

FUNDING INFORMATION

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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

To the Editor,

Allergen production by house dust mites is influenced directly by antibacterial compounds and digestive enzymes¹ 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 (Erban et al,⁵ Lee et al⁶), 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.

TABLE 1 Expression of confirmed and putative allergens in three different strains of *Tyrophagus putrescentiae*

Gr.	Allergen name	Transcriptome			Koppert			Phillips		Dog		Kop./ Phil.		Phil./ Dog
		Sequence	Contigs	Region	HMMER Identifier	Mean	Stderr	Mean	Stderr	Mean	Stderr	P-value	P-value	
gr2	NPC2 family ^{a,b,c}	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 ^{a,c}	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 ^{b,c}	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 ^{b,c}	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 ^c	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
		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
gr7	Bactericidal-permeability increasing-like protein ^{b,c}	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
		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
gr8	Glutathione S-transferase ^c	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

(Continues)

TABLE 1 (Continued)

Gr.	Allergen name	Transcriptome		Region	HMMER Identifier	Koppert		Phillips		Dog		Kop./Phil.		Phil./Dog
		Sequence	Contigs			Mean	Stderr	Mean	Stderr	Mean	Stderr	P-value	P-value	
gr10	Tropomyosin ^{a,b,c}	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 ^c	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 ^{a,b,c}	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
gr16	Gelsolin/villin	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
		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 ^c	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	Triosephosphate isomerase ^c	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
gr27	Serpins ^c	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

(Continues)

TABLE 1 (Continued)

Gr.	Allergen name	Transcriptome		Region	HMMER Identifier	Koppert		Phillips		Dog		Kop./Phil.		Phil./Dog
		Sequence	Contigs			Mean	Stderr	Mean	Stderr	Mean	Stderr	P-value	P-value	
gr28	Heat shock protein	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
		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
gr30	Ferritin ^c	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
gr31	Cofilin ^c	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
gr33	alpha-Tubulin	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
		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 ^a	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
gr35	Aldehyde dehydrogenase	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
		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	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
		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
gr38	Bacterial lytic enzyme ^c	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
		K_42057	contig_15866	(69..503)	NILPC_P60	0.0007	0.0002	0.0004	0.0001	0.0002	0.0000	0.033	0.125	0.089
		K_01620	contig_440	(1482..1997)	NILPC_P60	0.0004	0.0001	0.0005	0.0000	0.0003	0.0001	0.341	0.205	0.014

Note: 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).

^aKnown allergen (WHO/IUIS—*T. putrescentiae*) in GenBank.

^bSimilarity to *T. putrescentiae* sequences in GenBank.

^cSimilarity to the GenBank genomic assembly GCF_001687245.1 (*Rhagoletis zephyria* contaminated with *T. putrescentiae*).

^dWHO/IUIS differences between Tyr p 36 (profilin) and Der f 36 (unknown protein), nd: unidentified by HMME.

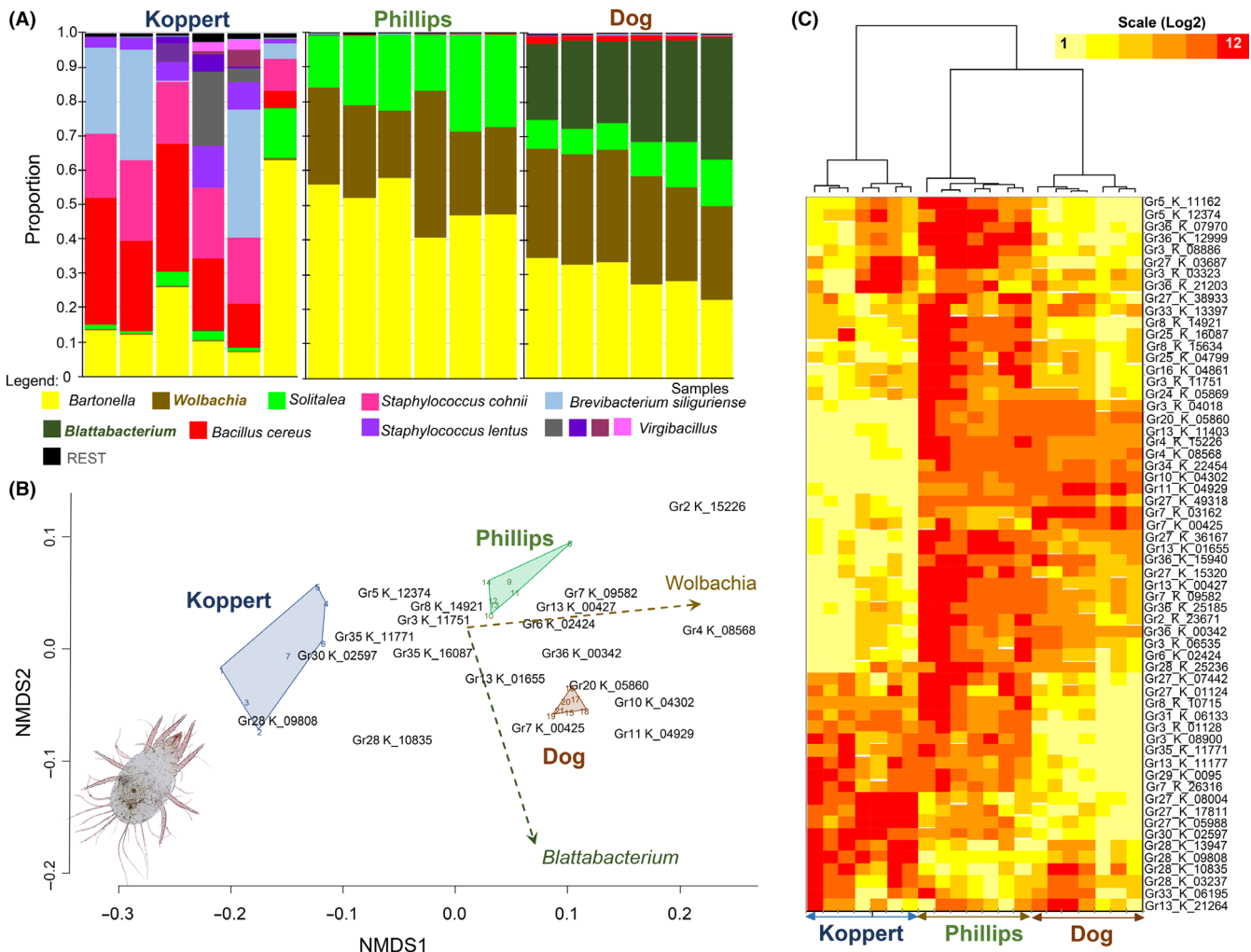


FIGURE 1 Allergen expression in three strains of the mite *Tyrophagus putrescentiae* with different microbiome compositions. The mite microbiomes were characterized based on V1-V3 16s RNA barcode sequencing (A). Differences in transcript expression among the mite strains were visualized by nonmetric 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)

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) (Table S1). The reads were processed and assembled in CLC Genomic Workbench v. 11 (Qiagen) (Tables 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 Supplementary Material and methods).

We obtained 21 transcriptomes (seven biological replicates per every strain) and found 78 transcripts belonging to the eight known allergens of *T. putrescentiae* (see Supplementary 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 surprising 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, for example, 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 al⁶

demonstrated that the microbiome profiles of *Dermatophagoides farinae*, *Dermatophagoides 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.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors declare that they have no relevant conflicts of interest.

KEYWORDS

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13922

Inadequate knowledge of allergen immunotherapy among athletes with allergic rhinitis: A post hoc analysis

To the Editor:

In our recent paper-based questionnaire survey of 636 German professional and hobby athletes, we observed a high prevalence of allergic rhinitis ($n = 269$; 42.6% in total, 36.1% confirmed by a physician, 6.5% self-diagnosed).¹ We presented evidence suggesting that this condition substantially impairs athletes' physical fitness. The negative impacts of allergic rhinitis on sport performance can be lessened by allergen immunotherapy (AIT), whereas symptomatic medications elicit only modest effects on sport performance and physical fitness in this particular group of allergic rhinitis sufferers.¹

This post hoc analysis was conducted in the above-mentioned 269 athletes to better understand (a) athletes' knowledge of AIT, (b) the reasons why most athletes had not been treated with AIT, and (c) the demographic and diagnostic factors influencing the athletes' decision to undergo AIT.

Methods of the study and questionnaire are presented in Online Repository.

Although the majority of athletes (230 out of 265 athletes who responded to this question; 86.8%) had "heard" about AIT, most (213; 80.4%) were familiar with the conventional subcutaneous

immunotherapy. The noninvasive route of administration with its better safety and tolerability profile was largely unknown to them: 57 out of 265 (21.5%) of athletes were aware of sublingual immunotherapy with drops and 37 (14.0%) with tablets. About half of the athletes (110 out of 265, 41.5%) knew about short-term subcutaneous immunotherapy comprising 4-7 injections per year. Even fewer athletes were familiar with pre-co-seasonal sublingual immunotherapy administered before and during the pollen season: 27 out of 265 (10.2%) were aware of drops and 19 (7.2%) of tablets.

The athletes' knowledge about AIT came mostly from physicians (69.7%). Hardly any athletes obtained information from public channels (academic literature: 6.1%; the Internet: 5.3%; newspaper: 4.8%; television/radio: 3.1%; pharmacy: 3.1%; German Allergy and Asthma Association: 1.8%). Only 15.4% of athletes evaluated their physician's consultation regarding AIT as being "good" (score 6-7), whereas 50.4% gave a "moderate" (score 3-5) and 34.2% "poor" rating (score 1-2). These results mirrored the athletes' knowledge of AIT; only 8.3% of athletes rated their knowledge as being "good" (score 6-7), 56.4% as "moderate" (score 3-5), and 35.3% as "poor" (score 1-2).

Differential allergen expression in three *Tyrophagus putrescentiae* strains influenced by microbiome composition

Jan Hubert, Marta Nesvorna, Pavel Klimov, Scot E. Dowd, Bruno Sopko, Tomas Erban

Supplementary figures S1–S26

Legend: ^{#1} – known allergen (WHO/IUIS) in GenBank; ^{#2} – similarity to the GenBank genomic assembly GCF_001687245.1 *Rhagoletis zephyria* contaminated with *Tyrophagus putrescentiae* (blue color); *T. putrescentiae* sequences are indicated by red color; the numbers indicated support values for the branches; the used GenBank sequences and references are provided in Table S5.

FIGURE S1 The dendrogram of NPC2 family of proteins (group 2) and NPC2-like proteins.

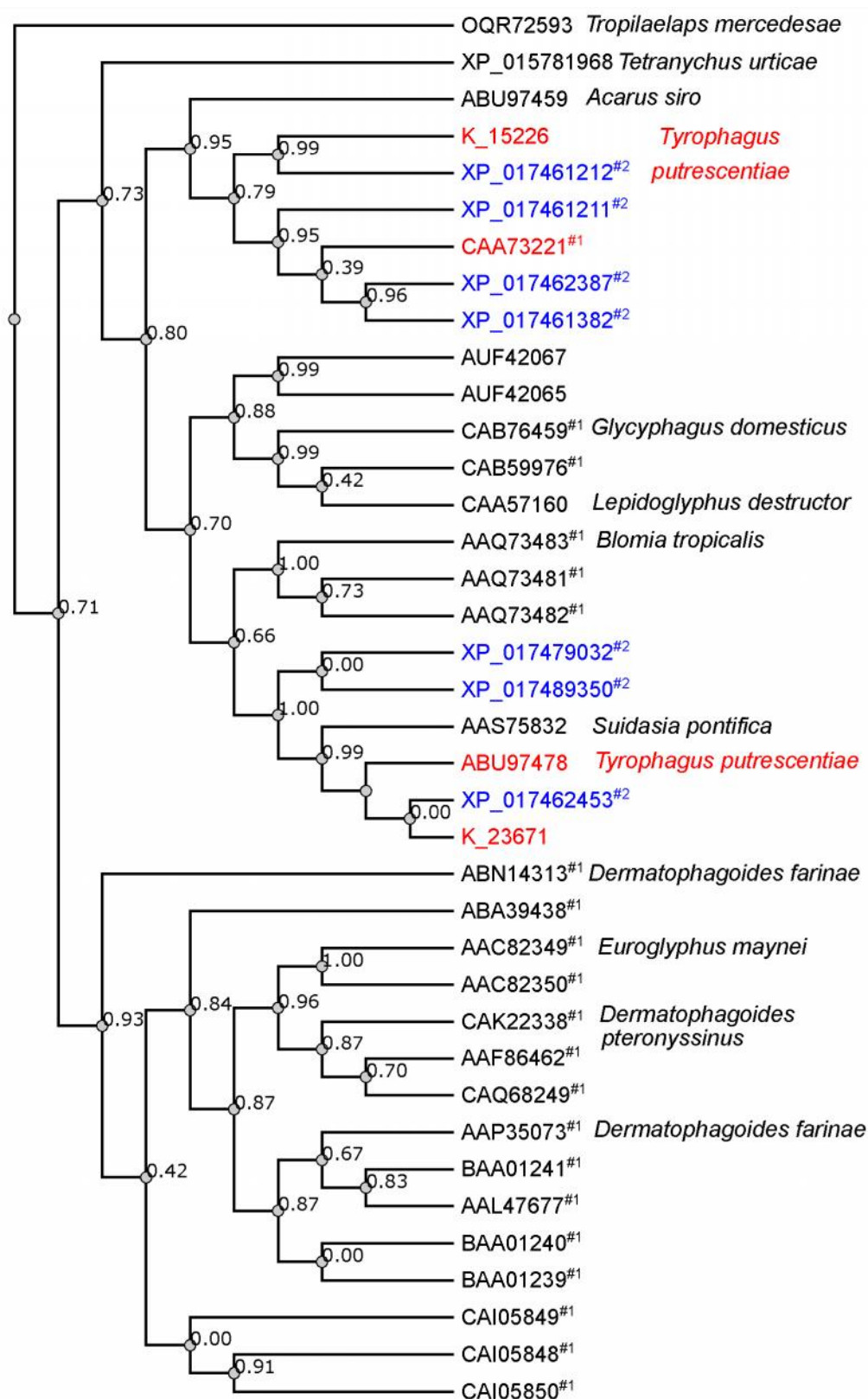


FIGURE S2 The dendrogram of trypsin (group 3) and trypsin-like proteins.

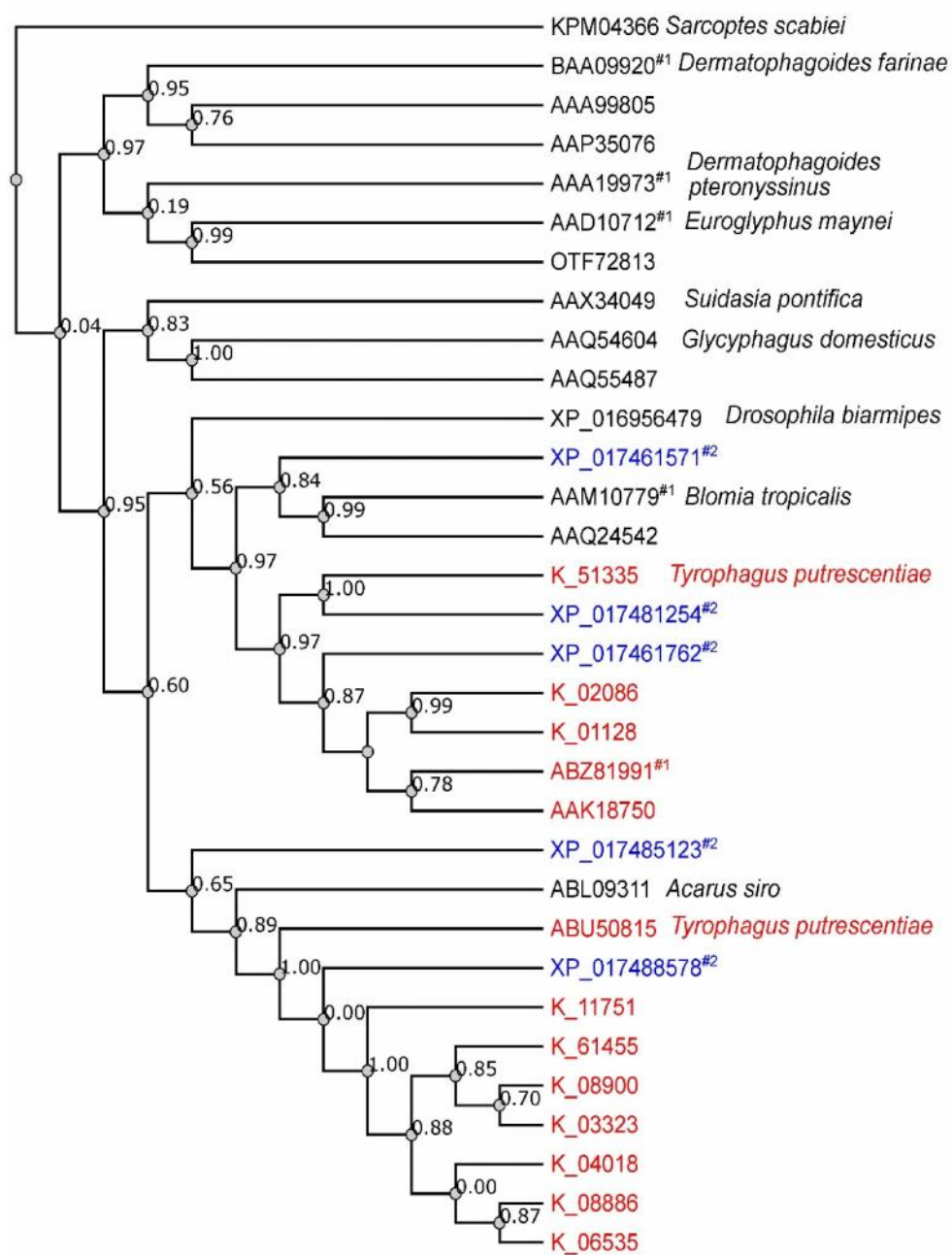


FIGURE S3 The dendrogram of alpha amylases (group 4) sequences.

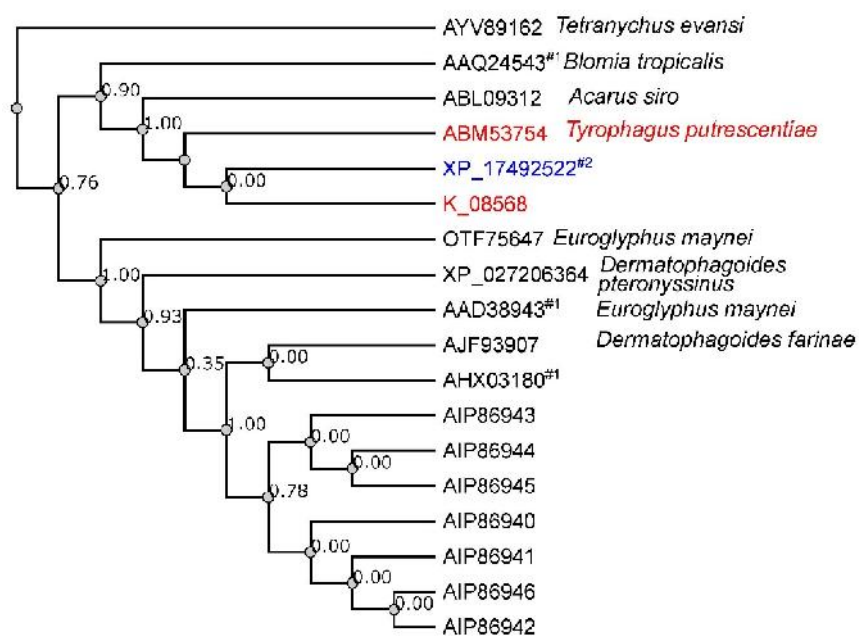


FIGURE S4

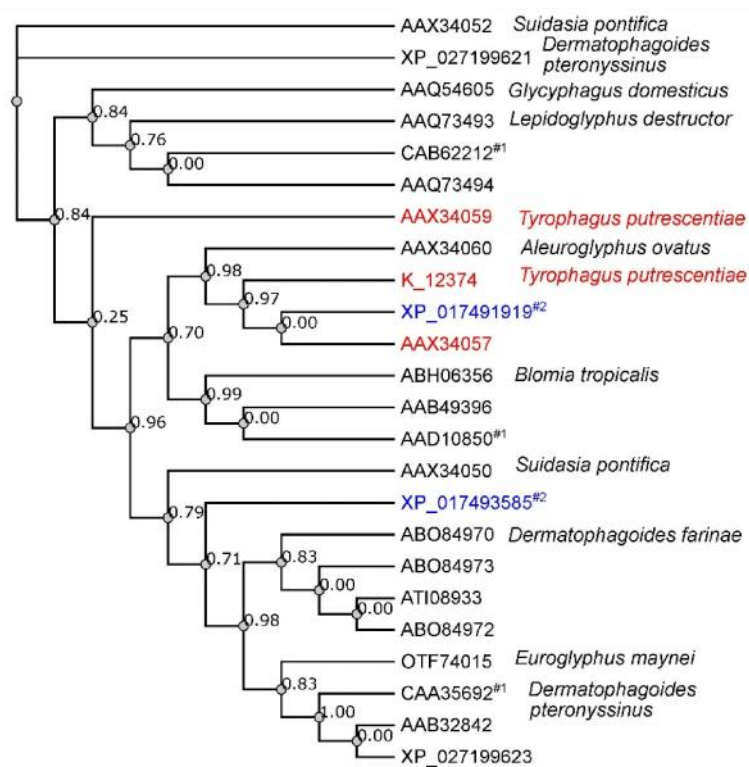


FIGURE S5 The dendrogram of chymotrypsin (group 6 allergen) and chymotrypsin-like proteins.

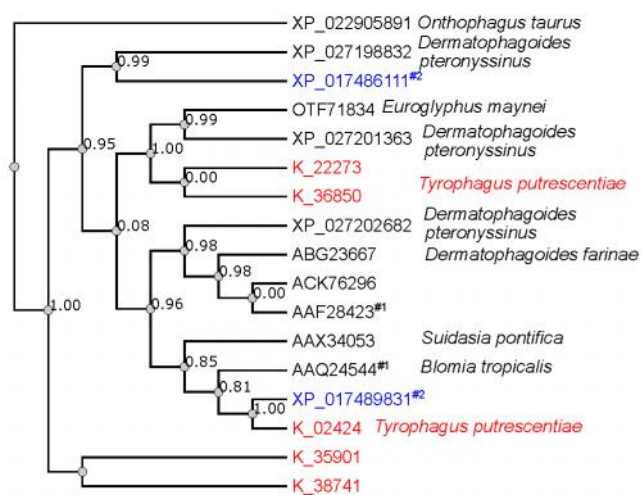


FIGURE S6 Bactericidal permeability-increasing like proteins (group 7) and similar proteins.

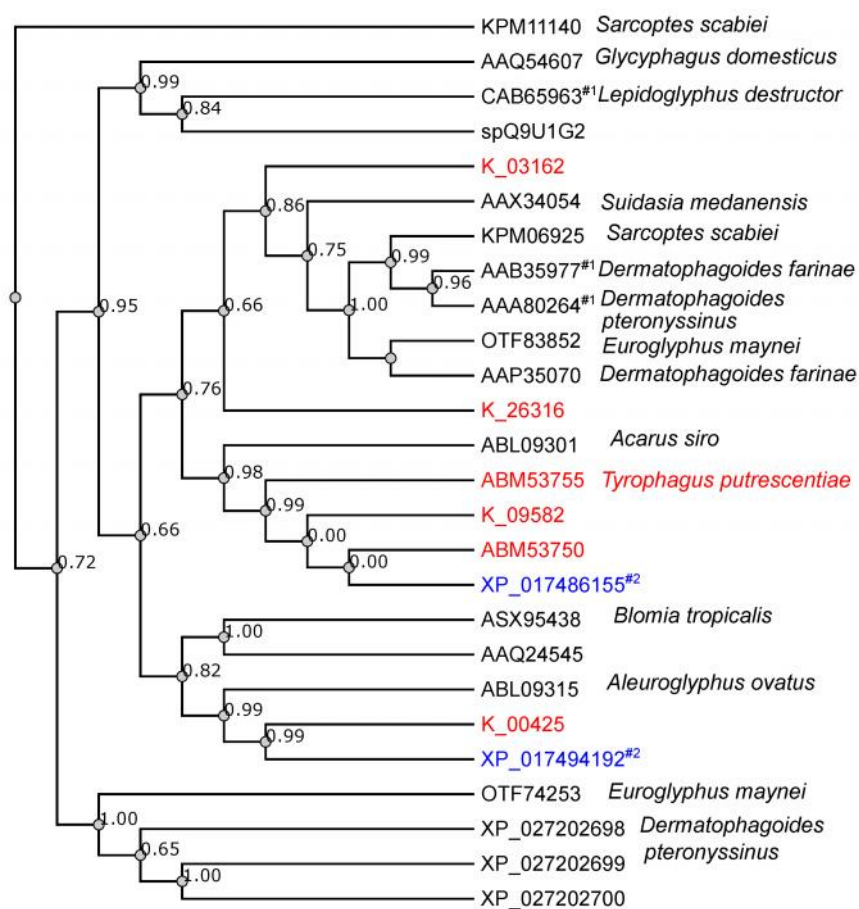


FIGURE S7 Glutathione S-transferase (group 8) proteins and next Glutathione S-transferase with putative allergen importance.

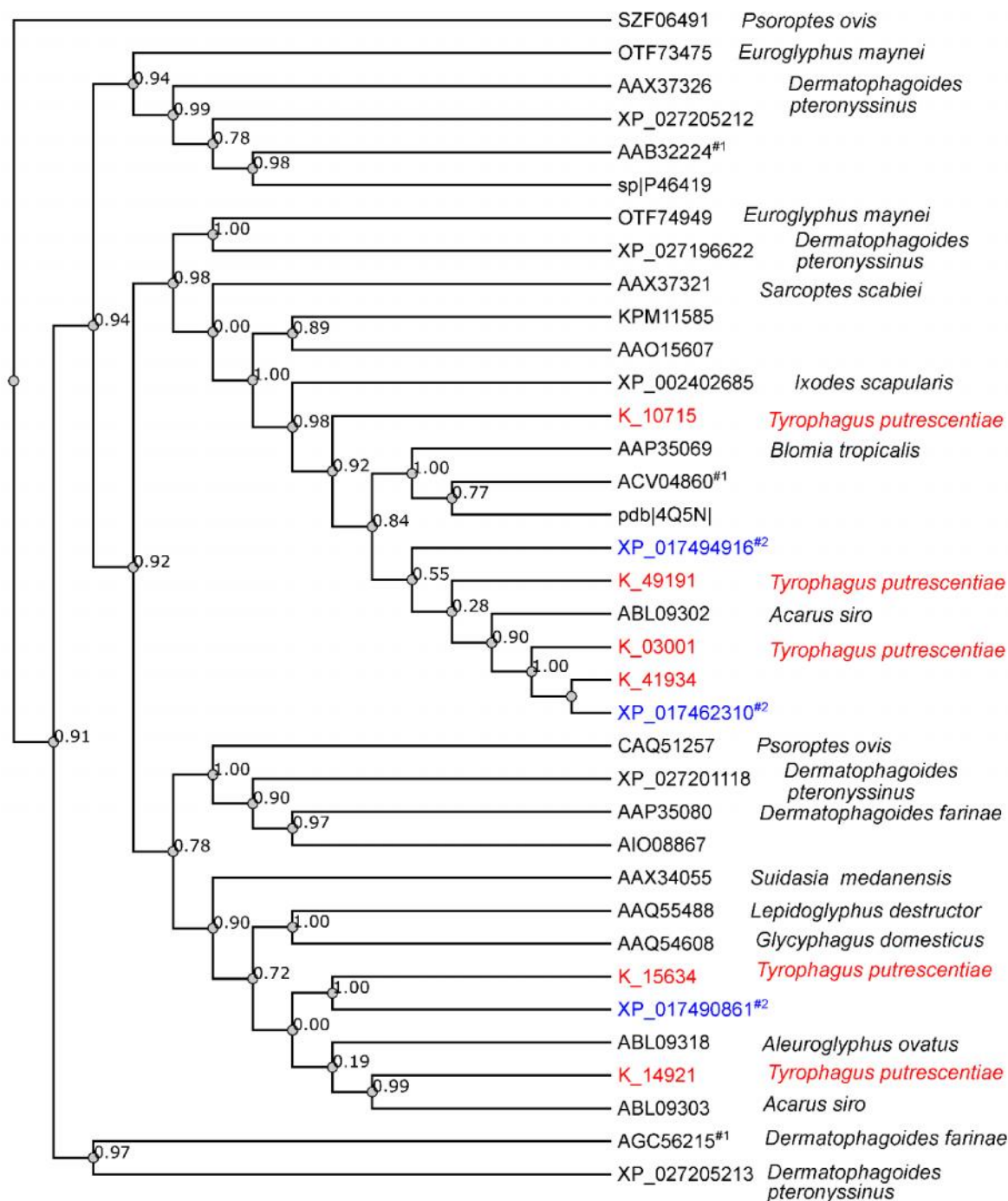


FIGURE S8 Tropomyosin (group 10) and tropomyosin-like proteins.

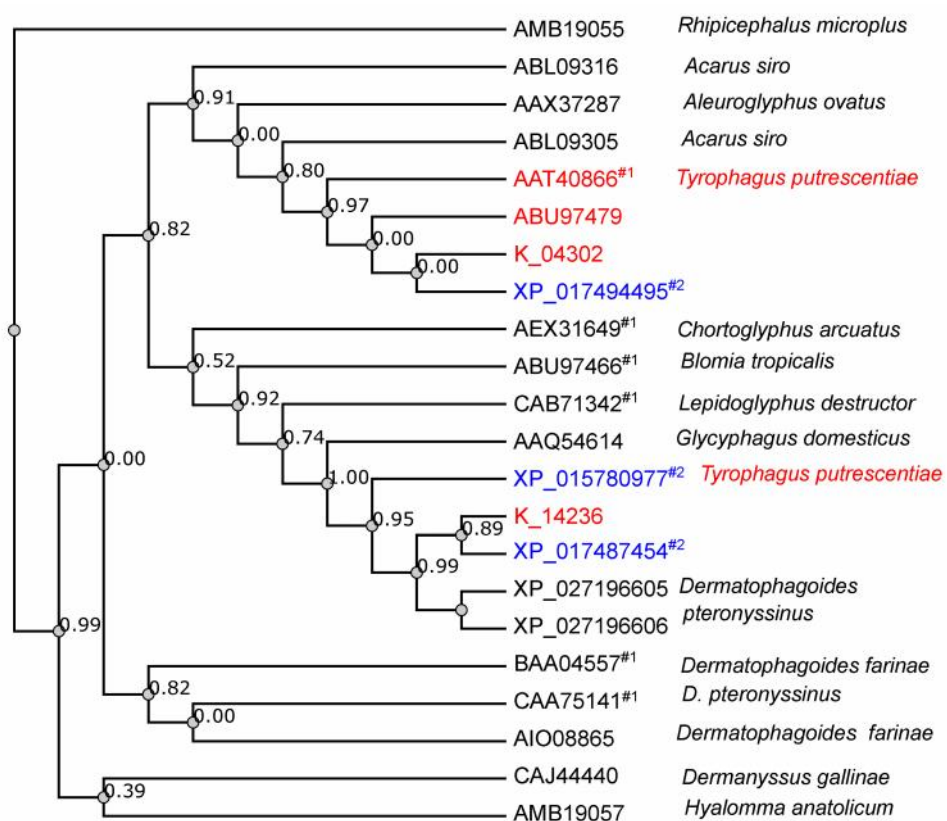


FIGURE S9 Paramyosin (group 11) and paramyosin-like proteins.

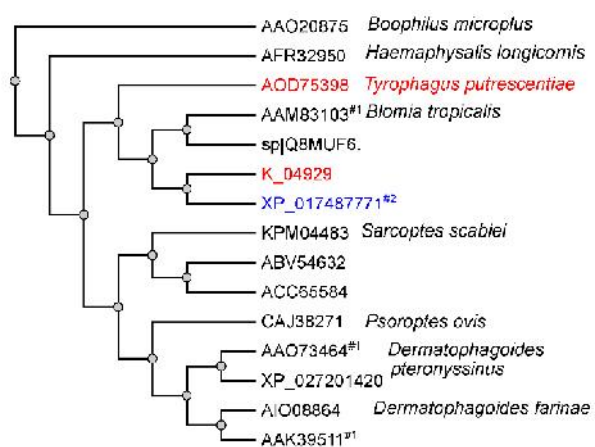


FIGURE S10 The sequences of fatty-acid binding (group 13) and fatty-acid binding-like proteins.

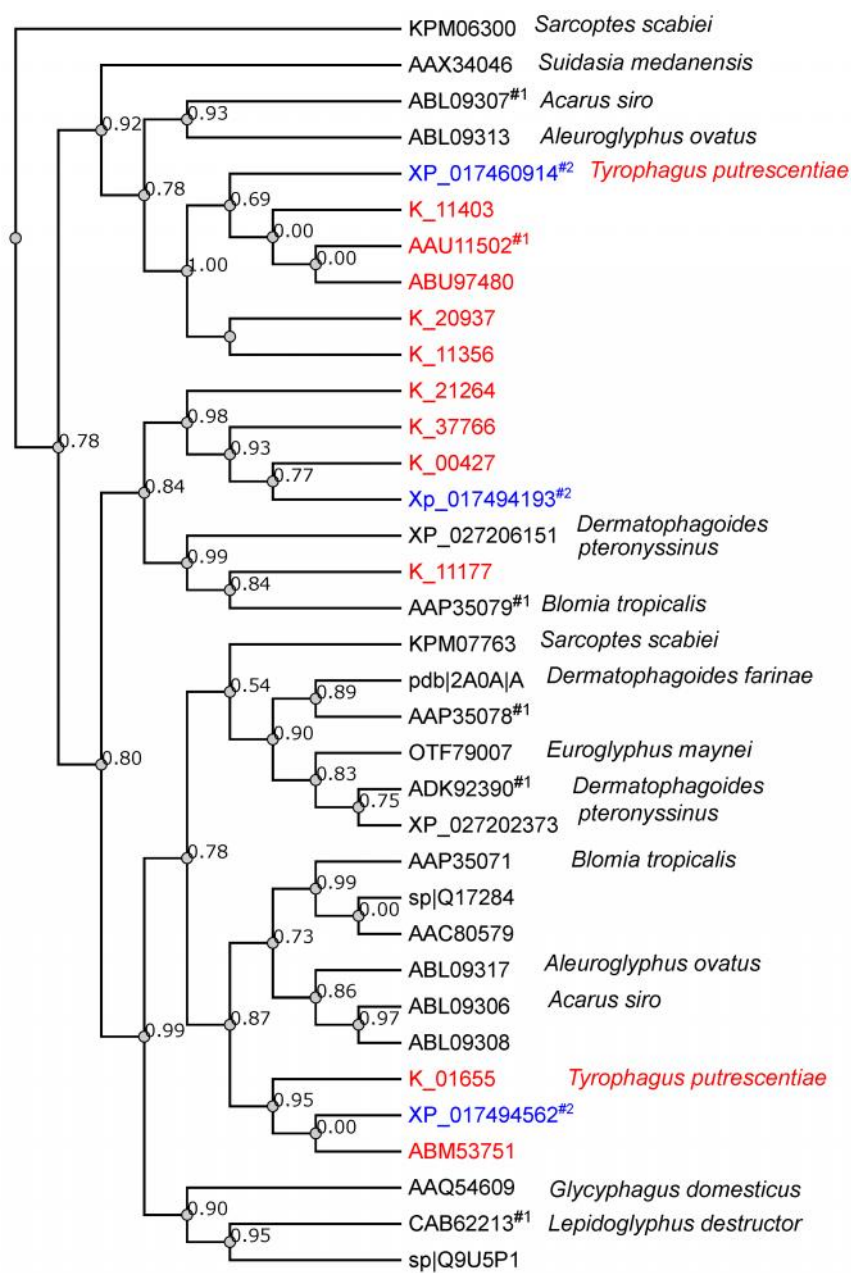


FIGURE S11 The gelsolin-like sequences (group 16) of allergen or putative allergen function.



FIGURE S12 The arginine-kinase sequences (group 20) of allergen or putative allergen function.

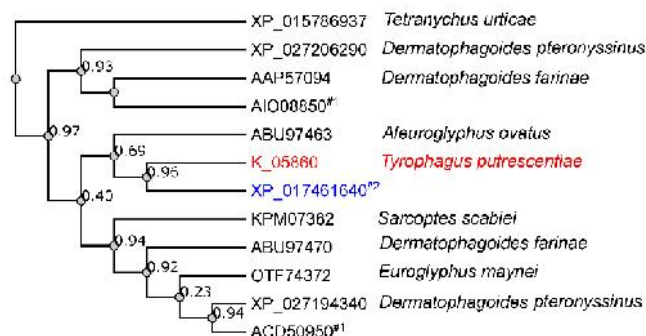


Figure S13. Ubiquinol-cytochrome c reductase binding protein-like protein (group 24) of allergen or putative allergen function.

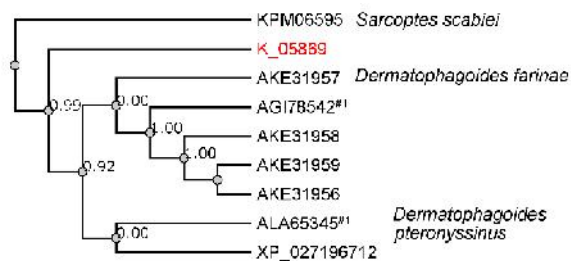


FIGURE S14 Triose-phosphate isomerase like proteins (group 25) of allergen or putative allergen function.



FIGURE S15 Serpin and serpin-like proteins sequences (group 27) of allergen or putative allergen function.

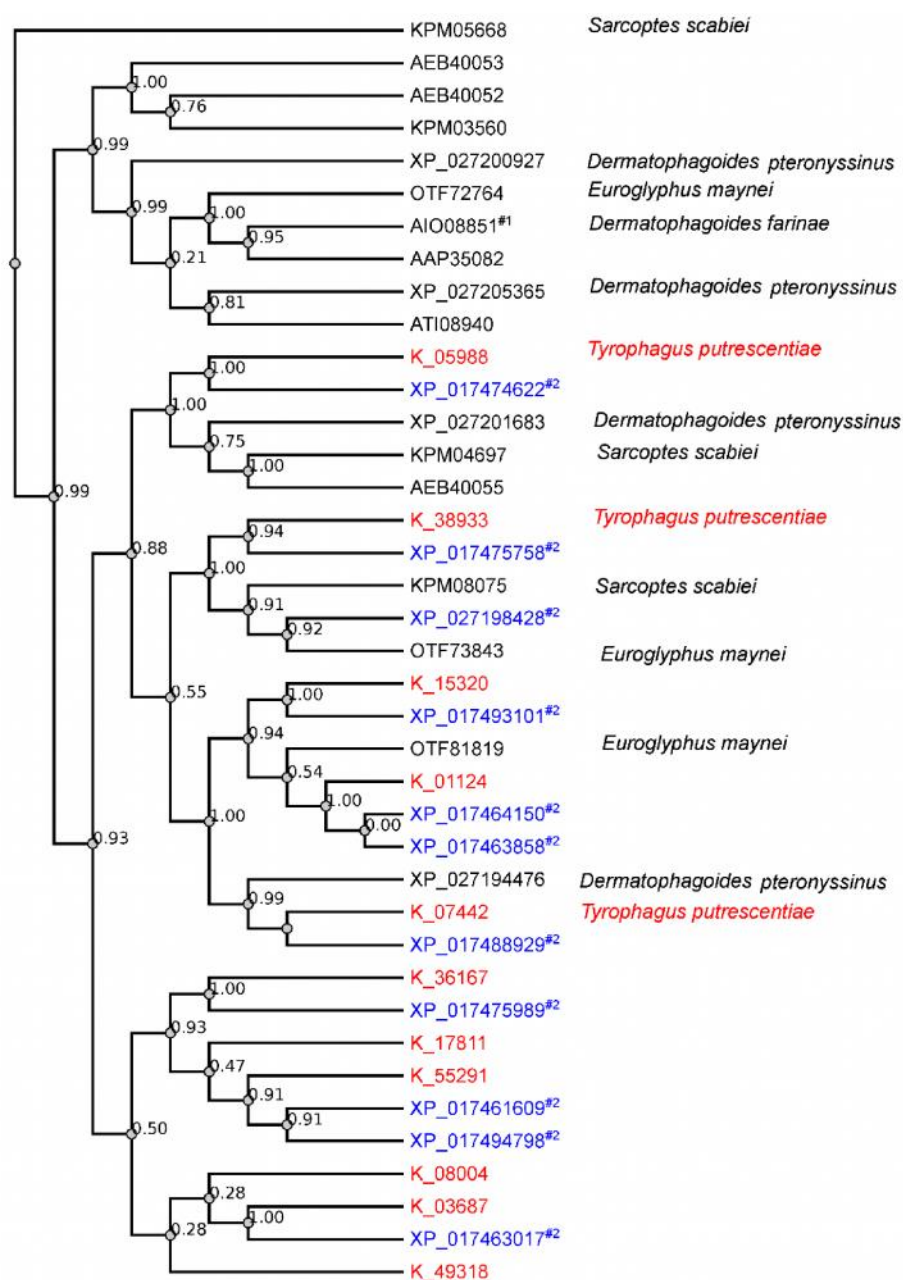


FIGURE S16 Heat shock proteins sequences (group 28) of allergen or putative allergen function.

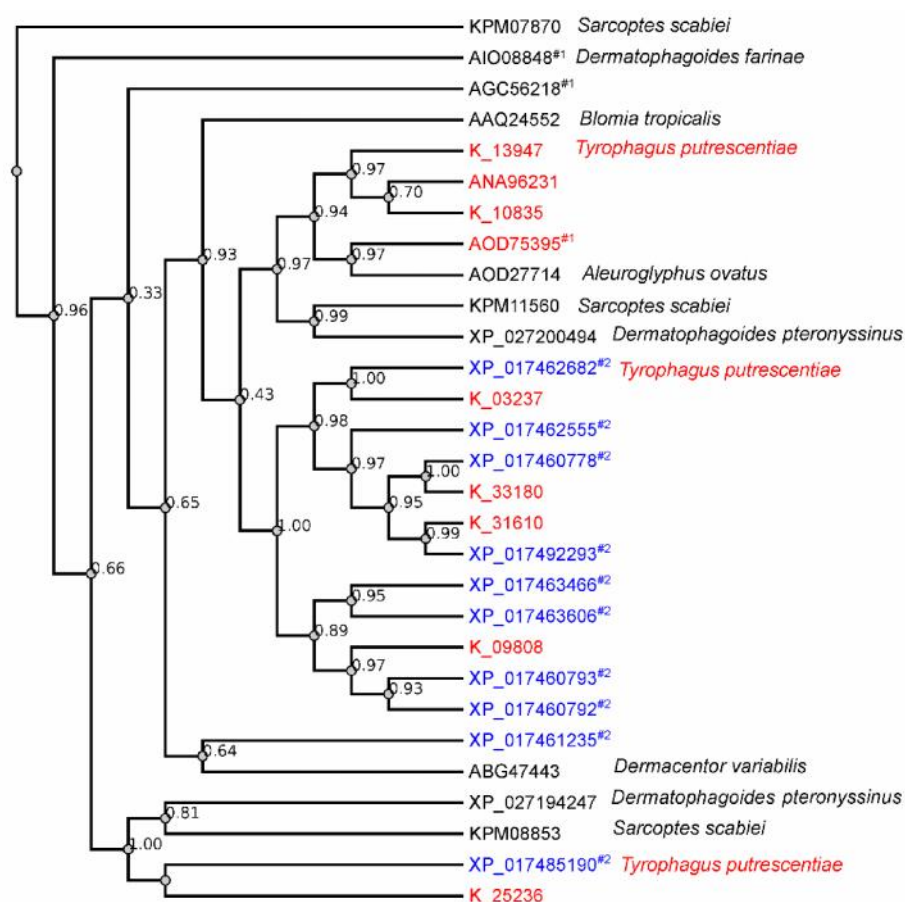


FIGURE S17 Peptidyl-prolyl cis-trans isomerase (cyclophilin)(group 29) of allergen or putative allergen function.

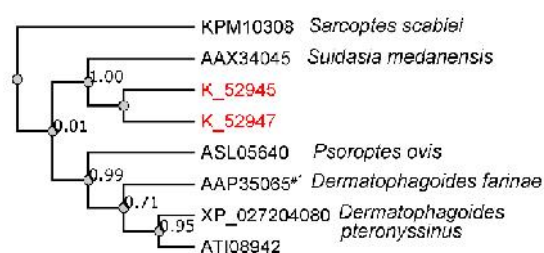


FIGURE S18 Ferritin (group 30) and similar proteins of allergen or putative allergen function.

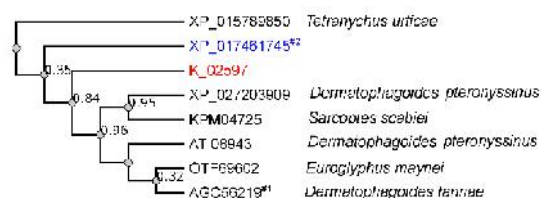


FIGURE S19 Cofilin (group 31) and similar proteins of allergen or putative allergen function.

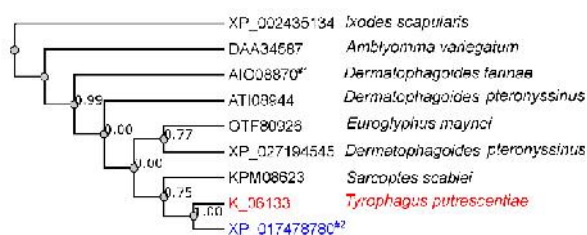


FIGURE S20 Alpha tubulin (group 33) and similar proteins. of allergen or putative allergen function.

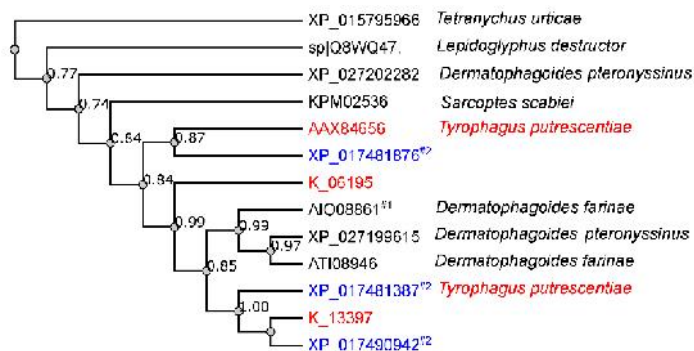


FIGURE S21 The enamine/imine deaminase (group 34/ *Dermatophagoides farinae*) and similar proteins of allergen or putative allergen function.

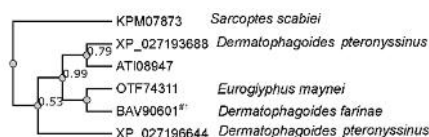


FIGURE S22 Troponin C (group 34/ *Tyrophagus putrescentiae*) and similar proteins of allergen or putative allergen function.

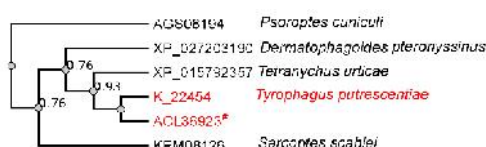


FIGURE S23 The aldehyde dehydrogenase-like proteins (group 35/ *Tyrophagus putrescentiae*) and similar proteins of allergen or putative allergen function, comparing to the position of Der f 35 and similar proteins (green color).

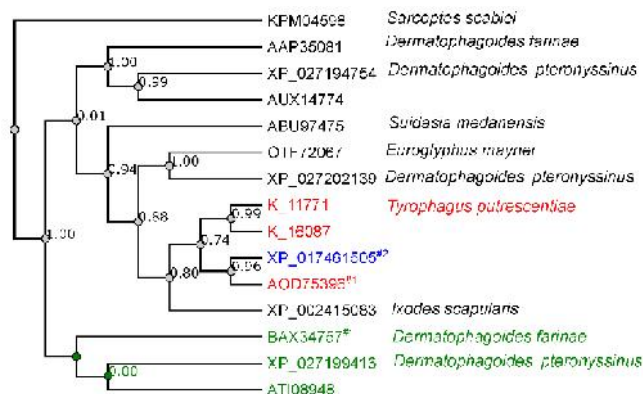


FIGURE S24 The “unknown biochemical functional” allergens Der p36 and Der f36 and similar proteins of allergen or putative allergen function.

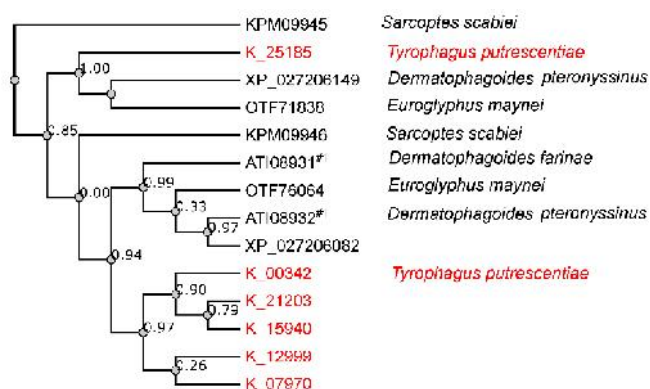


FIGURE S25 The profilin allergen Tyr p36 and similar proteins of allergen or putative allergen function.

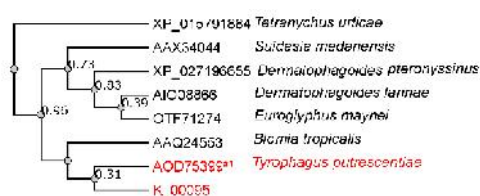
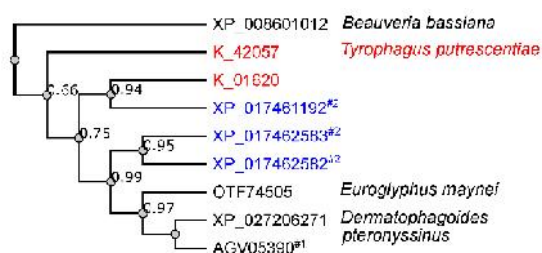


FIGURE S26 The LytFM protein Der p 38 and similar proteins of allergen or putative allergen function.



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

Jan Hubert, Marta Nesvorna, Pavel Klimov, Scot E. Dowd, Bruno Sopko, Tomas Erban

Supplementary data:

SUPPLEMENTARY MATERIALS AND METHODS

Mite population

Three strains of *Tyrophagus putrescentiae* (Schrank 1781) were selected; their microbiomes were characterized previously by 16S DNA barcoding.^{5,11,12} Koppert population (no endosymbionts), Phillips and Dog populations (with endosymbionts; *Wolbachia* abundant in both, *Blattabacterium*-like bacteria present in Dog but absent in Phillips). The Dog population was sampled from dog food in the United States by J. Hubert in 2007; the Koppert population was obtained from a grain-derived diet by E. van Baal in the Netherlands in 2012, and the Phillips population was obtained from dog food by T. Phillips in Manhattan, KS, USA, in 2014. The mites were mass reared in Iwaki flasks and collected as previously described;¹³ different rearing incubators were used to prevent culture cross-contamination. The mites used in the experiments were reared on wheat germ derived diet (SPMd) composed from as blended oat flakes, wheat germ, and Mauripan dried yeast extract (*Saccharomyces cerevisiae*) (10:10:1 w/w).¹³

Sampling

The mites included the mixture of adults and juvenile stadia were sampled from approximately 28 days of old cultures. It corresponds to mite culture with exponential growth commonly used for allergenic extracts.^{14,15} Live mites were collected from the surfaces of the flasks and plugs by brush and placed into sterile Eppendorf tubes. This method is considered as more suitable than flotation of mites and protect to contamination.^{16,17} Every strain was kept in 10 flasks, for sampling, we selected 7 flasks and every flask was sampled separately to obtain 7 biological replicates per strain. Altogether, we obtained 21 samples. We modified our previously used protocol¹⁸ as follows: (i) manipulations with mites or RNA were performed on ice; (ii) mites were weighed to obtain 30-40 mg samples; (iii) mites were placed into 100% ethanol and vortexed for 5 s, followed by centrifugation (13,000 ×g for 1 min); (iv) the supernatant was replaced by 1 : 10 bleach (sodium hypochlorite 5%): ddH₂O solution, vortexed for 5 s and centrifuged for 2 min at 13,000 ×g. Then, the cleaning solution was removed, the mites were washed in ddH₂O, and the previous step was repeated.

RNA extraction

RNA isolation was performed using a NucleoSpin RNA kit (cat. no. 740984.50, Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions, with the following modifications: (i) the mites were placed in 500 µL of lysis buffer and then homogenized for 30 s in a glass tissue grinder; (ii) the sample was centrifuged (2000 ×g for 3 s); (iii) in the sample, DNA was degraded by DNase I at 37 °C according to the manufacturer's protocol (Riboclear Plus, cat. No 313-50, GeneAll), and the quality of RNA was assessed on a Nanodrop spectrophotometer (NanoDrop One, Thermo Scientific) and Bioanalyzer (Agilent Bioanalyzer); (iv) the RNA samples were sent on dried ice to the MR DNA laboratory (<http://www.mrdnalab.com/>) for downstream processing and sequencing.

RNA processing and sequencing

The total RNA concentration was determined using the Qubit[®] RNA Assay Kit (Life Technologies). For rRNA depletion, 1 µg of total RNA was first used to remove DNA contamination using Baseline-ZERO™ DNase

(Epicentre) according to the manufacturer's instructions, followed by purification using RNA Clean & Concentrator⁻⁵ columns (Zymo Research). From these DNA-free RNA samples, rRNA was removed using the Ribo-Zero™ Gold rRNA Removal Kit (Epidemiology; Illumina), and final purification was performed using the RNA Clean & Concentrator⁻⁵ columns (Zymo Research). The rRNA-depleted samples were used for library preparation using the KAPA mRNA HyperPrep Kit (Kapa Biosystems) according to the manufacturer's instructions. For each library, DNA concentration was measured using the Qubit® dsDNA HS Assay Kit (Life Technologies), and the average fragment size was determined with Agilent 2100 Bioanalyzer (Agilent Technologies). The libraries were then pooled in equimolar ratios of 2 nM, and 10.0 pM of the library pool was clustered using a cBot (Illumina) and subjected to paired-end (PE) sequencing for 500 cycles using the HiSeq 2500 system (Illumina).

Reads processing

The reads were deposited in the SRA database under accession number SUB4527480, project PRJNA493156. A list of samples is provided in Table S1. Read processing was performed in CLC Genomic Workbench v. 11 (Qiagen, Venlo, Netherlands). The PE reads were trimmed to remove adapter sequences and then length- and quality-filtered, resulting in the removal of 2 million reads (of the initial 688 million reads). The remaining reads were used for *de novo* assembly according to the CLC manual.¹⁹

Contig processing

De novo assembly resulted in 31,158 contigs ($N_{50}=2,470$), with 6.85×10^8 reads mapped back to the assembly. The contigs were annotated using Prokka^{7,20} installed on the Galaxy server;²¹ 67,354 loci were predicted.

Mapping: The trimmed reads were mapped against the annotated assembly in CLC Workbench. Low-coverage loci (lower than 100 mapped reads) were removed, resulting in 2.5×10^7 protein-coding sequences.

Protein identification: Our final set of 2.5×10^7 predicted proteins were submitted to a BLAST search against the GenBank non-redundant protein sequence database (12/2018). After the search, we selected the protein sequences with the highest similarity to known allergens according to database if the official site for the systematic allergen nomenclature that is approved by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee⁸ or allergen-like sequences (GenBank) of mites; the sequences were then aligned in T-coffee (<http://tcoffee.crg.cat/apps/tcoffee/index.html>),²² and maximum likelihood phylogenetic trees were inferred in PhyML 3.0 (LG substitution model, SPR and NNI tree searching and aLRT SH-like branch support).²³ The phylograms were visualized in FigTree v.1.4.3.²⁴ Protein domains were annotated using HMMER.²⁵

Statistical analyses

The number of reads per identified transcript was standardized to the total number of reads and to the number of reads according to the minimum per sample. The analogical protocol is used for microbiome analyses.²⁶ The differences among the mite strains were evaluated by analysis of similarities (ANOSIM) using the Bray–Curtis distance.²⁷ Then, the results were visualized by nonmetric multidimensional scaling (NMDS). We applied variable forward selection to identify which bacterial taxa contributed to the expression level variability among mite strains,²⁸ and Similarity Percentage (SIMPER)²⁷ analysis to identify the sequences having the highest effect on the differences among the mite strains using the Bray–Curtis matrix. The levels of gene expression were compared using METASTATS analyses²⁹ in MOTHUR.³⁰ The other analyses were performed in R³¹ (vegan³² and adespatial³³ packages) and PAST.³⁴

SUPPLEMENTARY RESULTS

Identification of sequences based on their comparison to allergen sequences in GenBank

The used sequences of allergen and putative allergens are presented in Table S5. In addition, we included the sequences of genomic assembly GCF_001687245.1 originated from *Rhagoletis zephyria* contaminated with *T. putrescentiae* mites. The dendrograms from the alignment of sequences are shown in Figures S1–S26.

The analyses of NPC2 family of proteins (group 2) showed different clusters between house dust mites *Dermatophagoides* spp., *Euroglyphus maynei* and stored product mites *Blomia tropicalis*, *Glycyphagus domesticus*, *Lepidoglyphus destructor* and *T. putrescentiae*. The obtained sequences formed two clusters outside domestic mites, but with stored product mites. One predicted sequence (K_15226) were similar to identified *T. putrescentiae* allergen (GenBank Access No: CAA73221).³⁵ The second predicted sequence (K_23671) formed cluster of similarity to protein *T. putrescentiae* (GenBank Access No: ABU97478) (Figure S1).

Similar situation was observed for trypsin (group 3), when the predicted sequences formed two clusters. Three predicted sequences (K_51335, K_02086, K_01128) formed cluster with known Tyr p 3 allergen (GenBank Access No: ABZ81991)³⁶ and *T. putrescentiae* serine proteinase protein (GenBank Access No: AAK18750). Seven predicted sequences (K_11751, K_61455, K_08900, K_03323, K_04018, K_08886, K_06535) formed cluster with sequence of *T. putrescentiae* (GenBank Access No: ABU50815) and *Acarus siro* (GenBank Access No: ABL09311) (Figure S2). Both these clustered were outside house dust mite trypsin cluster.

The dendrogram of alpha-amylases (group 4) sequences showed the separation of the house dust mite alpha-amylase from stored product mites. The predicted sequence (K_08568) clustered to stored product mites altogether with know Blo t 4 allergen (GenBank Access No: AAQ24543) and *T. putrescentiae* protein (Figure S3).

The dendrogram of unknown allergens of group 5 showed the high similarity of obtained sequence (K_12374) to allergen-like portions of *T. putrescentiae* (GenBank Access No: AAX34057 and AAX34059), protein from *Aleuroglyphus ovatus* (GenBank Access No: AAX34060) and Blo t 5 allergen³⁷ (Figure S4).

The dendrogram of chymotrypsin (group 6) and similar sequences indicated two predicted sequences of *T. putrescentiae* (K_22273 and K_36850) that are similar to predicted chymotrypsin sequences of *Dermatophagoides pteronyssinus*³⁸ and *E. maynei*. One sequence (K_02424) formed separate cluster from previous with group 6 allergens of *D. farinae*³⁹ and *B. tropicalis* altogether with chymotrypsin of *Suidasia pontifica* (GenBank Access No: AAX34053). The last two predicted sequences (K_35901 and K_38741) were out grouped to mite chymotrypsin (Figure S5).

Bactericidal permeability-increasing like proteins (group 7) formed three clusters when predicted sequences of *T. putrescentiae* were presented. Two sequences (K_03162 and K_26316) formed cluster with house dust mites allergens Der p7 and Der f 7⁴⁰ and altogether with group 7 like proteins of *E. maynei*, *Suidasia medanensis* and *Sarcoptes scabiei*⁴¹ (Figure S6). The next cluster was formed from the predicted sequences (K_09582) and sequences of group 7 like proteins of *T. putrescentiae* (GenBank Access No: ABM53755 and ABM53750) *A. siro* (GenBank Access No: ABL09301). The last sequence (K_00425) clustered with group 7 like proteins of *B. tropicalis* (GenBank Access No: ASX95438 and AAQ24545) and *A. ovatus* (GenBank Access No: ABL09315).

Glutathione S-transferase (group 8) proteins formed more clusters. House dust mite allergens Der f 8 (GenBank Access No: AGC56215) and Der p 8⁴² were outgrouped from Blo t 8 (GenBank Access No: ACV04860). However another glutathione S-transferase of house dust mites (*D. pteronyssinus*: GenBank Access No: XP_027196622 and *E. maynei*: GenBank Access No: OTF74949) showed higher similarity to Blo t 8 than Der p 8 and Der f 8 (Figure S7). Four predicted *T. putrescentiae* sequences (K_10715, K_49191, K_03001 and K_41934) clustered altogether with Blo t 8. While next two sequences (K_15634 and K_14921) formed separate cluster altogether with stored product mites (*S. medanensis*, *L. destructor*, *G. domesticus*, *A. ovatus* and *A. siro*).

Tropomyosin (group 10) sequences formed three clusters, Der f 10⁴³ and Der p 10⁴⁴ allergens formed separate cluster with other tropomyosins of *D. farinae*.⁴⁴ While the predicted *D. pteronyssinus* sequences (GenBank Access No: XP_027196605 and XP_027196606) (ref were more similar to stored product mite tropomyosin. The tropomyosin proteins of stored product mite formed two clusters and both contain predicted *T. putrescentiae* tropomyosin sequences. One sequence (K_14236) formed cluster to Cho a 10 (GenBank Access No: AEX31649), Blo t10 (GenBank Access No: ABU97466) and Lep 10⁴⁵ allergens. The second sequences (K_04302) clustered to Tyr p 10 (GenBank Access No: AAT40866) and next tropomyosin of *T. putrescentiae* (GenBank Access No: ABU97479) and tropomyosin of *A. siro* and *A. ovatus* (Figure S8).

The comparison of paramyosin (group 11) sequences provided similar results to tropomyosin, because Der p11 and Der f 11⁴⁶⁻⁴⁸ were separated from Blo t 11. The predicted *T. putrescentiae* sequence clustered with

sequences of paramyosin (GenBank Access No: AOD75398)⁴⁹ from *T. putrescentiae* to Blo t11 allergen⁵⁰ (Figure S9).

The sequences of fatty-acid-binding protein (group 13) showed a separation of house dust allergens Der p 13 (GenBank Access No: ADK92390), Der f 13 and proteins of predicted protein of *E. maynei* (GenBank Access No: OTF79007) from stored product mites. The sequences of fatty-acid-binding proteins and fatty-acid binding-like proteins formed smaller clusters irregularly containing predicted sequences of *T. putrescentiae*. Tyr p 13⁵¹ and Aca s 13 (GenBank Access No: ABL09307) allergens clustered to next protein of *T. putrescentiae* (GenBank Access No: ABU97480), the protein of *A. ovatus* (GenBank Access No: ABL09313) and predicted sequences (K_11403, K_20937, K_11356, K_21264, K_37766). Next cluster was formed from Blo t t13 (GenBank Access No: AAP35079), predicted *D. pteronyssinus* protein (XP_027206151) and predicted *T. putrescentiae* sequence (K_11177). The last cluster contains proteins of *B. tropicalis*, *A. ovatus* and *A. siro* altogether with *T. putrescentiae* (GenBank Access No: ABM53751) and predicted *T. putrescentiae* sequence (K_01655) (Figure S10).

Der f 16 (Gelsolin/villin)⁵² formed one cluster outgrouped by *S. scabiei* (GenBank Access No: KPM04580) with predicted *T. putrescentiae* (K_04861), predicted sequences of *D. pteronyssinus* and *E. maynei* (Figure S11).

The Arginine kinase (group 20) clustered to three clusters outgrouped by predicted arginine kinase of *Tetranychus urticae*.⁵³ Der f 20 (GenBank Access No: AIO08850) and Der p 20 (GenBank Access No: ACD50950) formed separate clusters and the last cluster was composed of sequences of *A. ovatus* (GenBank Access No: ABU97463) and predicted sequence of *T. putrescentiae* (K_05860) (Figure S12).

Ubiquinol-cytochrome c reductase binding protein-like protein (group 24), Der p 24 and Der f 24 formed cluster outgrouped by *S. scabiei* (KPM06595) and including predicted sequence of *T. putrescentiae* (K_05869) (Figure S13).

The sequences of triose-phosphate isomerase, Der f 25 (GenBank Access No: AGC56216) clustered altogether with predicted sequences of *T. putrescentiae* (K_04799) and *D. pteronyssinus* (GenBank Access No: XP_027198629), when they are outgrouped by *S. scabiei* (GenBank Access No: KPM10468) sequences (Figure S14).

The sequence (GenBank Access No: AIO08851) of serpin (Der f27) belonged to the large group of serpin-like sequences from *D. pteronyssinus*, *E. maynei*, and *S. scabiei*. These sequences clustered altogether with predicted sequences of *T. putrescentiae* (K_05988, K_38933, K_15320, K_01124, K_07442, K_36167, K_17811, K_55291, K_08004, K_03687 and K_49318) and predicted sequences of genomic assembly GCF_001687245.1 originated from *R. zephyria* contaminated by *T. putrescentiae* (Figure S15).

Heat Shock Proteins (group 28) sequences of Der f 28 (GenBank Access No: AIO08848 and AGC56218) separated from Tyr p 28 (GenBank Access No: AOD75395). Tyrp 28 clustered with the next sequences of *T. putrescentiae*, *B. tropicalis*, *A. ovatus* and predicted sequences of *D. pteronyssinus* (GenBank Access No: XP_027200494) and 5 predicted sequences from *T. putrescentiae* (K_13947, K_10835, K_03237, K_33180, K_61610, K_09808). It was supported by the presence of predicted sequences of genomic assembly GCF_001687245.1 originated from *R. zephyria* contaminated by *T. putrescentiae* (Figure S16). One predicted sequences of *T. putrescentiae* (K_25236) was outside of this cluster to show similarity to predicted sequences of *S. scabiei* (GenBank Access No: KPM08853) and *D. pteronyssinus* (GenBank Access No: XP_027194247).

Der f 29 peptidyl-prolyl cis-trans isomerase (cyclophilin) (GenBank Access No: AAP35065) formed cluster with sequences of *Psoroptes ovis* (GenBank Access No: ASL05640) and *D. pteronyssinus* (GenBank Access No: ATI08942). There is low support for the conception to predicted peptidyl-prolyl cis-trans isomerases sequences of *T. putrescentiae* (K_52945 and K_52947) and "Mal s 6" allergen of *S. medanensis* (GenBank Access No: AAX34045) (Figure S17). Therefore we did not consider these predicted proteins as allergen-like.

Der f 30 (GenBank Access No: AGC56219) is ferritin and the sequences clustered sequences of *D. pteronyssinus*, *E. maynei*, *S. scabiei* and predicted sequences of *T. putrescentiae* (K_02597) (Figure S18).

Der f 31 (GenBank Access No: AIO08870) is cofilin and the sequences clustered altogether with *D. pteronyssinus*, *E. maynei*, *S. scabiei* and predicted sequences of *T. putrescentiae* (K_06133) (Figure S19).

Der f 33 (GenBank Access No: AIO08861) is alpha-tubulin and the sequences clustered altogether with *D. pteronyssinus*, *E. maynei*, *S. scabiei*, *L. destructor*, *T. putrescentiae*⁵⁴ and predicted sequences of *T. putrescentiae* (K_06133 and K_13397) (Figure S20).

Der f 34⁵⁵ is enamine/imine deaminase which is similar to protein from *D. pteronyssinus*⁵⁶ and predicted proteins from *D. pteronyssinus* (GenBank Access No: XP_027193688 and XP_027196644) and *E. maynei* (GenBank Access No: OTF74311), but not to Tyr p 34 allergen (Fig S21),^{51,57} Tyr p34 is a different protein, i.e. Troponin C. Troponin C of *T. putrescentiae* (GenBank Access No: ACL36923) has similar predicted sequence's in *D. pteronyssinus* (GenBank Access No: XP_027203190) and *T. urticae*⁵³ and predicted sequence of our transcriptome (K_22454) (Figure S22).

The similar situation is in the group of 35 allergens, when proteins with different biochemical functions are included. The proteins of "unknown biochemical function" Der f 35⁵⁸ (GenBank Access No: BAX34757) and *D. pteronyssinus* proteins (GenBank Access No: ATI08948)⁵⁶ formed a separate cluster from incorporated aldehyde dehydrogenase-like proteins (Figure S23). The incorporated aldehyde dehydrogenase-like proteins included Tyr p 35 (GenBank Access No: AOD75396) and the predicted sequences of the transcriptome of *T. putrescentiae* (K_16087 and K_11771) and similar sequences in *D. pteronyssinus* (GenBank Access No: XP_027202139, XP_027194754, AUX14774), *D. farinae* (GenBank Access No: AAP35081), *S. medanensis* (GenBank Access No: ABU97475) and *E. maynei* (GenBank Access No: OTF72067).

Also the group allergens 36 incorporated two groups of proteins with different biochemical function, the "unknown biochemical function" Der p 36⁵⁶ and Der f 36⁵⁶ and profilin Tyr p 36 (GenBank Access No: AOD75399). The Der p36 (GenBank Access No: ATI08932) and Der f 36 (ATI08931) formed cluster to proteins from *S. scabiei*⁴¹, *E. maynei* (GenBank Access No: OTF71838 and OTF76064), predicted proteins from *D. pteronyssinus* (XP_027206082) and six predicted proteins from *T. putrescentiae* transcriptome (K_25185, K_00342, K_21203, K_15940, K_12999 and K_07970) (Figure S24).

Profilin-like allergen Tyr p 36 (GenBank Access No: AOD75399) clustered altogether with predicted protein from *T. putrescentiae* transcriptome (K_00095), *B. tropicalis* (GenBank Access No: AAQ24553), *E. maynei* (GenBank Access No: OTF71274), *D. farinae* (GenBank Access No: AIO08866), *S. medanensis* (GenBank Access No: AAX34044) and predicted protein of *D. pteronyssinus* (GenBank Access No: XP_027196655) (Figure S25).

LytFM protein Der p 36 (GenBank Access No: AGV05390) is recently described protein with antibacterial function.⁵⁹ Der p 36 clustered to the predicted proteins of *T. putrescentiae* (K_01620 and K_42057). The presence of the proteins in *T. putrescentiae* is confirmed by predicted sequences of genomic assembly GCF_001687245.1 originated from *R. zephyria* contaminated by *T. putrescentiae*. The next similar sequences originated from *E. maynei* (GenBank Access No: OTF74505) and predicted *D. pteronyssinus* (GenBank Access No: XP_027206271) (Figure S26).

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