Supplementary Information:

Transcriptomic Profiling of the Medicinal Plant *Clitoria ternatea*: Identification of Potential Genes in Cyclotide Biosynthesis

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Supplementary Figure S1: Orthology analysis of *Clitoria ternatea* predicted proteome with other plant proteomes using OrthoVenn 52. (A) Venn diagram showing shared and distinct protein clusters (orthogroups) between *C. ternatea* (green), *A. thaliana* (brown), *M. truncatula* (blue), *G. max* (red), *V. unguiculata* (orange) and *P. vulgaris* (yellow). (B) Horizontal bar chart depicting top 20 Gene Ontology (GO) annotation categories of *C. ternatea*-specific proteins. GO categories included BP = biological process, MF = molecular function, CC = cellular component. In *C. ternatea*, the highly represented GO categories of biological process were those of cellular process, macromolecular metabolic process, response to stimulus, etc. The highly represented GO categories of molecular function were those of transferase activity, nucleic acid binding, oxidoreductase activity, etc.

Supplementary Figure S2: Comparative Venn diagram of the number of cyclotides identified from *Clitoria ternatea* by Prof. David Craik’s group, Prof. James Tam’s group and our group (current study).
Supplementary Figure S3: MEME logos predicted for the cyclotide precursors distributed across three structural clades (A-C) in the cyclotide phylogeny (main text, Fig. 3) using MEME Suite 62. (A) MEME logo for ER-signal peptide, cyclotide domain and loop 5 in cluster A (B) MEME logo for ER-signal peptide, cyclotide domain and loop 5 in cluster B (C) MEME logo for ER-signal peptide, cyclotide domain and loop 5 in cluster C.
**Supplementary Figure S4**: Mass detected analytical LC ESI-MS profile (ion chromatogram) of the stems, leaves, pods and flower crude extract from *C. ternatea*. Masses corresponding to cyclotides (>3000 Da) were detected in the retention time range 25-55 min. Further fractionation of these extracts using semi-preparative HPLC is illustrated in Supplementary Fig. S5.

**Supplementary Figure S5**: Semi-prep HPLC profiles of cyclotide extracts obtained from leaf, pod, stem and flower tissues of *Clitoria ternatea*. Late-eluting peaks were separated into five fractions (A-E). Cyclotides were distributed in fractions B-E and these are labeled. For cyclotide transcript and peptide IDs, and molecular weight (monoisotopic masses) refer to Supplementary Table S6.
Supplementary Figure S6: MALDI-TOF mass-spectra of HPLC-purified cyclotide-rich fractions (B-E) from C. ternatea (A) stem, (B) leaf, (C) pod and (D) flower tissues. (Insets) The expanded isotopic multiplets for M+H masses that correspond to predicted cyclotide transcripts (peptide identifiers are marked) are shown.
Supplementary Figure S7: Heatmap of differential expression of asparaginyl endopeptidase genes, (highlighted in pink font) across four tissues of *C. ternatea de novo* transcriptome. The heatmap was generated using average TPM values along with Pearson correlation coefficient clustering method and complete linkage on genes. Positive (red) and negative (blue) values correspond to up-and down-regulated clusters respectively.
Supplementary Figure S8: Multiple sequence alignment of protein disulphide isomerase (PDIs) transcripts from C. ternatea transcriptome, Conus geographus conotoxin-specific PDI (Cg_csPDI; GenBank accession no. KT874567) and Conus geographus canonical PDI (Cg_PDI; GenBank accession no. KT874559). Domain architecture of canonical PDI sequences is displayed on top. a- and a'- domains containing the active site “CXXC” motif, and the C-terminal ER-retention signal is highlighted.
Supplementary Figure S9: Heatmap of differential expression of protein disulphide genes (highlighted in blue font) and ER-oxidoreductin 1 gene (highlighted in red font) across four tissues of *C. ternatea de novo* transcriptome. The heatmap was generated using average TPM values along with Pearson correlation coefficient clustering method and complete linkage on genes. Positive (red) and negative (blue) values correspond to up-and down-regulated clusters respectively.
Supplementary Figure S10:
Heatmap of differential expression of peptidyl prolyl cis-trans isomerase (PPIase) across four tissues of *C. ternatea de novo* transcriptome. The heatmap was generated using average TPM values along with Pearson correlation coefficient clustering method and complete linkage on genes. Positive (red) and negative (blue) values correspond to up- and down-regulated clusters respectively.
## Supplementary Table S1: Raw reads summary

| Sl. No | Sample                  | Read orientation | Mean read quality (Phred score) | Number of reads | % GC | % Q < 10 | % Q 10–20 | % Q 20–30 | % Q > 30 | Number of bases (MB) | Mean read length (bp) |
|--------|-------------------------|------------------|--------------------------------|-----------------|------|---------|----------|----------|---------|----------------------|----------------------|
| 1      | C.ternatea-Leaf-1-A     | R1               | 33.51                          | 16868898        | 44.42| 0.02    | 8.87     | 8.35     | 82.76   | 1686.89              | 100                  |
|        |                         | R2               | 34.68                          | 16868898        | 44.63| 0.32    | 5.38     | 5.09     | 89.21   | 1686.89              | 100                  |
| 2      | C.ternatea-Leaf-1-B     | R1               | 33.62                          | 15579380        | 46.85| 0.02    | 8.49     | 8.11     | 83.38   | 1557.94              | 100                  |
|        |                         | R2               | 34.70                          | 15579380        | 46.91| 0.32    | 5.23     | 5.09     | 89.36   | 1557.94              | 100                  |
| 3      | C.ternatea-Leaf-2-A     | R1               | 34.04                          | 19273240        | 44.64| 0.02    | 7.28     | 7.10     | 85.60   | 1927.32              | 100                  |
|        |                         | R2               | 34.62                          | 19273240        | 44.74| 0.32    | 5.51     | 5.24     | 88.92   | 1927.32              | 100                  |
| 4      | C.ternatea-Leaf-2-B     | R1               | 33.88                          | 17517155        | 44.94| 0.02    | 7.76     | 7.46     | 84.76   | 1751.72              | 100                  |
|        |                         | R2               | 34.65                          | 17517155        | 45.09| 0.32    | 5.43     | 5.16     | 89.09   | 1751.72              | 100                  |
| 5      | C.ternatea-Flower-1-A   | R1               | 34.09                          | 17695012        | 42.66| 0.02    | 7.21     | 6.99     | 85.78   | 1769.50              | 100                  |
|        |                         | R2               | 34.77                          | 17695012        | 42.54| 0.30    | 5.17     | 4.89     | 89.64   | 1769.50              | 100                  |
| 6      | C.ternatea-Flower-1-B   | R1               | 34.01                          | 16868324        | 43.82| 0.02    | 7.38     | 7.23     | 85.37   | 1686.83              | 100                  |
|        |                         | R2               | 34.89                          | 16868324        | 43.57| 0.30    | 4.71     | 4.68     | 90.31   | 1686.83              | 100                  |
| 7      | C.ternatea-Flower-2-A   | R1               | 33.74                          | 23074480        | 43.62| 0.02    | 8.26     | 7.80     | 83.92   | 2307.45              | 100                  |
|        |                         | R2               | 35.08                          | 23074480        | 43.52| 0.30    | 4.22     | 4.16     | 91.32   | 2307.45              | 100                  |
| 8      | C.ternatea-Flower-2-B   | R1               | 33.66                          | 18826377        | 43.53| 0.02    | 8.52     | 7.97     | 83.48   | 1882.64              | 100                  |
|        |                         | R2               | 35.01                          | 18826377        | 43.45| 0.30    | 4.43     | 4.34     | 90.93   | 1882.64              | 100                  |
| 9      | C.ternatea-Pod-1-A      | R1               | 33.64                          | 18422283        | 43.81| 0.02    | 8.55     | 8.04     | 83.38   | 1842.23              | 100                  |
|        |                         | R2               | 35.14                          | 18422283        | 43.91| 0.30    | 4.02     | 4.08     | 91.61   | 1842.23              | 100                  |
| 10     | C.ternatea-Pod-1-B      | R1               | 33.85                          | 17781619        | 43.47| 0.02    | 7.91     | 7.55     | 84.52   | 1778.16              | 100                  |
|        |                         | R2               | 34.92                          | 17781619        | 43.70| 0.30    | 4.64     | 4.58     | 90.48   | 1778.16              | 100                  |
| 11     | C.ternatea-Pod-2-A      | R1               | 34.08                          | 21853026        | 44.44| 0.02    | 7.17     | 7.02     | 85.79   | 2185.30              | 100                  |
|        |                         | R2               | 34.59                          | 21853026        | 44.52| 0.32    | 5.59     | 5.32     | 88.77   | 2185.30              | 100                  |
| 12     | C.ternatea-Pod-2-B      | R1               | 33.56                          | 19212475        | 44.40| 0.02    | 8.71     | 8.21     | 83.06   | 1921.25              | 100                  |
|        |                         | R2               | 34.69                          | 19212475        | 44.54| 0.32    | 5.31     | 5.08     | 89.29   | 1921.25              | 100                  |
| 13     | C.ternatea-Stem-1-A     | R1               | 33.78                          | 21671955        | 43.10| 0.02    | 8.15     | 7.68     | 84.15   | 2167.20              | 100                  |
|        |                         | R2               | 34.91                          | 21671955        | 43.11| 0.30    | 4.71     | 4.58     | 90.41   | 2167.20              | 100                  |
| 14     | C.ternatea-Stem-1-B     | R1               | 33.41                          | 16434353        | 43.22| 0.02    | 9.22     | 8.61     | 82.15   | 1643.44              | 100                  |
|        |                         | R2               | 34.90                          | 16434353        | 43.29| 0.30    | 4.74     | 4.62     | 90.34   | 1643.44              | 100                  |
| 15     | C.ternatea-Stem-2-A     | R1               | 33.76                          | 17054426        | 43.45| 0.02    | 8.21     | 7.73     | 84.03   | 1705.44              | 100                  |
|        |                         | R2               | 34.83                          | 17054426        | 43.41| 0.30    | 4.94     | 4.76     | 90.00   | 1705.44              | 100                  |
| 16     | C.ternatea-Stem-2-B     | R1               | 33.95                          | 14732524        | 43.43| 0.02    | 7.64     | 7.30     | 85.04   | 1473.25              | 100                  |
|        |                         | R2               | 34.92                          | 14732524        | 43.37| 0.30    | 4.68     | 4.58     | 90.44   | 1473.25              | 100                  |
Supplementary Table S2: TPM values across biological replicates of all the transcripts discussed in this work.

(a) Cyclotide precursor genes

| Transcript_ID | Flower_1 | Flower_2 | Leaf_1 | Leaf_2 | Pod_1 | Pod_2 | Stem_1 | Stem_2 |
|---------------|----------|----------|--------|--------|-------|-------|--------|--------|
| ctr28192_c2_g1_i2 | 0.15 | 0.99 | 2.25 | 2.08 | 40.06 | 4.84 | 24.53 | 17.51 |
| ctr28192_c2_g1_i7 | 1.43 | 1.74 | 7.00 | 12.52 | 2.78 | 0.66 | 159.18 | 123.67 |
| ctr28192_c2_g2_i1 | 0.30 | 0.10 | 0.25 | 0.00 | 22.65 | 0.53 | 1.07 | 0.97 |
| ctr28192_c2_g3_i3 | 63.79 | 116.87 | 14276.88 | 28807.20 | 12415.99 | 13892.23 | 11362.00 | 10765.10 |
| ctr28192_c2_g4_i3 | 121.51 | 220.90 | 870.84 | 1119.08 | 131.09 | 271.43 | 3333.16 | 3225.11 |
| ctr28192_c2_g6_i1 | 713.84 | 753.83 | 491.17 | 1936.65 | 3590.99 | 4684.75 | 3270.64 | 3624.38 |
| ctr28192_c2_g6_i2 | 2.07 | 5.47 | 0.00 | 0.57 | 20.54 | 11.64 | 6.34 | 4.93 |
| ctr28495_c0_g2_i1 | 114.66 | 96.79 | 1125.24 | 2203.40 | 1139.05 | 1097.28 | 437.42 | 349.29 |
| ctr28495_c0_g2_i2 | 18.02 | 31.49 | 1331.48 | 2848.55 | 1252.17 | 1250.82 | 1056.21 | 908.77 |
| ctr28495_c0_g3_i1 | 0.00 | 0.51 | 0.66 | 0.24 | 0.52 | 0.10 | 0.00 | 0.16 |
| ctr28495_c0_g4_i1 | 17.17 | 26.48 | 0.00 | 0.00 | 49.41 | 8.03 | 1.37 | 0.00 |
| ctr28495_c0_g5_i3 | 0.26 | 1.06 | 35.22 | 61.27 | 6.27 | 10.18 | 2.18 | 1.60 |
| ctr28495_c0_g7_i1 | 0.00 | 0.00 | 0.00 | 0.00 | 404.51 | 0.00 | 0.00 | 0.00 |
| ctr28495_c0_g7_i3 | 19707.33 | 24483.00 | 5966.39 | 8060.99 | 21976.51 | 22768.29 | 2822.98 | 4884.34 |
| ctr28841_c1_g1_i3 | 0.16 | 0.00 | 0.52 | 2.44 | 1.77 | 0.85 | 4702.12 | 4642.01 |
| ctr28841_c1_g1_i4 | 0.00 | 1.04 | 3.09 | 0.45 | 2.75 | 1.36 | 32.03 | 23.04 |
| ctr28841_c1_g1_i5 | 2.81 | 3.23 | 15.57 | 4.53 | 115.08 | 91.97 | 41.25 | 52.81 |
| ctr28841_c1_g1_i6 | 0.00 | 0.20 | 0.55 | 0.00 | 6.08 | 4.33 | 1.90 | 1.86 |
| ctr28841_c1_g1_i7 | 0.04 | 0.56 | 32.18 | 1.73 | 316.81 | 389.80 | 176.13 | 156.18 |
| ctr28841_c1_g1_i8 | 35.85 | 50.21 | 64.44 | 77.43 | 511.29 | 637.77 | 216.41 | 206.34 |
| ctr28841_c1_g2_i1 | 954.63 | 1697.22 | 1805.76 | 5792.96 | 3858.11 | 1108.97 | 7744.54 | 5133.19 |
| ctr28841_c1_g3_i1 | 0.37 | 1.77 | 0.00 | 0.00 | 34.18 | 8.52 | 14.63 | 14.14 |
| ctr28841_c1_g4_i1 | 0.00 | 0.00 | 0.00 | 2.48 | 2.39 | 1.18 | 1780.64 | 1597.27 |
| ctr28841_c2_g3_i1 | 174.77 | 207.17 | 494.23 | 714.35 | 1276.97 | 409.45 | 989.62 | 959.27 |
| ctr28841_c2_g4_i2 | 17.70 | 55.80 | 657.34 | 766.34 | 63.69 | 248.89 | 3625.87 | 3362.48 |
| ctr28926_c1_g1_i1 | 4.34 | 11.84 | 1995.71 | 3296.19 | 1509.06 | 1037.23 | 1106.65 | 818.95 |
| ctr28926_c1_g1_i2 | 5.95 | 7.86 | 1636.11 | 3471.18 | 1491.08 | 949.49 | 1139.77 | 782.01 |
| ctr28926_c1_g1_i3 | 0.97 | 1.36 | 92.17 | 211.71 | 24.68 | 40.67 | 28.52 | 25.30 |
| ctr28926_c1_g1_i4 | 0.46 | 1.78 | 386.32 | 623.61 | 276.08 | 194.26 | 189.91 | 150.18 |
| ctr28926_c1_g1_i6 | 0.00 | 0.00 | 93.82 | 181.43 | 62.20 | 32.33 | 41.04 | 24.44 |
| ctr28926_c1_g2_i1 | 926.54 | 1176.53 | 1234.29 | 2029.37 | 4573.74 | 1627.36 | 6406.79 | 6228.02 |
| ctr28926_c1_g3_i1 | 0.00 | 4.95 | 604.35 | 1014.65 | 465.62 | 291.31 | 395.62 | 303.25 |
| ctr28926_c1_g3_i2 | 18.62 | 49.18 | 15340.37 | 25515.53 | 9048.21 | 6944.78 | 5877.14 | 4467.25 |
| ctr28926_c2_g1_i1 | 1025.50 | 1540.48 | 8128.47 | 8429.72 | 793.87 | 2785.64 | 17031.04 | 16555.49 |
| ctr28926_c2_g3_i3 | 17.02 | 38.97 | 1087.79 | 2135.36 | 666.29 | 467.58 | 1602.96 | 1849.74 |
| ctr29379_c2_g2_i1 | 49.88 | 46.88 | 5.57 | 9.14 | 508.35 | 9.55 | 64.29 | 36.91 |
### (b) Asparaginyl endopeptidase

| Transcript_ID | Flower_1 | Flower_2 | Leaf_1 | Leaf_2 | Pod_1 | Pod_2 | Stem_1 | Stem_2 |
|---------------|----------|----------|--------|--------|-------|-------|--------|--------|
| ctr17958_c0_g1_i1 | 0.00 | 0.00 | 0.00 | 0.00 | 26.90 | 0.00 | 0.00 | 0.00 |
| ctr24604_c1_g1_i1 | 10.58 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr24604_c1_g2_i2 | 94.13 | 1.87 | 0.00 | 0.00 | 0.00 | 0.00 | 0.09 | 0.02 |
| Transcript_ID | Flower_1 | Flower_2 | Leaf_1 | Leaf_2 | Pod_1 | Pod_2 | Stem_1 | Stem_2 |
|---------------|----------|----------|--------|--------|-------|-------|--------|--------|
| ctr27109_c1_g4_i1 | 0.59 | 2.86 | 2.72 | 4.64 | 6.56 | 4.25 | 7.77 | 8.07 |
| ctr28924_c0_g1_i1 | 0.40 | 0.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr28924_c0_g1_i2 | 5.20 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr29014_c2_g1_i2 | 4.36 | 7.73 | 38.06 | 57.99 | 6.27 | 27.62 | 24.39 | 30.54 |

(c) Protein disulphide isomerase

| Transcript_ID | Flower_1 | Flower_2 | Leaf_1 | Leaf_2 | Pod_1 | Pod_2 | Stem_1 | Stem_2 |
|---------------|----------|----------|--------|--------|-------|-------|--------|--------|
| ctr16675_c0_g1_i1 | 0.62 | 2.36 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| ctr21421_c0_g1_i1 | 0.10 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.55 | 0.38 |
| ctr23276_c0_g1_i1 | 0.20 | 1.86 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr23512_c0_g1_i5 | 51.39 | 86.65 | 51.30 | 38.27 | 91.22 | 56.33 | 90.71 | 99.42 |
| ctr24519_c0_g1_i1 | 8.75 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr24519_c0_g1_i2 | 0.06 | 0.21 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr24519_c0_g2_i1 | 7.62 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr24519_c0_g3_i1 | 4.98 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr26632_c5_g4_i3 | 36.49 | 0.44 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr28710_c1_g1_i4 | 8.79 | 16.31 | 7.67 | 6.54 | 18.05 | 16.16 | 23.41 | 24.20 |
| ctr29427_c2_g3_i1 | 2.75 | 0.71 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr29427_c2_g3_i2 | 111.03 | 1.58 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 |
| ctr30222_c3_g6_i1 | 17.28 | 24.59 | 19.32 | 13.92 | 59.02 | 24.03 | 33.87 | 38.37 |
| ctr39989_c0_g1_i1 | 0.10 | 0.85 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr6173_c0_g1_i1 | 25.91 | 0.72 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 |

(d) Endoplasmic reticulum oxidoreductin-1

| Transcript_ID | Flower_1 | Flower_2 | Leaf_1 | Leaf_2 | Pod_1 | Pod_2 | Stem_1 | Stem_2 |
|---------------|----------|----------|--------|--------|-------|-------|--------|--------|
| ctr203_c0_g1_i1 | 4.87 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

(e) Peptidylprolyl cis-trans isomerase/Cyclophilin

| Transcript_ID | Flower_1 | Flower_2 | Leaf_1 | Leaf_2 | Pod_1 | Pod_2 | Stem_1 | Stem_2 |
|---------------|----------|----------|--------|--------|-------|-------|--------|--------|
| ctr10137_c0_g1_i1 | 10.99 | 0.21 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr11074_c0_g1_i1 | 1.87 | 3.38 | 3.40 | 2.63 | 12.51 | 5.24 | 11.56 | 12.96 |
| ctr15543_c0_g1_i1 | 20.08 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr15837_c0_g2_i1 | 14.42 | 0.24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr17099_c0_g1_i1 | 6.93 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 |
| ctr17583_c0_g1_i1 | 8.72 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr18375_c0_g1_i1 | 17.46 | 0.26 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr18912_c0_g1_i1 | 7.73 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 |
| ctr19397_c0_g1_i1 | 6.46 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr20383_c0_g1_i1 | 6.71 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr22690_c0_g1_i4 | 0.01 | 0.04 | 0.27 | 0.00 | 0.00 | 0.02 | 0.03 | 0.01 |
| ctr22690_c0_g1_i5 | 31.89 | 54.30 | 12.75 | 14.91 | 32.25 | 21.40 | 24.71 | 26.77 |
| ctr22957_c0_g1_i1 | 104.57 | 1.79 | 0.00 | 0.00 | 0.00 | 0.00 | 0.08 | 0.00 |
| ctr22957_c0_g1_i2 | 0.00 | 1.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr22957_c0_g1_i3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr24033_c0_g1_i1 | 7.12 | 9.03 | 44.21 | 40.12 | 6.59 | 16.25 | 16.87 | 15.65 |
| ctr24500_c0_g1_i2 | 0.35 | 0.90 | 0.73 | 0.92 | 2.23 | 2.50 | 1.85 | 2.21 |
| ctr24674_c0_g1_i1 | 0.31 | 2.57 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr24674_c0_g1_i2 | 0.80 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr24674_c0_g1_i3 | 0.04 | 0.46 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr24700_c0_g1_i1 | 6.19 | 10.33 | 9.11 | 9.50 | 11.49 | 9.98 | 16.20 | 16.41 |
| ctr24700_c0_g1_i2 | 0.37 | 0.88 | 1.19 | 0.92 | 1.01 | 1.24 | 1.59 | 1.73 |
| ctr25168_c0_g1_i1 | 0.44 | 0.25 | 4.36 | 2.97 | 2.15 | 1.87 | 1.12 | 1.49 |
| ctr25226_c0_g1_i11 | 0.22 | 0.27 | 0.19 | 0.27 | 0.07 | 0.31 | 0.48 | 0.48 |
| ctr25226_c0_g1_i13 | 0.10 | 0.27 | 0.39 | 0.40 | 0.26 | 0.58 | 0.93 | 0.93 |
| ctr25226_c0_g1_i15 | 0.48 | 0.63 | 0.95 | 0.71 | 2.24 | 1.85 | 1.70 | 2.79 |
| ctr25226_c0_g1_i12 | 0.07 | 0.48 | 0.29 | 0.10 | 0.27 | 0.09 | 0.28 | 0.20 |
| ctr25239_c0_g1_i1 | 0.12 | 0.95 | 0.00 | 0.00 | 0.00 | 0.00 | 4.07 | 2.70 |
| ctr25252_c0_g1_i1 | 0.00 | 0.04 | 0.26 | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 |
| ctr25252_c0_g1_i2 | 5.36 | 12.11 | 6.21 | 7.77 | 10.62 | 21.37 | 15.60 | 18.25 |
| ctr25252_c0_g1_i4 | 0.52 | 0.35 | 0.38 | 0.37 | 0.32 | 0.42 | 0.41 | 0.56 |
| ctr26423_c0_g1_i5 | 50.51 | 94.82 | 35.42 | 28.06 | 314.06 | 122.13 | 157.35 | 157.42 |
| ctr26897_c1_g2_i13 | 9.15 | 13.86 | 15.24 | 13.09 | 9.66 | 17.12 | 18.76 | 21.61 |
| ctr27050_c0_g3_i1 | 5.44 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr27345_c1_g1_i1 | 0.09 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.80 | 0.53 |
| ctr27345_c3_g2_i22 | 0.00 | 0.86 | 0.09 | 0.22 | 0.21 | 0.40 | 0.00 | 0.11 |
| ctr27355_c0_g1_i1 | 0.00 | 0.01 | 0.00 | 0.00 | 0.02 | 0.01 | 0.14 | 0.00 |
| ctr27355_c0_g1_i10 | 0.28 | 0.08 | 0.02 | 0.00 | 0.09 | 0.00 | 0.11 | 0.43 |
| ctr27652_c1_g2_i7 | 7.87 | 12.73 | 14.52 | 16.90 | 18.65 | 17.46 | 22.17 | 25.15 |
| ctr27784_c2_g1_i14 | 20.03 | 39.14 | 15.16 | 14.54 | 13.57 | 32.44 | 23.38 | 26.48 |
| ctr27784_c2_g1_i20 | 0.00 | 0.00 | 0.04 | 0.14 | 0.26 | 0.01 | 0.06 | 0.02 |
| ctr27784_c2_g1_i28 | 0.92 | 2.94 | 1.41 | 2.62 | 0.41 | 1.62 | 2.91 | 4.01 |
| ctr27856_c0_g2_i1 | 0.01 | 0.17 | 0.31 | 0.58 | 0.21 | 0.47 | 0.51 | 0.21 |
| ctr27930_c3_g13_i1 | 9.45 | 0.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr28568_c0_g5_i4 | 10.09 | 17.36 | 2.86 | 2.33 | 10.11 | 8.67 | 14.34 | 15.24 |
| ctr28930_c1_g3_i5 | 15.99 | 24.98 | 21.42 | 28.31 | 17.68 | 18.46 | 21.04 | 21.36 |
| ctr28936_c0_g2_i2 | 176.41 | 4.22 | 0.00 | 0.00 | 0.00 | 0.00 | 0.19 | 0.03 |
| ctr28936_c0_g2_i3 | 0.00 | 1.55 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr28936_c0_g3_i12 | 5.00 | 8.63 | 6.02 | 6.52 | 5.61 | 3.26 | 24.88 | 18.19 |
| ctr28936_c0_g4_i4 | 2.37 | 6.50 | 129.69 | 101.54 | 10.68 | 33.68 | 7.69 | 12.97 |
| ctr28936_c0_g4_i6 | 15.04 | 27.94 | 473.64 | 394.74 | 54.54 | 109.57 | 32.12 | 34.68 |
| ctr28936_c0_g7_i1 | 30.50 | 0.93 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ctr29069_c0_g1_i11 | 0.00 | 0.05 | 0.00 | 0.09 | 0.00 | 0.02 | 0.02 | 0.15 |
TPM value is averaged for technical replicates per biological replicate per plant tissue.

**Supplementary Table S3: Summary of de novo transcriptome assembly.**

| Assembly details                        | Summary statistics   |
|-----------------------------------------|----------------------|
| Total size of transcriptome             | 402,275,420 bp       |
| Total number of reads                   | 281,871,893          |
| Reads mapped to transcriptome assembly  | 94.44%               |
| Mean contig length                      | 1441.89 bp           |
| Median contig length                    | 988 bp               |
| Contig N50                               | 2437 bp              |
| Total trinity genes                     | 120,067              |
| Total trinity transcripts               | 278,991              |
| Percent GC                              | 38.92                |
| Average number of reads in Leaf tissue replicates | 16.6 million     |
| Average number of reads in Pod tissue replicates | 18.57 million       |
| Average number of reads in Stem tissue replicates | 16.82 million   |
| Average number of reads in Flower tissue replicates | 18.45 million     |

*after quality filtering with Trimmomatic*
**Supplementary Table S4: Top 20 enriched GO terms from highly expressed transcripts**

| Count | GO term                                         | Class | P-value    |
|-------|-------------------------------------------------|-------|------------|
| 240   | regulation of transcription, DNA-templated      | BP    | 7.70E-24   |
| 149   | RNA splicing                                    | BP    | 1.05E-04   |
| 148   | metal ion binding                              | MF    | 1.97E-04   |
| 137   | transmembrane transport                         | BP    | 4.89E-11   |
| 129   | rRNA processing                                 | BP    | 1.10E-04   |
| 120   | protein ubiquitination                          | BP    | 1.98E-12   |
| 117   | response to salt stress                         | BP    | 2.33E-05   |
| 92    | response to water deprivation                   | BP    | 1.11E-06   |
| 90    | viral process                                   | BP    | 1.73E-04   |
| 84    | recognition of pollen                           | BP    | 3.54E-04   |
| 76    | lipid catabolic process                         | BP    | 1.46E-07   |
| 76    | tricarboxylic acid cycle                        | BP    | 5.25E-05   |
| 75    | defense response                                | BP    | 8.38E-20   |
| 73    | response to wounding                            | BP    | 1.56E-04   |
| 70    | response to oxidative stress                    | BP    | 2.95E-05   |
| 65    | plasma membrane                                 | CC    | 1.04E-11   |
| 64    | DNA recombination                               | BP    | 1.48E-14   |
| 61    | serine-type endopeptidase activity              | MF    | 2.79E-04   |
| 54    | oxidoreductase activity                         | MF    | 6.54E-04   |
| 48    | DNA integration                                 | BP    | 8.19E-08   |
Supplementary Table S5: Cyclotide transcript sequences determined in the current transcriptome assembly of *C. ternatea*.

| Sl. No. | Transcript ID | GenBank Accession Numbers | Cyclotide Mature Domain Sequence | Topology | Tissue Abundance | Previous Evidence (“Cter” – Craik group) (“Cliootide – Tam group”) |
|--------|---------------|--------------------------|---------------------------------|----------|-----------------|---------------------------------------------------------------|
| 1      | ctr28192_c2_g1_i2/ ctr28192_c2_g1_i7 | MT468661 MT468662 | ---GIPCGESCVFIPCTITALLGCSCKS-KVCYKN | B | P/S | Cter 14; CliotideT40 |
| 2      | ctr28192_c2_g2_i1 | MT468663 | ---GIPCGESCVFIPCLTT-VVGCSCKN-KVCYNN | B | P | Cliotide T9 |
| 3*     | ctr28192_c2_g3_i3 | MT468664 | --GRPTCGETCFKTCKYTP---GCSCS-YPICKKN | M | L | |
| 4*     | ctr28192_c2_g4_i3 | MT468665 | -GDALKCGETCFGCTCYTP---GCSCD-YPICKKN | M | S | |
| 5*     | ctr28192_c2_g6_i1 | MT468666 | ---------------FKTCKYTP---GCSCS-YPVCKRN | partial | P | |
| 6*     | ctr28192_c2_g6_i2 | MT468667 | -GCLPICGETCFKTKCYTK---GCSCS-YPICKKN | M; unusual | P | |
| 7      | ctr28495_c0_g2_i1 | MT468668 | --- | | | |
| 8*     | ctr28495_c0_g2_i2 | MT468669 | ---GDALKCGETCFKTKCYTK---GCSCS-YPVCKRN | M | L | Cliotide T15; Cter24 |
| 9      | ctr28495_c0_g3_i1 | MT468670 | --- | | | |
| 10     | ctr28495_c0_g4_i1 | MT468671 | ---GDPLACGETCFGCTCYTP---GCVDPWPICTKN | M | P | |
| 11*    | ctr28495_c0_g5_i3 | MT468672 | -ANIPMTCGPCLTDECWTP---GCEYH-CKYCKNS | H; unusual; acyclic | L | |
| 12*    | ctr28495_c0_g7_i1/ ctr28495_c0_g7_i3 | MT468673/ MT468674 | ---LPTCGETCGFTCYTP---DCSCS-WPICMK | M | P/F | |
| 13     | ctr28841_c1_g1_i3 | MT468675 | ---KIPCGESCWIPCFTS-AFGCQCS-KVCYHS | B; acyclic | S | Cliotide T38 |
| 14*    | ctr28841_c1_g1_i4/ ctr28841_c1_g1_i5/ ctr28841_c1_g1_i6 | MT468676/ MT468677/ MT468678 | --------SCVWIPCITG-AIGCSCKN-RVCYRN | partial | S/P/P | |
| 15     | ctr28841_c1_g1_i7 | MT468679 | ---DTTPCGESCWIVPCVSS-IVGSCQKN-KVCYQN | B | P | Cliotide T13; Cter 23 |
| 16     | ctr28841_c1_g1_i8 | MT468680 | ---GFNSCEASYVLPFQRS---GCSCKK-RQCYKN | H | P | Cter 34; CliotideT33 |
| 17     | ctr28841_c1_g2_i1 | MT468681 | ---GIVPCESCVFIPCTIST-VIGCSCKN-KVCYRN | B | S | Cter A |
| 18*    | ctr28841_c1_g3_i1 | MT468682 | ---GSCSGESCVFIPCTST-IAGCSCKN-KVCYLN | B | P | |
| 19*    | ctr28841_c1_g4_i1 | MT468683 | ---------------ESCWIPCSTLTG-YFGCQCS-KVCYRN | partial | S | |
| 20*    | ctr28841_c2_g3_i1 | MT468684 | ---CVIPCGESRVFIPCTIG-AIGCSCKN-KVCYRN | B; unusual | P | |
| 21*    | ctr28841_c2_g4_i2 | MT468685 | ---ARIPCGESCWIVPCITALTGVCAK--- | partial | S | |
|    |sequence ID/  |  |Accession numbers |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|
| 22*| ctr28926_c1_g1_i1/  | MT468686/  | -GSITCGGGGCLLGRGICYP---GCTCV-RRICRRN  |  | H  |  |  |  |
| 23 | ctr28926_c1_g1_i2/  | MT468687/  | -GSAIRCGERCLLGRGHCYP---GCTCV-RRICRRN  |  | B  |  | L/L  | Cter 13 |
| 24*| ctr28926_c1_g1_i3  | MT468688  | -GSAIRCGERCLLGRGHCYP---GCTCV-RRICRRN  |  | H  |  | L  |  |
| 25 | ctr28926_c1_g2_i1  | MT468691  | -GDLFKGEGTFGGCTYTP---GCSCD-YPICKNN  |  | M  |  | S  | Cliotide T32 |
| 26*| ctr28926_c1_g3_i1  | MT468692  | GSSVTCGETCLRGCYTP---GCTCV-RPICKKN  |  | M  |  | L  |  |
| 27 | ctr28926_c1_g3_i2  | MT468693  | -GSVIGCGETCLRGCYTP---GCTCD-HGICKKN  |  | H  |  | L  | Cliotide T16 |
| 28 | ctr28926_c2_g2_i1  | MT468694  | --GLPICGETCFTGTCTYTP---GCTCS-YPVCKKN  |  | M  |  | S  | Cliotide T18; Cter 6 |
| 29*| ctr28926_c2_g3_i3  | MT468695  | -GDPFKGESCFAKGKTYP---GCTCS-RPICKKN  |  | M  |  | L  |  |
| 30*| ctr29379_c2_g2_i1/  | MT468696/  | -GSPCGERCIFIPCIST-VIGCSCKN-KCYKN  |  | B  |  | P/P  |  |
| 31*| ctr29379_c3_g1_i2  | MT468698  | ---GIPCGERCIFIPCTVATLGCSCKN-KVCYKN  |  | B  |  | P  |  |
| 32 | ctr29379_c3_g2_i2  | MT468699  | --DTIPCGERCWSICISS-ILGCESCKD-KVCYKN  |  | B  |  | S  | Cliotide T14 |
| 33*| ctr29379_c3_g2_i10/  | MT468700/  | --GIPCGERCIFISCTVTATLGCSCKD-KVCYKN  |  | B  |  | S/S  |  |
| 34*| ctr29379_c3_g2_i12  | MT468702  | --GIPCGERCIFISCTVTATLGCSCKD-KVCYKN  |  | B  |  | S/S  |  |
| 35 | ctr29379_c3_g2_i3  | MT468703  | --GIPCGERCIFISCTVTATLGCSCKD-KVCYKN  |  | B  |  | S  | Cliotide T18; Cter R |
| 36*| ctr29379_c3_g2_i4  | MT468704  | --GIPCGERCIFISCTVTATLGCSCKD-KVCYKN  |  | B  |  | F  |  |
| 37 | ctr29379_c3_g2_i7/  | MT468707/  | --GIPCGERCIFISCTVTATLGCSCKD-KVCYKN  |  | B  |  | S/S  | Cter 29; Cliotide T44 |
| 38*| ctr29379_c3_g2_i9/  | MT468709/  | --GIPCGERCIFISCTVTATLGCSCKD-KVCYKN  |  | B  |  | S/S  |  |
| 39 | ctr29379_c3_g2_i11  | MT468724  | --GIPCGERCIFISCTVTATLGCSCKD-KVCYKN  |  | B  |  | S  | Cliotide T18; Cter 21 |
| 40 | ctr29609_c1_g1_i2/  | MT468711/  | --SYIPCGERCIFIPCTVATLGCSCKD-KVCYKN  |  | B  |  | F/S  | Cter 10; Cliotide T34 |
| 41 | ctr29609_c1_g2_i11  | MT468713  | --DLICSSTCLHTPKAS---VYCK-NAVCYKN  |  | H  |  | P  | Cliotide T18; Cter 43 |
| 42 | ctr29609_c1_g2_i13  | MT468714/  | --DLQACETVHSCIGP---CYKCHVICYKN  |  | H  |  | S/P  | Cliotide T31; Cliotide T46 |
|   | Accession | Sequence 1 | Sequence 2 | Sequence 3 | Tissue Abundance | Cterm |
|---|------------|------------|------------|------------|------------------|-------|
| 43*| ctr29609_c1_g3_i1 | MT468716 | ---GIPCGESCVFIPCFI---IPGCSCKD-KVCYLN | B | P |
| 44 | ctr29609_c1_g4_i1 | MT468717 | ---VDGFCLETVCILPCFSS-VAGCYCHG-STCMRG | B; acyclic | F |
| 45 | ctr29746_c1_g1_i2/ ctr29746_c1_g1_i6/ ctr29746_c1_g1_i10 | MT468720/ MT468719/ MT468718 | ---GIPCGESCVFIPCISSS-VVGCSCKS-KVCYNN | B | P/P/S | Cliotide T37 |
| 46 | ctr29746_c1_g2_i1/ ctr29746_c1_g2_i6 | MT468721/ MT468726 | ---GIPCGESCVYIPCTVTALLGCSCKN-KVCYRN | B | P/S | Cliotide T8 |
| 47* | ctr29746_c1_g2_i2 | MT468722 | ---GIPCVESCVFIPCTVTALLGCSCKD-KVCYKN | B | S |
| 48* | ctr29746_c1_g2_i3/ ctr29746_c1_g2_i5 | MT468723/ MT468725 | ---GVPCGESCVYIPCTVTALLGCSCKN-KVCYRN | B | S/S |
| 49 | ctr29746_c1_g3_i1/ ctr29746_c1_g3_i2/ ctr29746_c1_g3_i4 | MT468727/ MT468728/ MT468730 | ---SIPCGESCVYIPCLTT-IVGCSCKS-NVCYSN | B | P/S/P | Cter 19 |
| 50* | ctr29746_c1_g3_i3 | MT468729 | ---------ALPIST-IVGCSCKS-NVCYSN | partial | F |
| 51 | ctr29746_c1_g4_i1 | MT468731 | ---GVPCAESCVWIPCTVTALLGCSCKD-KVCYLN | B | P | Cter B |

*Precursor sequences with novel cyclotides domains identified in the current study.

*Tissue abundance in the current transcriptome assembly using untransformed TPM values. P: pod; L: leaf; S: stem; F: flower.
Supplementary Table S6: Identification of cyclotide sequences in purified fractions of *C. ternatea* stems, leaves, pods and flowers crude extracts by MALDI-TOF MS (See Supplementary Fig. S)

| Sl. No. | Transcript ID | Peptide ID | Calc. Mass (Monoisotopic) [Da] | Evidence in Tissue Fractions |
|--------|---------------|------------|-------------------------------|-----------------------------|
| 1      | ctr28192_c2_g1_i2/ ctr28192_c2_g1_i7 | ctr pep 1 | 3212.5 | S(C), L(E), P(C) |
| 2      | ctr28192_c2_g2_i1 | ctr pep 2 | 3126.4 | P(D), P(E) |
| 3*     | ctr28192_c2_g3_i3 | ctr pep 3 | 3150.4 | S(B), L(B), L(C) |
| 4*     | ctr28192_c2_g4_i3 | ctr pep 4 | 3109.3 | S(D), S(E), L(D), L(E), P(D), P(E), F(E) |
| 5*     | ctr28192_c2_g6_i1 | ctr pep 5 | 2262.0 | |
| 6*     | ctr28192_c2_g6_i2 | ctr pep 6 | 3253.5 | |
| 7      | ctr28495_c0_g2_i1 | ctr pep 7 | 3164.5 | P(D) |
| 8*     | ctr28495_c0_g2_i2 | ctr pep 8 | 3195.4 | L(C), L(D), L(E) |
| 9      | ctr28495_c0_g3_i1 | ctr pep 9 | 3162.3 | S(B) |
| 10     | ctr28495_c0_g4_i1 | ctr pep 10 | 3183.3 | P(C) |
| 11*    | ctr28495_c0_g5_i3 | ctr pep 11 | 3342.4 | |
| 12*    | ctr28495_c0_g7_i1/ ctr28495_c0_g7_i3 | ctr pep 12 | 3000.2 | P(B) |
| 13     | ctr28841_c1_g1_i3 | ctr pep 13 | 3321.4 | P(E) |
| 14*    | ctr28841_c1_g1_i4/ ctr28841_c1_g1_i5/ ctr28841_c1_g1_i6 | ctr pep 14 | 2623.2 | |
| 15     | ctr28841_c1_g1_i7 | ctr pep 15 | 3298.4 | F(C) |
| 16     | ctr28841_c1_g1_i8 | ctr pep 16 | 3247.4 | |
| 17     | ctr28841_c1_g2_i1 | ctr pep 17 | 3267.6 | P(D) |
| 18*    | ctr28841_c1_g3_i1 | ctr pep 18 | 3057.4 | S(C), P(D), F(D) |
| 19*    | ctr28841_c1_g4_i1 | ctr pep 19 | 2880.2 | |
| 20*    | ctr28841_c2_g3_i1 | ctr pep 20 | 3281.6 | P(E) |
| 21*    | ctr28841_c2_g4_i2 | ctr pep 21 | 2837.3 | |
| 22*    | ctr28926_c1_g1_i1/ ctr28926_c1_g1_i4 | ctr pep 22 | 3162.5 | S(B) |
| 23     | ctr28926_c1_g1_i2/ ctr28926_c1_g1_i6 | ctr pep 23 | 3390.7 | |
| 24*    | ctr28926_c1_g1_i3 | ctr pep 24 | 3376.7 | |
| 25     | ctr28926_c1_g2_i1 | ctr pep 25 | 3171.3 | F(D) |
| 26*    | ctr28926_c1_g3_i1 | ctr pep 26 | 3252.5 | |
| 27     | ctr28926_c1_g3_i2 | ctr pep 27 | 3106.4 | P(B), F(B) |
| 28     | ctr28926_c2_g2_i1 | ctr pep 28 | 3021.3 | S(E), P(C) |
| 29*    | ctr28926_c2_g3_i3 | ctr pep 29 | 3175.4 | |
| 30*    | ctr29379_c2_g2_i1/ ctr29379_c2_g2_i3 | ctr pep 30 | 3142.4 | L(D) |
| 31*    | ctr29379_c3_g1_i2 | ctr pep 31 | 3253.5 | |
| 32     | ctr29379_c3_g2_i1 | ctr pep 32 | 3348.5 | |
| 33*    | ctr29379_c3_g2_i10/ ctr29379_c3_g2_i12 | ctr pep 33 | 3216.5 | |
| 34*    | ctr29379_c3_g2_i3/ ctr29379_c3_g2_i4/ ctr29379_c3_g2_i9/ ctr29746_c1_g2_i4 | ctr pep 34 | 3308.4 | P(C) |
| 35     | ctr29379_c3_g2_i5 | ctr pep 35 | 3226.5 | P(D), F(D) |
|   | GenBank Accession Numbers | Transcript ID | *Gene Name | AA Length | Tissue Abundance |
|---|--------------------------|---------------|------------|-----------|-----------------|
| 36* | MT468732 | ctr29379_c3_g2_i6 | CtAEP7 | 442 | P(E) |
| 37 | MT468733 | ctr29379_c3_g2_i7/ctr29379_c3_g2_i11 | ctr pep 37 | 3346.5 | P(E) |
| 38* | MT468734 | ctr29379_c3_g2_i8 | ctr pep 38 | 3280.4 | P(B), F(B), F(C) |
| 39 | MT468735 | ctr29609_c0_g1_i1 | ctr pep 39 | 3240.5 | L(D), F(C) |
| 40 | MT468736 | ctr29609_c1_g1_i2/ctr29609_c1_g1_i6 | ctr pep 40 | 3393.5 | P(B) |
| 41 | MT468737 | ctr29609_c1_g2_i1 | ctr pep 41 | 3042.4 |  |
| 42 | MT468738 | ctr29609_c1_g2_i2/ctr29609_c1_g2_i3 | ctr pep 42 | 3059.3 |  |
| 43* | MT468739 | ctr29609_c1_g3_i1 | ctr pep 43 | 3083.4 | P(D) |
| 44 | MT468740 | ctr29609_c1_g4_i1 | ctr pep 44 | 3233.4 | F(D), F(E) |
| 45 | MT468741 | ctr29746_c1_g1_i2/ctr29746_c1_g1_i6/ctr29746_c1_g1_i10 | ctr pep 45 | 3071.4 | S(D), S(E), L(B), L(C), L(D), P(C), P(D), F(D), F(E) |
| 46 | MT468742 | ctr29746_c1_g2_i1/ctr29746_c1_g2_i6 | ctr pep 46 | 3269.5 |  |
| 47* | MT468743 | ctr29746_c1_g2_i2 | ctr pep 47 | 3268.6 |  |
| 48* | MT468744 | ctr29746_c1_g2_i3/ctr29746_c1_g2_i5 | ctr pep 48 | 3255.5 |  |
| 49 | MT468745 | ctr29746_c1_g3_i1/ctr29746_c1_g3_i2/ctr29746_c1_g3_i4 | ctr pep 49 | 3118.4 | S(E) |
| 50* | MT468746 | ctr29746_c1_g3_i3 | ctr pep 50 | 2038.0 |  |
| 51 | MT468747 | ctr29746_c1_g4_i1 | ctr pep 51 | 3250.5 | P(E), F(D) |

*Precursor sequences with novel cyclotides domains identified in the current study.

Stems: S; Leaves: L; Pods: P; Flowers: F. Letters within parentheses indicate fraction ID (Refer to Supplementary Fig. S)

Supplementary Table S7: AEP transcript sequences determined in the current transcriptome assembly of C. ternatea.

| GenBank Accession Numbers | Transcript ID | *Gene Name | AA Length | Tissue Abundance |
|--------------------------|---------------|------------|-----------|-----------------|
| MT468732 | ctr17958_c0_g1_i1 | CtAEP7 | 442 | P |
| MT468733 | ctr24604_c1_g1_i1 | CtAEP8 | 457 | F |
| MT468734 | ctr24604_c1_g2_i2 | CtAEP9 | 448 | F |
| MT468735 | ctr27109_c1_g4_i1 | CtAEP10 | 376 | S |
| MT468736 | ctr29014_c2_g1_i2 | CtAEP12 | 478 | F |
| MT468737 | ctr29014_c2_g1_i2 | CtAEP12 | 478 | F |
| MT468738 | ctr29014_c2_g1_i2 | CtAEP12 | 478 | F |

*CtAEP sequences identified in the current study.

Tissue abundance in the current transcriptome assembly using untransformed TPM values. P: pod; L: leaf; S: stem; F: flower.
Supplementary Table S8: PDI transcript sequences determined in the current transcriptome assembly of *C. ternatea*.

| GenBank Accession Numbers | Transcript ID          | *Gene Name | Length | Domain Composition | Active Site Motifs | C-terminal Retention Motif | Tissue Abundance |
|---------------------------|------------------------|------------|--------|--------------------|-------------------|---------------------------|-----------------|
| MT468739                  | ctr16675_c0_g1_i1      | CtPDI1     | 479    | a-b-b'-a'          | CGHC, CGHC        | KDEL                      | F               |
| MT468740                  | ctr21421_c0_g1_i1      | CtPDI2     | 487    | a-b-b'-a'          | CGHC, CGFC        | HDEL                      | S               |
| MT468741                  | ctr23276_c0_g1_i1      | CtPDI3     | 470    | a-b-b'-a'          | CGHC, CGHC        | KEEL                      | F               |
| MT468742                  | ctr23512_c0_g1_i5      | CtPDI4     | 336    | a°-a-d             | CGHC, CGHC        | STYV                      | S               |
| MT468743                  | ctr24519_c0_g1_i1      | CtPDI5     | 477    | a-b-b'-a'          | CGHC, CGHC        | KEEL                      | F               |
| MT468744                  | ctr24519_c0_g1_i2      | CtPDI6     | 562    | a-b-b'-a'          | CGHC, CGHC        | KEEL                      | F               |
| MT468745                  | ctr24519_c0_g2_i1      | CtPDI7     | 473    | a-b-b'-a'          | CGHC, CGHC        | KEEL                      | F               |
| MT468746                  | ctr24519_c0_g3_i1      | CtPDI8     | 413    | a°-a-b             | CGHC, CGHC        | KEEL                      | F               |
| MT468747                  | ctr26632_c5_g4_i3      | CtPDI9     | 487    | a-b-b'-a'          | CGHC, CGHC        | KDEL                      | F               |
| MT468748                  | ctr28710_c1_g1_i4      | CtPDI10    | 498    | c-a-b-b'-a'        | CPRS, CINC        | KDEL                      | S               |
| MT468749                  | ctr29427_c2_g3_i1      | CtPDI11    | 470    | a-b-b'-a'          | CGHC, CGHC        | KDEL                      | F               |
| MT468750                  | ctr29427_c2_g3_i2      | CtPDI12    | 470    | a-b-b'-a'          | CGHC, CGHC        | KDEL                      | F               |
| MT468751                  | ctr30222_c3_g6_i1      | CtPDI13    | 410    | a°-a-b             | CGHC, CGHC        | KDQI                      | P               |
| MT468752                  | ctr39989_c0_g1_i1      | CtPDI14    | 415    | a°-a-b             | CGHC, CGHC        | KDEL                      | F               |
| MT468753                  | ctr6173_c0_g1_i1       | CtPDI15    | 416    | a°-a-b             | CGHC, CGHC        | KEEL                      | L               |

* CtPDI sequences identified in the current study.

*Putative domain composition include the a-type (a, a’ a°) and b-type (b, b’) domains, N-terminal acidic domain (c) and Erp29c domain (d).

*Tissue abundance in the current transcriptome assembly using untransformed TPM values. P: pod; L: leaf; S: stem; F: flower.
Supplementary Table S9: PPIase transcript sequences determined in the current transcriptome assembly of *C. ternatea.*

| GenBank Accession Numbers | Transcript ID         | **Gene Name** | **AA length** | **Domain Type** | Predicted localization | Tissue Abundance |
|---------------------------|-----------------------|---------------|---------------|-----------------|------------------------|-----------------|
| MT468755                  | ctr10137_c0_g1_i1     | CtCYP1        | 338           | MD              | M, ER, ER, Cy, G, F   |                 |
| MT468756                  | ctr11074_c0_g1_i1     | CtCYP2        | 360           | MD              | Cy, Cy, Cy, ER, Cy, S |                 |
| MT468757                  | ctr15543_c0_g1_i1     | CtCYP3        | 175           | SD              | Cy, Cy, Cy, Cy, F    |                 |
| MT468758                  | ctr15837_c0_g2_i1     | CtCYP4        | 184           | SD              | Cy, Cy, Cy, Cy, F    |                 |
| MT468759                  | ctr17099_c0_g1_i1     | CtCYP5        | 497           | SD              | N, Cy, Pr, N, Pr, F  |                 |
| MT468760                  | ctr17583_c0_g1_i1     | CtCYP6        | 165           | SD              | Cy, Cy, Cy, Cy, F    |                 |
| MT468761                  | ctr18375_c0_g1_i1     | CtCYP7        | 209           | SD              | P, M, M, Cy, M, F    |                 |
| MT468762                  | ctr18912_c0_g1_i1     | CtCYP8        | 548           | MD              | N, Cy, N, N, N, F    |                 |
| MT468763                  | ctr19397_c0_g1_i1     | CtCYP9        | 624           | MD              | N, Cy, Cy, Cy, F    |                 |
| MT468764                  | ctr20383_c0_g1_i1     | CtCYP10       | 521           | MD              | N, Cy, Cy, Cy, F    |                 |
| MT468765                  | ctr22690_c0_g1_i4     | CtCYP11       | 196           | SD              | E, PM, E, PM, ER, L  |                 |
| MT468766                  | ctr22690_c0_g1_i5     | CtCYP12       | 226           | SD              | ER, ER, V, Cy, V, F  |                 |
| MT468767                  | ctr22957_c0_g1_i1     | CtCYP13       | 220           | SD              | ER, ER, ER, Cy, ER, F|                 |
| MT468768                  | ctr22957_c0_g1_i2     | CtCYP14       | 250           | SD              | P, ER, ER, Cy, ER, F |                 |
| MT468769                  | ctr22957_c0_g1_i3     | CtCYP15       | 250           | SD              | ER, ER, ER, Cy, ER, F|                 |
| MT468770                  | ctr24033_c0_g1_i1     | CtCYP16       | 243           | SD              | P, M, Cy, Cy, Ch, L  |                 |
| MT468771                  | ctr24500_c0_g1_i2     | CtCYP17       | 339           | SD              | G, G, E, Cy, E, P    |                 |
| MT468772                  | ctr24674_c0_g1_i1     | CtCYP18       | 253           | SD              | P, ER, ER, Cy, ER, F |                 |
| MT468773                  | ctr24674_c0_g1_i2     | CtCYP19       | 229           | SD              | ER, Cy, ER, Cy, ER, F|                 |
| MT468774                  | ctr24674_c0_g1_i3     | CtCYP20       | 229           | SD              | ER, Cy, ER, Cy, ER, F|                 |
| MT468775                  | ctr24700_c0_g1_i1     | CtCYP21       | 615           | MD              | N, Cy, Cy, Cy, Cy, S |                 |
| MT468776                  | ctr24700_c0_g1_i2     | CtCYP22       | 231           | SD              | M, ER, ER, Pr, ER, S |                 |
| MT468777                  | ctr25168_c0_g1_i1     | CtCYP23       | 361           | MD              | Cy, Cy, Cy, Cy, Cy, L|                 |
| MT468778                  | ctr25226_c0_g1_i11    | CtCYP24       | 666           | SD              | N, Cy, N, N, N, S    |                 |
| MT468779                  | ctr25226_c0_g1_i13    | CtCYP25       | 196           | SD              | Cy, Cy, Cy, Cy, Cy, S|                 |
| MT468780                  | ctr25226_c0_g1_i15    | CtCYP26       | 575           | SD              | N, Cy, N, N, N, S    |                 |
| MT468781                  | ctr25226_c0_g1_i2     | CtCYP27       | 552           | SD              | N, Cy, N, N, N, F    |                 |
| MT468782                  | ctr25239_c0_g1_i1     | CtCYP28       | 210           | SD              | M, M, M, Cy, M, S    |                 |
| MT468783                  | ctr25252_c0_g1_i1     | CtCYP29       | 519           | MD              | Cy, Cy, M, Cy, L     |                 |
| MT468784                  | ctr25252_c0_g1_i2     | CtCYP30       | 598           | MD              | N, Cy, Cy, Cy, Cy, P  |                 |
| MT468785 | ctr25252_c0_g1_i4 | CYP31   | 546 | MD | Cy | Cy | Cy | Cy | P |
| MT468786 | ctr26423_c0_g1_i5 | CYP32   | 200 | SD | ER | ER | ER | Cy | ER | P |
| MT468787 | ctr26897_c1_g2_i13| CYP33   | 430 | SD | N  | Cy | N  | N  | N  | S |
| MT468788 | ctr27050_c0_g3_i1  | CYP34   | 470 | MD | N  | Cy | N  | N  | N  | F |
| MT468789 | ctr27345_c1_g1_i1  | CYP35   | 227 | SD | P  | ER | ER | E  | ER | S |
| MT468790 | ctr27345_c3_g2_i22 | CYP36   | 171 | SD | Cy | ER | ER | M  | ER | F |
| MT468791 | ctr27355_c0_g1_i1  | CYP37   | 157 | SD | Cy | Cy | M  | Cy | S |
| MT468792 | ctr27355_c0_g1_i10 | CYP38   | 164 | SD | Cy | Cy | M  | Cy | F |
| MT468793 | ctr27652_c1_g2_i7  | CYP39   | 223 | SD | ER | ER | ER | Cy | ER | S |
| MT468794 | ctr27784_c2_g1_i14 | CYP40   | 229 | SD | M  | ER | M  | M  | G  | F |
| MT468795 | ctr27784_c2_g1_i20 | CYP41   | 274 | SD | M  | PM | M  | PM | G  | P |
| MT468796 | ctr27856_c0_g2_i1  | CYP42   | 229 | SD | ER | G  | M  | Cy | M  | S |
| MT468797 | ctr27930_c3_g13_i1 | CYP43   | 174 | SD | Cy | M  | Cy | Cy | L |
| MT468798 | ctr28568_c0_g5_i4  | CYP44   | 542 | MD | Cy | N  | Cy | N  | Pr | F |
| MT468799 | ctr28568_c0_g5_i4  | CYP45   | 341 | SD | G  | PM | PM | E  | PM | F |
| MT468800 | ctr28936_c0_g3_i5  | CYP46   | 160 | SD | Cy | Cy | Cy | M  | Cy | L |
| MT468801 | ctr28936_c0_g2_i2  | CYP47   | 209 | SD | M  | Ch | M  | M  | M  | F |
| MT468802 | ctr28936_c0_g2_i3  | CYP48   | 209 | SD | M  | Ch | M  | PM | M  | F |
| MT468803 | ctr28936_c0_g3_i2  | CYP49   | 255 | SD | P  | Ch | Cy | Cy | Ch | S |
| MT468804 | ctr28936_c0_g4_i4  | CYP50   | 197 | SD | E  | ER | ER | Cy | ER | L |
| MT468805 | ctr28936_c0_g4_i4  | CYP51   | 185 | SD | P  | Cy | Cy | Cy | Cy | L |
| MT468806 | ctr28936_c0_g7_i1  | CYP52   | 164 | SD | Cy | Cy | Cy | Cy | Cy | F |
| MT468807 | ctr29069_c0_g1_i11 | CYP53   | 336 | SD | N  | Cy | Cy | N  | Cy | S |
| MT468808 | ctr29069_c0_g1_i13 | CYP54   | 493 | SD | N  | Cy | N  | N  | N  | L |
| MT468809 | ctr29069_c0_g1_i2  | CYP55   | 256 | SD | Cy | Cy | Cy | Cy | Cy | S |
| MT468810 | ctr29069_c0_g1_i3  | CYP56   | 496 | SD | N  | Cy | N  | N  | N  | P |
| MT468811 | ctr29069_c0_g1_i6  | CYP57   | 496 | SD | N  | Cy | N  | N  | N  | S |
| MT468812 | ctr29069_c0_g1_i7  | CYP58   | 493 | SD | N  | Cy | N  | N  | N  | P |
| MT468813 | ctr29098_c0_g1_i3  | CYP59   | 221 | SD | Cy | Cy | Cy | Cy | Cy | L |
| MT468814 | ctr29582_c2_g1_i1  | CYP60   | 190 | SD | Cy | Cy | Cy | Cy | Cy | F |
| MT468815 | ctr29752_c0_g3_i1  | CYP61   | 368 | MD | P  | Ch | M  | N  | M  | L |
| MT468816 | ctr30170_c0_g1_i12 | CYP62   | 396 | MD | N  | Cy | N  | Cy | N  | S |
| MT468817 | ctr30170_c0_g1_i15 | CYP63   | 606 | MD | N  | Cy | N  | N  | N  | S |
| MT468818 | ctr30170_c0_g1_i9 | CtCYP64 | 315 | MD | Cy | Cy | Cy | Cy | Cy | P |
|----------|-----------------|---------|-----|----|----|----|----|----|----|---|
| MT468819 | ctr30172_c0_g1_i1 | CtCYP65 | 163 | SD | Cy | Ch | Cy | Cy | Cy | L |
| MT468820 | ctr31571_c0_g1_i1 | CtCYP66 | 360 | MD | Cy | Cy | Cy | PM | Cy | F |
| MT468821 | ctr44502_c0_g1_i1 | CtCYP67 | 257 | SD | Cy | Cy | Cy | Cy | Cy | F |
| MT468822 | ctr68387_c0_g1_i1 | CtCYP68 | 161 | SD | Cy | Cy | Cy | Cy | Cy | F |
| MT468823 | ctr79517_c0_g1_i1 | CtCYP69 | 161 | SD | Cy | Cy | Cy | E  | Cy | F |
| MT468824 | ctr8964_c0_g1_i1 | CtCYP70 | 206 | SD | ER | ER | ER | Cy | ER | F |

*CtCYP sequences identified in the current study.

aSD: single-domain; MD: multi-domain

bCy: cytosol; Ch: chloroplast; E: extra cellular; N: nucleus; M: mitochondria; V: vacuole; P: plastid; Pr: peroxisomal; ER: endoplasmic reticulum; G: Golgi apparatus; PM: plasma membrane
cTissue abundance in the current transcriptome assembly using untransformed TPM values.
P: pod; L: leaf; S: stem; F: flower.