	Pseudomonadaceae	*Pseudomonas*	100		2			4		2		5	
Pasteurellales	*Pasteurellaceae*	*Haemophilus*	100							1			
Xanthomonadales	*Xanthomonadaceae*	*Stenotrophomonas*	100				1						
Number of sequences				**13**	**17**	**68**	**18**	**74**	**9**	**67**	**10**	**71**	**17**
Number of taxa				**6**	**6**	**4**	**5**	**15**	**5**	**14**	**4**	**5**	**4**

Supplementary Fig. S1.
Phylogenetic analysis of *Enterobacteriales* clones related to the genera *Xenorhabdus* and *Providencia* with their closest neighbors available in public databases.

Phylogeny was inferred by Bayesian analysis of 16S rRNA gene sequences of 23 clones from *L. destructor* (Ld), and *D. farinae* (Df), and 18 type-strain sequences of the related species.
Branch lengths correspond to mean posterior estimates of evolutionary distances (scale bar, 0.05). For selected branches, the branch labels indicate the Bayesian posterior probability and supporting bootstrap values from maximum-likelihood and neighbor-joining analyses. The phylograms were outgrouped using the *E. coli* sequence U00096.



Supplementary Fig. S2.
Phylogenetic analysis of clones and isolates belonging to *Bacillales* together with their closest neighbors available in public databases.

Phylogeny was inferred by Bayesian analysis of 135 16S rRNA gene sequences of 115 clones from *L. destructor* (Ld), *D. farinae* (Df), *A. siro* (As), and *T. putrescentiae* (Tp), 20 cultured isolates (marked as isol.) from mite whole body homogenates or mite diets HDMd and SPMd, and 16 selected type-strain sequences from the genera *Bacillus* and *Staphylococcus*.
Branch lengths correspond to mean posterior estimates of evolutionary distances (scale bar, 0.5). Branch labels indicate the Bayesian posterior probability and for selected branches also supporting bootstrap values from maximum-likelihood and neighbor-joining analyses. The phylograms were outgrouped using the *E. coli* sequence U00096.

