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Letter to the Editor

Genomic analysis reveals novel allergens of *Blomia tropicalis*

Dear Editor,

Blomia tropicalis was considered a storage mite but is now identified as an important species of house dust mite (HDM) and causes a series of allergic diseases, especially in tropical regions such as Singapore.[1] Compared with the major HDM species *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, *B. tropicalis* has much fewer reported allergen groups.[2,3] As an important HDM, *B. tropicalis* has only fourteen groups of allergens reported in the WHO/IUIS Allergen Nomenclature database,[2] which limits our knowledge to its allergen components.

With the advent of omics era, genome-based approaches have been applied to the study of mite allergens.[4,5] Based on the high-quality genome of *B. tropicalis*,[6] our genome-wide analysis predicted a comprehensive putative allergen profile of thirty-seven allergen groups (up to group 42), which covered a wide range of allergen homologs (Table 1). The reported group 19 allergen of *B. tropicalis*, Blo t 19, could not be found in the genome. Consistent with a previous report,[6] tandem gene duplication caused gene family expansion and result in multiple homologs (Supplementary Fig. 1), such as five tandemly arrayed homologous genes of Blo t 8 (glutathione S-transferase). Two cysteine proteases of *B. tropicalis* have been identified as isoallergens in group 1. In the allergen profile, many homologs of *B. tropicalis* allergens were predicted to be allergens (Table 1).

In the transcriptomic analysis, Blo t 13.0101 unexpectedly presented the highest expression level in transcriptome analysis (Fig. 1A), which was similarly observed in *Tyrophagus putrescentiae*.[7] After Blo t 13.0101, two major allergens of *B. tropicalis*, Blo t 5 and Blo t 21, were the two most highly expressed allergens (Fig. 1A). However, the application of advanced high-resolution proteomics methods can be instrumental in quantifying the abundance of allergen proteins and clarifying the potential clinical importance.

For the major allergen groups 5 and 21 of *B. tropicalis*,[8–10] we noticed that Blo t 5.0101 and Blo t 21.0101 are tandemly arrayed homologs that share 41.9 % identity (Supplementary Fig. 2). To gain a deeper understanding of the complex allergen profile, further comparative analysis was conducted on significant allergen groups. More details on this analysis can be found in the Supplementary Notes, particularly those in relation to allergens with chitin-binding domains (including the group 12, 15, and 18 allergens).

Although our genome-wide analysis revealed a comprehensive profile of both identified and predicted allergen components of *B. tropicalis* (Table 1), serological evidence is still needed to confirm the allergenicity of the putative allergens. Therefore, recombinant proteins were expressed and subjected to ELISA experiment using patient serum samples. Serum samples were collected in Hong Kong from allergic patients for testing and healthy individuals as controls (Supplementary Table 1). When at least class 3 allergy (over 3.5 kUA/L of IgE) was considered positive, 58 % (29/50) of those tested positive for HDM (Der p 1, *D. pteronyssinus*) were identified to be positive for *B. tropicalis*, while only one sample (BC0699 out of 20 samples) was negative for HDM (class 0) but positive for *B. tropicalis* (class 4).

In subsequent ELISA experiments, 26 serum samples were collected from allergic patients who tested positive for *B. tropicalis* (BT-allergic). Nine recombinant proteins of *B. tropicalis* were expressed and purified (Supplementary Fig.13), including three reported allergens (rBlo t 5, rBlo t 21 and rBlo t 12) and six putative allergens (rpBlo t 18, rpBlo t 23, rpBlo t 24, rpBlo t 25, rpBlo t 26 and rpBlo t 31). The major allergens rBlo t 5 and rBlo t 21 were used as positive controls (Fig. 1B, C). According to the allergen-specific IgE levels in BT-allergic sera, rpBlo t 18, rpBlo t 23 and rpBlo t 26 were suggested as novel allergens of *B. tropicalis* (Fig. 1D-F), while rpBlo t 24, rpBlo t 25 and rpBlo t 31 were not confirmed to be novel allergens, because of their low IgE levels (Supplementary Fig. 14). The different ELISA results necessitate further investigation within a larger sample size. Unexpectedly, rBlo t 12 presented significantly higher IgE levels in BT-allergic sera, but the low absorbance values (all below 0.15) could not well confirm its allergenicity (Supplementary Fig. 14A), which was similarly observed in rpBlo t 24 (Supplementary Fig. 14B). The IgE levels of two major allergens, rBlo t 5 and rBlo t 21 (Fig. 1B, C), were highly dispersed with high maximum absorbance values (above 1.0). Intriguingly, regression analysis revealed good correlations among the IgE levels of rBlo t 5, rBlo t 21, rpBlo t 23 and rpBlo t 26, but not rpBlo t 18, and suggested the co-sensitization of these allergens (Supplementary Fig. 15).

For the sensitization rates of specific allergens (Fig. 1G), unexpectedly, rpBlo t 26 and rpBlo t 18 presented high positive percentages of 88.46 % and 73.08 %, respectively, while two major allergens

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Table 1
Summary of all the genes that encode proteins predicted to be allergens.

Allergen ID	Isoallergen ID	Gene locus	Biochemical function	No. of exons	No. of amino acids	TPM value (Avg ± Stdev)	Homologue (Protein identity)
Blo t 1	Blo t 1.0101[†]	BT_013135.03	Cysteine protease	4	331	18744 ± 2261	AAK58415 Blo t 1.0101 (98.2 %)
	Blo t 1.0201[†]	BT_013137.01	Cysteine protease	4	333	24102 ± 3762	AAQ24541 Blo t 1.0201 (98.8 %)
	pBlo t 1.0301[‡]	BT_013135.02	Cysteine protease	4	333	757 ± 582	AAQ24541 Blo t 1.0201 (63.8 %)
	pBlo t 1.0401[‡]	BT_013135.04	Cysteine protease	2	330	509 ± 158	AAQ24541 Blo t 1.0201 (39.0 %)
	pBlo t 1.0501[‡]	BT_013144.01	Cysteine protease	3	369	650.575 ± 127	AAQ24541 Blo t 1.0201 (32.9 %)
Blo t 2	Blo t 2.0101[†]	BT_003416.02	ML-domain protein	2	142	42029 ± 16214	AAQ73483 Blo t 2.0101 (93.8 %)
	pBlo t 2.0201[‡]	BT_007270.01	ML-domain protein	2	144	40370 ± 13402	AAQ73483 Blo t 2.0101 (50.0 %)
	pBlo t 2.0301[‡]	BT_007271.01	ML-domain protein	3	145	372 ± 57	AAQ73483 Blo t 2.0101 (42.5 %)
	pBlo t 2.0401[‡]	BT_007142.01	ML-domain protein	2	151	5289 ± 838	CAI05848 Blo t 2.0101 (41.9 %)
Blo t 3	Blo t 3.0101[†]	BT_006831.01	Trypsin	2	266	10277 ± 880	AAM10779 Blo t 3.0101 (98.9 %)
	pBlo t 3.0201[‡]	BT_015438.01	Trypsin	2	269	2075 ± 267	AAM10779 Blo t 3.0101 (55.6 %)
Blo t 4	Blo t 4.0101[†]	BT_008084.02	Alpha-amylase	1	515	5328 ± 1077	AAQ24543 Blo t 4.0101 (99.2 %)
	Blo t 4.0201[†]	BT_011038.02	Alpha-amylase	1	515	5328 ± 1077	AAQ24543 Blo t 4.0101 (99.0 %)
Blo t 5	Blo t 5.0101[†]	BT_005893.01	Unknown	2	134	111590 ± 10602	AAD10850 Blo t 5.0101 (100 %)
Blo t 6	Blo t 6.0101[†]	BT_006509.01	Chymotrypsin	3	275	5203 ± 695	AAQ24544 Blo t 6.0101 (100 %)
Blo t 7	Blo t 7.0101[†]	BT_006271.01	Bactericidal/permeability-increasing protein	2	212	24757 ± 7825	ASX95438.1 Blo t 7.0101 (99.5 %)
Blo t 8	Blo t 8.0101[†]	BT_006863.04	Glutathione S-transferase	2	236	7738 ± 5410	ACV04860 Blo t 8.0101 (97.0 %)
	pBlo t 8.0201[‡]	BT_006863.05	Glutathione S-transferase	2	236	1463 ± 414	ACV04860 Blo t 8.0101 (90.3 %)
	pBlo t 8.0301[‡]	BT_006865.01	Glutathione S-transferase	2	236	5687 ± 1686	ACV04860 Blo t 8.0101 (80.9 %)
	pBlo t 8.0401[‡]	BT_006863.03	Glutathione S-transferase	2	236	452 ± 256	ACV04860 Blo t 8.0101 (45.5 %)
	pBlo t 8.0501[‡]	BT_006863.02	Glutathione S-transferase	2	237	133 ± 25	ACV04860 Blo t 8.0101 (43.5 %)
Blo t 9	pBlo t 9.0101[‡]	BT_003514.02	Serine protease	2	272	7454 ± 1549	AAP57077 Der p 9 (57.5 %)
	pBlo t 9.0201[‡]	BT_016173.01	Serine protease	3	274	368 ± 156	AAP57077 Der p 9 (53.8 %)
	pBlo t 9.0301[‡]	BT_001003.01	Serine protease	2	288	173 ± 58	AAP57077 Der p 9 (49.8 %)
Blo t 10	Blo t 10.0101[†]	BT_015321.01	Tropomyosin	9	284	12444 ± 1051	ABU97466 Blo t 10.0101 (99.6 %)
	pBlo t 10.0201[‡]	BT_006475.01	Tropomyosin	9	353	2500 ± 863	ABU97466 Blo t 10.0101 (59.1 %)
Blo t 11	Blo t 11.0101[†]	BT_008464.01	Paramyosin	10	875	3119 ± 379	AAM83103 Blo t 11.0101 (99.7 %)
Blo t 12	Blo t 12.0101[†]	BT_016677.01	Chitin-binding domain containing protein	2	143	19241 ± 2339	AAA78904 Blo t 12.0101 (92.4 %)
Blo t 13	Blo t 13.0101[†]	BT_008106.02	Fatty acid-binding protein	4	130	136948 ± 13042	AAC80579 Blo t 13.0101 (99.2 %)
	pBlo t 13.0201[‡]	BT_008106.03	Fatty acid-binding protein	2	131	32933 ± 8181	AAC80579 Blo t 13.0101 (60.8 %)
	pBlo t 13.0301[‡]	BT_005106.01	Fatty acid-binding protein	3	131	16078 ± 7775	AAC80579 Blo t 13.0101 (56.3 %)
pBlo t 14[‡]	pBlo t 14.0101[‡]	BT_005757.01	Apolipophorin	6	1700	9095 ± 2545	AF373221_1 Der p 14 (46.3 %)
pBlo t 15[‡]	pBlo t 15.0101[‡]	BT_016675.01	Chitinase	6	575	8653 ± 2836	AAY84565 Der p 15 (59.8 %)
pBlo t 16[‡]	pBlo t 16.0101[‡]	BT_003239.01	Gelsolin/villin	8	484	767 ± 284	AAM64112 Der f 16 (57.0 %)
pBlo t 18[‡, §]	pBlo t 18.0101[‡]	BT_010400.01	Chitinase	4	461	12893 ± 7857	AAM19082 Der f 18 (60.9 %)
pBlo t 20[‡]	pBlo t 20.0101[‡]	BT_008259.02	Arginine kinase	3	357	9153 ± 1347	ABU97470 Der f 20 (86.0 %)

(*continued on next page*)

Table 1 (*continued*)

Allergen ID	Isoallergen ID	Gene locus	Biochemical function	No. of exons	No. of amino acids	TPM value (Avg ± Stdev)	Homologue (Protein identity)
	pBlo t 20.0201[‡]	BT_008259.03	Arginine kinase	4	356	3159 ± 955	ABU97470 Der f 20 (83.1 %)
	pBlo t 20.0301[‡]	BT_012279.01	Arginine kinase	2	358	134 ± 10	ABU97470 Der f 20 (68.2 %)
Blo t 21	Blo t 21.0101[†]	BT_005892.01	Unknown	2	129	105276 ± 23261	AAX34047 Blo t 21.0101 (99.2 %)
pBlo t 23[‡, §]	pBlo t 23.0101[‡]	BT_016256.01	Peritrophin-like protein	1	113	4870 ± 2707	ACB46292 Der p 23 (40.0 %)
pBlo t 24[‡]	pBlo t 24.0101[‡]	BT_008155.01	UQCRB protein	3	118	9069 ± 1578	AGI78542 Der f 24 (72.9 %)
pBlo t 25[‡]	pBlo t 25.0101[‡]	BT_013681.01	Triosphosphate isomerase	6	419	6001 ± 1630	AIO08860 Der f 25 (80.9 %)
pBlo t 26[‡, §]	pBlo t 26.0101[‡]	BT_007095.02	Myosin light-chain	6	158	26093 ± 5323	AIO08852 Der f 26 (82.7 %)
pBlo t 28[‡]	pBlo t 28.0101[‡]	BT_002686.01	Heat shock protein 70	1	650	1895 ± 644	AIO08848 Der f 28 (80.5 %)
	pBlo t 28.0201[‡]	BT_001717.01	Heat shock protein 70	1	647	1310 ± 121	AIO08848 Der f 28 (80.2 %)
	pBlo t 28.0301[‡]	BT_000218.01	Heat shock protein 70	1	650	16851 ± 1947	AIO08848 Der f 28 (79.4 %)
	pBlo t 28.0401[‡]	BT_003376.01	Heat shock protein 70	1	658	10802 ± 517	AIO08848 Der f 28 (79.2 %)
	pBlo t 28.0501[‡]	BT_011597.01	Heat shock protein 70	1	635	23005 ± 14241	AIO08848 Der f 28 (78.0 %)
pBlo t 29[‡]	pBlo t 29.0101[‡]	BT_014409.01	Cyclophilin	2	254	12008 ± 686	AAP35065 Der f 29 (83.5 %)
pBlo t 30[‡]	pBlo t 30.0101[‡]	BT_008038.02	Ferritin	3	174	27150 ± 883	AGC56219 Der f 30 (74.3 %)
	pBlo t 30.0201[‡]	BT_008039.02	Ferritin	2	276	15119 ± 16669	AGC56219 Der f 30 (66.1 %)
	pBlo t 30.0301[‡]	BT_008039.03	Ferritin	2	173	2487 ± 566	AGC56219 Der f 30 (75.5 %)
pBlo t 31[‡]	pBlo t 31.0101[‡]	BT_016210.01	Cofilin	4	147	16453 ± 4806	AIO08870 Der f 31 (80.1 %)
pBlo t 32[‡]	pBlo t 32.0101[‡]	BT_003614.02	Inorganic pyrophosphatase	2	296	1544 ± 335	AIO08849 Der f 32 (67.7 %)
pBlo t 33[‡]	pBlo t 33.0101[‡]	BT_003250.01	Alpha-tubulin	1	450	9708 ± 578	AIO08861 Der f 33 (81.3 %)
	pBlo t 33.0201[‡]	BT_000606.02	Alpha-tubulin	3	450	290 ± 76	AIO08861 Der f 33 (82.0 %)
	pBlo t 33.0301[‡]	BT_005979.01	Alpha-tubulin	1	448	2357 ± 496	AIO08861 Der f 33 (81.1 %)
	pBlo t 33.0401[‡]	BT_002245.02	Alpha-tubulin	1	449	12065 ± 172	AIO08861 Der f 33 (79.4 %)
pBlo t 34[‡]	pBlo t 34.0101[‡]	BT_004919.01	Rid-like protein	1	136	8805 ± 900	BAV90601 Der f 34 (58.4 %)
pBlo t 36[‡]	pBlo t 36.0101[‡]	BT_000325.01	Unknown	3	221	13971 ± 3133	ATI08931 Der f 36 (53.4 %)
pBlo t 37[‡]	pBlo t 37.0101[‡]	BT_008575.02	Chitin binding protein	2	190	9952 ± 2395	QBF67839 Der f 37 (50.9 %)
	pBlo t 37.0201[‡]	BT_008575.03	Chitin binding protein	2	186	2977 ± 1032	QBF67839 Der f 37 (37.1 %)
pBlo t 38[‡]	pBlo t 38.0101[‡]	BT_009281.01	Bacteriolytic enzyme	1	153	5043 ± 2717	QHQ72282 Der f 38 (56.2 %)
pBlo t 39[‡]	pBlo t 39.0101[‡]	BT_015114.01	Troponin C	5	153	7377 ± 2066	QBF67841 Der f 39 (95.4 %)
pBlo t 40[‡]	pBlo t 40.0101[‡]	BT_000785.01	Thioredoxin-like protein	3	105	49617 ± 18539	UJQ69787 Der f 40 (71.7 %)
pBlo t 41[‡]	pBlo t 41.0101[‡]	BT_002783.03	Aldehyde dehydrogenase	3	483	1206 ± 352	AOD75396.1 Tyr p 35 (85.4 %)
	pBlo t 41.0201[‡]	BT_002783.02	Aldehyde dehydrogenase	3	481	8888 ± 2370	AOD75396.1 Tyr p 35 (73.3 %)
pBlo t 42[‡]	pBlo t 42.0101[‡]	BT_008117.01	Profilin	3	130	16035 ± 11211	AOD75399 Tyr p 36 (94.7 %)

[†] These genes were suggested as the identical allergen genes by the high identity to those reported in the WHO/IUIS Allergen Nomenclature database. In the naming rules, we used the first and last two digits after the decimal point to differentiate the different genes and isoforms, respectively.

[‡] These genes shared high similarities with the allergens reported in the WHO/IUIS Allergen Nomenclature database. The names of the putative allergens were indicated with an additional prefix p in this study with no official approval by the WHO/IUIS Allergen Nomenclature Sub-Committee.

[§] Three putative allergens, pBlo t 18, pBlo t 23 and pBlo t 26, have been approved by WHO/IUIS Allergen Nomenclature Sub-Committee on November 28, 2023 and were officially named as Blo t 18, Blo t 41 and Blo t 26, respectively.

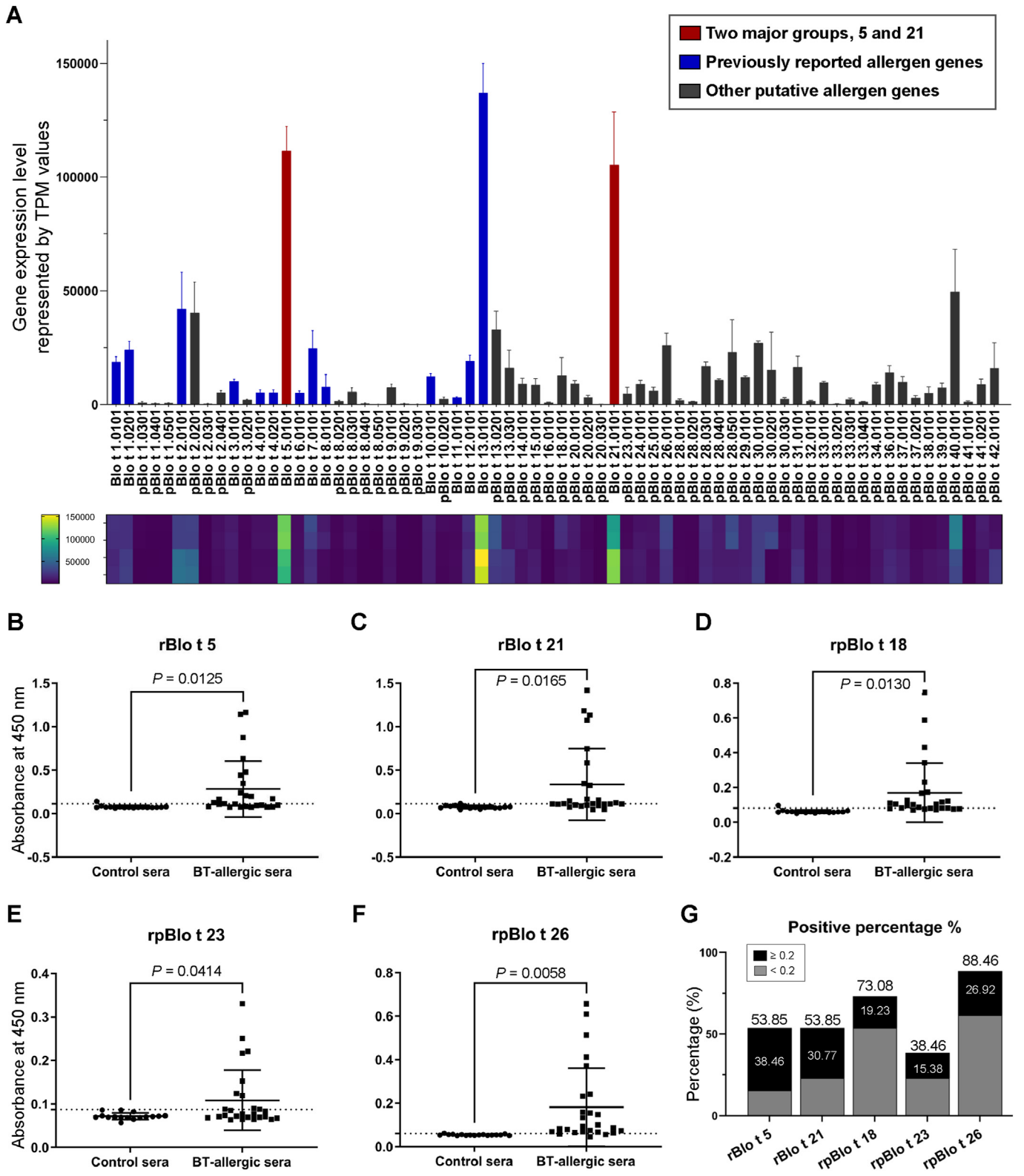

Fig. 1. Genomic analyses of *B. tropicalis* allergens and ELISA results of recombinant proteins. **(A)** Expression level of all the allergens genes predicted in the *B. tropicalis* genome (Table 1). The gene expression level was represented by transcript per million (TPM) values. In the bar chart, error bar means standard deviation (SD). **(B–F)** The IgE antibody binding to recombinant proteins of *B. tropicalis* was evaluated by ELISA using serum samples from 26 patients positive for *B. tropicalis* (BT-allergic sera) for testing and 17 healthy individuals as control sera (Supplementary Table 1). The absorbance values at 450 nm of recombinant proteins were recorded to represent the IgE levels of specific allergens, which are presented on the Y-axis. The lines in dot plots were set as the mean with a standard deviation (SD) and the dotted lines indicated the cutoff value, mean + 2*SD of the healthy controls (Supplementary Table 2). The allergenicity was evaluated for five recombinant proteins as follows: rBlo t 5, rBlo t 21, rpBlo t 18, rpBlo t 23, rpBlo t 26. Unpaired two-tailed *t*-test *P*-values indicate statistical significance (all below 0.05). **(G)** Positive percentage of five recombinant proteins for the allergens of *B. tropicalis*. The absorbance values equal to or higher than 0.2 were regarded as high IgE and allergy levels and their percentages were highlighted in columns.

rBlo t 5 and rBlo t 21 demonstrated only 53.85 %, and the chitin-binding protein rpBlo t 23 showed low sensitization rates 38.46 %. The significant positivity observed for the putative allergens, particularly pBlo t 26 and pBlo t 18, underscores the importance of including them in the component-resolved diagnosis of *B. tropicalis* allergy. However, the two major allergens exhibited significantly higher maximum absorbance values, exceeding 1.0, whereas none of the other allergens reached a value above 0.8. To gain a deeper understanding of the IgE binding activities of these recombinant proteins, we considered absorbance values equal to or higher than 0.2 as indicative of high IgE and allergy levels based on the value distribution. Our findings revealed that the two major allergens, rBlo t 5 and rBlo t 21, exhibited higher percentages of 38.46 % and 30.77 %, respectively (Fig. 1G). The high proportion of high allergy levels to rBlo t 5 and rBlo t 21 indicated the importance of these two major allergens. Considering that nearly all the samples (BT-allergic) were also positive for *D. pteronyssinus* (Supplementary Table 1), we cannot exclude the influence of cross-reactivity in the ELISA results. A significant limitation of this study is the expression of only nine recombinant proteins. Additionally, performing a proteomic analysis of IgE-binding proteins in the whole extract of *B. tropicalis* will offer a comprehensive understanding of the potential allergens.

In summary, this genome-wide analysis has provided valuable insights into the allergen profile of *B. tropicalis*. Furthermore, the comparative analysis has revealed significant divergences within allergen gene families, while the immunoassay experiments conducted on recombinant proteins have supplied serological evidence for novel allergens. Although further experiments examining allergen function and cross-reactivity are warranted, this comprehensive genomic analysis of *B. tropicalis* has significantly enriched our understanding to the allergens of this important mite species.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2023.11.004.

Conflict of interest

The authors have no conflict of interest to declare.

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