

Supplementary Materials: Comparative Transcriptome Analysis Identifies Genes Putatively Involved in 20-Hydroxyecdysone Biosynthesis in *Cyanotis arachnoidea*

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Figure S1: Size distribution of assembled unigenes in the *C. arachnoidea* transcriptome.

Figure S2: Species and *E*-value distributions of BLASTX data. (a) Distribution among species of BLASTX unigene matches made against the NR protein database (cutoff *E* value $\leq 1.0 \times e^{-5}$) and the percentage assigned of each species. Different colors represent different species in the right-hand pie chart. (b) *E*-value distributions of unigenes giving top BLAST hits in the NR database.

Figure S3: (a) Venn diagram of annotated unigenes using different databases and (b) different expression genes (DEGs) between leaf and root samples.

Figure S4: KEGG classification of all unigenes. The ordinate is the name of the pathway, and the abscissa is the proportion of unigenes belonging to this pathway. Unigenes were divided into five branches: cellular processes, environment information processing, genetic information processing, metabolism, and organismal systems.

Figure S5: Map of the steroid biosynthesis pathway generated by KEGG analysis. Green boxes denoted unigenes that were identified in the pathway.

Table S1: List of primers used for the validation of expression through RT-qPCR.

Table S2: List of DEGs between leaves and roots of *C. arachnoidea*.

Table S1. List of primers used for validation of expression through RT-qPCR.

Unigene ID	Description	Forward primers (5'-3')	Reverse primers (5'-3')
actin	Reference gene	AGGCCGTCCCTCTCCCTTAT	GCGGTCGTCGTGAAAGAGTA
comp36620_c0_seq1	cytochrome P450 94C1-like	AGCAGGGTCGACAAGAACTG	CCTCAATCCATCTCTCCGGC
comp56632_c0_seq1	cytochrome P450 90B1-like	CGCGTATCGAAAAGCTGTG	GCGAAAGAGCAATAGCACCG
comp60467_c1_seq1	cytochrome P450 710A1-like	CCTTGACCTCCCTGGCTTT	ATCTCTGGAGTGCCTGTG
comp70580_c0_seq1	cytochrome P450 704C1	ACCACAAGGCTATGCTCACT	ACTTGAAGCATCATCCAGCTCA
comp28108_c0_seq1	cytochrome P450 CYP736A12	TTGGCGGAAAGCTTAGTGAG	TCCCCTCACGTCCCTTAAT
comp30002_c0_seq1	cytochrome P450 90A1 isoform X2	CAGCTACCCGACCTCCATC	GGCCAGGCGATTGATAGTCAT
comp67837_c0_seq4	cytochrome P450 86B1-like isoform X2	GATCGATCCGGGTGTCTTC	CGTCCAAGTACCTCAACCCC
comp57563_c0_seq2	cytochrome P450 71A1-like	ATGTTAGCGCTGGAACCGA	GGCGTGATTGATCTTGCC
comp61518_c0_seq1	cytochrome P450 90A1-like	TCGATCTACAGGAGCACCGA	CCAATCCAAGGCCTCGTTCT
comp71681_c0_seq1	cytochrome P450 71A1-like	GCACTGCCCTTTTAAGC	CACGTGTGATCCTCTCCGTC
comp69723_c0_seq2	cytochrome P450 86B1-like	CATGTCGTTCAATTGGGGC	GAGAGCTTAGGCTCGACGAC
comp60312_c0_seq1	cytochrome P450 734A1-like	AGCTATGGGCGAAAATGCT	TCCAATGCACACTCTAGGGC
comp60108_c0_seq3	cytochrome P450 90B1-like	TCCTGGCGCAGATTAGCAA	GTAAACCCCCATGGAAGCCA
comp60517_c0_seq1	cytochrome P450 734A6-like	TTGGTTAGTGGCCTGGGG	GTTTGTGGCACCATTGGCT
comp66356_c0_seq1	cytochrome P450 71A1-like	TCCCTCATCGTCCCTAGCA	CTTGGTGTGGGATGAGACC
comp70580_c1_seq4	cytochrome P450 704C1-like	CTATCCGCCGATTGCTGGAA	ATGGCTCAACTCTCCCTTGC
comp60316_c0_seq2	cytochrome P450 714B3-like	CGCTCGGTGGGATTCACATA	GAACTCATGCACGTCTTCGC
comp66583_c0_seq3	cytochrome P450 714D1-like	GGGACATAGGCCAGCACTTT	TCGCCCAAATAATGGCCTT
comp67012_c0_seq1	cytochrome P450 734A6-like	GAGCGAAGGTGGACGTAGAG	TCGGGACCCAAAACCTAGC
comp70760_c4_seq11	Cytochrome P450 86A1	CGTCGAGAGAGCAATCGTCA	CATCGCGACGACGTACTTA
comp70319_c0_seq8	acetyl-CoA acetyltransferase (AACT)	CATCACGTTTCGCCGTCA	CGTGCAACACCAACAACACA
comp69323_c0_seq6	hydroxymethylglutaryl-CoA synthase (HMGS)	TCAGAAAGCGCGAAGGATG	CCCTTGCTGCCCATCATA
comp68996_c0_seq2	3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR)	CCATCACTTGAGGTGGGAC	ACCAGCAACGATGGTAGCAA
comp65552_c0_seq7	phosphomevalonate kinase (PMK)	CAATGCAGCTGGGTTCTA	ACTGAGTCGCTTGCATCTCC
comp33484_c0_seq1	mevalonate diphosphate decarboxylase (PMD)	TCCACCACAATCGGAAACTGA	CTCCCAACTGTGGTGCAGAT

comp29765_c0_seq1	isopentenyl-diphosphate Delta-isomerase I (IDI)	CCAGAGGCAGCTGACATGAA	ACATTGACGAGCTAAGCA
comp59095_c0_seq1	1-deoxy-D-xylulose-5-phosphate synthase (DXS)	CAGGTTTCATTGCCCACTGC	TCACGGAGCTTGCCTAGTT
comp47417_c0_seq1	1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR)	GGGACAAGCCCAAGCCTATT	CTGATCAGCTAACAGAGCGCGA
comp33211_c0_seq1	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (HDS)	TGGATGATGCAACTGATGCC	CTGCACGATGTCTGCACAAAA
comp28732_c0_seq1	farnesyl pyrophosphate synthase (FDS)	CGGAGTTGCTCCAAGATCCA	AACAACTGACAGTCCGCGAT
comp68019_c0_seq1	squalene synthase (SQS)	TCGAAAGCGTTGGAGGGAA	CCACAAGACAGCCGATCAGA
comp35298_c0_seq1	squalene monooxygenase (SQLE)	CCAACGGTGCTGAAATCCAC	AAATTGCAGCGACGAGAACG
comp67692_c1_seq3	obtusifoliol 14-alpha demethylase (CYP51)	TGACTTTGCTGCCACTCCAA	ACAAACAGATAACCCGCAGCA
comp52043_c0_seq5	delta(14)-sterol reductase (ERG24)	TTCGTGGACTTCTGTCGTCG	CAGCTACAACCGTAGGCGAT
comp48599_c0_seq1	3-beta-hydroxysteroid-dehydrogenase (ERG26)	CCTAGGAAGCGGGAAAGTGG	AGCTTAGCCATTGCCGTGAT
comp48248_c0_seq1	delta(24)-sterol reductase (DHCR24)	CGGATGCGTCGTCAGATGTA	CTACCCGTCATAAGGACCAC
comp47084_c0_seq3	3-beta-hydroxysteroid-Delta(8),Delta(7)-isomerase (EBP)	ACTCCGCGATTGTGAGTGTT	TTTCGTTGCAATCGCGTACC
comp50441_c0_seq1	delta(7)-sterol-C5(6)-desaturase (ERG3)	TTGGTTGGGCAGCCTATGTT	TGAGTTGCGTGGAGATGCTT

Figure S1

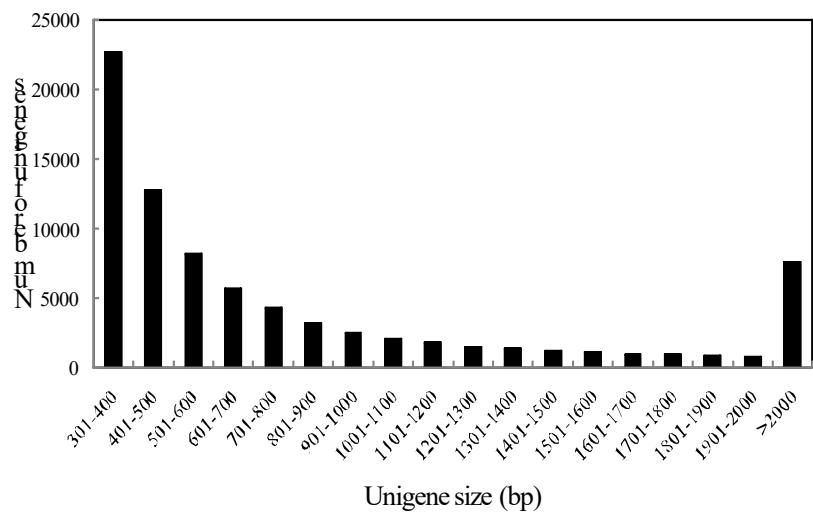


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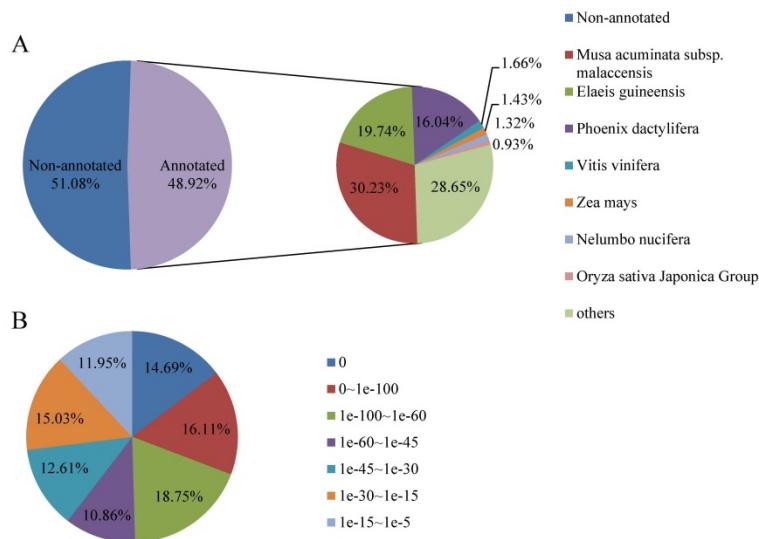


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Figure S3

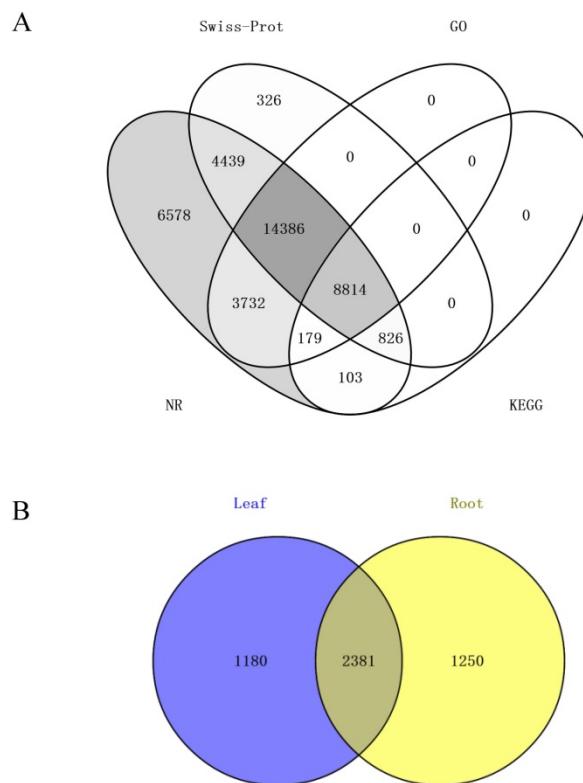


Figure S3. Venn diagram of annotated unigenes using different databases (A) and different expression genes (DEGs) between leaf and root samples (B).

Figure S4

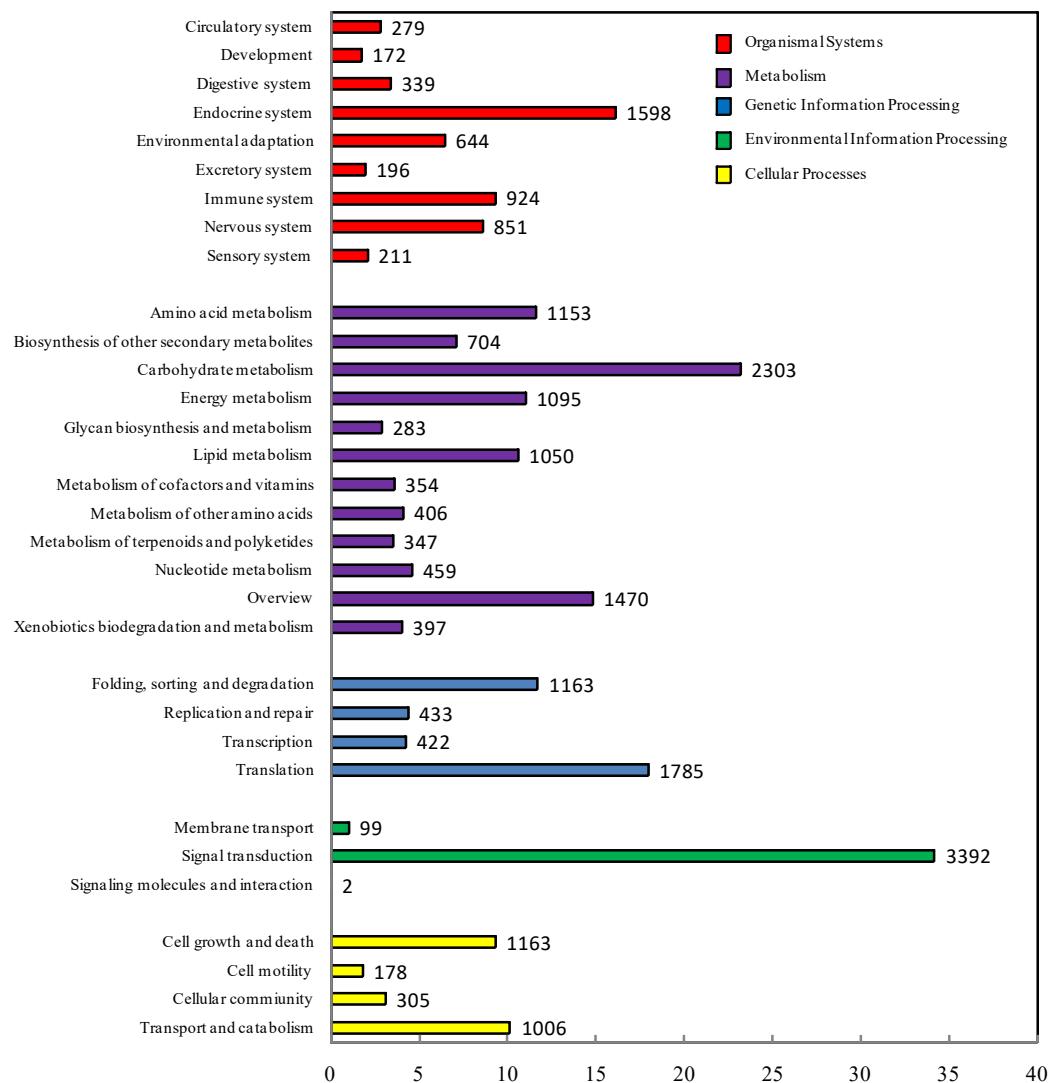


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Figure S5

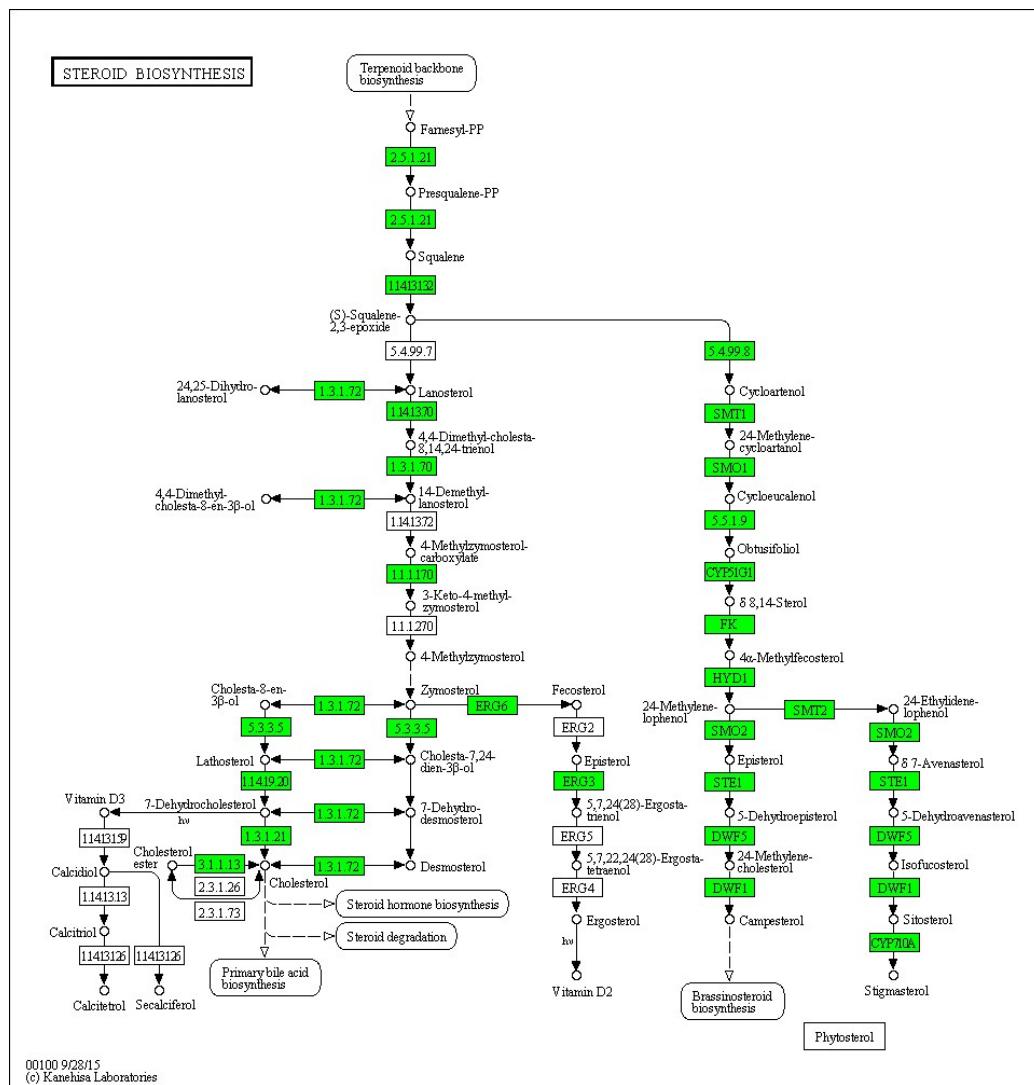


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