Species Identification of Necrophagous Insect Eggs Based on Amino Acid Profile Differences Revealed by Direct Analysis in Real Time-High Resolution Mass Spectrometry

SUPPORTING INFORMATION

Justine E. Giffen[1], Jennifer Y. Rosati[2], Cameron M. Longo[1] and Rabi A. Musah[1*]

[1]Department of Chemistry, University at Albany, State University of New York, 1400 Washington Avenue, Albany, NY 12222, USA

[2]John Jay College of Criminal Justice, 524 West 59th St, New York, NY, 10019, USA

*Corresponding author: rmusah@albany.edu

This document contains 30 additional figures and 1 table of supporting information pertaining to the entitled article. The figures show DART-HRMS in-source CID spectral data of aqueous ethanol egg extracts and amino acid standards, MALDI-MS/MS spectral data of fly eggs, and an image of a TLC plate. The table contains information on fly egg spectra, including relative abundances and accurate masses of various peaks.

Figures

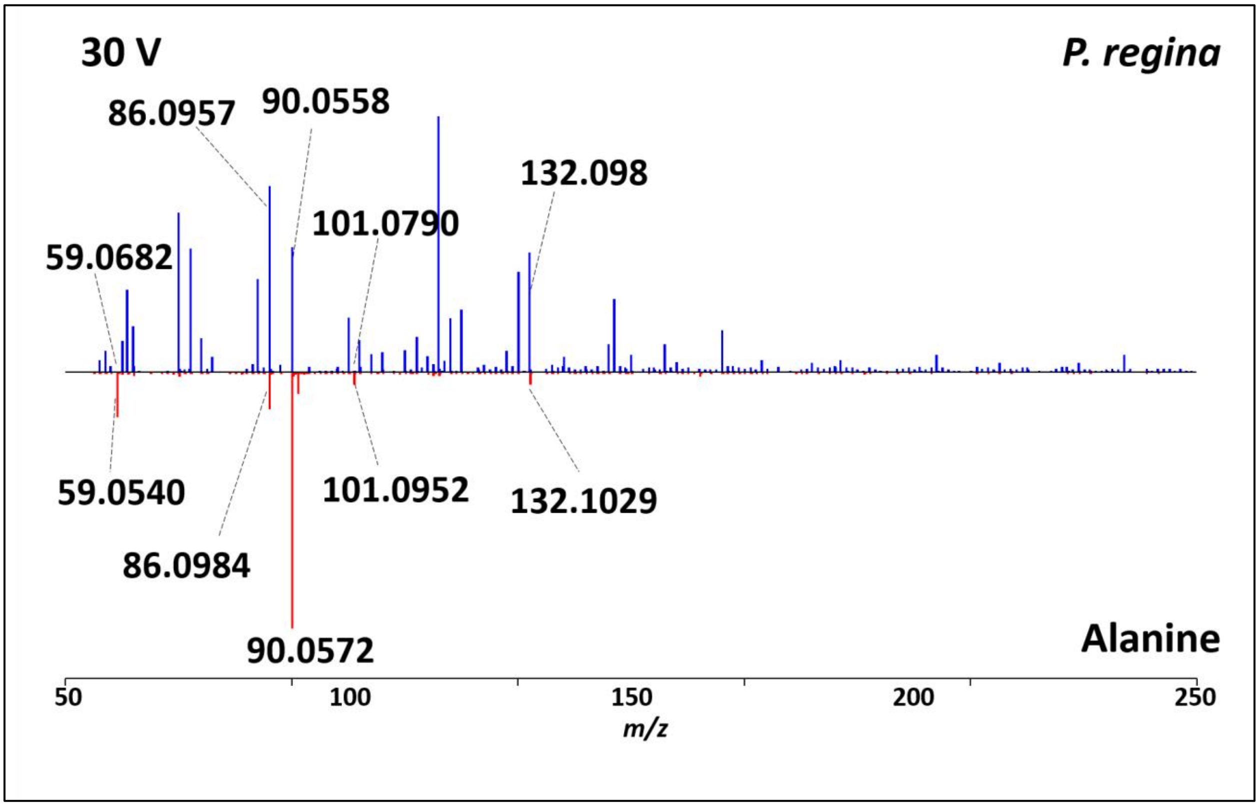

Figure S1. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid alanine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 30 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the alanine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

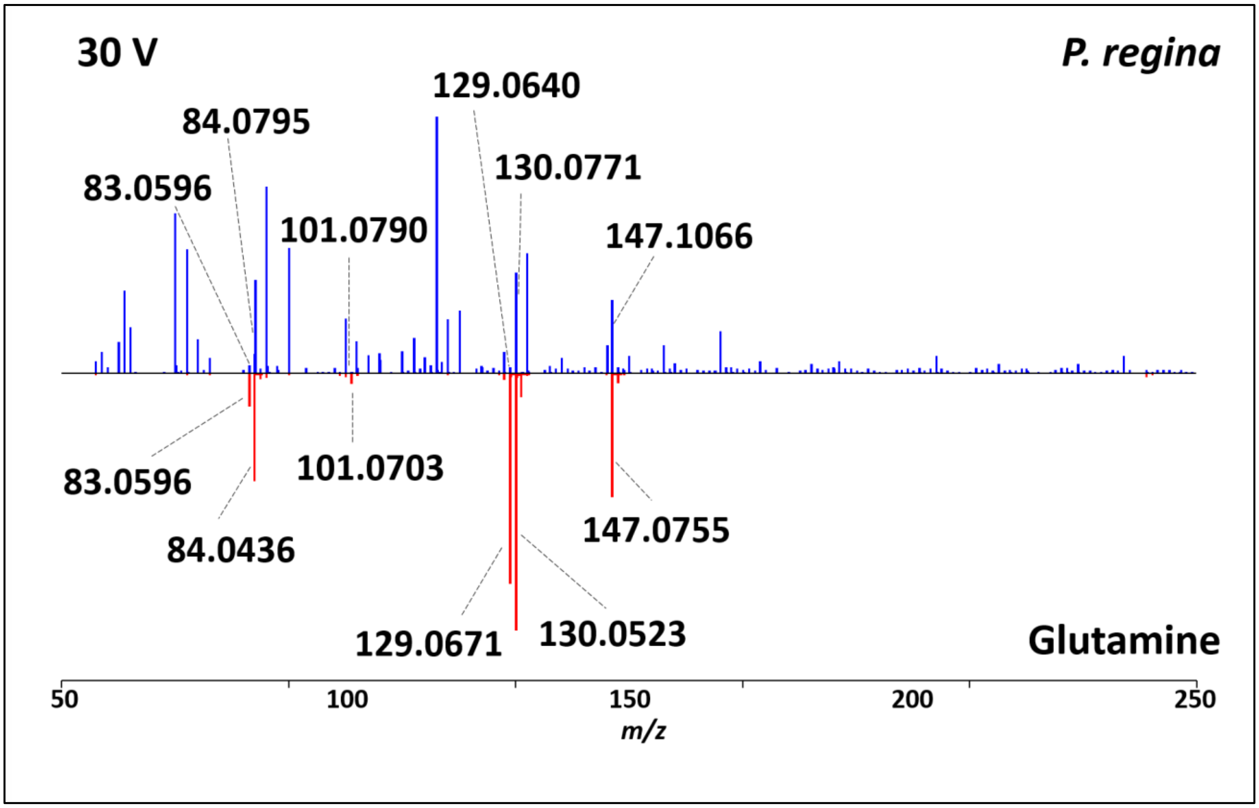

Figure S2. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid glutamine in the *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 30 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the glutamine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

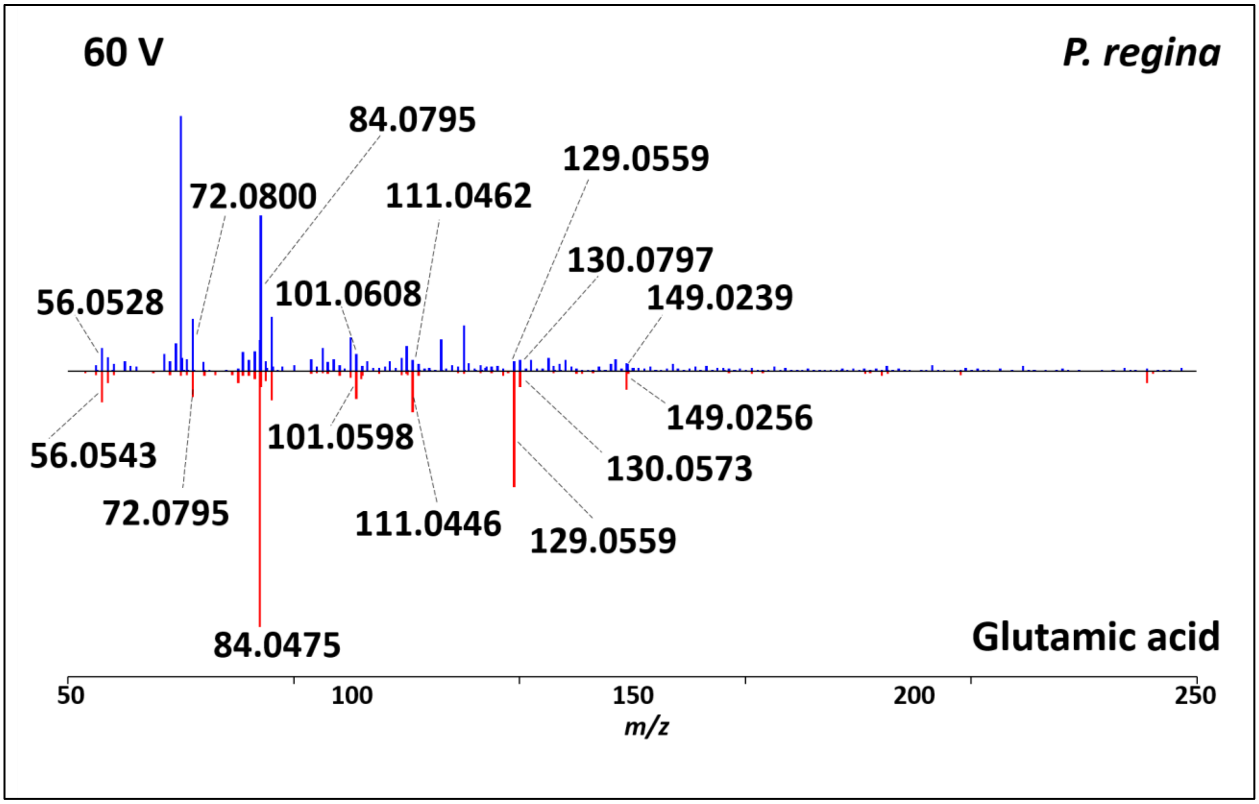

Figure S3. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid glutamic acid in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the glutamic acid standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

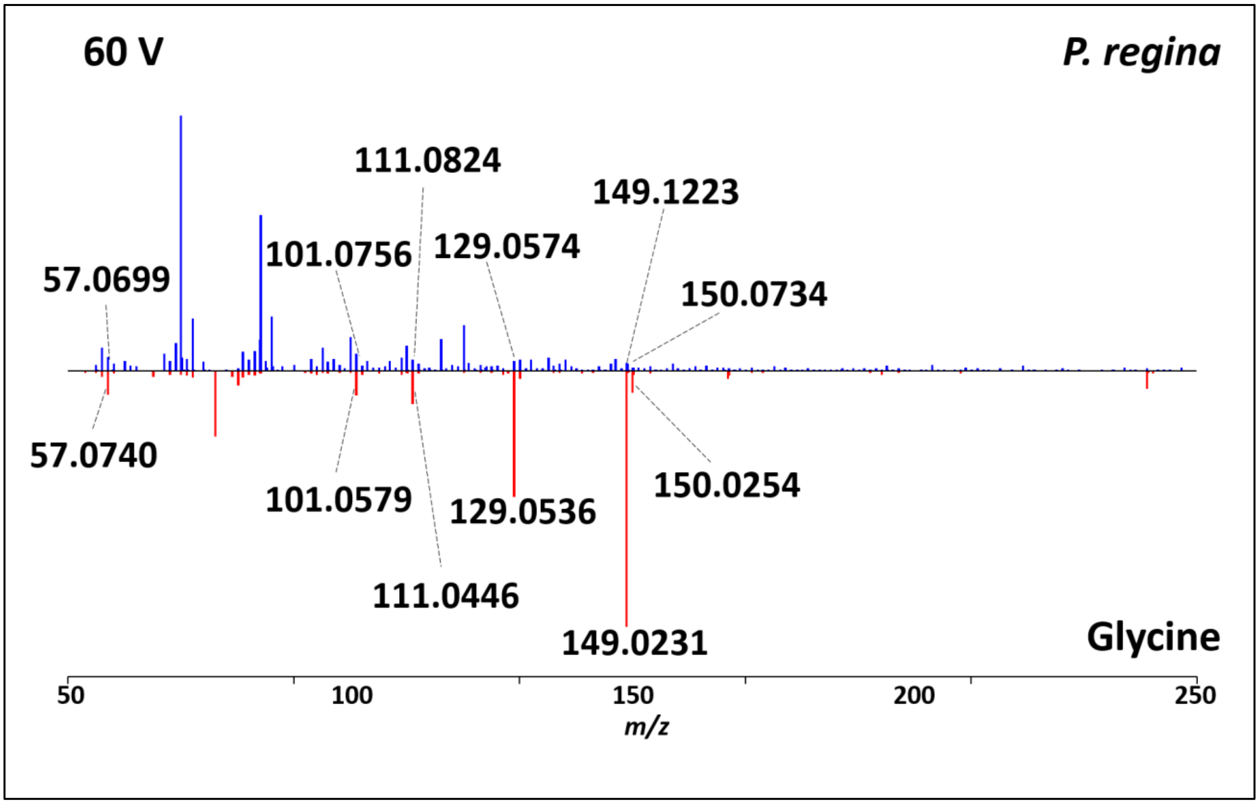

Figure S4. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid glycine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the glycine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

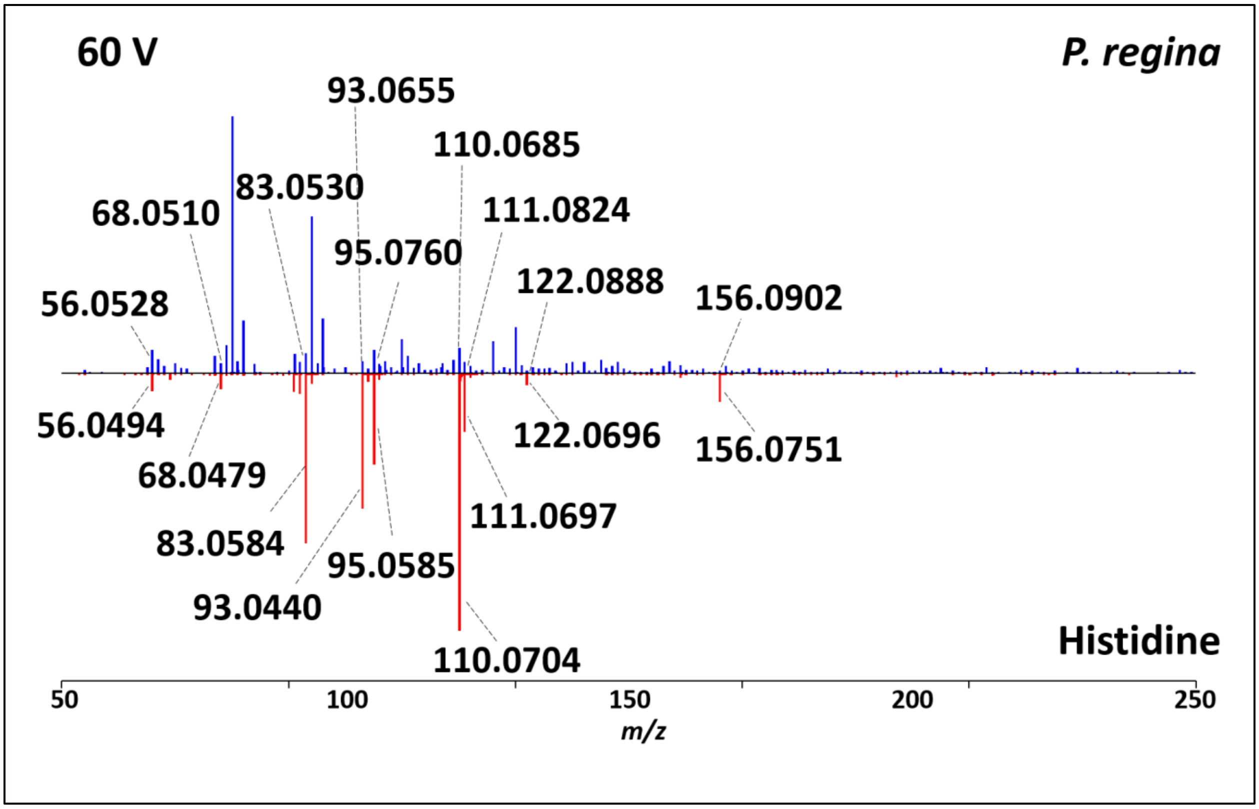

Figure S5. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid histidine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the histidine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

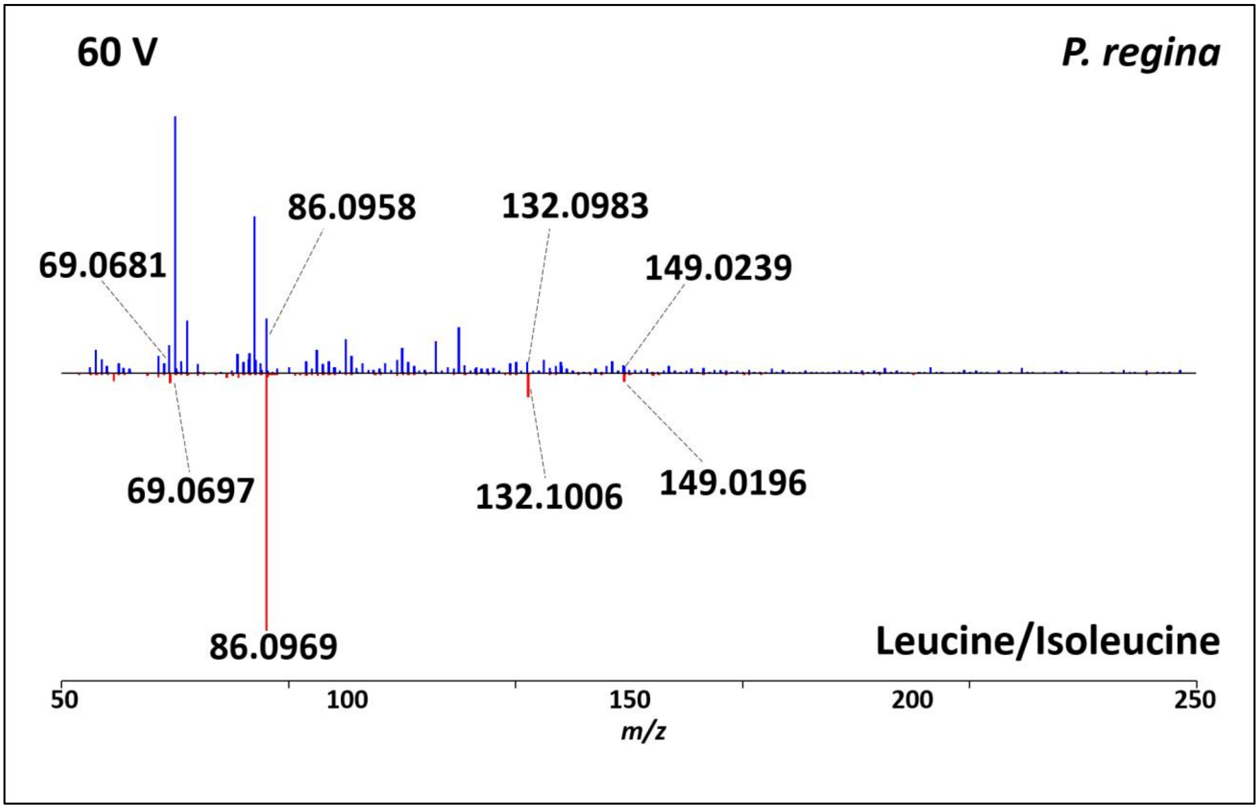

Figure S6. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid leucine/isoleucine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the leucine/isoleucine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

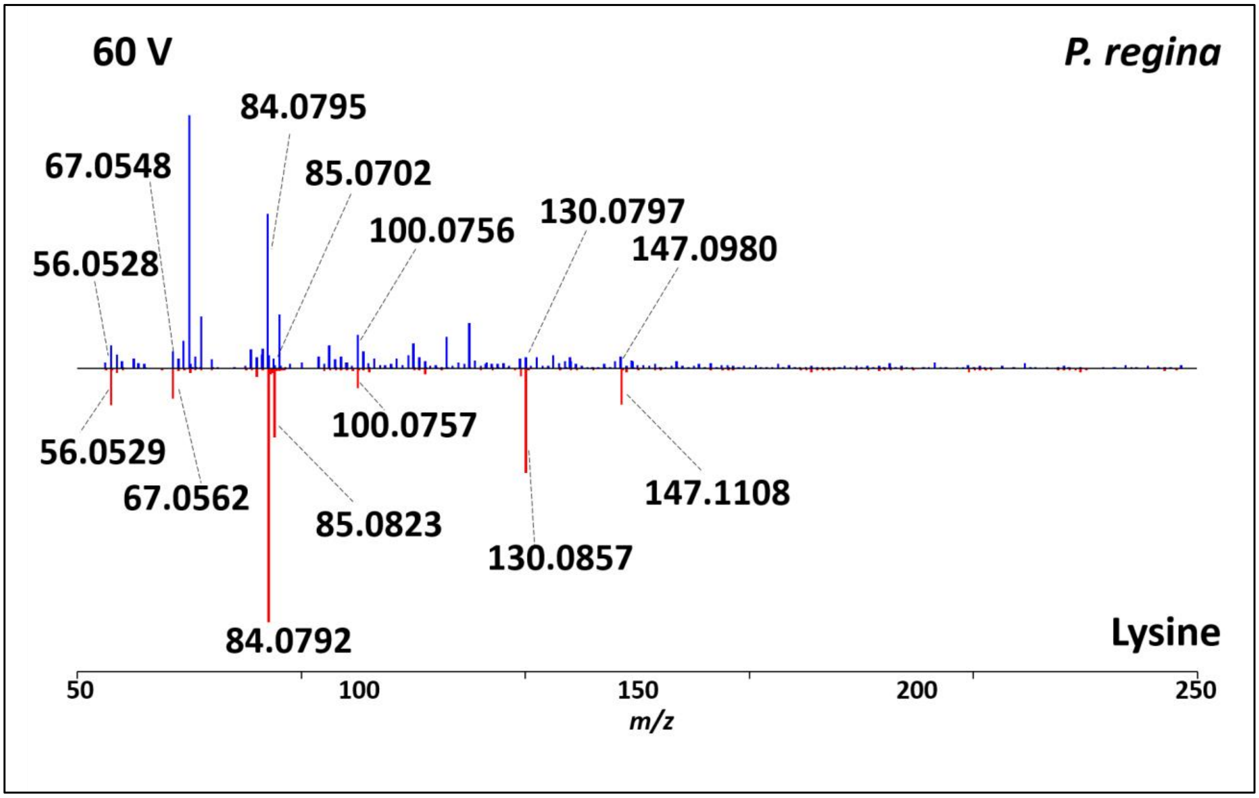

Figure S7. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid lysine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the lysine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

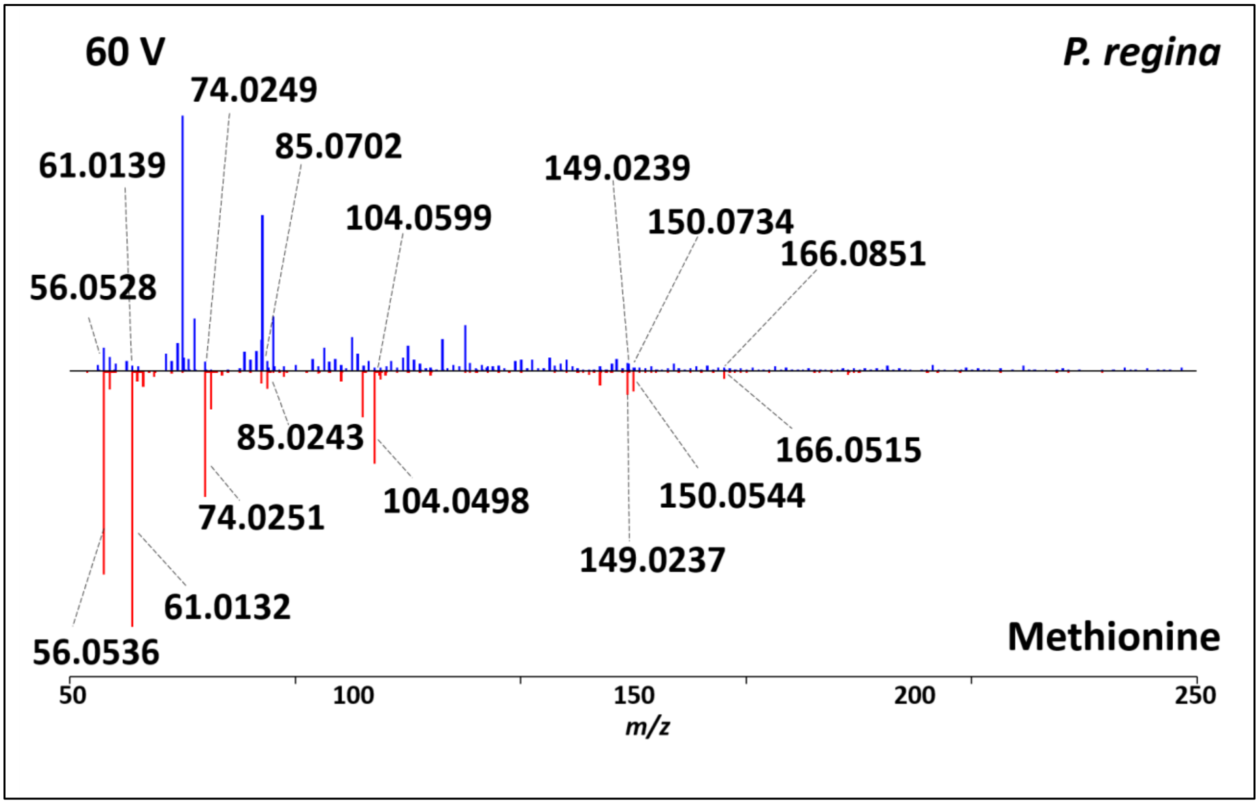

Figure S8. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid methionine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the methionine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

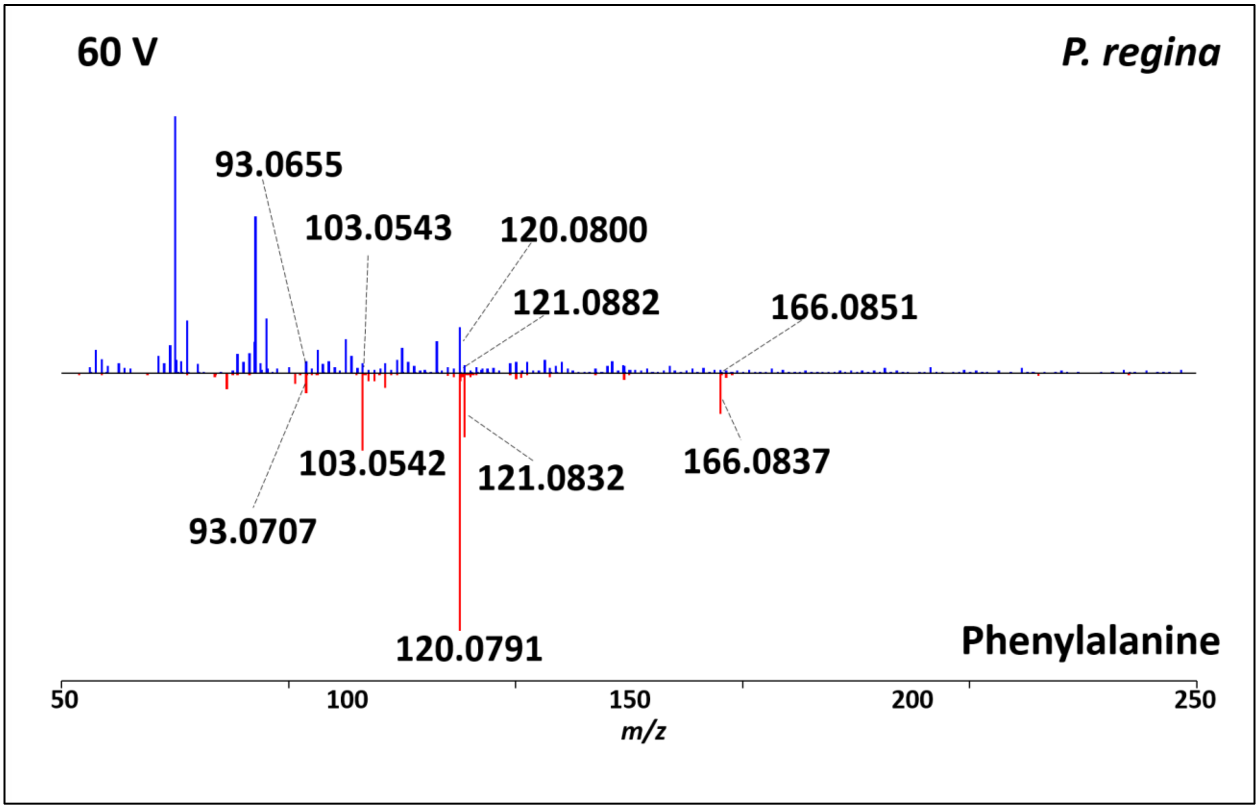

Figure S9. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid phenylalanine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the phenylalanine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

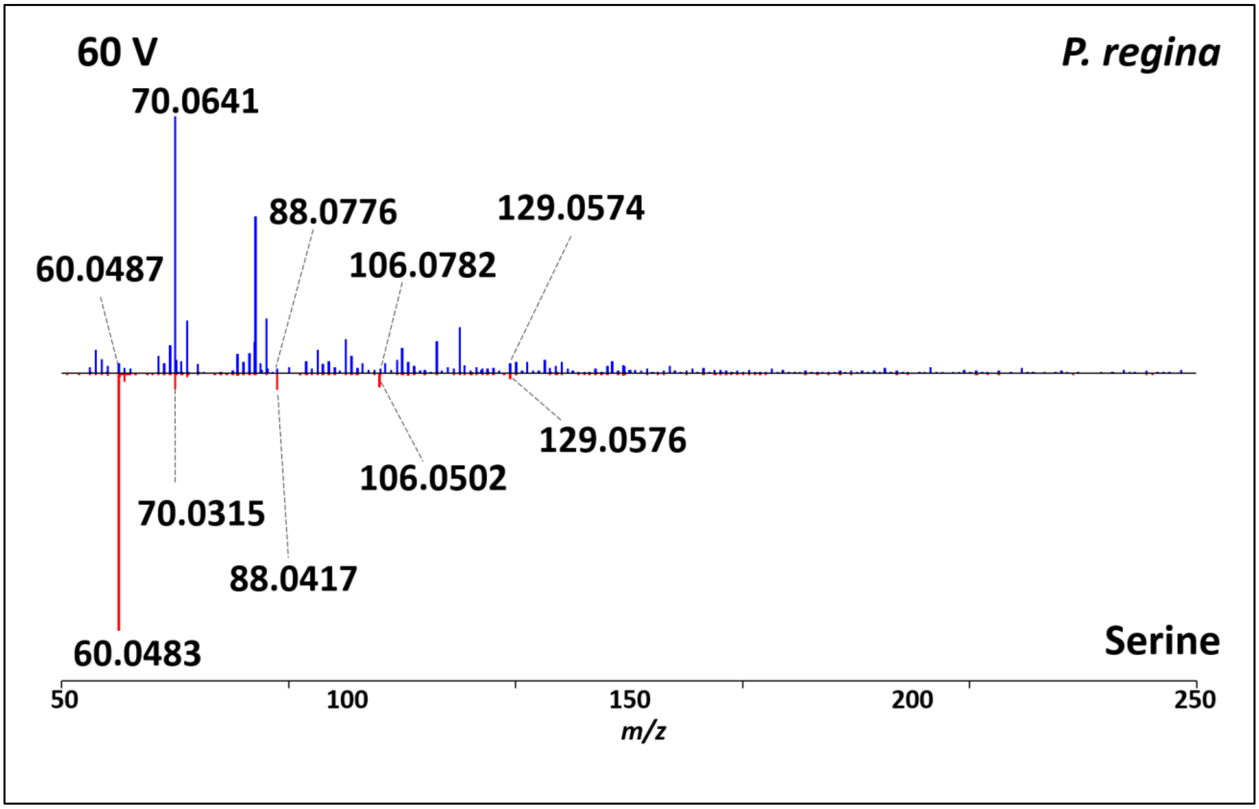

Figure S10. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid serine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the serine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

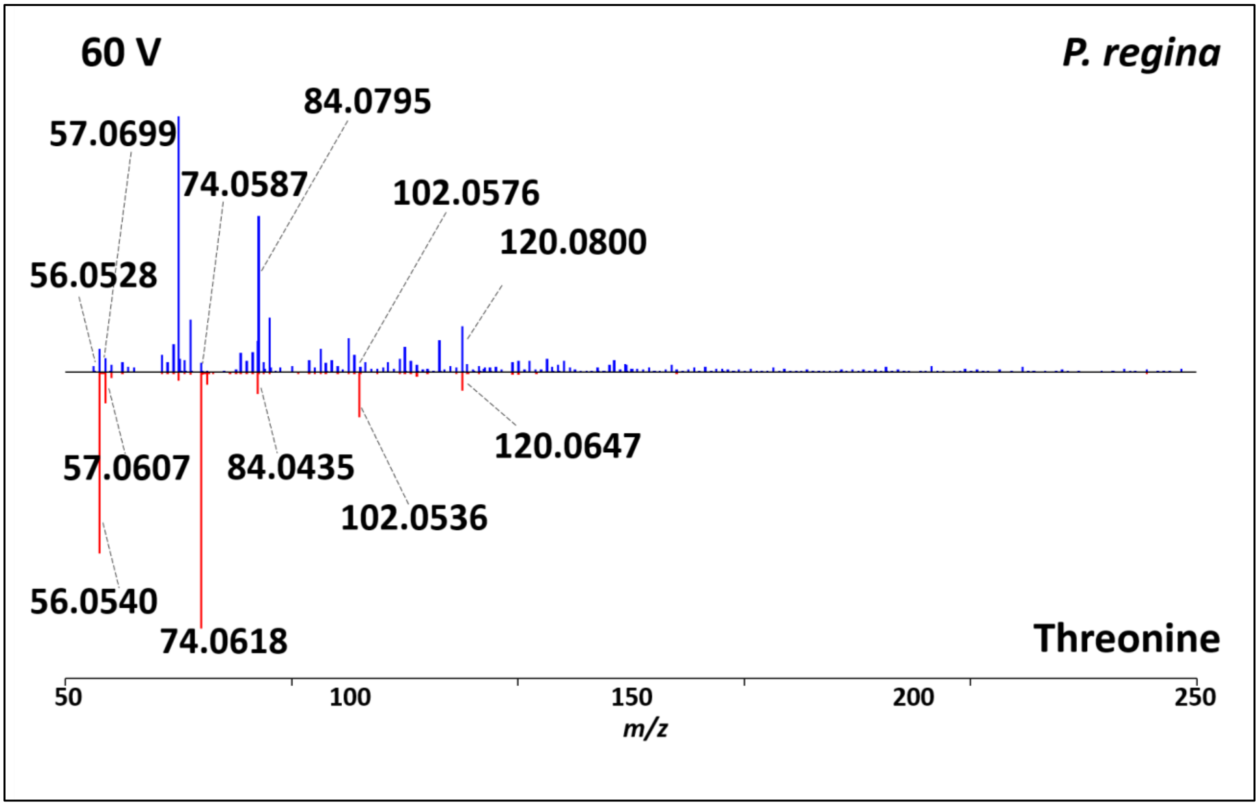

Figure S11. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid threonine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 60 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the threonine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

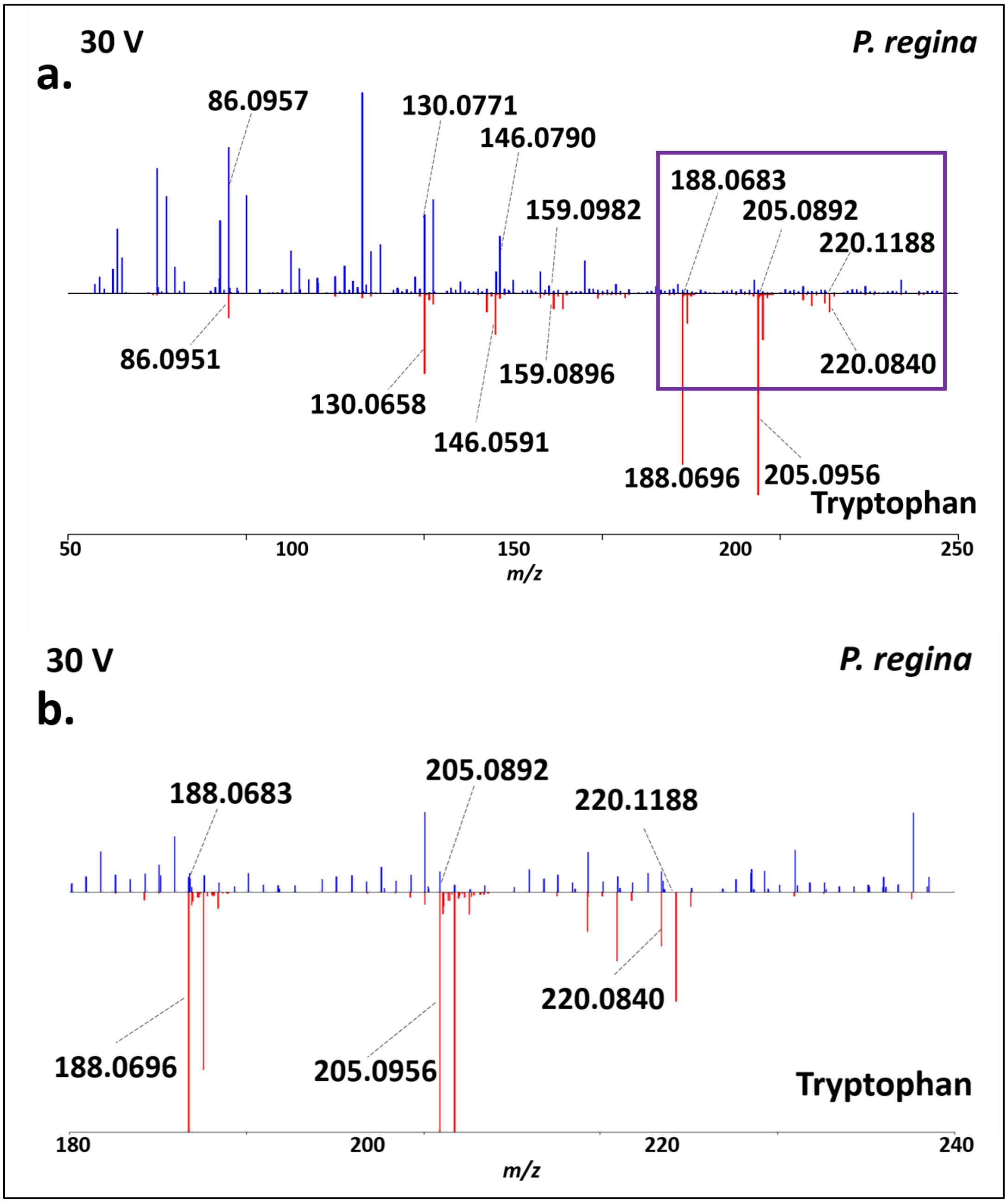

Figure S12. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid tryptophan in *P. regina* eggs. Panel a shows the full spectra and Panel b depicts a magnified section of the *m/z* range shown in the purple box to highlight several ions of low abundance. In both panels, the top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 30 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the tryptophan standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

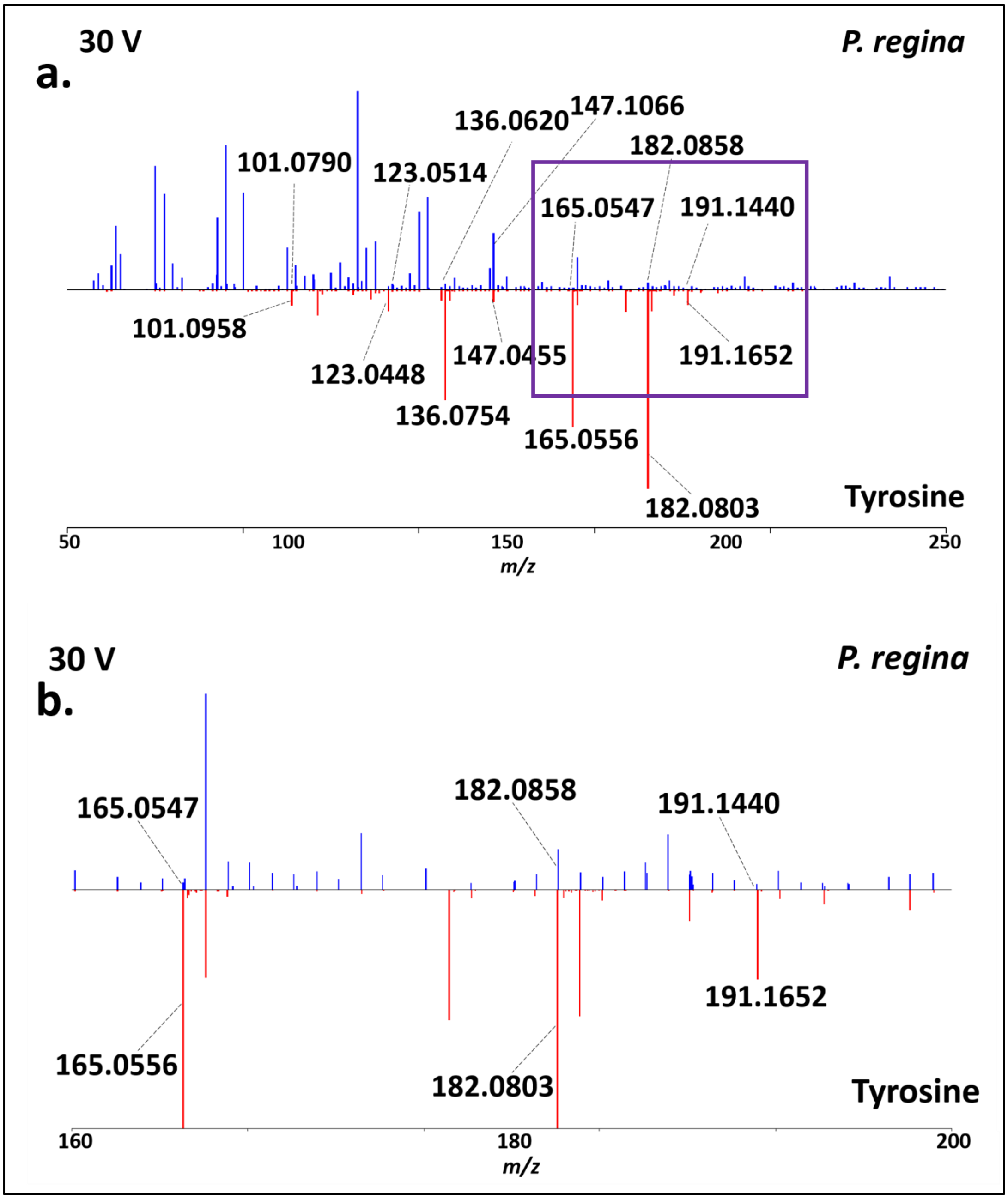

Figure S13. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid tyrosine in *P. regina* eggs. Panel a shows the full spectra and Panel b depicts a magnified section of the *m/z* range shown in the purple box to highlight several ions of low abundance. In both panels, the top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 30 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the tyrosine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

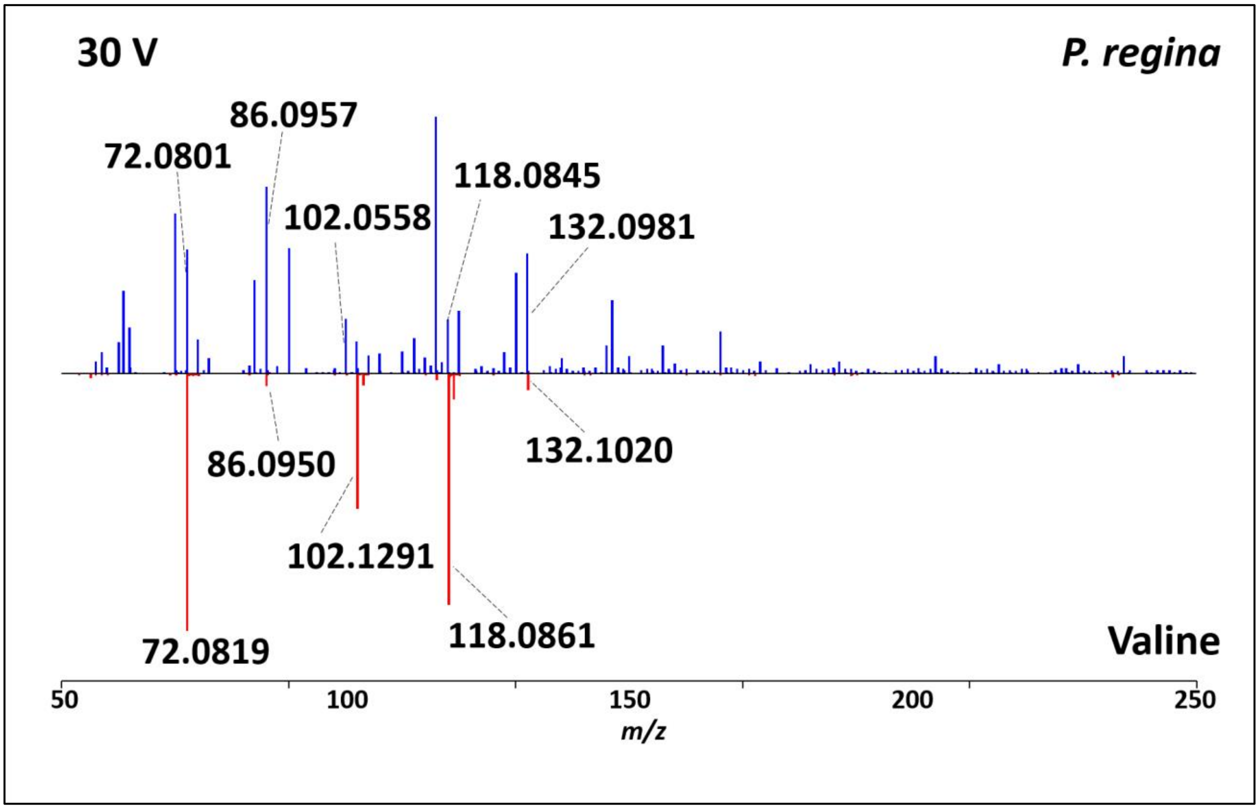

Figure S14. Head-to-tail plot displaying the results of the in-source CID experiments performed to tentatively confirm the presence of the amino acid valine in *P. regina* eggs. The top spectrum shows the analysis of the ethanol extract of the egg sample and the bottom, that of the amino acid standard. Both spectra were recorded at 30 V, which was determined to be the optimal voltage to induce fragmentation while still retaining the protonated precursor ion. The presence in the egg spectrum of the peaks from the valine standard indicates that the amino acid is found within the eggs. Both the top and bottom spectra represent an average of 3 individual analyses.

Arginine

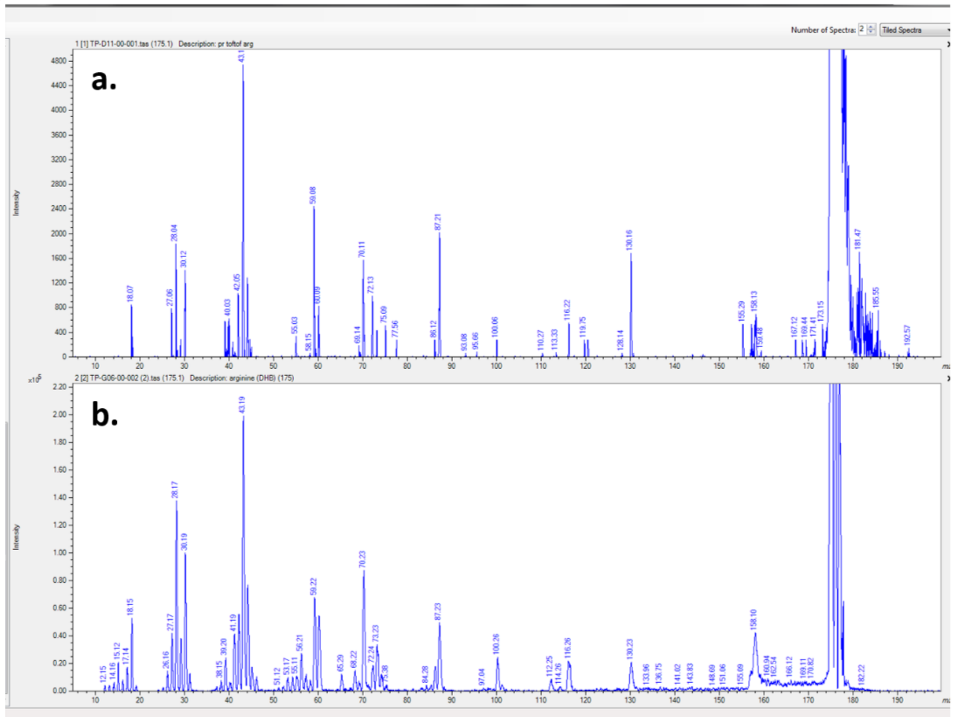

Figure S15. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid arginine (*m/z* 175.12) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the arginine authentic standard.

Asparagine

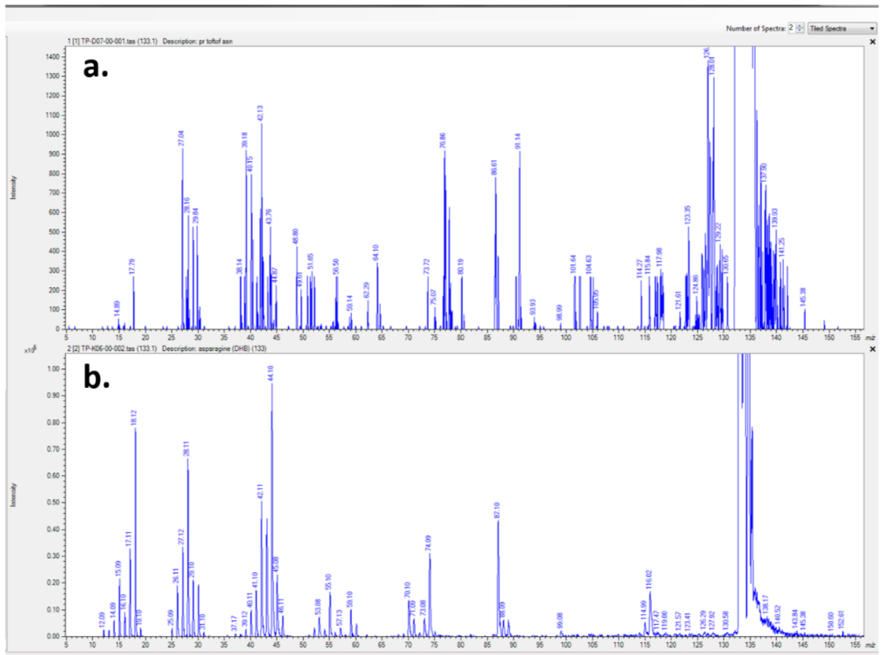

Figure S16. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid asparagine (*m/z* 133.06) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the asparagine authentic standard.

Aspartic acid

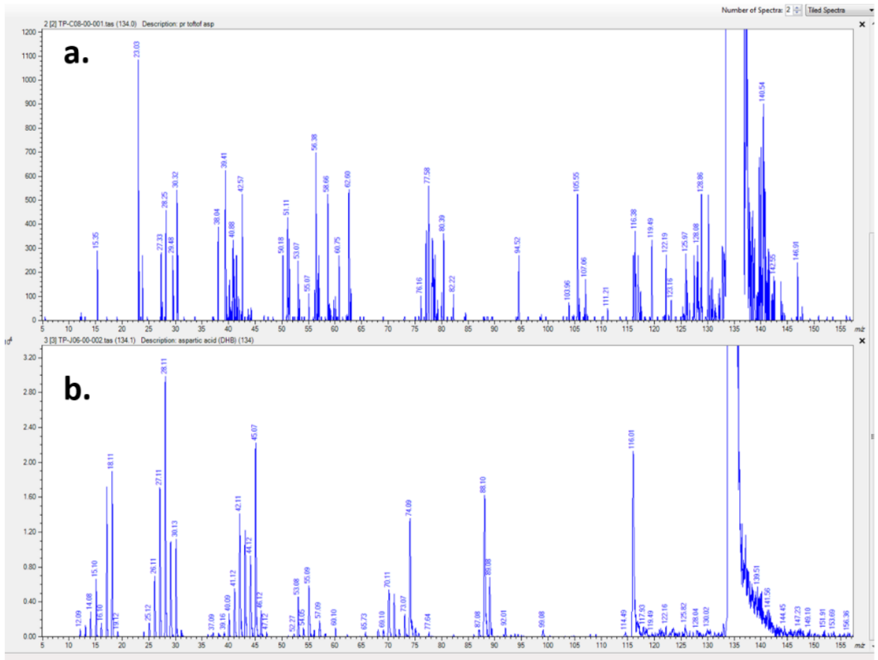

Figure S17. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid aspartic acid (*m/z* 134.05) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the aspartic acid authentic standard.

Glutamine

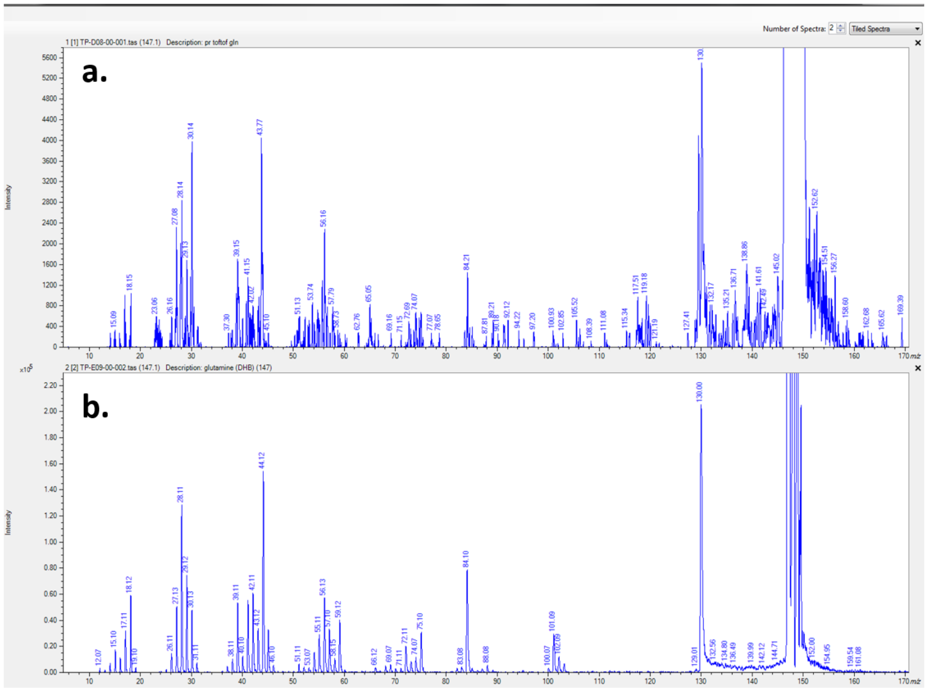

Figure S18. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid glutamine (*m/z* 147.06) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the glutamine authentic standard.

Glutamic acid

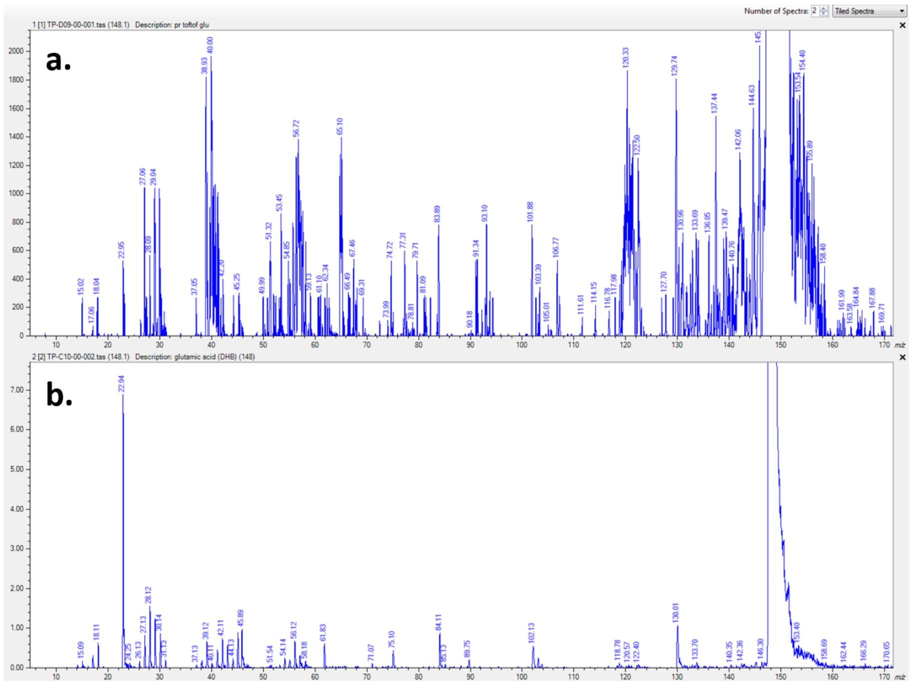

Figure S19. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid glutamic acid (*m/z* 148.08) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the glutamic acid authentic standard.

Histidine

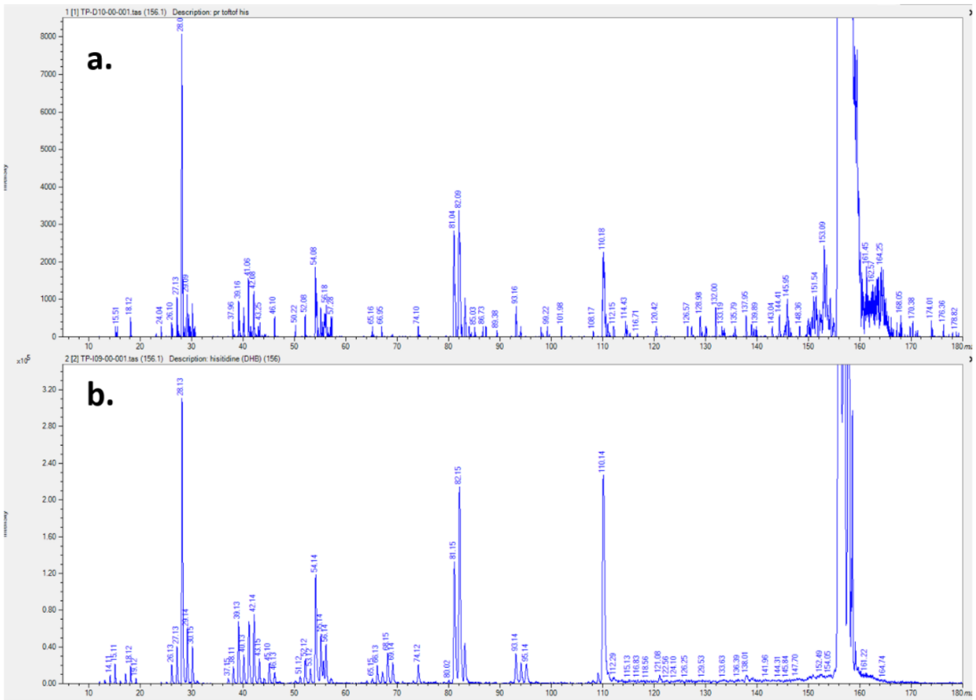

Figure S20. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid histidine (*m/z* 156.08) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the histidine authentic standard.

Isoleucine/Leucine

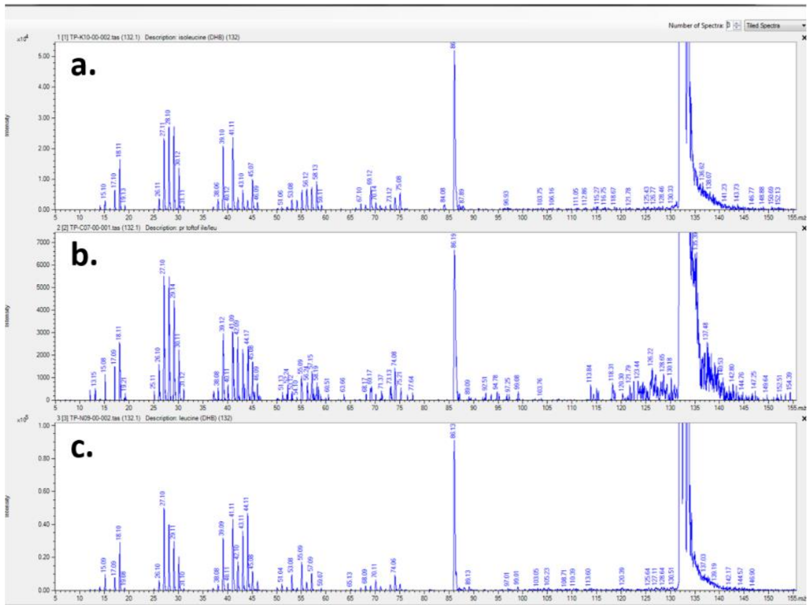

Figure S21. MALDI-MS/MS analyses of *P. regina* blow fly eggs and authentic standards to confirm the presence of the amino acid isoleucine and/or leucine (*m/z* 132.10) in the eggs. Panel a shows the product-ion spectrum of the amino acid isoleucine, Panel d shows the egg sample and Panel c, that of the amino acid leucine.

Lysine

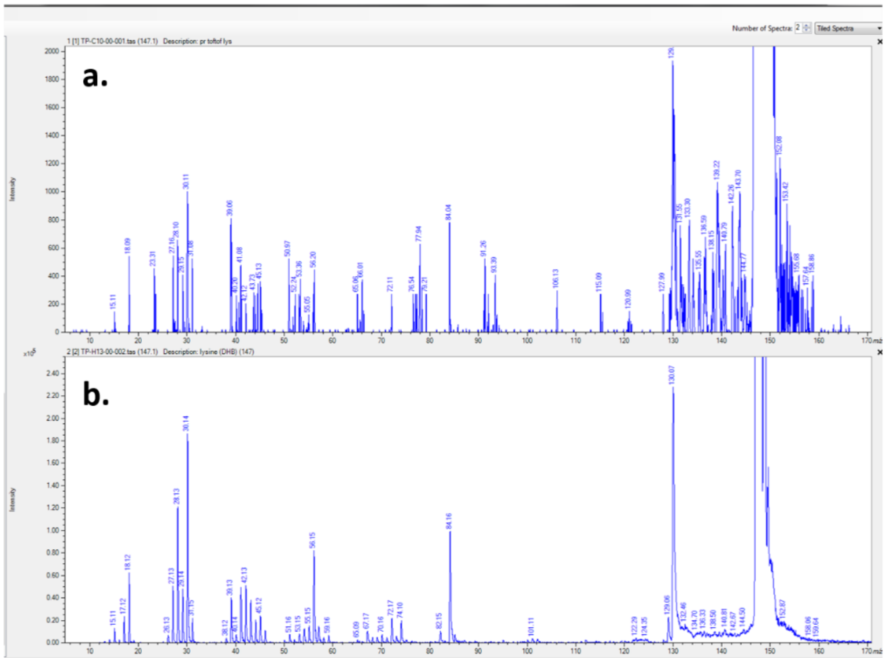

Figure S22. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid lysine (*m/z* 147.11) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the lysine authentic standard.

Methionine

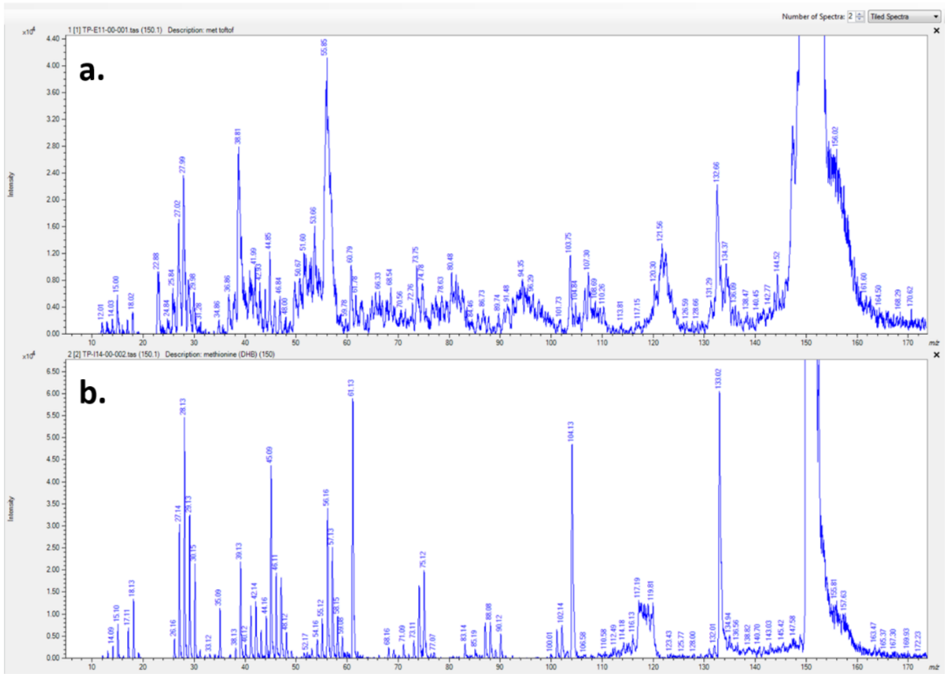

Figure S23. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid methionine (*m/z* 150.06) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the methionine authentic standard.

Phenylalanine

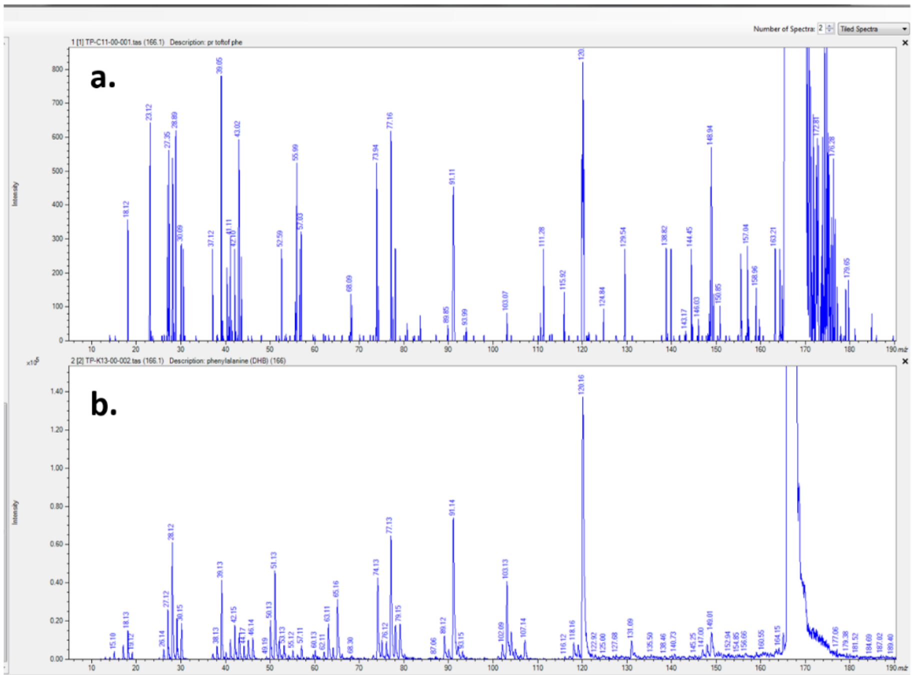

Figure S24. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid phenylalanine (*m/z* 166.09) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the phenylalanine authentic standard.

Proline

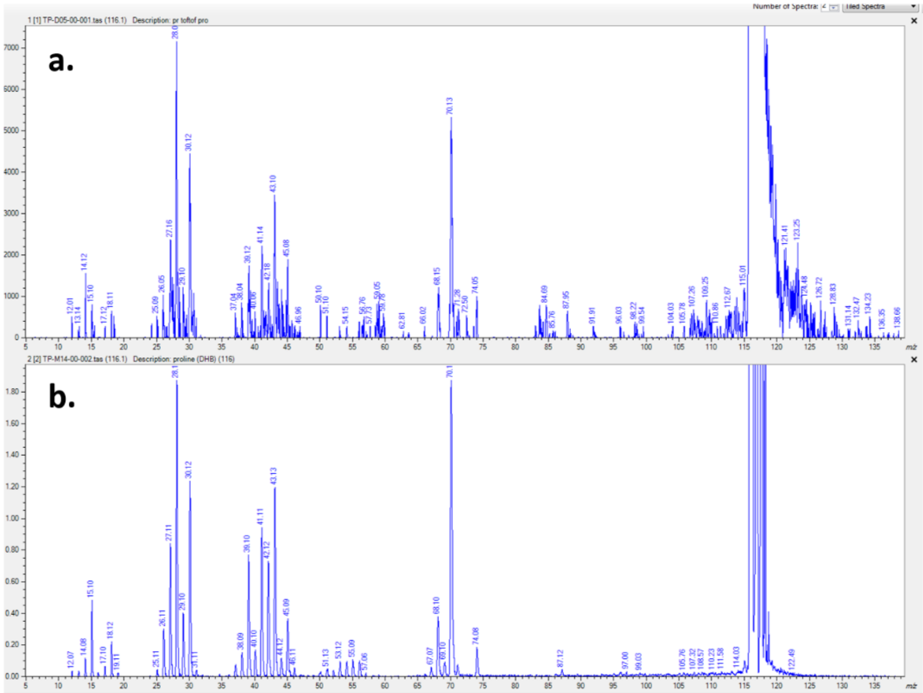

Figure S25. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid proline (*m/z* 116.07) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the proline authentic standard.

Serine

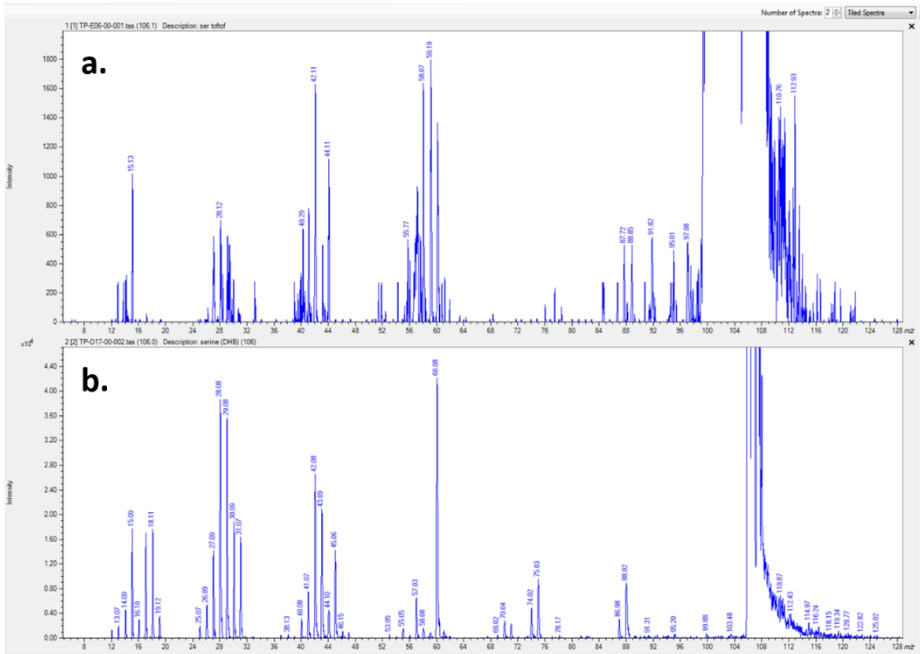

Figure S26. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid serine (*m/z* 106.05) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the serine authentic standard.

Tryptophan

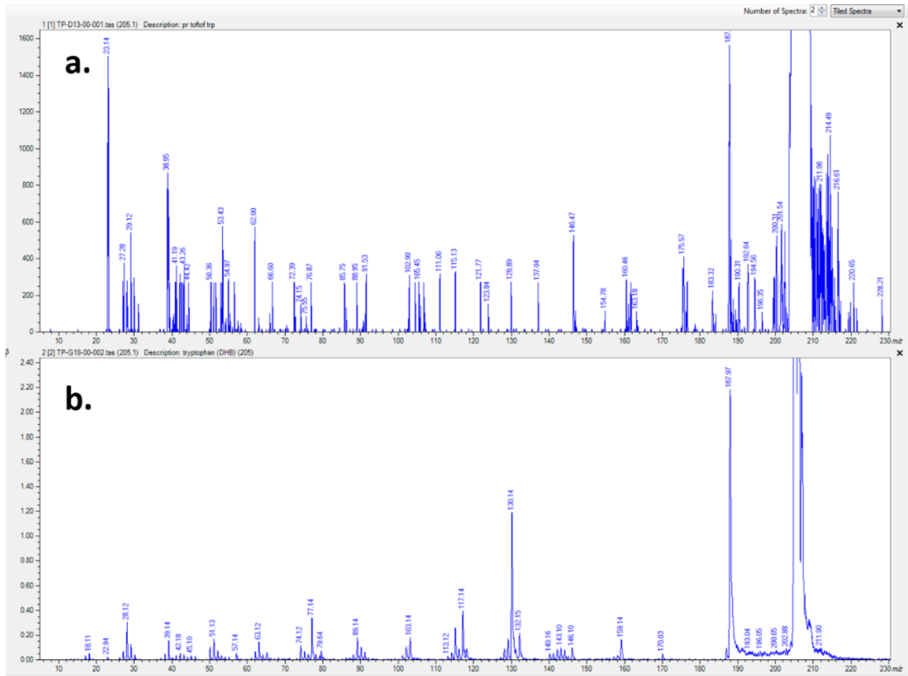

Figure S27. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid tryptophan (*m/z* 205.10) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the tryptophan authentic standard.

Tyrosine

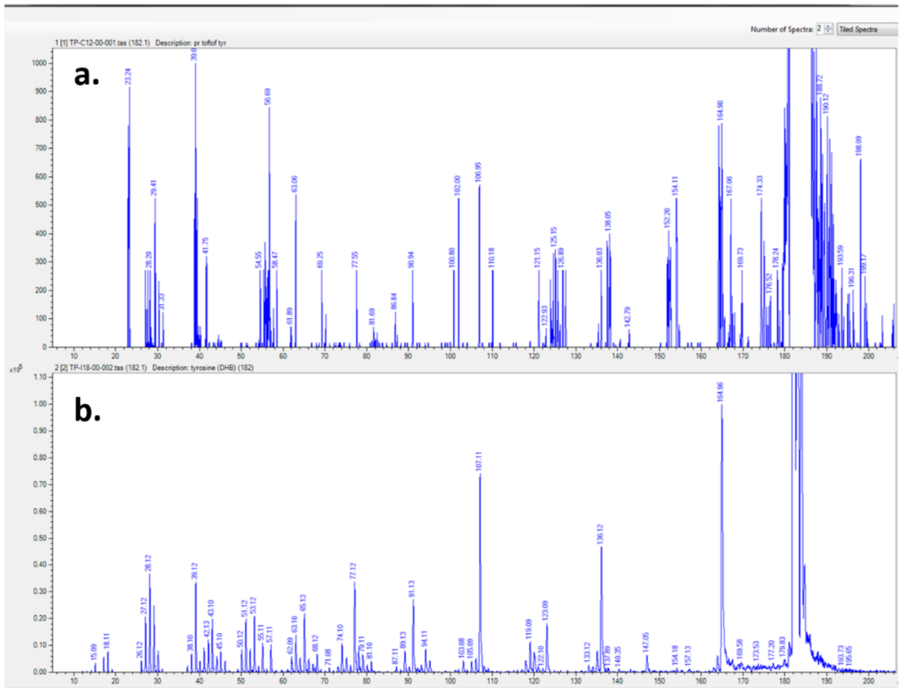

Figure S28. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid tyrosine (*m/z* 182.08) in the eggs. Panel a of the product-ion spectrum of the egg sample and Panel b, that of the tyrosine authentic standard.

Valine

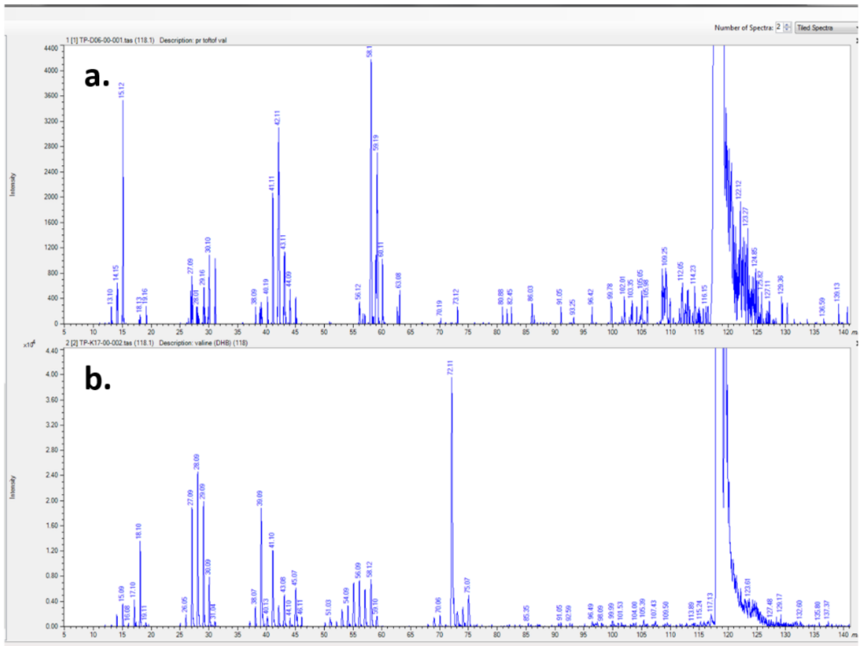

Figure S29. MALDI-MS/MS analyses of *P. regina* blow fly eggs and an authentic standard to confirm the presence of the amino acid valine (*m/z* 118.09) in the eggs. Panel a shows the product-ion spectrum of the egg sample and Panel b, that of the valine authentic standard.

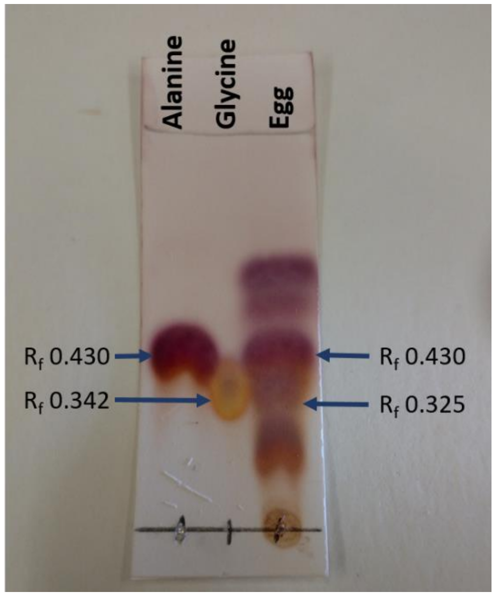

Figure S30. Image of the TLC analysis of *P. regina* eggs and two amino acid standards. The R_f values of the spots observed for the alanine and glycine standards are 0.430 and 0.342, respectively. The spot for alanine appears deep red and that representing glycine is yellow in color. Within the egg sample, 6 spots were observed. The third spot on the plate has an R_f value 0.325 and is yellow, and the fourth spot has an R_f value of 0.430 and appears dark red. The color and R_f values of these spots are consistent with the presence of the amino acids alanine and glycine.

Tables

Table S1. Results of the positive-ion mode DART-HRMS analysis of the six species of blow fly eggs featured in Figure 2. All reported peaks were above a 2% relative abundance threshold unless otherwise noted.

C. vicina		*L. coeruleiviridis*		*L. sericata*		*P. regina*		*Phoridae* spp.		*Sarcophagidae* spp.	
m/z	Rel. Int.	*m/z*	Rel. Int.	*m/z*	Rel. Int.	*m/z*	Rel. Int.	*m/z*	Rel. Int.	*m/z*	Rel. Int.
62.0588	2.01	61.0418	3.34	72.0790	2.50	70.0677	5.90	61.0411	8.77	61.0399	3.77
65.0580	2.68	70.0671	8.50	75.0774	3.72	72.0815	9.83	61.0683	3.03	83.0827	9.17
70.0630	10.53	72.0814	13.74	81.0655	2.25	74.0613	3.22	72.0806	2.58	90.0551	7.52
72.0783	9.80	74.0609	7.06	83.0843	9.57	75.0810	2.99	75.0806	5.90	93.0901	100.00
75.0768	4.06	75.0802	3.71	85.0604	2.33	76.0419	3.36	83.0855	15.09	93.1725	2.05
76.0375	2.05	76.0417	3.45	90.0554	2.77	83.0862	2.22	85.0633	3.17	94.0929	5.61
84.0796	7.59	83.0855	4.35	93.0896	100.00	84.0807	8.21	93.0926	100.00	100.0732	23.75
85.0262	11.30	84.0461	14.18	93.1702	3.18	86.0596	3.52	94.0948	6.81	101.0832	7.19
86.0934	18.74	84.0800	10.52	94.0917	5.29	86.0964	15.16	95.0871	3.29	107.0682	24.01
90.0522	40.09	85.0310	2.75	95.0840	5.28	88.0779	2.80	97.0645	2.09	107.1057	30.14
93.0885	100.00	85.0613	2.23	97.0998	4.20	90.0555	65.08	97.1007	2.57	116.0688	2.70
93.1703	2.60	86.0568	5.61	100.0734	18.08	93.0903	100.00	99.0465	3.20	119.0987	3.71
94.0885	5.93	86.0970	27.56	101.0603	3.25	94.0922	5.46	99.0814	2.76	121.1215	2.70
97.0268	5.37	88.0415	3.78	101.0941	8.24	100.0752	6.87	100.0705	3.87	124.0944	9.41
99.0419	4.77	88.0766	3.77	107.1046	32.70	100.1120	2.76	101.0600	4.74	132.0937	2.32
100.0685	4.70	90.0557	100.00	108.0951	2.19	101.0934	2.81	101.0957	8.38	139.1250	9.49
102.0521	5.46	93.0916	74.04	109.0999	4.43	102.0556	5.44	102.0924	2.38	153.1329	8.05
104.0650	8.97	94.0903	4.72	111.1125	3.01	102.0915	4.52	107.0682	5.13	163.1333	3.66
106.0476	8.85	98.0634	2.86	114.0814	2.43	104.0676	7.60	107.1058	28.42	279.1621	3.23
107.0995	4.14	99.0476	2.81	115.0692	2.73	106.0485	14.07	109.0988	2.98	288.2514	19.16
110.0881	2.54	100.0427	4.10	115.1088	2.06	107.1047	14.69	111.1136	2.38	289.2488	2.46
111.0421	2.12	100.0770	12.81	116.0712	1.85*	112.0802	5.43	113.0546	2.68		
112.0483	7.67	100.1086	4.25	118.0837	1.49*	114.0792	6.52	114.0827	3.23		
113.0327	9.10	101.0614	3.41	121.1142	3.38	115.0739	2.40	115.0693	3.55		
113.0664	5.50	101.0970	12.81	123.1200	2.46	116.0694	71.06	115.1075	2.05		
114.0609	9.55	102.0554	11.79	125.0911	2.97	116.1324	3.65	118.0831	2.56		
115.0759	2.49	102.0904	5.12	127.0738	2.32	117.0703	3.86	119.0997	3.99		
116.0663	75.75	104.0656	7.10	127.1120	2.49	118.0932	27.71	121.1221	4.98		
117.0626	5.18	106.0488	22.93	129.0882	3.22	119.0851	3.05	124.0961	5.64		
118.0830	22.07	107.1052	10.26	132.0945	2.08	120.0659	22.11	125.1034	3.38		
120.0634	12.70	110.0652	4.57	137.1132	2.05	121.1221	2.93	127.0741	2.20		
127.0406	16.02	112.0733	11.86	139.1249	14.67	128.0712	7.39	127.1157	2.39		
129.0594	6.15	113.0361	5.57	141.0900	3.68	129.0863	2.01	129.0857	2.72		
130.0514	29.32	113.0634	4.73	141.1282	2.14	130.0528	8.83	139.1128	9.67		
132.0991	47.40	114.0583	7.92	143.1023	4.01	132.1011	59.37	141.0886	2.66		
136.0622	3.97	115.0698	4.49	145.1099	2.39	136.0863	2.17	143.0990	2.98		
137.1101	16.04	116.0690	68.17	153.1320	11.31	139.1150	3.71	153.1320	6.81		
138.0994	2.64	117.0681	3.84	155.1048	3.97	146.0796	10.58	161.0988	2.25		
139.1108	3.68	118.0851	32.13	155.1443	2.24	147.0831	6.51	171.1403	3.97		
142.0844	5.11	118.1514	2.37	157.1147	2.23	147.1081	42.70	324.2552	2.14		
144.0679	2.70	119.0822	2.42	167.1590	3.00	148.0932	3.42				
144.1046	2.90	120.0666	32.76	169.1208	2.00	150.0602	9.22				
145.0506	16.78	123.0576	3.42	171.1385	3.92	153.1193	2.50				
146.0803	46.50	126.0603	42.51	173.1201	2.40	156.0783	8.02				
147.0955	4.76	126.0923	2.56	191.1590	2.86	158.1082	2.38				
148.0648	3.88	127.0511	4.04	199.6070	2.45	159.1112	2.51				
150.0608	9.44	127.1124	2.47	237.2101	2.55	161.0973	3.88				
153.0446	2.44	128.0726	17.27	255.2283	13.36	166.0765	17.92				
154.0835	2.93	129.0644	4.22	257.2398	8.27	167.0765	2.22				

156.0831	2.67	129.0999	3.96	271.2241	5.37	171.1380	2.10				
158.1069	4.10	130.0551	26.80	272.2486	2.25	173.1219	6.97				
162.0747	3.57	130.9010	22.25	279.1607	2.67	182.0840	2.39				
163.0618	4.48	132.1013	71.99	311.2876	5.16	187.1079	4.59				
164.0905	8.04	136.0625	3.94			205.0852	3.95				
166.0831	10.24	137.0513	5.69			215.1575	2.51				
169.0937	2.18	137.1267	2.83			229.1493	2.76				
171.1278	2.05	139.0552	2.04			250.1265	4.07				
172.0948	6.00	139.1284	5.35			255.2245	9.21				
173.1131	3.07	141.0825	2.24			271.2202	2.10				
174.1005	2.25	142.0792	2.13			272.2446	2.78				
180.0880	7.86	143.0893	2.60			311.2865	5.13				
182.0900	4.77	144.0709	3.42								
183.0931	4.05	146.0826	16.48								
187.1035	2.53	147.1076	34.71								
190.1042	2.31	148.0643	3.90								
192.0963	2.00	148.1138	3.09								
197.1176	2.05	149.0831	4.79								
198.0958	3.38	150.0602	14.11								
199.1098	3.37	153.1108	4.40								
201.1146	2.19	156.0786	12.16								
202.1048	2.23	157.0908	2.27								
211.1307	3.19	158.1023	2.97								
213.1174	2.74	159.1020	2.26								
215.1318	3.03	162.0748	2.37								
216.1196	2.90	163.1341	2.45								
220.1144	3.17	166.0790	21.31								
227.1330	3.74	167.0904	3.19								
228.1209	2.11	171.1351	3.30								
229.1379	3.47	173.1181	3.71								
232.1300	2.06	182.0842	6.97								
246.1274	5.92	189.1203	8.23								
255.2290	3.16	191.1521	3.05								
273.1438	2.85	220.1173	2.63								
311.2932	2.43	255.2207	3.75								
		269.2251	2.06								
		279.1622	2.38								
		288.2506	12.32								
		289.2452	2.38								
		311.2862	7.43								
		369.3491	2.37								

***** Mass was below a 2% relative abundance threshold.