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Differential allergen expression in three *Tyrophagus putrescentiae* strains inhabited by distinct microbiome

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SHORT SUMMARY

The domestic mite, *Tyrophagus putrescentiae,* is a medically important, ubiquitous pest that commonly occurs in stored food and house dust, causing allergies. We analyzed the transcriptomes of three strains of *T. putrescentiae*, each harboring a different microbiome. These mite strains were analyzed at a level representative of a natural population using seven biological replicates. We identified 78 transcripts belonging to 8 allergen groups known in *T. putrescentiae* (2, 3, 10, 13, 28, 34, 35 and 36) and identified 16 allergen candidates previously unknown for this mite species (4, 5, 6, 7, 8, 11, 14, 16, 20, 24, 25, 27, 30, 31, 33 and 38). We documented polymorphism and differences in the expression level of these transcripts. Most importantly, the expression of several allergens (group 4, 7, 10, 11, 13, 20 and 36) correlated with the presence of common intracellular bacterial symbionts, *Wolbachia* and *Blattabacterium*-like bacteria. The largest differences in allergen expression among the strains were found in known allergens Tyr p 2, 10, and 13 and putative allergens Tyr p 4 and 14. We suggest that allergen production in *T. putrescentiae* may be affected by its microbiome composition.

Key words

Blattabacterium, *Wolbachia*, *Bartonella*, intracellular symbionts, stored product mite, house dust mite

To the Editor,

Allergen production by house dust mites is influenced directly by antibacterial compounds and digestive enzymes[1] or indirectly *via* interactions with nutrients linked to associated microorganisms.[1] Importantly, heat-stable lipopolysaccharides (endotoxins) from associated Gram-negative bacteria can affect the efficacy of allergen immunotherapy sera produced from house dust mites.[1,2] House dust mites serve as carriers of bacteria and it is possible that these bacteria are responsible for the induction of IgE sensitization to microbial antigens.[3] For this reason, the microbiomes of allergen-producing mites have recently become the subject of intensive research.[4] Unfortunately, the existence of variability in microbiome composition among different mite populations or strains is insufficiently considered (e.g., Erban et al.,[5] Lee et al.[6]), despite the evidence that different strains of mites may harbor different and persistent microbial communities. In *Tyrophagus putrescentiae*, for example, these communities include the following bacteria: *Wolbachia*, *Cardinium*, *Solitalea*, *Blattabacterium*-like (intracellular), *Bartonella*-like, and *Bacillus* sp. (gut-associated).[5] These observations open the question of whether mite strains harboring different bacterial communities differ in allergen production.

Until now, only eight allergens, Tyr p 2, 3, 10, 13, 28, 34, 35 and 36, have been described in *T. putrescentiae* (IUIS/WHO allergen database). In this study, we investigated the expression of these allergens (and additional putative allergens predicted by us *in silico*) in three different strains of *T. putrescentiae* with known microbiomes.[5] We used V1-V3 16S RNA sequence data from the three strains of mites.[5] The transcriptomes were obtained by Illumina HiSeq 2500, and the raw reads were deposited in the SRA database (SUB4527480) (Tab. S1). The reads were processed and assembled in CLC Genomic Workbench v. 11 (Qiagen, Venlo, Netherlands) (Tab. S2-S4); the resulting assembly was then annotated by Prokka.[7] The identified cDNA and protein sequences were compared to sequences of known mite allergens using BLAST, and the sequences with the lowest E values were compared

manually to known and predicted allergen protein sequences (see Suppl. Material and methods).

We obtained 21 transcriptomes (seven biological replicates per every strain) and found 78 transcripts belonging to the eight allergen known allergens of *T. putrescentiae* (see Suppl. Results); we also found 16 putative allergens based on sequence similarity with other mite species (groups 4-8, 11, 14, 16, 20, 24, 25, 27, 30, 31, 33, and 38) (Table 1).[8] Our BLAST search showed high similarity of our transcriptomes to some contigs of the genomic assembly of the fly *Rhagoletis zephyria* from GenBank (GCF_001687245.1). Several independent GenBank sequences of *T. putrescentiae* allergens also showed high identities (99-100%) to the '*R. zephyria*' genome. These data suggest that the GenBank assembly of *R. zephyria* contains a significant portion of *T. putrescentiae* DNA (nearly 100 Mb), most likely due to inadvertent laboratory contamination. Such contamination is not suprising because *T. putrescentiae* commonly infects insect and fungal cultures in the laboratory. Nevertheless, the chimeric mite-fly GenBank '*R. zephyria*' genome facilitated our search for mite proteins (Table 1). We also detected polymorphisms in several allergens (Table 1). Coding sequences of some allergens were found in different contigs, e.g., 2, 3, 5, 6, 7, 8, 13, 27, 28, 35 and 36 (Table 1).

In this study, we employ an approach to continue on a recent description of the allergen-producing mite microbiome.[6] Lee et al.[6] demonstrated that the microbiome profiles of *Dermatophagoides farinae*, *D. pteronyssinus* and *T. putrescentiae* change after antibiotic treatment and bacteria that influence the endotoxin concentration.[6] In this study, we set out to show that bacteria not only affect the endotoxin concentration but also their presence is correlated with the mite allergen expression level. The authors did not consider the intraspecies variability of the microbiome. For example, *Solitalea*-like (Sphingobacteriaceae_JN236497) and *Bartonella*-like bacteria (*Bartonella*_JX001274) form the microbiome of the Korean strain of *T. putrescentiae*.[6] We previously observed that strains of *T. putrescentiae* are inhabited by unique microbial communities formed by intracellular (*Wolbachia*, *Cardinium*), putative intracellular (*Solitalea*, *Blattabacterium*-like bacteria), and gut-associated symbionts (*Bartonella*-like bacteria, *Bacillus* sp.).[5] All of these strains have unique and stable microbiome compositions with different proportions of the

abovementioned bacterial taxa, suggesting a question of whether the allergen production of strains with different symbionts is similar or different.

Expression analyses indicated significant differences in transcript abundances among the three tested mite strains ($ANOSIM_{perm.=1000}$; R=0.9518, P<0.001). Among all the mite strains, the differences in allergen transcript expression levels were strong (Figure 1), as shown by nonmetric multidimensional scaling (NMDS) (Table 1). The profiles of both intracellular symbionts (*Wolbachia* and *Blattabacterium*-like) significantly contributed (P<0.001) to the explained variability of transcript expression after forward variable selection (Figure 1A).

In the two mite strains with intracellular bacterial symbionts (Phillips and Dog), many immunogenic proteins showed increased expression levels (Figure 1B), as shown by NMDS, where these groups were separated from the group lacking these bacteria along the x-axis: alpha-amylase (group 4), chymotrypsin (group 6), bactericidal permeability-increasing like protein (group 7), tropomyosin (Tyr p 10), paramyosin (group 11), fatty acid binding protein (Tyr p 13), arginine kinase (group 20), and an unknown group 36 allergen. In contrast, the Koppert population (no intracellular bacteria) had higher expression of allergen group 5, heat shock protein (Tyr p 28), ferritin (group 30) and aldehyde dehydrogenase (Tyr p 35) than the mite populations having intracellular bacteria, Phillips and Dog (Figure 1). The Koppert population is connected to highly abundant *Bacillus cereus*, a bacterium previously shown to be associated with the feces of *T. putrescentiae*.[9] The y-axis of our NMDS analysis separates the two latter populations: the Dog population is characterized by higher expression of bactericidal permeability-increasing like protein (group 7), tropomyosin (Tyr p 10), paramyosin (group 11), and arginine kinase (group 20), while the relative expression of alpha-amylase (group 4), chymotrypsin (group 6), bactericidal permeability-increasing like protein (group 7) and fatty acid binding protein (Tyr p 13) is higher in the Phillips population. The increased expression of muscle proteins/allergens (tropomyosin and paramyosin) and digestive enzymes (amylase and chymotrypsin) suggests a correlation with increased mite population growth. The growing mite cultures produce more juveniles that can contain higher muscle proportion in their bodies than adults due to reduced reproductive organs.

Previous experiments have demonstrated that the allergen expression in *D. pteronyssinus* is quantitatively and/or qualitatively influenced by mite development, sex and environment.[10] Here, we demonstrate that differences among mite strains are correlated

with the presence and absence of intracellular symbionts in individuals taken from the same population. This sampling technique is used by the majority of mite allergen studies.

In conclusion, our results indicate that allergen expression is variable across different mite populations, and this phenomenon can be linked to differences in their microbiome compositions. It is possible that due to symbiotic microbes, the mite population in the natural conditions produces different levels of allergens, although belong to the same species of mites. However, establishing whether this relationship is causative or correlational requires further experiments. Mite microbiome composition appears to be an important factor that should be considered in allergen production.

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TABLE 1 Expression of confirmed and putative allergens in three different strains of *Tyrophagus putrescentiae*. The numbers are means and standard errors in percent. The statistical significance of differences in expression levels across the mite strains was estimated by METASTATS using 100,000 permutations and P-values for pairwise comparison after Bonferroni correction are shown. The significant differences are indicated by bold. Sequences marked with gray shading contributed 75% of the variability between the mite strains (SIMPER test).

		Transcriptome			HMMER	Koppert		Phillips		Dog		Kop./Dog	Kop./Phil.	Phil./Dog
Gr.	Allergen name	Sequence	Contigs	Region	Identifier	mean	stderr	mean	stderr	mean	stderr	p-value	p-value	p-value
gr2	NPC2 family[#1,2,3]	K_15226	contig_4836	(44..376)	E1_DerP2_DerF2	0.0018	0.0004	0.0282	0.0053	0.0118	0.0005	**0.000**	**0.001**	**0.010**
		K_23671	contig_7944	(801..1241)	E1_DerP2_DerF2	0.0039	0.0007	0.0079	0.0006	0.0044	0.0003	0.484	**0.001**	**0.000**
gr3	Trypsin[#1,3]	K_06535	contig_1898	(1254..1919)	Trypsin	0.0124	0.0009	0.0111	0.0006	0.0117	0.0006	0.502	0.251	0.495
		K_02086	contig_555	(1861..2442)	Trypsin	0.0007	0.0001	0.0001	0.0000	0.0001	0.0000	**0.000**	**0.000**	**0.200**
		K_61455	contig_27351	(130..1011)	Trypsin	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	**0.001**	**0.037**	**0.002**
		K_51335	contig_20689	(221..835)	Trypsin	0.0002	0.0000	0.0003	0.0000	0.0001	0.0000	0.083	0.064	**0.002**
		K_04018	contig_1132	(1215..2051)	Trypsin	0.0033	0.0004	0.0078	0.0006	0.0087	0.0006	**0.000**	**0.000**	0.271
		K_08900	contig_2652	(847..1731)	Trypsin	0.0035	0.0004	0.0018	0.0002	0.0009	0.0001	**0.000**	**0.004**	**0.003**
		K_01128	contig_292	(1012..1611)	Trypsin	0.0077	0.0007	0.0045	0.0003	0.0010	0.0001	**0.000**	**0.002**	**0.000**
		K_11751	contig_3628	(537..1346)	Trypsin	0.0199	0.0010	0.0193	0.0017	0.0131	0.0004	**0.000**	0.748	**0.005**
		K_03323	contig_920	(6..1211)	nd.	0.0050	0.0007	0.0026	0.0002	0.0031	0.0001	**0.023**	**0.007**	**0.022**
		K_08886	contig_2647	(357..1211)	Trypsin	0.0013	0.0002	0.0015	0.0001	0.0008	0.0000	**0.008**	0.339	**0.000**
gr4	alpha-Amylase[#2,3]	K_08568	contig_2537	(72..1628)	Alpha-amylase	0.0227	0.0028	0.1096	0.0067	0.0992	0.0054	**0.000**	**0.000**	0.246
gr5	unknown[#2,3]	K_12374	contig_3834	(649..1056)	Blo-t-5	0.0600	0.0066	0.0455	0.0041	0.0222	0.0003	**0.000**	0.083	**0.000**
		K_11162	contig_3408	(509..895)	Blo-t-5	0.0108	0.0007	0.0088	0.0007	0.0053	0.0002	**0.000**	0.067	**0.000**
gr6	Chymotrypsin[#3]	K_02424	contig_654	(959..1786)	Trypsin	0.0167	0.0011	0.0205	0.0020	0.0151	0.0008	0.269	0.118	**0.027**
		K_22273	contig_7404	(2029..2520)	Glyco_hydro_20	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	**0.013**	0.060	0.151
		K_36850	contig_13356	(412..1347)	Trypsin	0.0001	0.0000	0.0001	0.0000	0.0001	0.0000	0.095	0.105	0.642
		K_35901	contig_12940	(10..912)	Trypsin	0.0005	0.0001	0.0005	0.0000	0.0004	0.0000	0.276	0.746	0.183
		K_38741	contig_14263	(653..1441)	Trypsin	0.0007	0.0001	0.0004	0.0000	0.0003	0.0000	**0.013**	**0.045**	0.169
gr7	Bactericidal	K_26316	contig_8979	(202..861)	nd.	0.0039	0.0003	0.0019	0.0001	0.0013	0.0001	**0.000**	**0.000**	**0.002**

	permeability-increasing like protein[#2,3]	K_00425	contig_109	(152..799)	Grp7_allergen	0.0316	0.0036	0.0240	0.0021	0.0450	0.0024	**0.011**	0.090	**0.000**
		K_03162	contig_881	(87..899)	Grp7_allergen	0.0006	0.0001	0.0013	0.0002	0.0052	0.0004	**0.000**	**0.005**	**0.000**
		K_09582	contig_2882	(369..1007)	Grp7_allergen	0.0218	0.0031	0.0465	0.0014	0.0338	0.0011	**0.006**	**0.000**	**0.000**

TABLE 1 continuation.

		Transcriptome			HMMER	Koppert		Phillips		Dog		Kop./Dog	Kop./Phil.	Phil./Dog
Gr.	**Allergen name**	**Sequence**	**Contigs**	**Region**	**Identifier**	**mean**	**stderr**	**mean**	**stderr**	**mean**	**stderr**	**p-value**	**p-value**	**p-value**
gr8	Glutathione S-transferase[#3]	K_10715	contig_3267	(46..738)	GST_C_3	0.0046	0.0002	0.0033	0.0002	0.0018	0.0001	**0.000**	**0.001**	**0.000**
		K_49191	contig_19504	(2291..2989)	GST_C_3	0.0005	0.0001	0.0003	0.0000	0.0001	0.0000	**0.004**	0.087	**0.006**
		K_03001	contig_838	(1454..1765)	GST_C_3	0.0004	0.0000	0.0008	0.0001	0.0003	0.0000	0.190	**0.001**	**0.000**
		K_14921	contig_4711	(1141..1797)	GST_C_3	0.0216	0.0010	0.0226	0.0009	0.0115	0.0004	**0.000**	0.476	**0.000**
		K_15634	contig_4972	(937..1608)	GST_N	0.0054	0.0003	0.0063	0.0006	0.0045	0.0002	**0.018**	0.170	**0.011**
		K_41934	contig_15817	(2077..2487)	GST_C_3	0.0009	0.0001	0.0010	0.0001	0.0005	0.0000	**0.002**	0.300	**0.000**
gr10	Tropomyosin[#1,2,3]	K_04302	contig_1207	(390..1244)	Tropomyosin	0.0235	0.0007	0.0509	0.0025	0.0680	0.0014	**0.000**	**0.000**	**0.000**
gr11	Paramyosin[#2.3]	K_04929	contig_1384	(206..2944)	Myosin_tail_1	0.0466	0.0019	0.0793	0.0061	0.1422	0.0049	**0.000**	**0.000**	**0.000**
gr13	Fatty-acid binding protein[#1,2,3]	K_01655	contig_449	(624..1316)	Lipocalin	0.1154	0.0068	0.1128	0.0063	0.1133	0.0022	0.781	0.783	0.938
		K_11403	contig_3502	(722..1117)	Lipocalin	0.0047	0.0004	0.0119	0.0014	0.0147	0.0007	**0.000**	**0.001**	0.097
		K_20937	contig_6885	(3..302)	fn3	0.0001	0.0000	0.0002	0.0000	0.0002	0.0000	0.187	0.110	0.536
		K_37766	contig_13786	(1487..1660)	nd.	0.0001	0.0000	0.0001	0.0000	0.0005	0.0000	**0.000**	0.356	**0.000**
		K_00427	contig_109	(2063..2728)	Lipocalin	0.0119	0.0016	0.0200	0.0009	0.0148	0.0008	0.123	**0.001**	**0.001**
		K_21264	contig_6995	(3604..4167)	Lipocalin	0.0012	0.0001	0.0006	0.0000	0.0009	0.0001	0.067	**0.002**	**0.012**

		K_11177	contig_3412	(5869..6336)	Lipocalin	0.0105	0.0008	0.0048	0.0002	0.0040	0.0002	**0.000**	**0.000**	**0.024**
		K_11356	contig_3483	(3..302)	Lipocalin	0.0002	0.0000	0.0001	0.0000	0.0001	0.0000	**0.000**	**0.021**	**0.000**
gr16	Gelsolin/villin	K_04861	contig_1366	(10167..10985)	Gelsolin	0.0060	0.0004	0.0051	0.0002	0.0042	0.0002	**0.002**	0.076	**0.004**
gr20	Arginine kinase[#3]	K_05860	contig_1679	(199..1269)	ATP-gua_Ptrans	0.0374	0.0015	0.0568	0.0019	0.0723	0.0007	**0.000**	**0.000**	**0.000**
gr24	Ubiquinol-cytochrome c reductase binding protein	K_05869	contig_1683	(1453..1809)	UCR_14kD	0.0067	0.0002	0.0047	0.0002	0.0046	0.0002	**0.000**	**0.000**	0.750
gr25	Triosphosphate isomerase[#3]	K_04799	contig_1354	(1295..2038)	TIM	0.0177	0.0010	0.0155	0.0007	0.0137	0.0004	**0.004**	0.099	**0.041**

TABLE 1 continuation.

Gr.	Allergen name	Transcriptome			HMMER	Koppert		Phillips		Dog		Kop./Dog	Kop./Phil.	Phil./Dog
		Sequence	Contigs	Region	Identifier	mean	stderr	mean	stderr	mean	stderr	p-value	p-value	p-value
gr27	Serpin[#3]	K_07442	contig_2180	(922..2037)	Serpin	0.0014	0.0002	0.0013	0.0001	0.0004	0.0000	**0.002**	0.592	**0.000**
		K_08004	contig_2364	(109..972)	Serpin	0.0035	0.0002	0.0008	0.0001	0.0007	0.0001	**0.000**	**0.000**	0.553
		K_15320	contig_4863	(16..1209)	Serpin	0.0007	0.0001	0.0011	0.0001	0.0008	0.0001	0.447	**0.010**	**0.012**
		K_17811	contig_5752	(182..1435)	Serpin	0.0087	0.0006	0.0033	0.0003	0.0032	0.0002	**0.000**	**0.000**	0.823

		K_36167	contig_13049	(707..1570)	Serpin	0.0024	0.0003	0.0060	0.0004	0.0043	0.0003	**0.001**	**0.000**	**0.003**
		K_01124	contig_290	(4967..6148)	Serpin	0.0092	0.0005	0.0052	0.0003	0.0049	0.0002	**0.000**	**0.000**	0.546
		K_03687	contig_1032	(464..1738)	Serpin	0.0199	0.0019	0.0128	0.0018	0.0076	0.0004	**0.000**	**0.019**	**0.017**
		K_05988	contig_1725	(2519..3862)	Serpin	0.0042	0.0002	0.0017	0.0001	0.0016	0.0001	**0.000**	**0.000**	0.490
		K_38933	contig_14360	(655..1860)	Serpin	0.0011	0.0002	0.0009	0.0001	0.0007	0.0001	0.070	0.356	0.149
		K_49318	contig_19580	(305..1471)	Serpin	0.0002	0.0001	0.0027	0.0002	0.0018	0.0006	**0.017**	**0.000**	0.156
		K_55291	contig_23036	(1046..2068)	Serpin	0.0001	0.0000	0.0001	0.0000	0.0001	0.0000	0.830	0.485	0.239
gr28	Heat Shock	K_03237	contig_900	(102..1025)	HSP70	0.0035	0.0005	0.0011	0.0001	0.0020	0.0001	**0.011**	**0.000**	**0.000**
	Protein[#1,3]	K_09808	contig_2962	(158..1363)	HSP70	0.0303	0.0031	0.0057	0.0004	0.0090	0.0007	**0.000**	**0.000**	**0.001**
		K_10835	contig_3314	(2320..3324)	HSP70	0.0714	0.0044	0.0235	0.0015	0.0425	0.0032	**0.000**	**0.000**	**0.000**
		K_13947	contig_4387	(1051..1548)	HSP70	0.0217	0.0011	0.0068	0.0005	0.0095	0.0007	**0.000**	**0.000**	**0.008**
		K_25236	contig_8554	(190..1659)	HSP70	0.0037	0.0004	0.0030	0.0002	0.0029	0.0001	**0.043**	0.128	0.570
		K_31610	contig_11117	(258..2126)	HSP70	0.0003	0.0001	0.0005	0.0000	0.0003	0.0000	0.993	0.208	0.059
		K_33180	contig_11776	(273..2258)	HSP70	0.0003	0.0001	0.0002	0.0000	0.0002	0.0000	0.344	0.555	0.436
gr30	Ferritin[#3]	K_02597	contig_710	(2655..3182)	Ferritin	0.0655	0.0026	0.0248	0.0018	0.0163	0.0007	**0.000**	**0.000**	**0.001**
gr31	Cofilin[#3]	K_06133	contig_1780	(42..488)	Cofilin_ADF	0.0143	0.0003	0.0082	0.0002	0.0070	0.0002	**0.000**	**0.000**	**0.002**
gr33	alpha-Tubulin[#2,3]	K_06195	contig_1796	(228..1199)	Tubulin_C	0.0234	0.0021	0.0108	0.0002	0.0148	0.0007	**0.003**	**0.000**	**0.000**
gr33		K_13397	contig_4189	(1072..2208)	Tubulin_C	0.0035	0.0002	0.0023	0.0001	0.0028	0.0001	**0.021**	**0.001**	**0.002**
gr34	Troponin C[#1]	K_22454	contig_7471	(218..679)	FAM91_C	0.0062	0.0003	0.0112	0.0007	0.0122	0.0002	**0.000**	**0.000**	0.224

TABLE 1 continuation.

Gr.	Allergen name	Transcriptome			HMMER	Koppert		Phillips		Dog		Kop./Dog	Kop./Phil.	Phil./Dog
		Sequence	Contigs	Region	Identifier	mean	stderr	mean	stderr	mean	stderr	p-value	p-value	p-value
gr35	Aldehyde	K_11771	contig_3635	(907..2040)	Aldedh	0.0591	0.0062	0.0375	0.0021	0.0214	0.0008	**0.000**	**0.007**	**0.000**

	dehydrogenase[#1,3]	K_16087	contig_5142	(44..1084)	Aldedh	0.0389	0.0035	0.0289	0.0013	0.0223	0.0009	**0.001**	**0.021**	**0.001**
gr36	Profilin[#1,4]	K_00095	contig_29	(3952..4347)	Profilin	0.0180	0.0013	0.0091	0.0003	0.0070	0.0003	**0.000**	**0.000**	**0.000**
	unknown[#4]	K_07970	contig_2348	(1974..2471)	nd.	0.0011	0.0003	0.0044	0.0004	0.0003	0.0000	**0.022**	**0.000**	**0.000**
		K_12999	contig_4063	(6000..6710)	nd.	0.0034	0.0003	0.0032	0.0001	0.0018	0.0001	**0.001**	0.401	**0.000**
		K_15940	contig_5084	(5..712)	C2	0.0058	0.0004	0.0065	0.0004	0.0072	0.0003	**0.015**	0.220	0.265
		K_21203	contig_6969	(422..1216)	nd.	0.0086	0.0010	0.0045	0.0005	0.0050	0.0003	**0.007**	**0.004**	0.366
		K_00342	contig_86	(965..1636)	nd.	0.0207	0.0028	0.0264	0.0019	0.0300	0.0013	**0.014**	0.117	0.139
		K_25185	contig_8537	(844..1806)	nd.	0.0031	0.0003	0.0032	0.0000	0.0028	0.0001	0.383	0.794	**0.005**
gr38	Bacterial lytic	K_42057	contig_15866	(69..503)	NLPC_P60	0.0007	0.0002	0.0004	0.0001	0.0002	0.0000	**0.033**	0.125	0.089
	enzyme[#3]	K_01620	contig_440	(1482..1997)	NLPC_P60	0.0004	0.0001	0.0005	0.0000	0.0003	0.0001	0.341	0.205	**0.014**

Legend: [#1] – known allergen (WHO/IUIS – *T. putrescentiae*) in GenBank; [#2] – similarity to *T. putrescentiae* sequences in GenBank; [#3] – similarity to the GenBank genomic assembly GCF_001687245.1 (*Rhagoletis zephyria* contaminated with *T. putrescentiae*); [#4] – WHO/IUIS differences between Tyr p 36 (profilin) and Der f 36, Der p 36 (unknown protein), nd. – unidentified by HMMER.

FIGURE 1 Allergen expression in three strains of the mite *Tyrophagus putrescentiae* with different microbiome compositions. The mite microbiomes were characterized based on V1-V3 16s RNA barcode sequencing (**A**). Differences in transcript expression among the mite strains were visualized by nonmetric multidimensional scaling (**B**); only sequences contributing to at least 75% of the total variability (SIMPER test) between the mite strains are shown. Differences in the transcript expression of selected immunogenic proteins are shown on the heatmap (**C**).