

Supporting Information

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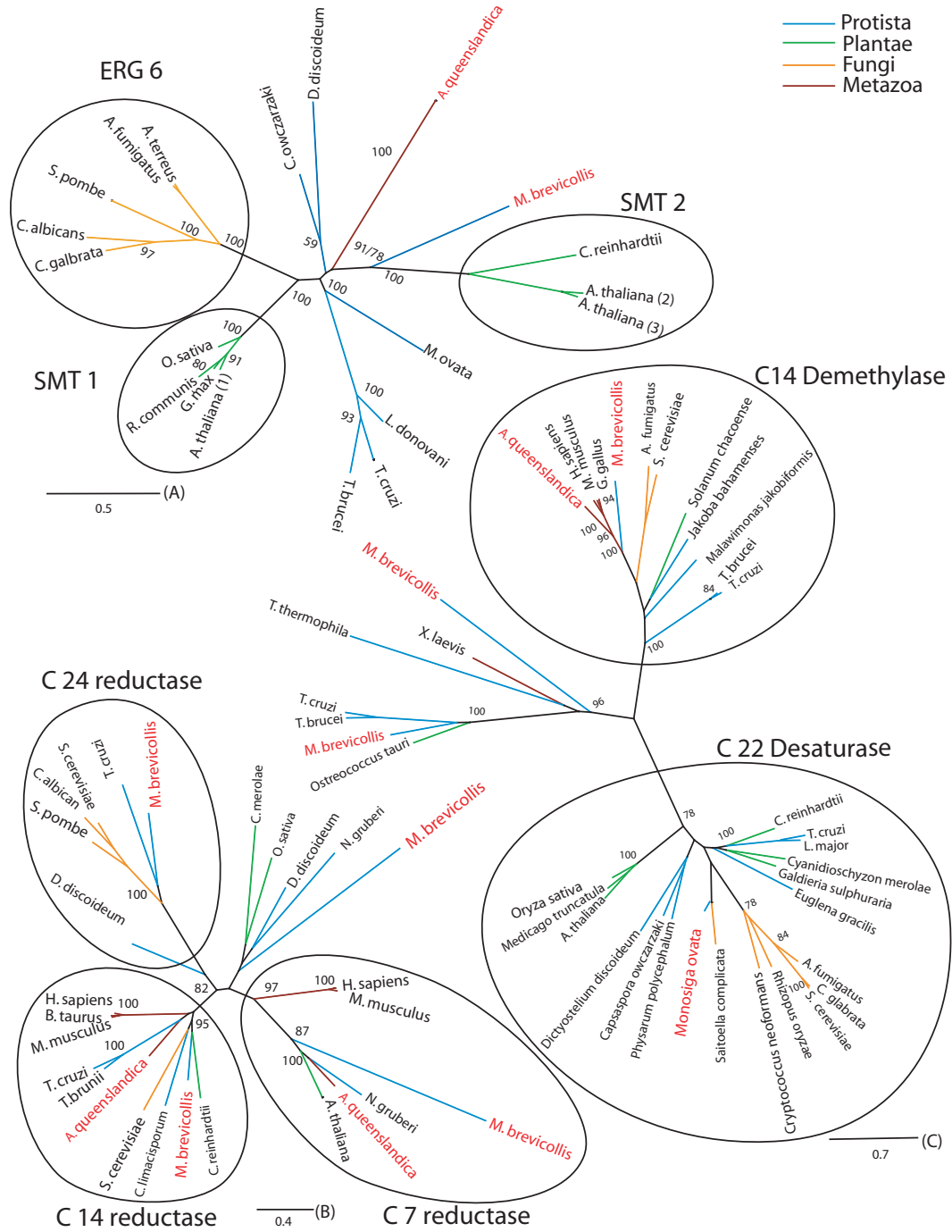


Fig. S1. Gene trees used to identify homology of sterol biosynthesis genes. This figure shows three that include genes from three gene families. Known genes are located within the circled area. Taxa branches are color-coded according to broad taxonomic affiliation. *M. brevicollis* and *A. queenslandica* are shown in red to highlight their positions. A scale bar for branch lengths is included for each tree. The upper left tree (A) contains the sterol methyl transferase genes (*SMT1*, *SMT2* defined for plants and *ERG 6* defined for fungi). The lower left tree (B) contains the reductase genes, and the tree at the right (C) includes C14 demethylase and C-22 desaturase as well as an unknown gene group that was identified from BLAST hits of a known C-22 desaturase to the *M. brevicollis* genome and then the reciprocal BLAST hits from GenBank.

Table S1. Reference spectra from C₂₇ trienes and tetraenes

Compound	Derivative	<i>m/z</i> (relative abundance) [fragment origin]	Ref.
C ₂₇ Δ ^{5,7,9(11),22}	TMS ether	452 (25) [M+], 437 (7) [M+-CH ₃], 362 (62) [M+-OTMS], 347 (17) [M+-OTMS-CH ₃], 325 (13), 251 (100) [M+-OTMS-SC], 249 (18), 235 (13), 111 (12)	1
C ₂₇ Δ ^{5,7,22}	Acetate	424 [M+], 364 [M+-OAc], 349 (17) [M+-OAc-CH ₃], 253 (100) [M+-OAc-SC]	2, 3
C ₂₇ Δ ^{5,7,22}	TMS ether	454 (48) [M+], 439 (6) [M+-CH ₃], 364 (20) [M+-OTMS], 349 (100) [M+-OTMS-CH ₃], 323 (64), 253 (27) [M+-OTMS-SC], 237 (17), 211 (22), 143 (28)	1
C ₂₇ Δ ^{5,8,22}	TMS ether	454 (49) [M+], 439(6) [M+-CH ₃], 364 (10) [M+-OTMS], 349 (100) [M+-OTMS-CH ₃], 323 (51), 253 (21) [M+-OTMS-SC], 237 (7), 211 (14), 143 (17)	1
C ₂₇ Δ ^{5,7, 24}	OH	367 (10) [M+-CH ₃], 364 [M+-H ₂ O], 349(100) [M+-OH-CH ₃], 323 (13), 251 (20)[M+-OH-SC], 209 (11)	4, 5, 6
C ₂₇ Δ ^{5,22, 24}	Acetate	424 (2) [M+], 364 (15) [M+-OAc], 349 (4) [M+-OAc-CH ₃], 213 (11), 282 (49), 267 (9), 255 (20) [M+-OAc-SC], 253 (42), 213 (8), 109 (100)	6, 7
C ₂₇ Δ ^{5,7, 9(11)}	Acetate	424 (4) [M+], 364 (100) [M+-OAc], 349 (62-49) [M+-OAc-CH ₃], 251 (46-38) [M+-OAc-SC], 235 (29-24), 209 (60-65), 195 (53)	7, 8
C ₂₇ Δ ^{5,7, 9(11)}	TMS ether	454 (16-11) [M+], 439 (10) [M+-CH ₃], 364 (100) [M+-OTMS], 349 (25) [M+-OTMS-CH ₃], 251 (26) [M+-OTMS-SC]	7, 9
C ₂₇ Δ ^{6,8,14}	Acetate	424 (32) [M+], 364 (82) [M+-OAc], 362 (28) [M+-OAc-CH ₃], 360 (22) [M+-OAc-SC], 349 (100), 345 (14), 313 (11), 311 (12), 225 (70) [M+-OAc-SC]	7
C ₂₇ Δ ^{6,8,14}	TMS ether	454 (49) [M+], 364(10) [M+-OTMS], 349 (100) [M+-OTMS-CH ₃], 323 (13), 251 (20) [M+-OTMS-SC], 209 (11)	7

Relative abundance and fragment origin included when available.

- Ruan BF, et al. (2002) Alternative pathways of sterol synthesis in yeast: Use of C-27 sterol tracers to study aberrant double-bond migrations and evaluate their relative importance. *Steroids* 67:1109–1119.
- Mulheirn LJ, Aberhart DJ, Caspi E (1971) Dehydrogenation of sterols by protozoan *Tetrahymena pyriformis*. *J Biol Chem* 246:6556–6559.
- Conner RL, Mallory FB, Landrey JR, Iyengar CWL (1969) Conversion of cholesterol to Δ^{5,7,22}-cholestatrien-3 β-ol by *Tetrahymena pyriformis*. *J Biol Chem* 244:2325–2333.
- Scallen T, Dean WJ, Laughran ED, Vora BV (1969) Isolation and chemical characterization of Δ^{5,7,24}-cholestatrien-3β-ol from pig tissues. *J Lipid Res* 10:121–127.
- Galli G, Maroni S (1967) Mass spectrometric investigations of some unsaturated sterols biosynthetically related to cholesterol. *Steroids* 10:189–197.
- Husselstein T, Gachotte D, Desperz T, Bard M, Benveniste P (1996) Transformation of *Saccharomyces cerevisiae* with a cDNA encoding a sterol C-methyltransferase from *Arabidopsis thaliana* results in the synthesis of 24-ethyl sterols. *FEBS Lett* 381:87–92.
- Ruan B, et al. (2001) Sterols in blood of normal and Smith-Lemli-Opitz subjects. *J Lipid Res* 42:799–811.
- Gerst N, Ruan B, Pang J, Wilson WK, Schroeppfer GJ (1997) An updated look at the analysis of unsaturated C₂₇ sterols by gas chromatography and mass spectrometry. *J Lipid Res* 38:1685–1701.
- Fabiani D, Caruso D, Cavaleri M, Kienle MG, Galli G (1996) Cholesta-5,7,9(11)-trien-3β-ol found in plasma of patients with Smith-Lemli-Opitz syndrome indicates formation of sterol hydroperoxide. *J Lipid Res* 37:2280–2287.

Table S2. Sequences used for phylogenetic trees in Fig. S1

Species	ID no.	Source	Gene product*
SMT			
<i>Amphimedon queenslandica</i>	See Table S3	NCBI/JGI	
<i>Arabidopsis thaliana SMT1</i>	NP 196875.1	NCBI	
<i>Arabidopsis thaliana SMT2</i>	NP 173458.1	NCBI	
<i>Arabidopsis thaliana SMT3</i>	NP 177736.1	NCBI	
<i>Aspergillus fumigatus</i>	EAL84512.1	NCBI	
<i>Aspergillus terreus</i>	EAU37134.1	NCBI	
<i>Candida albicans</i>	O74198	NCBI	
<i>Candida galbrata</i>	Q6FRZ7	NCBI	
<i>Capsaspora owczarzaki</i>	NUL00000815 (TBestDB)	TBestDB	
<i>Chlamydomonas reinhardtii</i>	Scaffold 11000008 [Chlre3:161904]	JGI	
<i>Dictyostelium discoideum</i>	ERG6 genome	NCBI	
Glycine max	AAB04057.1	NCBI	
<i>Leishmania donovani</i>	AY488059.1	NCBI	
<i>Monosiga brevicoli</i>	fgenes2 pg.C 230046 [Monbr1:33702]	JGI	
<i>Monosiga ovata</i>	MNL00000137 (TBestDB)	TBestDB	
<i>Oryza sativa subsp. japonica</i>	AAC34988.1	NCBI	
<i>Ricinus communis</i>	AAB62812.1	NCBI	
<i>Schizosaccharomyces pombe</i>	BAA13793.2	NCBI	
<i>Schizosaccharomyces pombe</i>	O14321	NCBI	
<i>Trypanosoma brucei</i>	EAN78102.1 and XP 822930.1	NCBI	
<i>Trypanosoma cruzi</i>	XP 802716.1 and EAN81270.1	NCBI	
<i>Trypanosoma cruzi</i>	XP 802864.1	NCBI	
<i>Trypanosoma cruzi</i>	EAN81365.1 9 and 802811.1	NCBI	
OSC			
<i>Dictyostelium discoideum</i>	AAF80384.1	NCBI/HGSP	
<i>Abies magnifica</i>	AAG44096.1	NCBI	
<i>Arabidopsis thaliana</i>	AAM15015.1	NCBI	
<i>Chlamydomonas reinhardtii</i>	gwW.1.10.1. [Chlre3:196409]	JGI	
<i>Cyanidioschyzon merolae</i>	c10f0001.	CMGSP	
<i>Gallus gallus</i>	XP_421893	NCBI	
<i>Homo sapiens</i>	NP_001001438.1	NCBI	
<i>Leishmania major</i>	CAJ02110.1	NCBI	
<i>Monosiga brevicollis</i>	scaffold_5 490669 496062(gwp gw1.C 50117)	JGI	
<i>Naegleria gruberi</i>	gw1.2.41.1. [Naegr1:29622]	JGI	
<i>Rattus norvegicus</i>	NP_112311.1	NCBI	
<i>Amphimedon queenslandica</i>	See Table S3	NCBI/JGI	
<i>Strongylocentrotus purpuratus</i>	ref XP_001193804.1 /Scaffold.v2.83908	NCBI/HGSP	
<i>Ricinus communis</i>	ABB76767	NCBI	
<i>Schizosaccharomyces pombe</i>	CAA93571.1	NCBI	
<i>Stigmatella aurantiaca</i>	ZP_01463642.1	NCBI	
<i>Aspergillus terreus</i>	EAU31178	NCBI	
<i>Trypanosoma brucei</i>	AAG26328.1	NCBI	
<i>Saccharomyces cerevisiae</i>	AAA16975	NCBI	
C7/C14/C24 Reductase			
<i>Amphimedon queenslandica</i>	2 seq. recovered, see Table S3	NCBI	
<i>Arabidopsis thaliana</i>	AAC49278 (C7 red)	NCBI	
<i>Arabidopsis thaliana</i>	BAE99303 (C7 red)	NCBI	
<i>Bos taurus</i>	AAK91505.1 (C14 red)	NCBI	
<i>Candida albicans</i>	EAK98700.1 (C24 red)	NCBI	
<i>Chlamydomonas reinhardtii</i>	Chlre3 126431 estExt.gwp.1H.C.70304	JGI	
<i>Corallochytrium limacisporum</i>	ABB20825 (C14 red)	NCBI	
<i>Cyanidioschyzon merolae</i>	gnl CMER CMI029C	CGD	
<i>Dictyostelium discoideum</i>	AAG30271.1 (C14 red)	NCBI	
<i>Dictyostelium discoideum</i>	EAL65252.1 (C4/C24 red)	NCBI	
<i>Homo sapiens</i>	NP_001351.2 (C7 red)	NCBI	
<i>Monosiga brevicollis</i>	Monbr1 26465 fgenes2.pg.scaffold.14000131	JGI	
<i>Monosiga brevicollis</i>	Monbr1 29161 fgenes2.pg.scaffold.32000022	JGI	
<i>Monosiga brevicollis</i>	Monbr1 37477 estExt.fgenes1.pg.C.130205	JGI	
<i>Monosiga brevicollis</i>	Monbr1 38824 estExt.fgenes1.pg.C.320049	JGI	
<i>Mus musculus</i>	AAQ05836.1 (C14 red)	NCBI	
<i>Mus musculus</i>	NP_031882.1 (C7 red)	NCBI	
<i>Mus musculus</i>	BAE27451.1 (C7 red)	NCBI	

Species	ID no.	Source	Gene product*
<i>Naegleria gruberi</i>	Scaffold.88 11103 12134 (C7 red)	JGI	
<i>Naegleria gruberi</i>	fgenesHG pg.scaffold 58000107	JGI	
<i>Naegleria gruberi</i>	Scaffold.1 1035149 1036204	JGI	
<i>Oryza sativa</i>	m (put C14 red)	NCBI	
<i>Saccharomyces cerevisiae</i>	AAB30203.1 (C14 red)	NCBI	
<i>Saccharomyces cerevisiae</i>	AAD13895.1(C24 red)	NCBI	
<i>Saccharomyces pombe</i>	CAA45113.1 (C24 red)	NCBI	
<i>Trypanosoma brucei</i>	XP 829611.1 (C14 red)	NCBI	
<i>Trypanosoma cruzi</i>	XP 814311.1	NCBI	
<i>Trypanosoma cruzi</i>	NP 014119.1	NCBI	
<i>Trypanosoma cruzi</i>	EAN92460.1 (C24 red)	NCBI	
<i>Trypanosoma cruzi</i>	XP 809497.1	NCBI	
C22 reductase/C14 demethylase/CYP 450 s			
<i>Amphimedon queenslandica</i>	See Table S3	NCBI	
<i>Arabidopsis thaliana</i>	NP 180996.1	NCBI	CYP710A1
<i>Aspergillus fumigatus</i>	XP 749134.1	NCBI	14- α -sterol demethylase Cyp51B
<i>Aspergillus fumigatus Af293</i>	XP 750145.1	NCBI	Cytochrome P450 sterol C-22 desaturase
<i>C. merolae</i>	gnl CMER CMJ284C	CMG	C-22 sterol desaturase
<i>Candida glabrata</i>	AAX73197.1	NCBI	C-22 sterol desaturase
<i>Capsaspora owczarzaki</i>	NUL00000946	TBestDB	
<i>Chlamydomonas reinhardtii</i>	genie.690.1 [chlre1:144202]	JGI	
<i>Cryptococcus neoformans</i>	XP 571445.1	NCBI	C-22 sterol desaturase
<i>Dictyostelium discoideum</i>	EAL73097.1	NCBI	Cytochrome p450-like protein
<i>Euglena gracilis</i>	ELL00000116	TBestDB	
<i>Galdieria sulphuraria</i>	contig_966.Oct13.2005	GSGP	
<i>Gallus gallus</i>	BAF30488.1	NCBI	Lanosterol 14- α -demethylase (Fragment)
<i>Homo sapiens</i>	AAC50951.1	NCBI	Cytochrome P450 51A1 (C-14 demethylase)
<i>Jakoba bahamensis</i>	JBL00000658	TBestDB	
<i>Leishmania major</i>	CAJ06868.1	NCBI	Cytochrome p450-like protein
<i>Malawimonas jakobiformis</i>	MJL00004550	TBestDB	
<i>Medicago truncatula</i>	ABC59097.1	NCBI	CYP710A15
<i>Monosiga brevicollis</i>	jgi Monbr1 33214 estExt.fgenes2.pg.C.170049	JGI	
<i>Monosiga brevicollis</i>	Ext fgenes1 pg.C 250046	JGI	C14 demethylase
<i>Monosiga ovata</i>	MNL00000309	TBestDB	
<i>Mus musculus</i>	AAF74562.1	NCBI	Lanosterol 14- α -demethylase
<i>Oryza sativa</i>	BAA96158.1	NCBI	Sterol C-22 desaturase-like
<i>Ostreococcus tauri</i>	CAL54893.1	NCBI	Cytochrome p450-like protein
<i>Physarum polycephalum</i>	PPL00001618	TBestDB	
<i>Rhizopus oryzae</i>	ROL00002494	TBestDB	
<i>Saccharomyces cerevisiae</i>	AAA34547.1	NCBI	Lanosterol 14- α -demethylase) (P450-14DM).
<i>Saccharomyces cerevisiae</i>	AAB06217.1	NCBI	Cytochrome P450 61 (C-22 sterol desaturase)
<i>Saitoella complicata</i>	SCL00000465	TBestDB	
<i>Solanum chacoense</i>	AAT12274.1	NCBI	14- α -demethylase
<i>Tetrahymena thermophila</i>	THERM.00101290.7.m00377	TIGR	
<i>Trypanosoma brucei</i>	XP 843664.1	NCBI	Cytochrome P450, putative
<i>Trypanosoma brucei</i> TREU927	XP 828695.1	NCBI	Lanosterol 14- α -demethylase
<i>Trypanosoma cruzi</i>	AAP33131.1	NCBI	14- α -demethylase
<i>Trypanosoma cruzi</i> , CL Brener	XP 810346.1	NCBI	Cytochrome P450
<i>Trypanosoma cruzi</i> , CL Brener	XP 818982.1	NCBI	Cytochrome p450-like protein
<i>Xenopus laevis</i>	BAD02914.1	NCBI	Cytochrome P450

*For the cytochrome P450 gene family.

Table S3. Sequences from trace archive of *Amphimedon queenslandica* used to assemble potential homologs for sterol biosynthesis gene

OSC (lanosterol synthase) from nucleotides	E value
gnl ti 1016575077 BAYA465351.y4	56.48e-05
gnl ti 1074245165 BHGO10092.b1	56.48e-05
gnl ti 1457616844 BAYA531728.b1	56.48e-05
gnl ti 1457638933 BAYB493476.g1	56.48e-05
gnl ti 1457841548 BAYB543343.b1	56.48e-05
gnl ti 1457955034 BAYS14793.g1	56.48e-05
gnl ti 1468821194 BAYB566073.b1	56.48e-05
gnl ti 1479526292 BAYB652071.b1	56.48e-05
gnl ti 1479655427 BAYB641943.b1	56.48e-05
gnl ti 859288707 BAYB22735.g1	56.48e-05
gnl ti 922428221 BAYB192514.b1	56.48e-05
gnl ti 922475967 BAYB214244.b1	56.48e-05
gnl ti 930303919 BAYA221473.y1	58.42e-05
gnl ti 930433153 BAYA329096.x1	56.48e-05
gnl ti 957170358 BAYA457494.x1	56.48e-05
gnl ti 957546467 BAYB246156.b2	56.48e-05
gnl ti 958585693 BAYB386446.b1	56.48e-05
gnl ti 958590446 BAYB389567.g1	56.48e-05
gnl ti 963856735 BAYA465351.y1 SMT/ERG 6	56.48e-05
gnl ti 1016575477 name:BAYB429955.b1	363 7e-99
gnl ti 858126285 name:BAYA1972.y1	313 1e-83
gnl ti 858485397 name:BAYA93391.y1	233 8e-60
gnl ti 859218754 name:BAYB4715.g1	368 e-100
gnl ti 859222209 name:BAYB7786.g1	369 e-100
gnl ti 859485758 name:BAYB69003.b2	258 4e-67
gnl ti 922270742 name:BAYA256709.y1	305 2e-81
gnl ti 922378536 name:BAYB156077.g1	263 1e-68
gnl ti 922384248 name:BAYB160445.b1	343 8e-93
gnl ti 922402151 name:BAYB175276.b1	356 9e-97
gnl ti 922417381 name:BAYB186570.b1	283 1e-74
gnl ti 929303397 name:BAYA268724.x1	305 2e-81
gnl ti 929509860 name:BAYB274372.b1	263 1e-68
gnl ti 930423799 name:BAYA322498.b1	266 2e-69
gnl ti 930424920 name:BAYA320451.b1	219 6e-60
gnl ti 931289437 name:BAYA348372.x1	293 7e-78
gnl ti 931445042 name:BAYB355052.b1	348 2e-94
gnl ti 931445161 name:BAYB355171.b1	300 1e-79
gnl ti 933366854 name:BAYB355052.g1	364 3e-99
gnl ti 933487781 name:BAYB365100.g1	251 5e-65
C-7 reductase/ C-14 reductase/ C-24 reductase	
gnl ti 1016575477 BAYB429955.b1 (C-24)	149 3e-35
gnl ti 858126285 BAYA1972.y1 (C-7)	155 5e-37
gnl ti 858485397 BAYA93391.y1 (C-7)	147 2e-34
gnl ti 859218754 BAYB4715.g1 (C-24)	149 6e-35
gnl ti 859218754 BAYB4715.g1 (C-7)	233 3e-60
gnl ti 859222209 BAYB7786.g1 (C-7)	142 5e-33
gnl ti 859222209 BAYB7786.g1 (C-24)	232 4e-60
gnl ti 922263890 BAYA248881.x1 (C-7)	151 1e-35
gnl ti 922264273 BAYA248881.y1 (C24)	155 8e-37
gnl ti 922270742 BAYA256709.y1 (C-7)	207 1e-52
gnl ti 922270742 BAYA256709.y1 (C14)	265 6e-70
gnl ti 922378536 BAYB156077.g1 (C-7)	170 2e-41
gnl ti 922378536 BAYB156077.g1 (C-24)	226 2e-58
gnl ti 922384248 BAYB160445.b1 (C-24)	150 2e-35
gnl ti 922384248 BAYB160445.b1 (C-7)	236 2e-61
gnl ti 922384248 BAYB160445.b1 (C-14)	304 7e-82
gnl ti 922402151 BAYB175276.b1 (C-24)	144 1e-33
gnl ti 922402151 BAYB175276.b1 (C-7)	240 2e-62
gnl ti 922402151 BAYB175276.b1 (C-14)	298 7e-80
gnl ti 922417381 BAYB186570.b1 (C-7)	152 7e-36
gnl ti 922417381 BAYB186570.b1 (C-14)	199 2e-50
gnl ti 929303397 BAYA268724.x1 (C24)	205 7e-52
gnl ti 929303397 BAYA268724.x1 (C-14)	260 1e-68

OSC (lanosterol synthase) from nucleotides	E value
gnl ti 929509860 BAYB274372.b1 (C-7)	1702e-41
gnl ti 930423799 BAYA322498.b1 (C-24)	1711e-41
gnl ti 930424920 BAYA320451.b1 (C-24)	1466e-36
gnl ti 930446160 BAYA356223.b1 (C-7)	1558e-37
gnl ti 930607560 BAYB297649.g1 (C-7)	1541e-36
gnl ti 930647190 BAYB313060.b1 (C-7)	1388e-32
gnl ti 931289437 BAYA348372.x1 (C-7)	1642e-39
gnl ti 931445042 BAYB355052.b1 (C-7)	2362e-61
gnl ti 931445161 BAYB355171.b1 (C-7)	1596e-38
gnl ti 933366854 BAYB355052.g1 (C-7)	2324e-60
C-22 desaturase	
gnl ti 913731319 BAYA127353.x1	651e-09
gnl ti 913731642 BAYA133821.x1	528e-06
gnl ti 913731642 BAYA133821.x1	652e-09
gnl ti 913745970 BAYA143206.x1	644e-09
gnl ti 913750203 BAYA138236.x1	666e-10
gnl ti 913814167 BAYA159320.x1	674e-10
gnl ti 913830254 BAYB124259.g1	644e-09
gnl ti 929291003 BAYA220774.x1	644e-09
gnl ti 929294991 BAYA238014.g1	652e-09

This table lists NCBI accession number from the trace archive of *Amphimedon queenslandica*. First number on the right is the bit score, and the second number is the e value. Highest scoring sequences are shown in table. The sequences for OSC genes were recovered with a discontinuous megablast on the NCBI database. All other sequences were recovered with a translated nucleotide BLAST search using local databases of the trace archives.