

Conodipine-P1-3, the first phospholipases A₂ characterized from injected cone snail-venom

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SUPPLEMENTAL FIGURE LEGENDS

Figure 1S. ESI-MS/MS spectra of digested fragments of fraction peak II. Fragments of α_1 -subunit are shown. Different states of proline hydroxylation (Hyp) were assigned. A) 2Hyp on Pro residues 14 and 18. MS/MS of $[M+6H]^{6+}=772.6710$ B) 3Hyp on Pro residues 14, 18 and 25. MS/MS of $[M+6H]^{6+}=775.3385$ C) 1Hyp on Pro residue 25. MS/MS of $[M+2H]^{2+}=1205.5145$ D) MS/MS of $[M+2H]^{2+}=499.2198$. The match tolerance for all spectra were 0.8 Da. All spectra represent key fragments characteristic only in α_1 -subunit. Fragment peptides colors were: Cys carbamethylation (green), Hyp (blue). Spectra assignments were directly labeled by Proteome Discoverer™ Software (Version 2.0) and/or Thermo BioPharma™ Finder™ (Version 2.0). The theoretical MS/MS values were included in the supplementary information.

Figure 2S. ESI-MS/MS spectra of digested fragments of fraction peak II. Fragments of β_1 -subunit are shown. A) Fragment that contains the N-terminus Pyro-Glu and a Hyp on Pro-7. MS/MS of $[M+3H]^{3+}=1052.8461$ B) MS/MS of $[M+H]^+=720.3338$ and C) MS/MS of $[M+2H]^{2+}=389.1759$. These three peptides are key fragments characteristic only in β_1 -subunit. The match tolerance for all spectra were 0.8 Da. Fragment peptides colors were: Cys carbamethylation (green), Hyp (blue), Pyro-Glu (Orange). Spectra assignments were directly labeled by Proteome Discoverer™ Software (Version 2.0) and/or Thermo BioPharma™ Finder™ (Version 2.0). The theoretical MS/MS values were included in the supplementary information.

Figure 3S. ESI-MS/MS spectra of digested fragments of fraction peak VI. Fragments of α_2 -subunit are shown. Different states of proline hydroxylation (Hyp) were assigned. A) Fragment characteristic only in α_2 -subunit with no Hyp. MS/MS of $[M+5H]^{5+}=569.0424$ B) 1Hyp on Pro residue 25. MS/MS of $[M+4H]^{4+}=715.2993$ C) Key fragment characteristic only in α_2 -subunit. MS/MS of $[M+3H]^{3+}=447.8742$ D) Key fragment characteristic only in α_2 -subunit with 1Hyp on Pro-18. MS/MS of $[M+2H]^{2+}=564.7593$. The match tolerance for all spectra were 0.8 Da. Fragment peptides colors were: Cys carbamethylation (green), Hyp (blue). Spectra assignments were directly labeled by Proteome Discoverer™ Software (Version 2.0) and/or Thermo BioPharma™ Finder™ (Version 2.0). The theoretical MS/MS values were included in the supplementary information.

Figure 4S. ESI-MS/MS spectra of digested fragments of fraction peak VI. Fragments of β_2 -subunit are shown. A) Fragment that contains the N-terminus Pyro-Glu with no Hyp. MS/MS of $[M+2H]^{2+}=890.4477$ B) Fragment that contains the N-terminus Pyro-Gln and a Hypo on Pro-7.

MS/MS of $[M+2H]^{2+}=898.4457$ C) MS/MS of $[M+3H]^{3+}=963.4370$, D) MS/MS of $[M+2H]^{2+}=414.2235$. Digested fragment NYHLAVK has a deamidation or ammonia loss from the asparagine side chain. All spectra represents key fragments characteristic only in β_2 -subunit. The match tolerance for all spectra were 0.8 Da. Fragment peptides colors were: Cys carbamethylation (green), Hyp (blue), Pyro-Gln (Orange). Spectra assignments were directly labeled by Proteome Discoverer™ Software (Version 2.0) and/or Thermo BioPharma™ Finder™ (Version 2.0). The theoretical MS/MS values were included in the supplementary information.

Figure 5S. Matched fragments obtained by Top-down proteomics of the reduced and alkylated α_1 , α_1 and β_2 -subunits of Cdpi-P. A) Matched ions obtained by ETHcD fragmentation of the charge $[M+10H]^{10+}= 862.1$ corresponding to the α_1 -subunit in fraction II. B) Matched ions obtained by ETHcD fragmentation of $[M+6H]^{6+}= 943.1$ corresponding to the α_1 -subunit in fraction II. C) Matched ions obtained by ETHcD fragmentation of $[M+5H]^{5+}= 1099.1$ corresponding to the β_2 -subunit in fraction VI.

Figure 6S. Direct assessment of sPLA₂s activities of by mass spectrometry. A) Control experiment with only DOPC. B) DOPC incubated with *Agkistrodon piscivorus piscivorus* (APP) venom. C) DOPC incubated with *Agkistrodon piscivorus locustoma* (APL) venom. D) DOPC incubated with *C. ermineus* injected venom F) DOPC incubated with *C. striatus* I.V. Notice that compared to the control, PLA₂s hydrolyzes the DOPC (786 Da) to form the lysophospholipid (522 Da). Sodium and potassium adducts could be observed.

Figure 7S. Phylogenetic tree of known conodipines from different cone snails species. Conodipines sequences were obtained by a Basic Local Alignment Search Tool (BLAST) search

against UniProtKB (91) where all cdpi-Ps were used. The following sequences were selected to construct the phylogenetic tree: *C. purpurascens*-1 to -6, which corresponded to Cdpi-P1 to -P6, *C. magus*-1, which corresponded to Conodipine-M alpha chain (UniProtKB Q9TWL9_CONMA), *C. lenavati*-1 to -3, which corresponded to the conopeptides (UniProtKB A0A0K8TUY3_CONLV, A0A0K8TUD7_CONLV, and A0A0K8TTR8_CONLV), *C. tribblei*-1 to -8, which corresponded to the conopeptides (UniProtKB A0A0C9RYL5_9CAEN, A0A0C9S5X4_9CAEN, A0A0C9SEP7_9CAEN, A0A0K8TU07_9CAEN, A0A0C9R765_9CAEN, A0A0K8TU01_9CAEN, A0A0C9SEK4_9CAEN, and A0A0C9SFM6_9CAEN), *C. geographus*-1 to -10, which corresponded to the conodipines-G 1-10 (UniProtKB W4VRW2_CONGE, W4VRZ7_CONGE, W4VSJ3_CONGE, W4VSC8_CONGE, W4VS60_CONGE, W4VRW0_CONGE, W4VRZ5_CONGE, W4VSJ2_CONGE, and W4VSC6_CONGE, W4VS57_CONGE), *C. victoriae*-1 to -3, which corresponded to the conodipine-Vc1 to -Vc3 (UniProtKB W4VSG8_CONVC, W4VSB8_CONVC, and W4VS19_CONVC), *C. monile*-1, which corresponded to the a conodipine fragment (UniProtKB A0A161J284_CONMO). The selected sequences were used to construct the phylogenetic tree by the multiple sequence alignment algorithm, MUSCLE (92). The scores shown in the phylogenetic tree correspond to a sequence distance measure, which is a representation the "length" of the branches and indicative of the evolutionary distance between the sequences.

Figure 8S. Homology modeling of Cdpi-alpha-P5. Homology modeling for all Cdpi-Ps alpha subunits was carried out using the SWISSMODEL online server (93). The known structures of two PLA2s, *Tuber brochii* (PDB 4aup.1.A) (94) and *Streptomyces violaceruber* (PDB 1it5.1.A) (95) were used as templates. Molecular graphics and related manipulations were carried out using UCSF Chimera beta version 1.11.2 (96). The models obtained with both templates were quite similar

for all Cdpi-Ps alpha subunits. All the models obtained using the structure 4aup.1.A as template included all cysteine residues but Cys69. The models using the 1it5.1.A as template included all cysteine residues but Cys4 and only conodipine-P alpha-5 included the Cys69. Accordingly, conodipine alpha-5 models were obtained with both templates and overlaid showing the predicted disulfide bridges. Cysteine side chains are labeled in green color. These models suggest that the disulfide bridges are between Cys33-Cys47, Cys11-Cys27, Cys4-Cys36, and Cys59-Cys69 (labeled in red color). It can be inferred that Cys19 would likely be involved in interchain disulfide bond with the beta-subunit. The overlay homology models shows the key residues of the active site Asp28, His30 and Asp31, which are within in an alpha helix and labeled in orange.

Figure 1S (Supplemental)

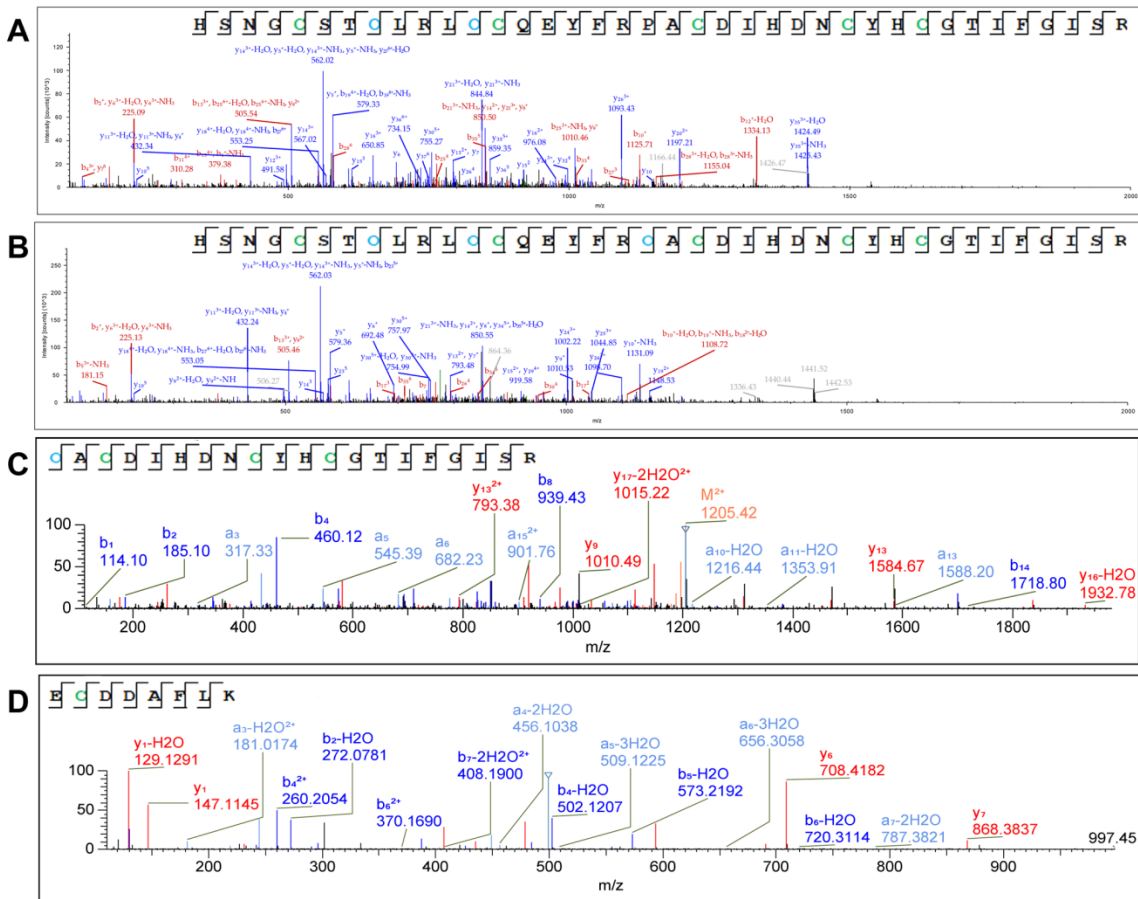


Figure 2S (Supplemental)

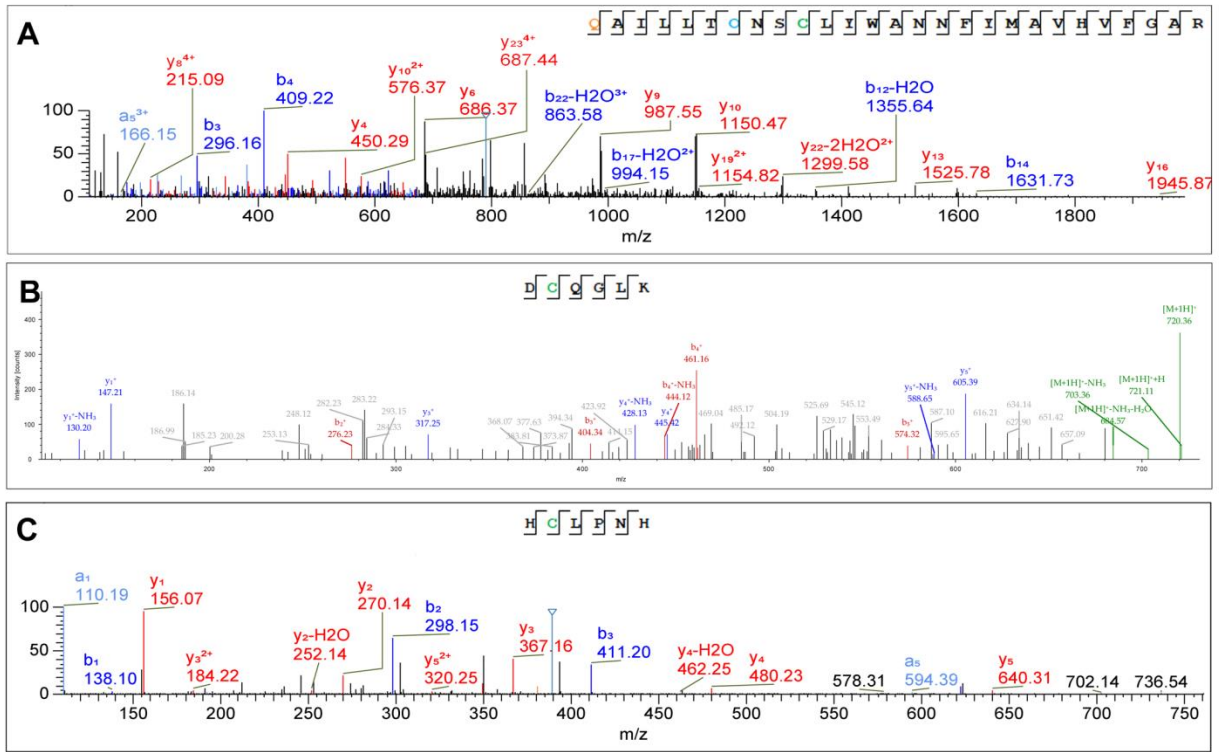


Figure 3S (Supplemental)

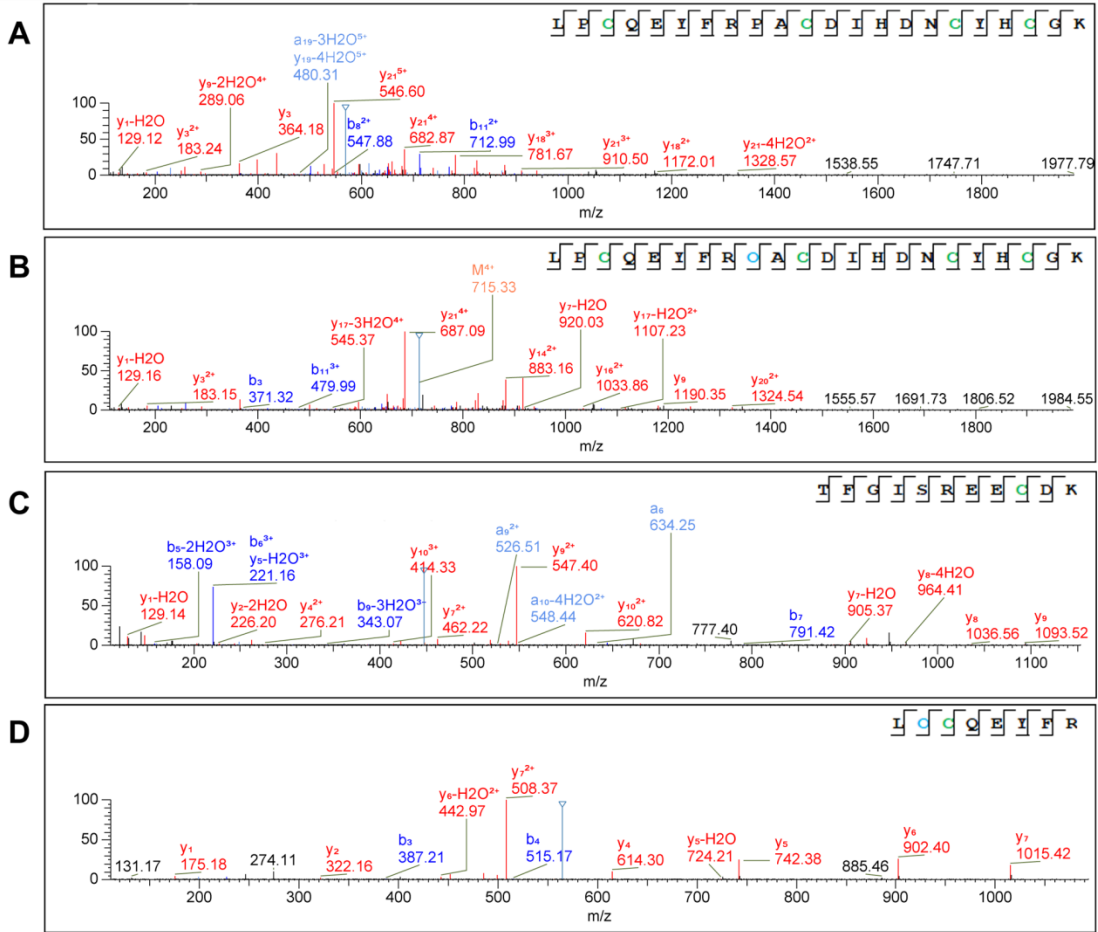


Figure 4S (Supplemental)

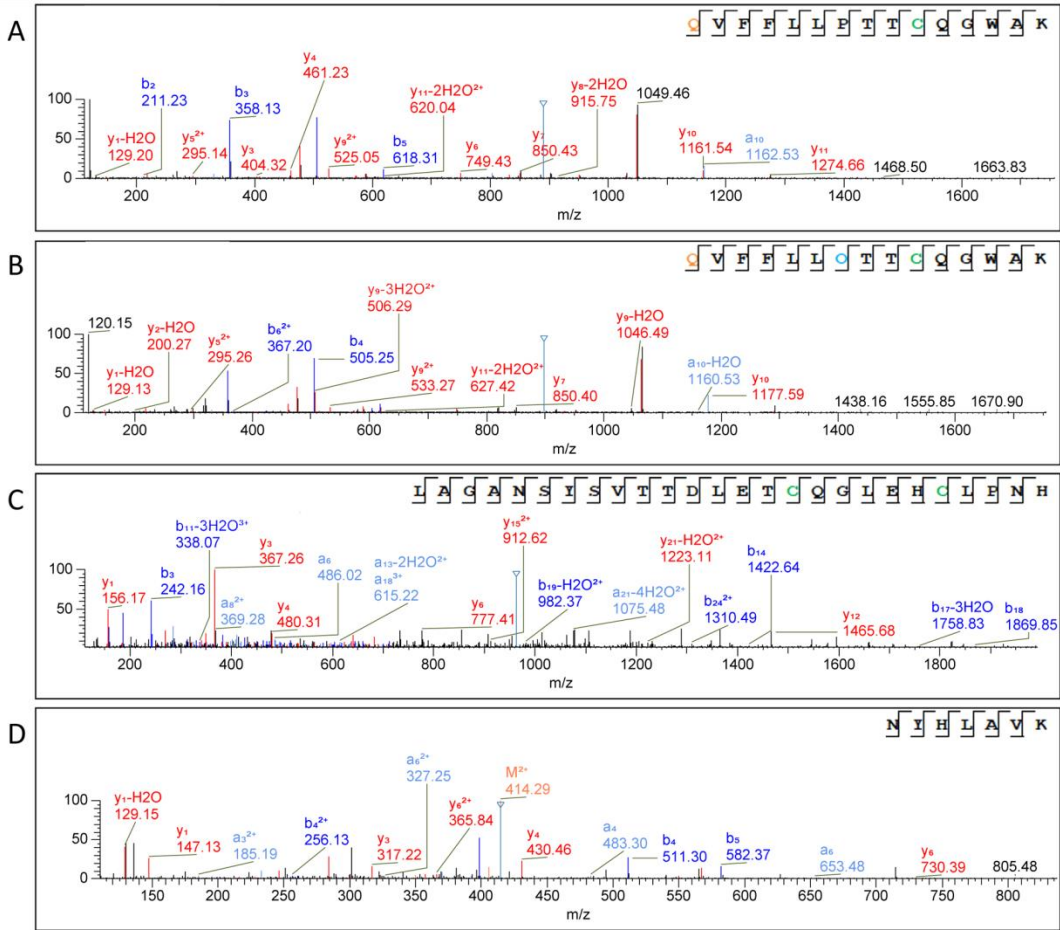


Figure 5S (Supplemental)

A

| Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) | Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) | Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) |
|------------|------------------|---------------|-----------------|------------|------------------|---------------|-----------------|------------|------------------|---------------|-----------------|
| C1 | 145.1213 | 145.0576 | -0.0636 | B11 | 1288.5289 | 1288.5935 | 0.0646 | Y8 | 818.3705 | 818.1486 | -0.2219 |
| B1 | 128.0950 | 128.1256 | 0.0307 | C11 | 1305.5552 | 1305.7466 | 0.1914 | Z8 | 802.3517 | 802.1813 | -0.1704 |
| C2 | 274.1639 | 274.1867 | 0.0229 | C12 | 1392.5872 | 1392.4854 | -0.1018 | Z9 | 916.3946 | 915.9886 | -0.4060 |
| B2 | 257.1376 | 257.3961 | 0.2586 | B12 | 1375.5609 | 1375.9524 | 0.3915 | Z9 | 916.3946 | 916.6153 | 0.2207 |
| B3 | 344.1696 | 344.2362 | 0.0667 | C13 | 1493.6349 | 1493.7129 | 0.0780 | Y9 | 932.4134 | 932.4556 | 0.0422 |
| C3 | 361.1959 | 361.3111 | 0.1152 | B13 | 1476.6086 | 1476.7359 | 0.1273 | Y10 | 1019.4454 | 1019.4122 | -0.0333 |
| B4 | 504.2002 | 503.9953 | -0.2049 | C14 | 1590.6876 | 1590.7151 | 0.0275 | Z10 | 1003.4267 | 1003.7041 | 0.2775 |
| C4 | 521.2265 | 521.2594 | 0.0329 | B14 | 1573.6613 | 1573.7655 | 0.1042 | Y11 | 1076.4669 | 1076.1264 | -0.3405 |
| C5 | 622.2742 | 622.0496 | -0.2246 | C15 | 1703.7717 | 1703.7298 | -0.0419 | Z11 | 1060.4481 | 1060.2895 | -0.1586 |
| B5 | 605.2479 | 605.3168 | 0.0689 | B15 | 1686.7454 | 1686.9265 | 0.1811 | Y12 | 1189.5510 | 1189.3711 | -0.1799 |
| C6 | 750.3692 | 750.1726 | -0.1966 | Y2 | 231.1331 | 231.2068 | 0.0737 | Z12 | 1173.5322 | 1173.7111 | 0.1789 |
| B6 | 733.3429 | 733.4093 | 0.0664 | Z3 | 286.1515 | 286.1940 | 0.0425 | Y13 | 1317.6459 | 1317.6797 | 0.0338 |
| C7 | 887.4281 | 887.4370 | 0.0089 | Y3 | 302.1702 | 302.3381 | 0.1679 | Z13 | 1301.6272 | 1301.7950 | 0.1678 |
| B7 | 870.4018 | 870.5696 | 0.1678 | Y4 | 399.2230 | 399.1950 | -0.0280 | Y14 | 1445.7409 | 1445.6187 | -0.1222 |
| B8 | 957.4338 | 957.3246 | -0.1092 | Z4 | 383.2042 | 383.2407 | 0.0364 | Y14 | 1445.7409 | 1446.1625 | 0.4216 |
| C8 | 974.4601 | 974.6408 | 0.1807 | Y5 | 559.2537 | 559.2613 | 0.0077 | Z14 | 1429.7221 | 1429.8603 | 0.1381 |
| B9 | 1071.4767 | 1071.0208 | -0.4559 | Z5 | 543.2349 | 543.4079 | 0.1730 | Y15 | 1605.7715 | 1605.7289 | -0.0426 |
| B9 | 1071.4767 | 1071.5491 | 0.0724 | Y6 | 660.3013 | 660.2310 | -0.0703 | Z15 | 1589.7528 | 1589.8926 | 0.1398 |
| C9 | 1088.5030 | 1088.5087 | 0.0056 | Z6 | 644.2826 | 644.4126 | 0.1300 | Y16 | 1718.8556 | 1718.6178 | -0.2377 |
| B10 | 1128.4982 | 1128.4762 | -0.0220 | Z7 | 715.3197 | 715.0111 | -0.3086 | Z16 | 1702.8368 | 1702.6538 | -0.1831 |
| B10 | 1128.4982 | 1128.9949 | 0.4967 | Z7 | 715.3197 | 715.5255 | 0.2058 | | | | |
| C10 | 1145.5245 | 1145.5128 | -0.0117 | Y7 | 731.3384 | 731.3807 | 0.0422 | | | | |

B

| Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) | Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) | Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) |
|------------|------------------|---------------|-----------------|------------|------------------|---------------|-----------------|------------|------------------|---------------|-----------------|
| C1 | 128.0583 | 128.2359 | 0.1776 | C12 | 1389.6960 | 1389.7706 | 0.0746 | Y7 | 904.4337 | 904.4582 | 0.0244 |
| B1 | 182.0691 | 182.3335 | 0.2644 | B13 | 1558.7490 | 1558.7157 | -0.0332 | Z8 | 1001.4990 | 1001.2530 | -0.2460 |
| C2 | 199.0954 | 199.4002 | 0.3048 | C13 | 1575.7753 | 1575.8470 | 0.0717 | Y9 | 1074.5393 | 1074.3691 | -0.1702 |
| B3 | 295.1532 | 295.2218 | 0.0686 | B14 | 1629.7861 | 1629.6075 | -0.1786 | Z9 | 1058.5205 | 1058.6210 | 0.1005 |
| B4 | 408.2373 | 408.3110 | 0.0737 | C14 | 1646.8124 | 1647.2718 | 0.4594 | Y10 | 1202.5978 | 1202.3870 | -0.2108 |
| C4 | 425.2636 | 425.3451 | 0.0816 | C15 | 1760.8553 | 1760.5221 | -0.3332 | Z10 | 1186.5791 | 1186.5444 | -0.0347 |
| C5 | 538.3476 | 538.3117 | -0.0359 | B15 | 1743.8290 | 1744.1490 | 0.3199 | Y11 | 1362.6285 | 1362.1827 | -0.4458 |
| B5 | 521.3213 | 521.4338 | 0.1124 | C16 | 1874.8983 | 1874.9139 | 0.0156 | Z11 | 1346.6097 | 1346.5724 | -0.0373 |
| C6 | 639.3953 | 639.2309 | -0.1644 | Z1 | 139.0507 | 139.0022 | -0.0485 | Y12 | 1477.6554 | 1477.2236 | -0.4319 |
| B6 | 622.3690 | 622.3432 | -0.0258 | Y1 | 155.0695 | 155.0934 | 0.0239 | Y12 | 1477.6554 | 1477.9615 | 0.3060 |
| B7 | 735.4167 | 735.4538 | 0.0372 | Z2 | 253.0936 | 253.1430 | 0.0494 | Z12 | 1461.6367 | 1462.0831 | 0.4464 |
| C7 | 752.4430 | 752.5517 | 0.1088 | Y2 | 269.1124 | 269.1912 | 0.0788 | Z13 | 1589.7316 | 1589.8259 | 0.0942 |
| C8 | 866.4859 | 866.3594 | -0.1265 | Z3 | 350.1464 | 350.2090 | 0.0626 | Y13 | 1605.7504 | 1606.1718 | 0.4214 |
| B8 | 849.4596 | 849.5065 | 0.0469 | Y3 | 366.1652 | 366.5333 | 0.3681 | Y14 | 1702.8032 | 1702.8173 | 0.0141 |
| C9 | 953.5179 | 953.4643 | -0.0536 | Y4 | 479.2492 | 479.2198 | -0.0294 | Z14 | 1686.7844 | 1687.2116 | 0.4272 |
| B9 | 936.4916 | 936.5580 | 0.0663 | Z4 | 463.2305 | 463.2168 | -0.0137 | Y15 | 1817.8301 | 1817.3483 | -0.4818 |
| B10 | 1096.5223 | 1096.5655 | 0.0432 | Y5 | 639.2799 | 639.2309 | -0.0490 | Z15 | 1801.8113 | 1801.7931 | -0.0182 |
| C10 | 1113.5486 | 1113.8194 | 0.2708 | Z5 | 623.2611 | 623.3041 | 0.0429 | Z16 | 1902.8590 | 1902.4426 | -0.4165 |
| B11 | 1209.6063 | 1209.2391 | -0.3673 | Z6 | 760.3200 | 760.2555 | -0.0645 | Y16 | 1918.8778 | 1918.7188 | -0.1590 |
| C11 | 1226.6326 | 1226.6591 | 0.0265 | Y6 | 776.3388 | 776.3322 | -0.0066 | | | | |
| B12 | 1372.6697 | 1372.3954 | -0.2742 | Z7 | 888.4150 | 888.0796 | -0.3353 | | | | |

C

| Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) | Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) | Ion | Theoretical Mass | Observed Mass | Delta Mass (Da) |
|------------|------------------|---------------|-----------------|------------|------------------|---------------|-----------------|------------|------------------|---------------|-----------------|
| C2 | 227.1533 | 227.2653 | 0.1120 | C16 | 1906.9714 | 1907.0103 | 0.0389 | Z14 | 1690.7317 | 1690.5974 | -0.1343 |
| C4 | 521.2901 | 520.9326 | -0.3575 | Y1 | 155.0695 | 155.1631 | 0.0936 | Y14 | 1706.7505 | 1706.7786 | 0.0281 |
| B4 | 504.2638 | 504.3756 | 0.1118 | Z2 | 253.0936 | 253.1025 | 0.0089 | Z15 | 1805.7586 | 1805.5376 | -0.2210 |
| B5 | 617.3479 | 617.5690 | 0.2211 | Y3 | 366.1652 | 366.0617 | -0.1034 | Y15 | 1821.7774 | 1822.1209 | 0.3434 |
| B6 | 730.4319 | 730.3138 | -0.1181 | Z3 | 350.1464 | 350.2513 | 0.1049 | Y16 | 1922.8251 | 1922.7148 | -0.1102 |
| B7 | 843.4847 | 843.4322 | -0.0525 | Z4 | 463.2305 | 463.3243 | 0.0938 | Z16 | 1906.8063 | 1907.0103 | 0.2039 |
| C7 | 860.5110 | 860.4885 | -0.0225 | Y5 | 639.2799 | 639.3889 | 0.1090 | | | | |
| C8 | 961.5587 | 961.3752 | -0.1834 | Z5 | 623.2611 | 623.6112 | 0.3501 | | | | |
| B8 | 944.5324 | 944.4802 | -0.0521 | Z6 | 760.3200 | 760.7527 | 0.4327 | | | | |
| C9 | 1062.6064 | 1062.5674 | -0.0390 | Y7 | 905.3814 | 905.1462 | -0.2352 | | | | |
| B10 | 1205.6107 | 1205.7688 | 0.1581 | Z7 | 889.3626 | 889.4723 | 0.1097 | | | | |
| B11 | 1333.6693 | 1333.3386 | -0.3307 | Z8 | 1002.4467 | 1002.4423 | -0.0044 | | | | |
| C11 | 1350.6956 | 1350.7842 | 0.0886 | Y8 | 1018.4654 | 1018.5010 | 0.0356 | | | | |
| B12 | 1390.6907 | 1390.5828 | -0.1080 | Y9 | 1075.4869 | 1075.3977 | -0.0892 | | | | |
| C12 | 1407.7170 | 1407.7041 | -0.0129 | Z9 | 1059.4681 | 1059.7560 | 0.2878 | | | | |
| B13 | 1576.7701 | 1576.5938 | -0.1763 | Z10 | 1187.5267 | 1187.4612 | -0.0655 | | | | |
| C14 | 1664.8335 | 1664.4785 | -0.3549 | Y11 | 1363.5761 | 1363.1621 | -0.4140 | | | | |
| B14 | 1647.8072 | 1647.6082 | -0.1990 | Y11 | 1363.5761 | 1363.9309 | 0.3548 | | | | |
| C15 | 1792.9284 | 1793.0215 | 0.0931 | Z12 | 1448.6050 | 1448.6660 | 0.0610 | | | | |
| B15 | 1775.9021 | 1776.3909 | 0.4887 | Y13 | 1593.6664 | 1593.2605 | -0.4059 | | | | |
| B16 | 1889.9451 | 1889.7815 | -0.1636 | Z13 | 1577.6476 | 1577.8105 | 0.1629 | | | | |

Figure 6S (Supplemental)

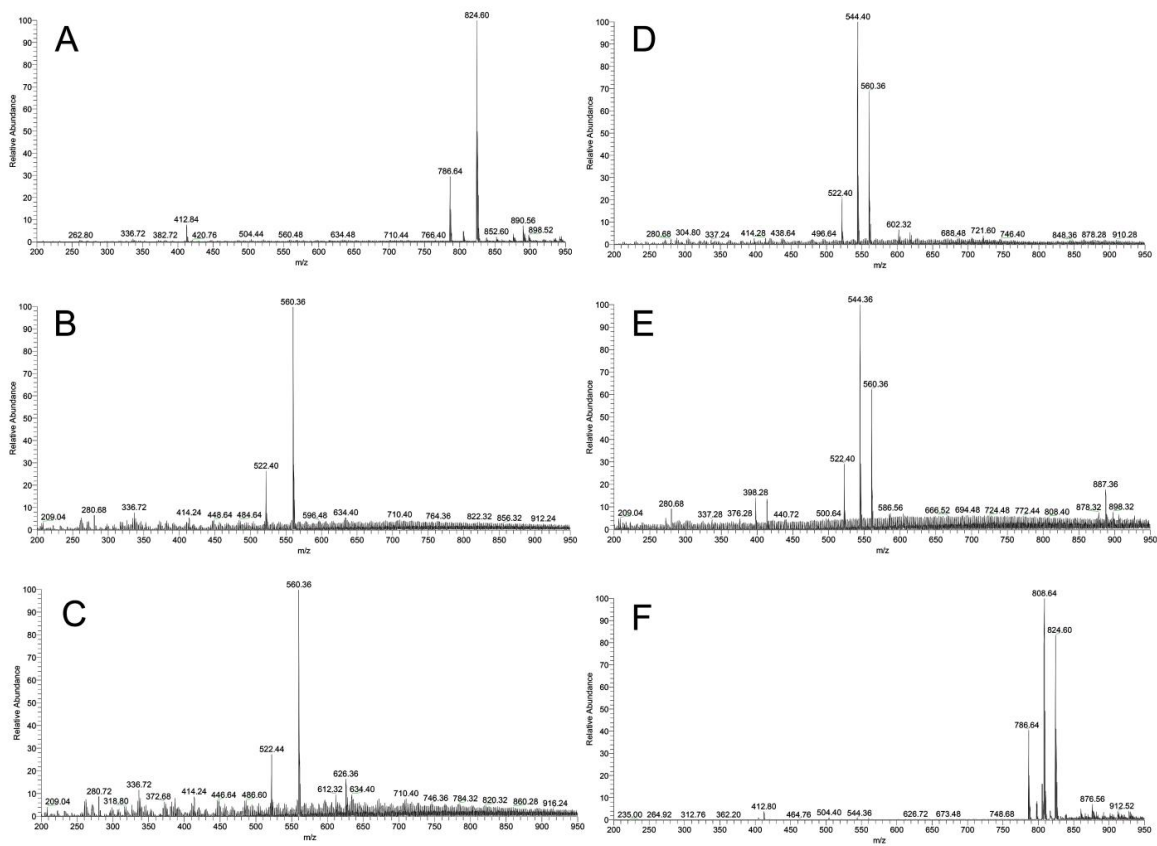


Figure 7S (Supplemental)

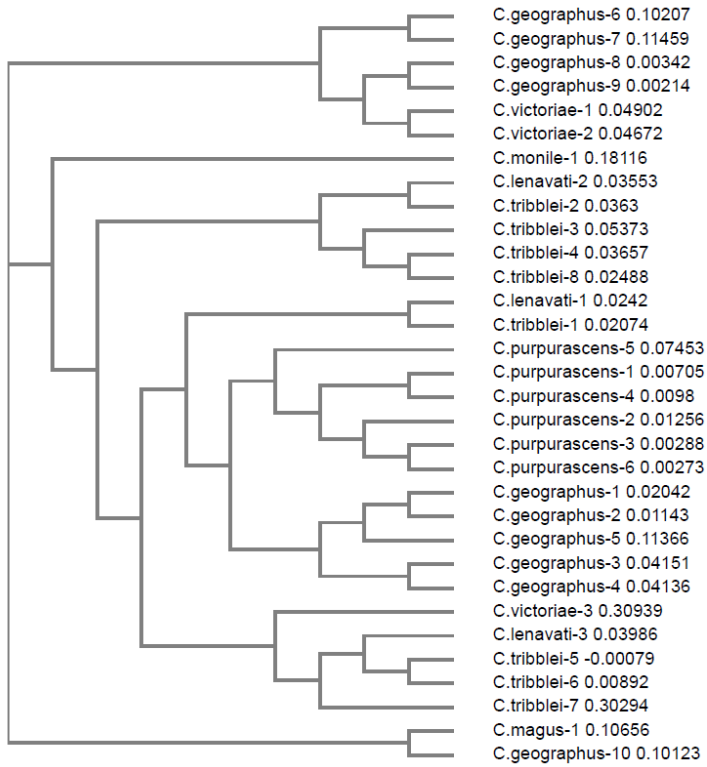


Figure 8S (Supplemental)

