

Supplementary data for article:

Smiljanic, K.; Apostolovic, D.; Trifunovic, S.; Ognjenovic, J.; Perusko, M.; Mihajlovic, L.; Burazer, L.; van Hage, M.; Cirkovic Velickovic, T. Subpollen Particles Are Rich Carriers of Major Short Ragweed Allergens and NADH Dehydrogenases: Quantitative Proteomic and Allergomic Study. *Clinical and Experimental Allergy* **2017**, *47* (6), 815–828.

<https://doi.org/10.1111/cea.12874>

Table S6. Protein spot identification lists from TOT, APE and SPP 2D gels analyzed by SEQUEST

Table S6 provides more details and information about protein identified from the spots of 2D S

Final Spot label as on the Figure 5 and Table 2	Green cells represent confidently identified officially recognized Amb allergens or IgE reactive proteins as determined by 2D Immunoblot.	Yellow cells represent confident		
Accession code	Protein Description from UniProt database preceded with the name of the raw file and initial spot labels	Score	Coverage	# Unique Peptides
SPOT 1	OB0343 / spot 1			
P27759	Pollen allergen Amb a 1.1 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA11_AMBAR]	209.59	31.06	6
Q54SK5	Hybrid signal transduction histidine kinase M OS=Dictyostelium discoideum GN=dhkM PE=3 SV=1 - [C	10.97	0.63	1
P49657	Serine/threonine-protein kinase minibrain OS=Drosophila melanogaster GN=mnbr PE=2 SV=2 - [MNB_	7.29	1.32	1
Q9P7D8	Uncharacterized rhomboid protein P4H10.10, mitochondrial OS=Schizosaccharomyces pombe (strain	2.35	4.34	1
P27761	Pollen allergen Amb a 1.3 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA13_AMBAR]	2.34	4.79	1
SPOT 2	OB0344 / spot 2			
P27759	Pollen allergen Amb a 1.1 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA11_AMBAR]	81.96	39.9	8
P27761	Pollen allergen Amb a 1.3 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA13_AMBAR]	10.48	28.72	4
SPOT 3	OB0367 / spot 25			
V5LU01	Cysteine protease OS=Ambrosia artemisiifolia PE=2 SV=1 - [V5LU01_AMBAR]	116.80	20.21	7
SPOT 4	OB2949 / spot 16			
V5LU01	Cysteine protease OS=Ambrosia artemisiifolia PE=2 SV=1 - [V5LU01_AMBAR]	216.0	31.87	10

SPOT 5		OB2953 / spot 9			
V5LU01	Cysteine protease OS=Ambrosia artemisiifolia PE=2 SV=1 - [V5LU01_AMBAR]		115.0	20.98	8
SPOT 6		OB0371 / spot 29			
P27760	Pollen allergen Amb a 1.2 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA12_AMBAR]		124.55	53.27	5
P27761	Pollen allergen Amb a 1.3 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA13_AMBAR]		89.73	38.29	5
P26519	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic OS=Petroselinum crispum GN=GAPC PE=2 SV=1		15.8	14.88	2
B3E846	50S ribosomal protein L18 OS=Geobacter lovleyi (strain ATCC BAA-1151 / DSM 17278 / SZ) GN=rpL8		11.67	11.48	1
Q0J8A4	Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic OS=Oryza sativa subsp. japonica GN=GAPC1		10.23	10.98	1
P46257	Fructose-bisphosphate aldolase, cytoplasmic isozyme 2 OS=Pisum sativum PE=1 SV=1 - [ALF2_PEA]		8.85	7.8	2
Q9SRT9	UDP-arabinopyranose mutase 1 OS=Arabidopsis thaliana GN=RGP1 PE=1 SV=1 - [RGP1_ARATH]		8.66	10.92	2
SPOT 7		OB0370 / spot 28			
P27760	Pollen allergen Amb a 1.2 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA12_AMBAR]		559.06	51.76	9
P27761	Pollen allergen Amb a 1.3 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA13_AMBAR]		182.73	32.24	3
Q7N7B1	Gamma-glutamyl phosphate reductase OS=Photobacterium luminescens subsp. laumondii (strain TT01)		4.89	6.24	1
P46257	Fructose-bisphosphate aldolase, cytoplasmic isozyme 2 OS=Pisum sativum PE=1 SV=1 - [ALF2_PEA]		4.47	7.52	1
SPOT 8		OB0365 / spot 23			
P27761	Pollen allergen Amb a 1.3 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA13_AMBAR]		519.78	67.25	10
P27760	Pollen allergen Amb a 1.2 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA12_AMBAR]		151.27	28.14	1
Q9HM19	DNA-binding protein Ta0052 OS=Thermoplasma acidophilum (strain ATCC 25905 / DSM 1728 / JCM 9)		35.41	11.3	1

Q54KN4	Alpha-mannosidase F OS=Dictyostelium discoideum GN=manF PE=3 SV=1 - [MANF_DICDI]	32.79	1.41	1
B7XK16	Probable isoleucine--tRNA ligase, cytoplasmic OS=Enterocytozoon bieneusi (strain H348) GN=EBI_228	18.95	1.36	1
O04015	Delta-1-pyrroline-5-carboxylate synthase OS=Actinidia deliciosa PE=2 SV=1 - [P5CS_ACTDE]	14.09	1.95	1
Q97EJ2	30S ribosomal protein S8 OS=Clostridium acetobutylicum (strain ATCC 824 / DSM 792 / JCM 1419 / L	12.15	13.64	1
P27759	Pollen allergen Amb a 1.1 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA11_AMBAR]	9.16	9.6	1
P46257	Fructose-bisphosphate aldolase, cytoplasmic isozyme 2 OS=Pisum sativum PE=1 SV=1 - [ALF2_PEA]	7.24	7.8	1
Q42962	Phosphoglycerate kinase, cytosolic OS=Nicotiana tabacum PE=1 SV=1 - [PGKY_TOBAC]	6.97	10.97	2
Q43321	Enolase OS=Alnus glutinosa GN=PGH1 PE=2 SV=1 - [ENO_ALNGL]	6.64	12.27	1
P28744	Pollen allergen Amb a 1.4 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA14_AMBAR]	6.07	7.91	2
P85920	Malate dehydrogenase (Fragments) OS=Pseudotsuga menziesii PE=1 SV=1 - [MDH_PSEMZ]	5.45	100	1
SPOT 9	OB0368 / spot 26			
P27761	Pollen allergen Amb a 1.3 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA13_AMBAR]	279.4	57.68	10
P46257	Fructose-bisphosphate aldolase, cytoplasmic isozyme 2 OS=Pisum sativum PE=1 SV=1 - [ALF2_PEA]	8.57	7.8	1
SPOT 10	OB0345 / spot 3			
P28744	Pollen allergen Amb a 1.4 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA14_AMBAR]	29.24	12.76	2
SPOT 11	OB0377 / spot 35			
P27762	Pollen allergen Amb a 2 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPAA2_AMBAR]	177.98	43.64	13
P27759	Pollen allergen Amb a 1.1 OS=Ambrosia artemisiifolia PE=1 SV=1 - [MPA11_AMBAR]	24.92	24.75	5

Q42961	Phosphoglycerate kinase, chloroplastic OS=Nicotiana tabacum PE=1 SV=1 - [PGKH_TOBAC]	6.79	11.43	2
SPOT 12				OB2941 / spot 1
D4III3	Ragweed homologue of Art v 1 (Fragment) OS=Ambrosia artemisiifolia GN=rha1 PE=2 SV=1 - [D4III3_	227.01	49.45	3
D4IIH1	Ragweed homologue of Art v 1 (Fragment) OS=Ambrosia artemisiifolia GN=rha1 PE=2 SV=1 - [D4IIH1	96.58	15.71	4
G5EN35	Actin OS=Chrysanthemum seticuspe f. boreale GN=CsActin PE=2 SV=1 - [G5EN35_9ASTR]	25.27	29.97	2
E1XUL2	Pectate lyase OS=Ambrosia artemisiifolia GN=amba1 PE=2 SV=1 - [E1XUL2_AMBAR]	23.25	15.4	2
P27759	Pectate lyase 5 OS=Ambrosia artemisiifolia PE=1 SV=1 - [PLY5_AMBAR]	23.25	15.4	2
SPOT 13				OB2942 / spot 2
D4III3	Ragweed homologue of Art v 1 (Fragment) OS=Ambrosia artemisiifolia GN=rha1 PE=2 SV=1 - [D4III3_	145.12	49.45	3
D4IIH1	Ragweed homologue of Art v 1 (Fragment) OS=Ambrosia artemisiifolia GN=rha1 PE=2 SV=1 - [D4IIH1	87.31	15.71	2
G5EN35	Actin OS=Chrysanthemum seticuspe f. boreale GN=CsActin PE=2 SV=1 - [G5EN35_9ASTR]	19.97	24.40	2
E1XUL2	Pectate lyase OS=Ambrosia artemisiifolia GN=amba1 PE=2 SV=1 - [E1XUL2_AMBAR]	18.75	15.4	2
P27759	Pectate lyase 5 OS=Ambrosia artemisiifolia PE=1 SV=1 - [PLY5_AMBAR]	18.75	15.4	2
SPOT 14				OB29433 / spot 3
D4III3	Ragweed homologue of Art v 1 (Fragment) OS=Ambrosia artemisiifolia GN=rha1 PE=2 SV=1 - [D4III3_	60.32	39.56	3
D4IIH1	Ragweed homologue of Art v 1 (Fragment) OS=Ambrosia artemisiifolia GN=rha1 PE=2 SV=1 - [D4IIH1	42.11	9.29	1
P48493	Triosephosphate isomerase, cytosolic (Fragment) OS=Lactuca sativa PE=2 SV=1 - [TPIS_LACSA]	126.94	68.21	7
SPOT 15				OB2944 / spot 4

D4IIH1	Ragweed homologue of Art v 1 (Fragment) OS=Ambrosia artemisiifolia GN=rha1 PE=2 SV=1 - [D4IIH_	78.06	15.71	2
D4III3	Ragweed homologue of Art v 1 (Fragment) OS=Ambrosia artemisiifolia GN=rha1 PE=2 SV=1 - [D4III3_	26.80	9.45	1
A0A068EQ30	Glyceraldehyde-3-phosphate dehydrogenase OS=Carthamus tinctorius GN=GAPDH PE=2 SV=1 - [A0A	22.65	19.35	2
P48493	Triosephosphate isomerase, cytosolic (Fragment) OS=Lactuca sativa PE=2 SV=1 - [TPIS_LACSA]	19.78	18.72	2
SPOT 16				
Q84ZX5	Major pollen allergen Art v 1 OS=Artemisia vulgaris PE=1 SV=1	62.64	14.1	1
SPOT group 17	SPOT GROUP 17 comprised of 17-21 labelled individual spots			
	OB0359 / spot 17			
Q43321	Enolase OS=Alnus glutinosa GN=PGH1 PE=2 SV=1 - [ENO_ALNGL]	90.53	19.32	1
P26301	Enolase 1 OS=Zea mays GN=ENO1 PE=1 SV=1 - [ENO1_MAIZE]	79.76	17.71	2
SPOT group 17	SPOT GROUP 17 comprised of 17-21 labelled individual spots			
	OB0360 / spot 18			
P19595	UTP--glucose-1-phosphate uridylyltransferase OS=Solanum tuberosum PE=1 SV=3 - [UGPA_SOLTU]	181.13	16.98	1
P57751	UTP--glucose-1-phosphate uridylyltransferase 1 OS=Arabidopsis thaliana GN=At5g17310 PE=2 SV=1 -	129.51	13.83	1
Q9LKG7	UTP--glucose-1-phosphate uridylyltransferase OS=Astragalus penduliflorus GN=UGP PE=1 SV=1 - [UG	42.85	8.28	1
O64459	UTP--glucose-1-phosphate uridylyltransferase OS=Pyrus pyrifolia PE=2 SV=1 - [UGPA_PYRPY]	22.07	7.01	1
Q43772	UTP--glucose-1-phosphate uridylyltransferase OS=Hordeum vulgare PE=2 SV=1 - [UGPA_HORVU]	9.76	10.15	1
SPOT group 17	SPOT GROUP 17 comprised of 17-21 labelled individual spots			
	OB0361 / spot 19			
Q43772	UTP--glucose-1-phosphate uridylyltransferase OS=Hordeum vulgare PE=2 SV=1 - [UGPA_HORVU]	2.31	3.81	1
SPOT group 17	SPOT GROUP 17 comprised of 17-21 labelled individual spots			
	OB0362 /spot 20			

P19595	UTP--glucose-1-phosphate uridylyltransferase OS=Solanum tuberosum PE=1 SV=3 - [UGPA_SOLTU]	54.29	16.98	3
O22818	Probable polygalacturonase At2g43860 OS=Arabidopsis thaliana GN=At2g43860 PE=2 SV=1 - [PGLR6	35.6	3.46	1
Q9LKG7	UTP--glucose-1-phosphate uridylyltransferase OS=Astragalus penduliflorus GN=UGP PE=1 SV=1 - [UG	23.97	8.28	1
SPOT group 17	SPOT GROUP 17 comprised of 17-21 labelled individual spots			
P19595	UTP--glucose-1-phosphate uridylyltransferase OS=Solanum tuberosum PE=1 SV=3 - [UGPA_SOLTU]	26.37	13.63	1
O22818	Probable polygalacturonase At2g43860 OS=Arabidopsis thaliana GN=At2g43860 PE=2 SV=1 - [PGLR6	24.86	3.46	1
P57751	UTP--glucose-1-phosphate uridylyltransferase 1 OS=Arabidopsis thaliana GN=At5g17310 PE=2 SV=1 -	23.97	7.45	1
Q9LKG7	UTP--glucose-1-phosphate uridylyltransferase OS=Astragalus penduliflorus GN=UGP PE=1 SV=1 - [UG	15.29	6.16	2
Q43772	UTP--glucose-1-phosphate uridylyltransferase OS=Hordeum vulgare PE=2 SV=1 - [UGPA_HORVU]	9.45	8.03	1
SPOT 18				
Q2KN23	Profilin OS=Ambrosia artemisiifolia PE=2 SV=1 - [Q2KN23_AMBAR]	34.64	36.84	3
Q64LH0	Profilin-3 OS=Ambrosia artemisiifolia GN=D03 PE=1 SV=1 - [PROF3_AMBAR]	27.20	27.07	2
SPOT group 19	SPOT GROUP 19 (comprised of 32,33,34)-			
Q9M9K1	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate mutase 2 OS=Arabidopsis thaliana	18.53	11.25	2
SPOT group 19	SPOT GROUP 19 (comprised of 32,33,34)-			
P93805	Phosphoglucomutase, cytoplasmic 2 OS=Zea mays PE=2 SV=2 - [PGMC2_MAIZE]	13.04	8.14	1
SPOT group 19	SPOT GROUP 19 (comprised of 32,33,34)- initially labelled spots			

No confident hits

SPOT 20		16A NRD_YO / spot 16			
P00304	Pollen allergen Amb a 3 OS=Ambrosia artemisiifolia var. elatior PE=1 SV=2 - [MPAA3_AMBEL]		189.84	34.65	1
SPOT 21		17A NRD_YO / spot 17			
P02878	Pollen allergen Amb a 5 OS=Ambrosia artemisiifolia var. elatior PE=1 SV=1 - [MPAA5_AMBEL]		44.37	73.33	4
SPOT 22		18ANRD_YO-01 / spot 18			
O04004	Non-specific lipid-transfer protein OS=Ambrosia artemisiifolia PE=1 SV=1 - [NLTP6_AMBAR]		55.27	40.68	5
SPOT 23		OB0347 / spot 5			
Q2KN26	Calcium-binding protein isoallergen 2 OS=Ambrosia artemisiifolia PE=2 SV=1		31.74	12	2
SPOT X1		20A_YO-01/ spot 20			
	No confident hits. Shown as IgE reactive in TOT and APE				
SPOT X2		OB2961 / spot 24A			
	No confident hits. No evident IgE reactivity at all. Present only in SPP fraction				
SPOT X3		31A NRD_YO-01 / spot 31			
	No confident hits. Shown as immunoreactive in TOT fraction				
SPOT X4		OB2962 / spot 27			
	No confident hits. Shown IgE immunoreactive in all three fractions.				

ST algorithm.

SDS-PAGE.

Identified proteins.

# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI	pI GEL	Mw GEL
9	169	396	42.7	5.90	5.9	43
1	21	2388	264.7	7.02		
1	11	908	95.8	8.85		
1	4	392	44.2	9.76		
1	1	397	42.9	6.10		
14	53	396	42.7	5.9	5.8	43
8	11	397	42.9	6.1		
7	48	386	43.13	6.9	6.4	41
15	85	386	43.13	6.9	6.6	41

					6.9	41
8	45	386	43.13	6.9		
					8.1	38
14	99	398	43.6	7.08		
12	52	397	42.9	6.1		
4	7	336	36.3	7.47		
1	18	122	13.1	10.68		
4	6	337	36.4	7.11		
3	4	359	38.5	7.25		
4	5	357	40.6	5.92		
					8.3	38
17	400	398	43.6	7.08		
10	121	397	42.9	6.1		
1	15	417	45.5	5.6		
2	3	359	38.5	7.25		
					6.1	38
18	291	397	42.9	6.1		
8	75	398	43.6	7.08		
1	19	115	14	8.6		

1	21	994	114.3	7.25		
1	21	953	112	7.37		
1	8	717	77.4	6.84		
1	15	132	14.7	9.67		
2	3	396	42.7	5.9		
3	4	359	38.5	7.25		
3	3	401	42.3	5.97		
3	4	440	47.6	5.58		
2	2	392	42.8	5.97		
2	2	23	2.5	4.75		
					6.3	38
16	200	397	42.9	6.1		
3	5	359	38.5	7.25		
					6.0	42
4	10	392	42.8	5.97		
					6.3-6.8	43
13	64	397	44.1	6.47		
7	10	396	42.7	5.9		

4	4	481	50.1	8.38		
					4.9	26
6	82	91	4.4	4.47		
4	37	140	13.3	5.05		
6	10	377	41.6	5.49		
4	8	396	42.7	5.90		
4	8	396	42.7	5.90		
					5.2	26
6	49	91	4.4	4.5		
4	30	140	13.3	5.1		
6	7	377	41.6	5.5		
4	6	396	42.7	5.9		
4	6	396	42.7	5.9		
					5.4	26
4	16	91	4.4	4.5		
2	11	140	13.3	5.1		
11	32	195	20.5	5.4		
					5.9	26

4	31	140	13.25	5.05		
2	13	91	4.4	4.5		
6	7	341	37.1	7.2		
4	10	195	20.5	5.4		
					9.0	27
2	20	132	13.4	7.62		
					5.9	56
8	77	440	47.6	5.58		
8	77	446	48	5.33		
					5.7	56
7	116	477	51.8	6		
6	87	470	51.9	6.01		
5	37	471	51.5	6.29		
4	13	471	51.8	6.39		
5	17	473	51.6	5.34		
					5.5	53
1	1	473	51.6	5.34		
					5.4	53

6	35	477	51.8	6		
2	23	405	43.4	6.86		
4	16	471	51.5	6.29		
					5.3	53
5	18	477	51.8	6		
2	25	405	43.4	6.86		
3	13	470	51.9	6.01		
3	6	471	51.5	6.29		
3	5	473	51.6	5.34		
					4.6-4.6	14
3	9	133	14.1	5.02		
2	8	133	14.3	5.17		
					6.7	62
4	6	560	60.7	5.85		
					6.6	62
4	5	583	63	5.71		
					6.3	62

3	59	101	11.4	6.52	6.6	10
4	12	45	5.0	7.87	8.0	5
5	21	118	12.78	8.56	8.5	11
2	2	83	9.3	4.15	4.3	10
					7.2	16
					9.1	16
					6.5	17
					5.9	10

