Fine-scale morphological, genomic, reproductive, and symbiont differences delimit the Caribbean octocorals *Plexaura homomalla* and *P. kükenthali*

SUPPLEMENTAL INFORMATION

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Table S1. *Plexaura homomalla* and *P. kükenthali* sample collection dates for reproductive analysis.

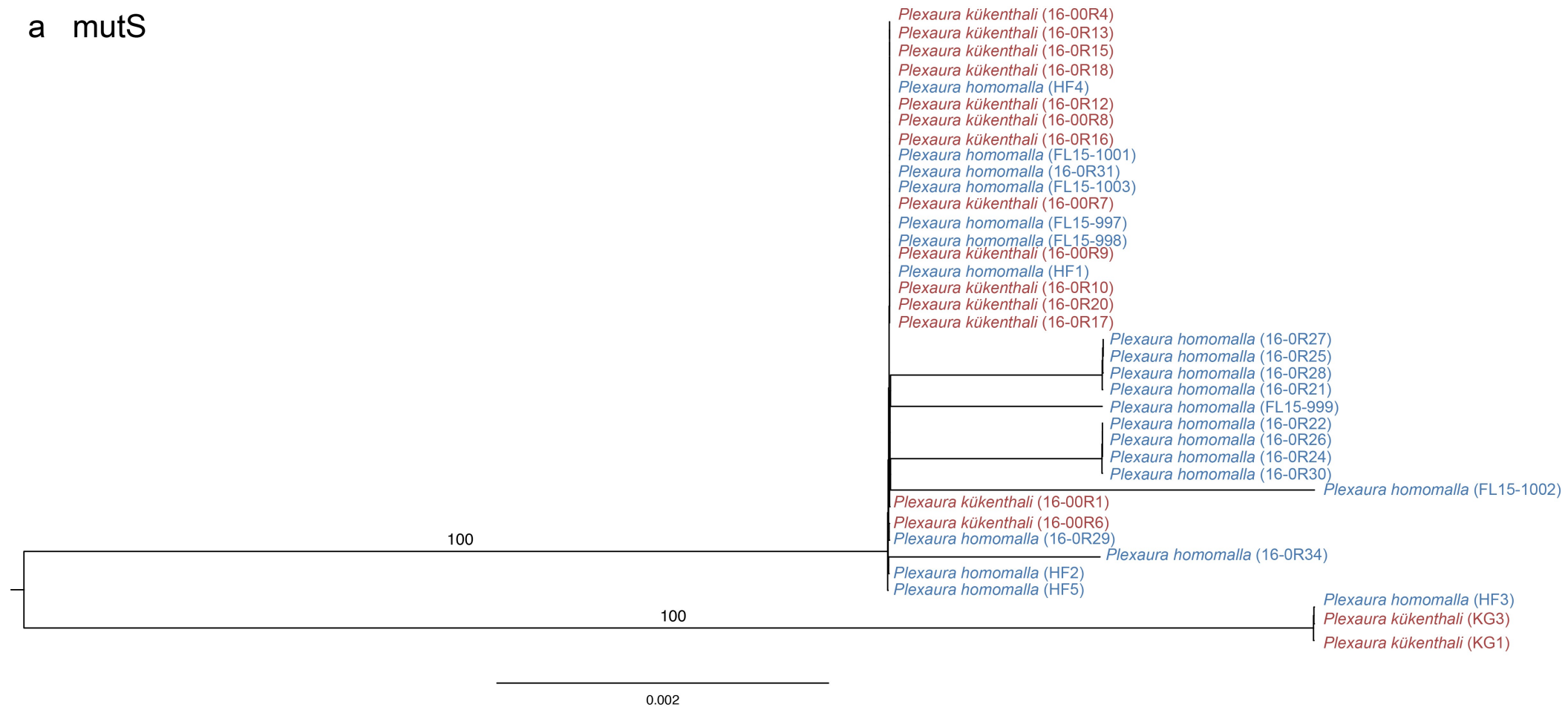
Lunar Day*	**4**	**5**	**6**	**15-Full Moon**	**17-19**	**20**	**21**
2018		17-Aug	20-June	28-July		4-July	
2019	7-July		6-Aug		**19-22 July** *P. homomalla* observed spawning		22-Aug

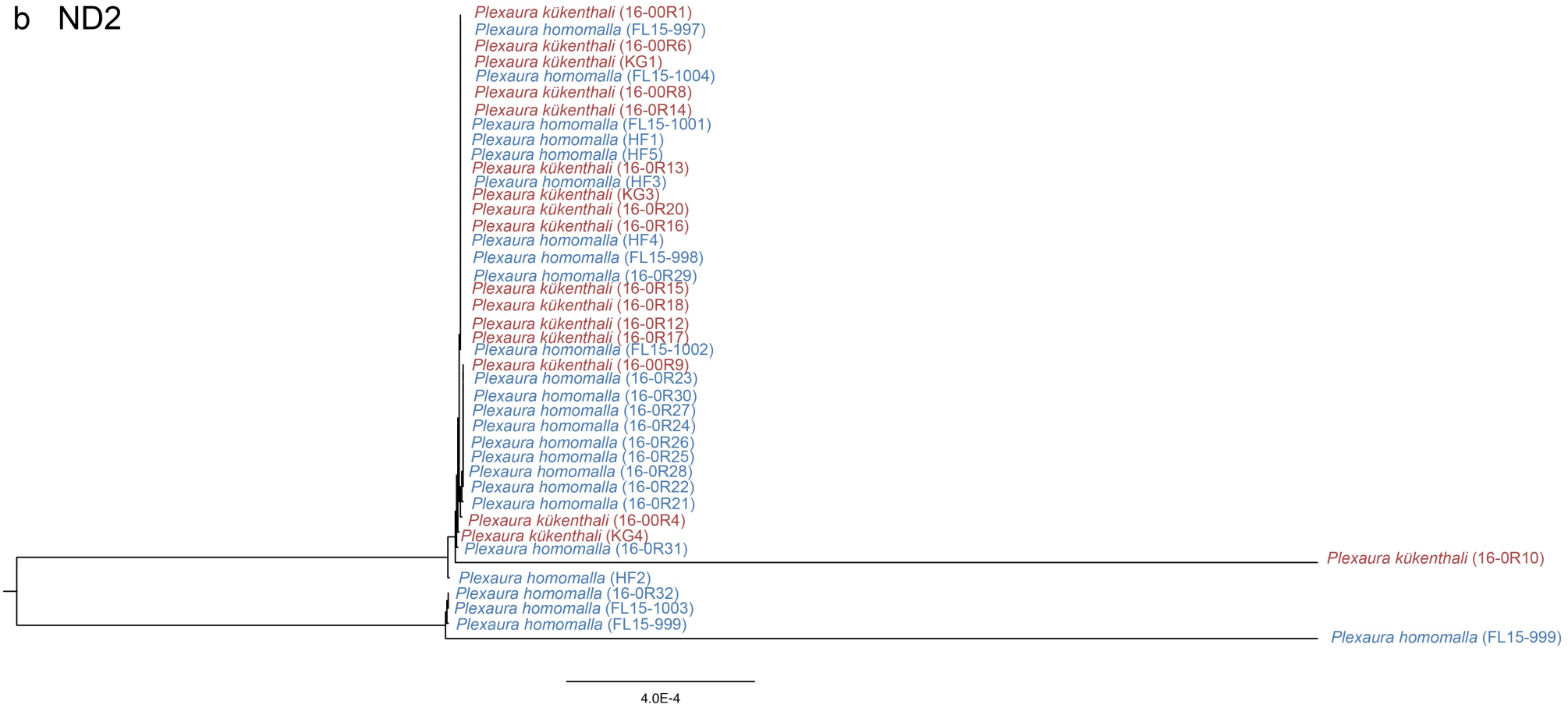
* Lunar Day - 0 represents the new moon of each month

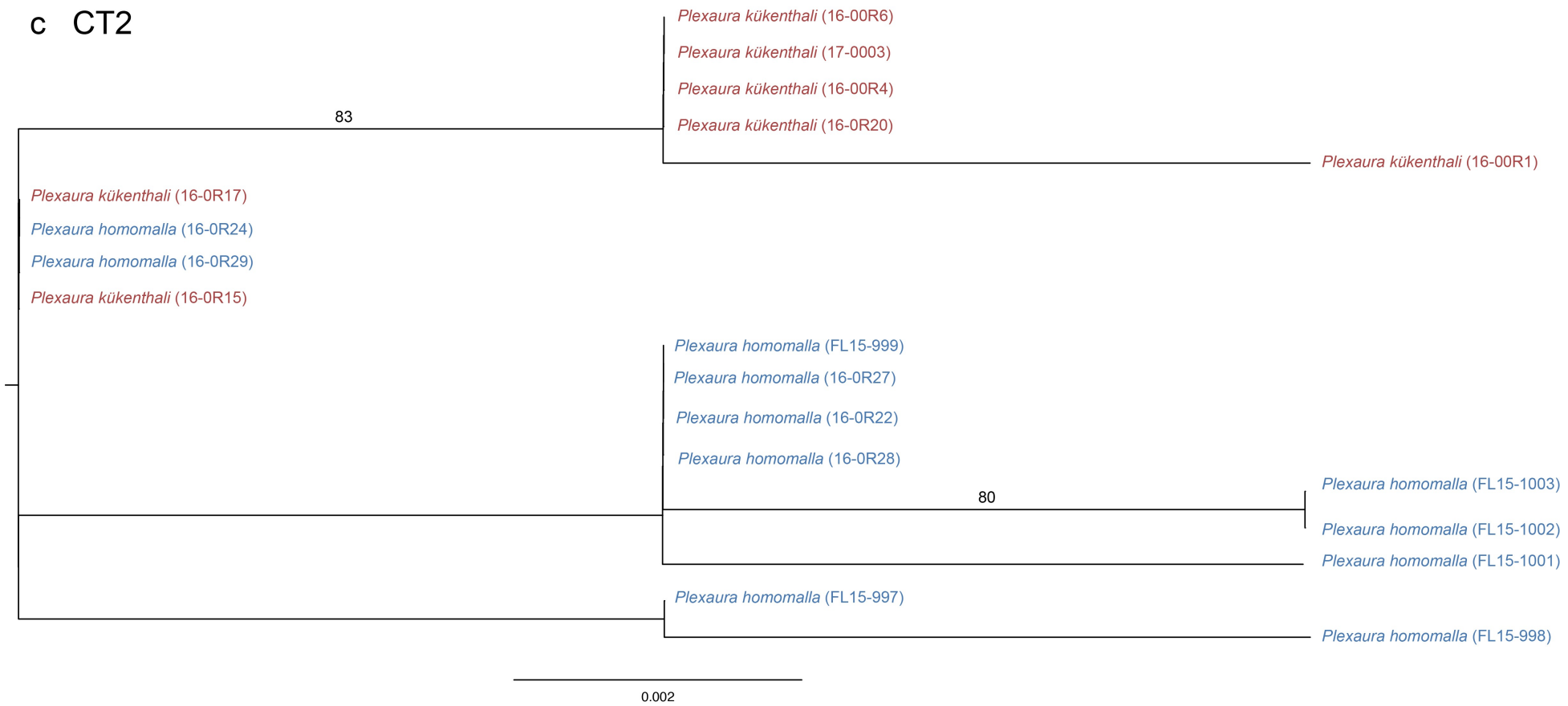
Table S2. Enriched GO terms from high-F_{ST} genes, with raw $P < 0.01$ Fisher's exact test. Note that no terms were significant ($P > 0.05$) after a multiple comparisons correction using Benjamini and Hochberg method.

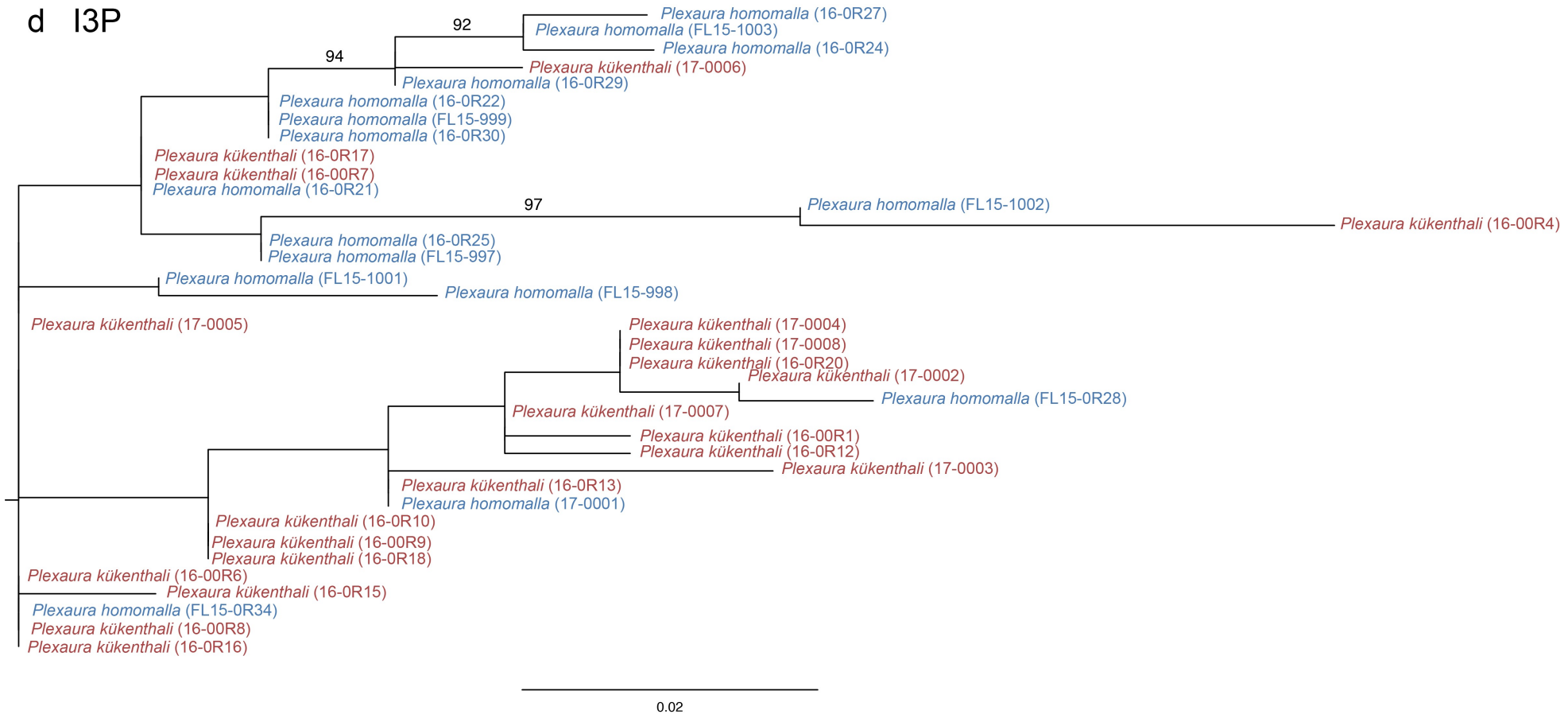
GO.ID	Term	Annotated	Significant	Expected	Raw p-value
Biological Process					
GO:0044262	cellular carbohydrate metabolic process	45	5	0.9	0.0018
GO:0006739	NADP metabolic process	14	3	0.28	0.0024
GO:0043632	modification-dependent macromolecule catabolic process	214	11	4.27	0.0033
GO:0000027	ribosomal large subunit assembly	18	3	0.36	0.005
GO:0044271	cellular nitrogen compound biosynthetic process	792	26	15.79	0.0053
GO:0044265	cellular macromolecule catabolic process	305	13	6.08	0.007
GO:0006511	ubiquitin-dependent protein catabolic process	206	10	4.11	0.0075
GO:0019941	modification-dependent protein catabolic process	210	10	4.19	0.0085
GO:1901575	organic substance catabolic process	505	18	10.07	0.0098
Cellular Component					
GO:0043229	intracellular organelle	2803	77	61.89	0.0025
GO:0043226	organelle	2831	77	62.51	0.0035
GO:0043227	membrane-bounded organelle	2412	67	53.26	0.006
GO:0005622	intracellular	3520	90	77.72	0.0068
GO:0044424	intracellular part	3520	90	77.72	0.0068
Molecular Function					
GO:0015103	inorganic anion transmembrane transporter activity	17	3	0.35	0.0046

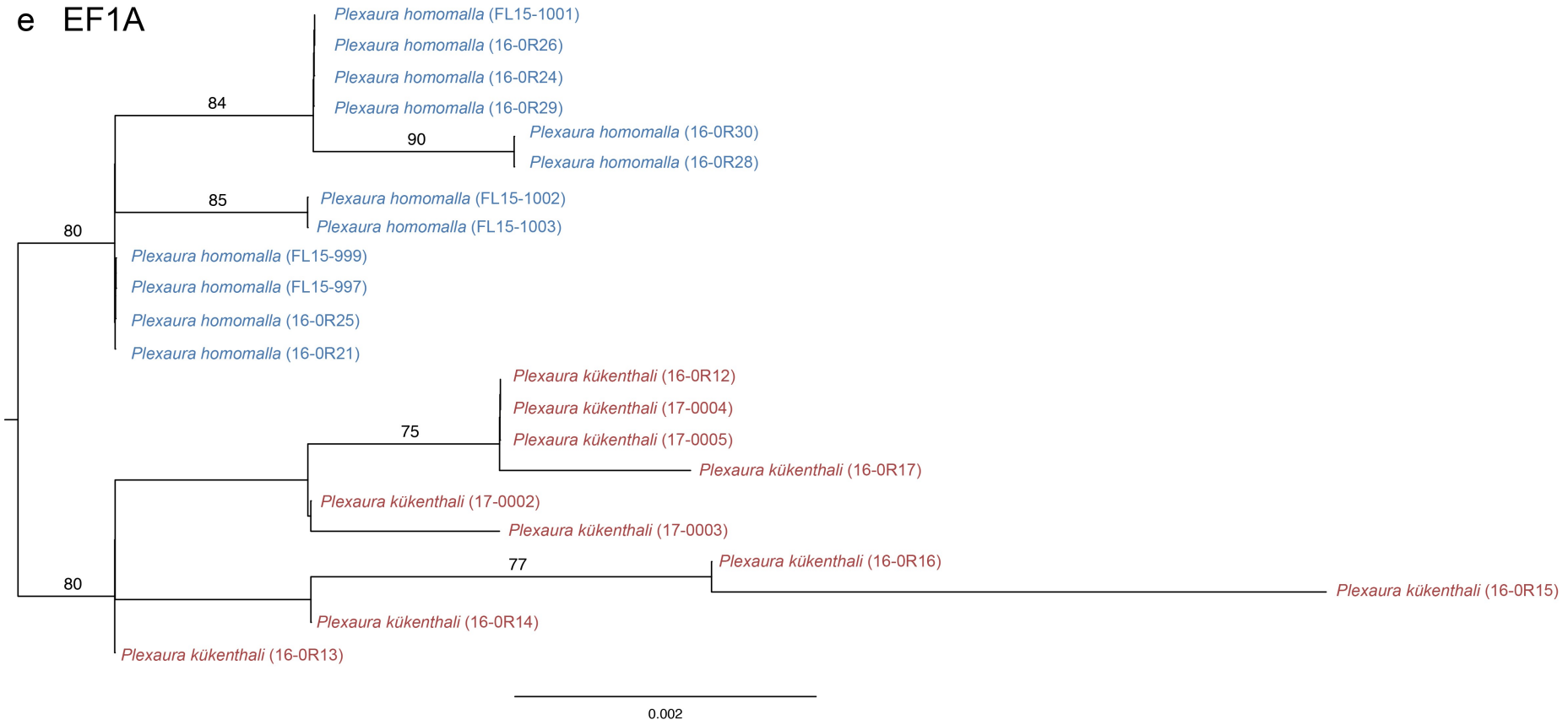
Figure S1 (below). Individual gene trees for A) mutS, B) ND2, C) CT2, D) I3P, and E) EF1a for samples of *Plexaura homomalla* (blue) and *P. kükenthali* (red). Branch supports are ML bootstraps; BS values < 75 are not shown.


a mutS
Plexaura kükenthali (16-00R4)
Plexaura kükenthali (16-0R13)
Plexaura kükenthali (16-0R15)
Plexaura kükenthali (16-0R18)
Plexaura homomalla (HF4)
Plexaura kükenthali (16-0R12)
Plexaura kükenthali (16-00R8)
Plexaura kükenthali (16-0R16)
Plexaura homomalla (FL15-1001)
Plexaura homomalla (16-0R31)
Plexaura homomalla (FL15-1003)
Plexaura kükenthali (16-00R7)
Plexaura homomalla (FL15-997)
Plexaura homomalla (FL15-998)
Plexaura kükenthali (16-00R9)
Plexaura homomalla (HF1)
Plexaura kükenthali (16-0R10)
Plexaura kükenthali (16-0R20)
Plexaura kükenthali (16-0R17)
Plexaura homomalla (16-0R27)
Plexaura homomalla (16-0R25)
Plexaura homomalla (16-0R28)
Plexaura homomalla (16-0R21)
Plexaura homomalla (FL15-999)
Plexaura homomalla (16-0R22)
Plexaura homomalla (16-0R26)
Plexaura homomalla (16-0R24)
Plexaura homomalla (16-0R30)
Plexaura homomalla (FL15-1002)
Plexaura kükenthali (16-00R1)
Plexaura kükenthali (16-00R6)
Plexaura homomalla (16-0R29)
Plexaura homomalla (16-0R34)
Plexaura homomalla (HF2)
Plexaura homomalla (HF5)
Plexaura homomalla (HF3)
Plexaura kükenthali (KG3)
Plexaura kükenthali (KG1)
100
100
0.002


b ND2
Plexaura kükenthali (16-00R1)
Plexaura homomalla (FL15-997)
Plexaura kükenthali (16-00R6)
Plexaura kükenthali (KG1)
Plexaura homomalla (FL15-1004)
Plexaura kükenthali (16-00R8)
Plexaura kükenthali (16-0R14)
Plexaura homomalla (FL15-1001)
Plexaura homomalla (HF1)
Plexaura homomalla (HF5)
Plexaura kükenthali (16-0R13)
Plexaura homomalla (HF3)
Plexaura kükenthali (KG3)
Plexaura kükenthali (16-0R20)
Plexaura kükenthali (16-0R16)
Plexaura homomalla (HF4)
Plexaura homomalla (FL15-998)
Plexaura homomalla (16-0R29)
Plexaura kükenthali (16-0R15)
Plexaura kükenthali (16-0R18)
Plexaura kükenthali (16-0R12)
Plexaura kükenthali (16-0R17)
Plexaura homomalla (FL15-1002)
Plexaura kükenthali (16-00R9)
Plexaura homomalla (16-0R23)
Plexaura homomalla (16-0R30)
Plexaura homomalla (16-0R27)
Plexaura homomalla (16-0R24)
Plexaura homomalla (16-0R26)
Plexaura homomalla (16-0R25)
Plexaura homomalla (16-0R28)
Plexaura homomalla (16-0R22)
Plexaura homomalla (16-0R21)
Plexaura kükenthali (16-00R4)
Plexaura kükenthali (KG4)
Plexaura homomalla (16-0R31)
Plexaura kükenthali (16-0R10)
Plexaura homomalla (HF2)
Plexaura homomalla (16-0R32)
Plexaura homomalla (FL15-1003)
Plexaura homomalla (FL15-999)
Plexaura homomalla (FL15-999)
4.0E-4


c CT2
Plexaura kükenthali (16-00R6)
Plexaura kükenthali (17-0003)
Plexaura kükenthali (16-00R4)
Plexaura kükenthali (16-0R20)
83
Plexaura kükenthali (16-00R1)
Plexaura kükenthali (16-0R17)
Plexaura homomalla (16-0R24)
Plexaura homomalla (16-0R29)
Plexaura kükenthali (16-0R15)
Plexaura homomalla (FL15-999)
Plexaura homomalla (16-0R27)
Plexaura homomalla (16-0R22)
Plexaura homomalla (16-0R28)
Plexaura homomalla (FL15-1003)
80
Plexaura homomalla (FL15-1002)
Plexaura homomalla (FL15-1001)
Plexaura homomalla (FL15-997)
Plexaura homomalla (FL15-998)
0.002


d I3P
92
94
97
Plexaura homomalla (16-0R27)
Plexaura homomalla (FL15-1003)
Plexaura homomalla (16-0R24)
Plexaura kükenthali (17-0006)
Plexaura homomalla (16-0R29)
Plexaura homomalla (16-0R22)
Plexaura homomalla (FL15-999)
Plexaura homomalla (16-0R30)
Plexaura kükenthali (16-0R17)
Plexaura kükenthali (16-00R7)
Plexaura homomalla (16-0R21)
Plexaura homomalla (FL15-1002)
Plexaura kükenthali (16-00R4)
Plexaura homomalla (16-0R25)
Plexaura homomalla (FL15-997)
Plexaura homomalla (FL15-1001)
Plexaura homomalla (FL15-998)
Plexaura kükenthali (17-0005)
Plexaura kükenthali (17-0004)
Plexaura kükenthali (17-0008)
Plexaura kükenthali (16-0R20)
Plexaura kükenthali (17-0002)
Plexaura homomalla (FL15-0R28)
Plexaura kükenthali (17-0007)
Plexaura kükenthali (16-00R1)
Plexaura kükenthali (16-0R12)
Plexaura kükenthali (17-0003)
Plexaura kükenthali (16-0R13)
Plexaura homomalla (17-0001)
Plexaura kükenthali (16-0R10)
Plexaura kükenthali (16-00R9)
Plexaura kükenthali (16-0R18)
Plexaura kükenthali (16-00R6)
Plexaura kükenthali (16-0R15)
Plexaura homomalla (FL15-0R34)
Plexaura kükenthali (16-00R8)
Plexaura kükenthali (16-0R16)
0.02


e EF1A
Plexaura homomalla (FL15-1001)
Plexaura homomalla (16-0R26)
Plexaura homomalla (16-0R24)
Plexaura homomalla (16-0R29)
Plexaura homomalla (16-0R30)
Plexaura homomalla (16-0R28)
Plexaura homomalla (FL15-1002)
Plexaura homomalla (FL15-1003)
Plexaura homomalla (FL15-999)
Plexaura homomalla (FL15-997)
Plexaura homomalla (16-0R25)
Plexaura homomalla (16-0R21)
Plexaura kükenthali (16-0R12)
Plexaura kükenthali (17-0004)
Plexaura kükenthali (17-0005)
Plexaura kükenthali (16-0R17)
Plexaura kükenthali (17-0002)
Plexaura kükenthali (17-0003)
Plexaura kükenthali (16-0R16)
Plexaura kükenthali (16-0R15)
Plexaura kükenthali (16-0R14)
Plexaura kükenthali (16-0R13)
84
90
85
80
75
77
80
0.002

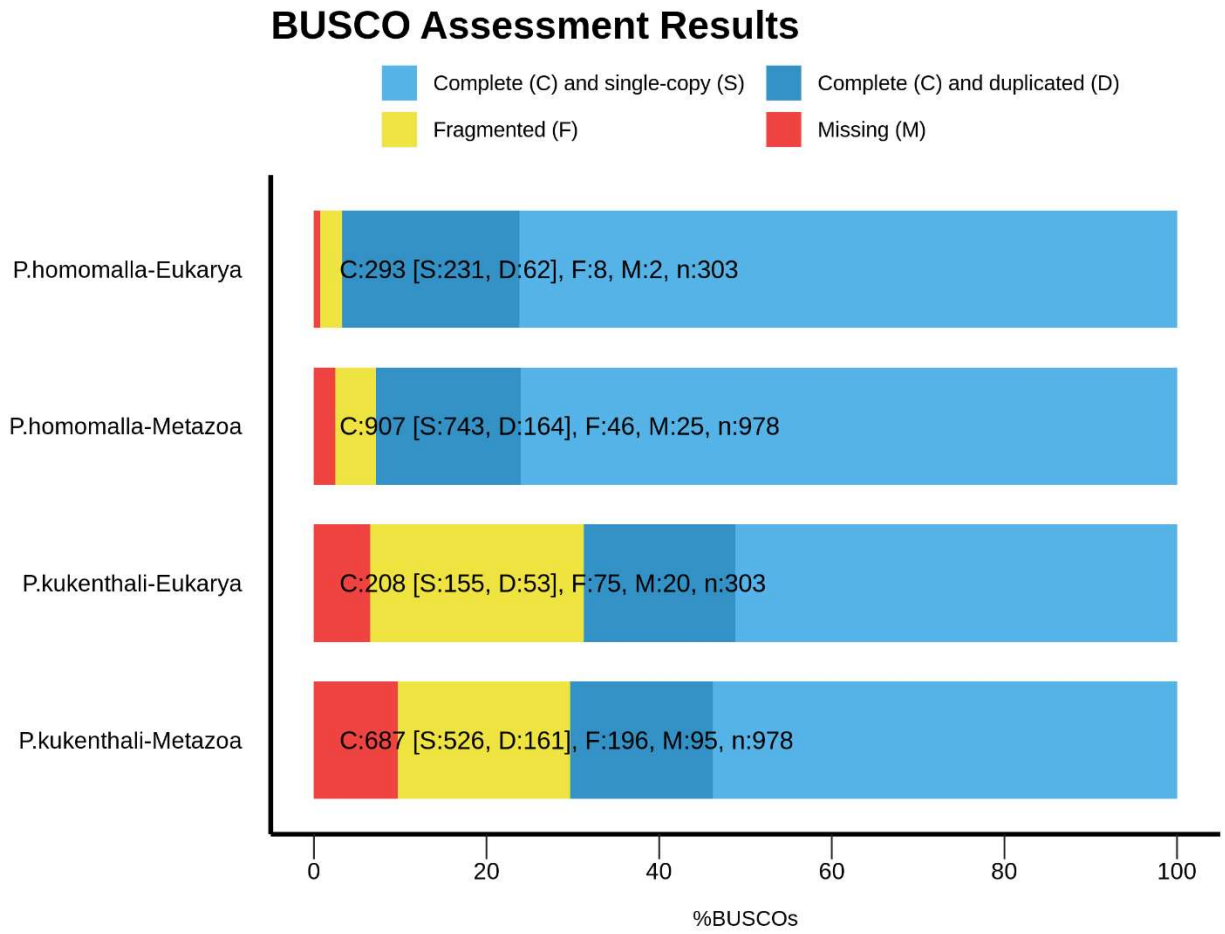


Figure S2. BUSCO results for *de novo* transcriptome assemblies of *P. homomalla* and *P. kükenthali* using Eukarya odb9 and Metazoa odb9 datasets.

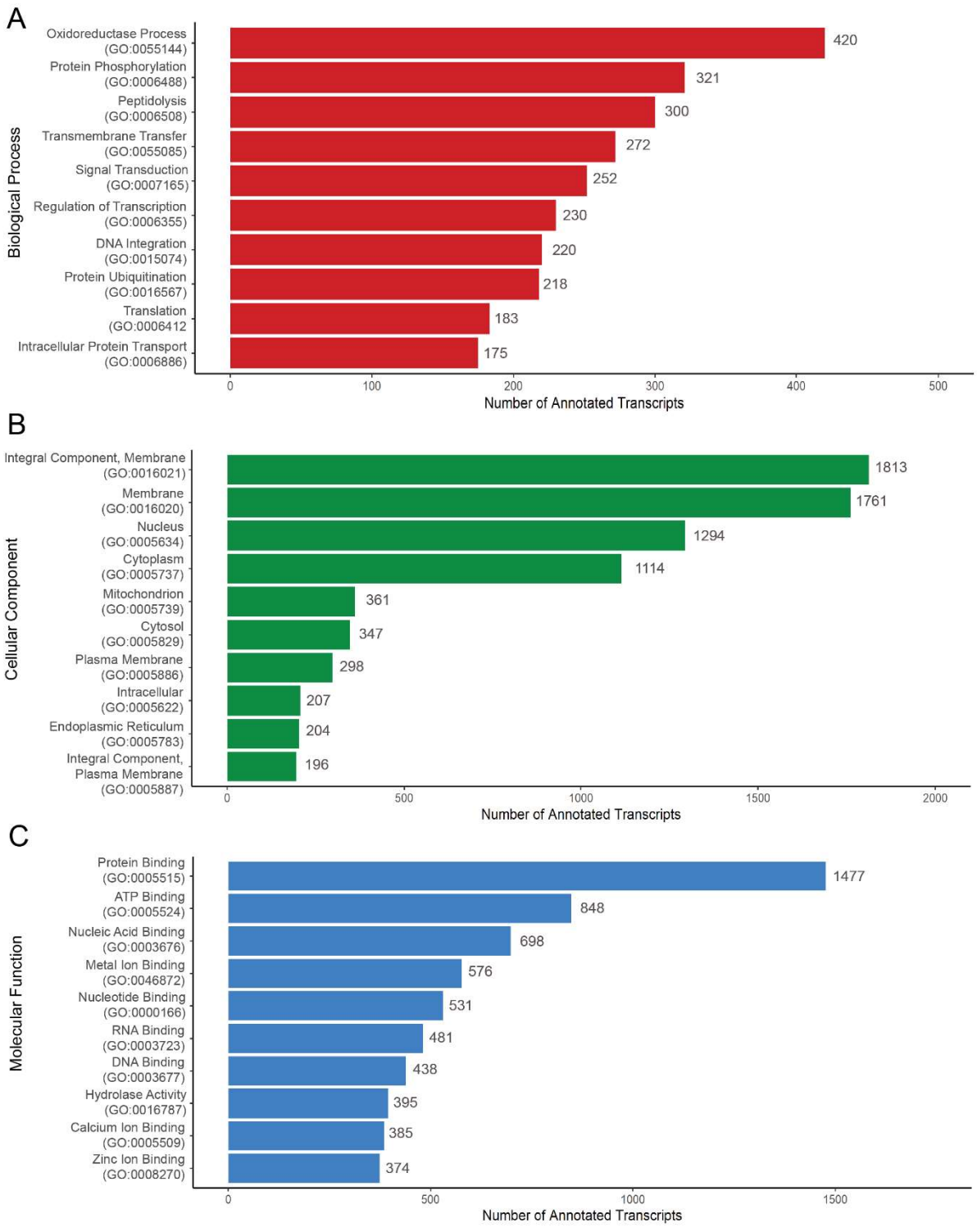


Figure S3. Top ten most frequent Gene Ontology terms in A) Biological Process, B) Cellular Component, and C) Molecular Function domains for the *de novo Plexaura homomalla* transcriptome.

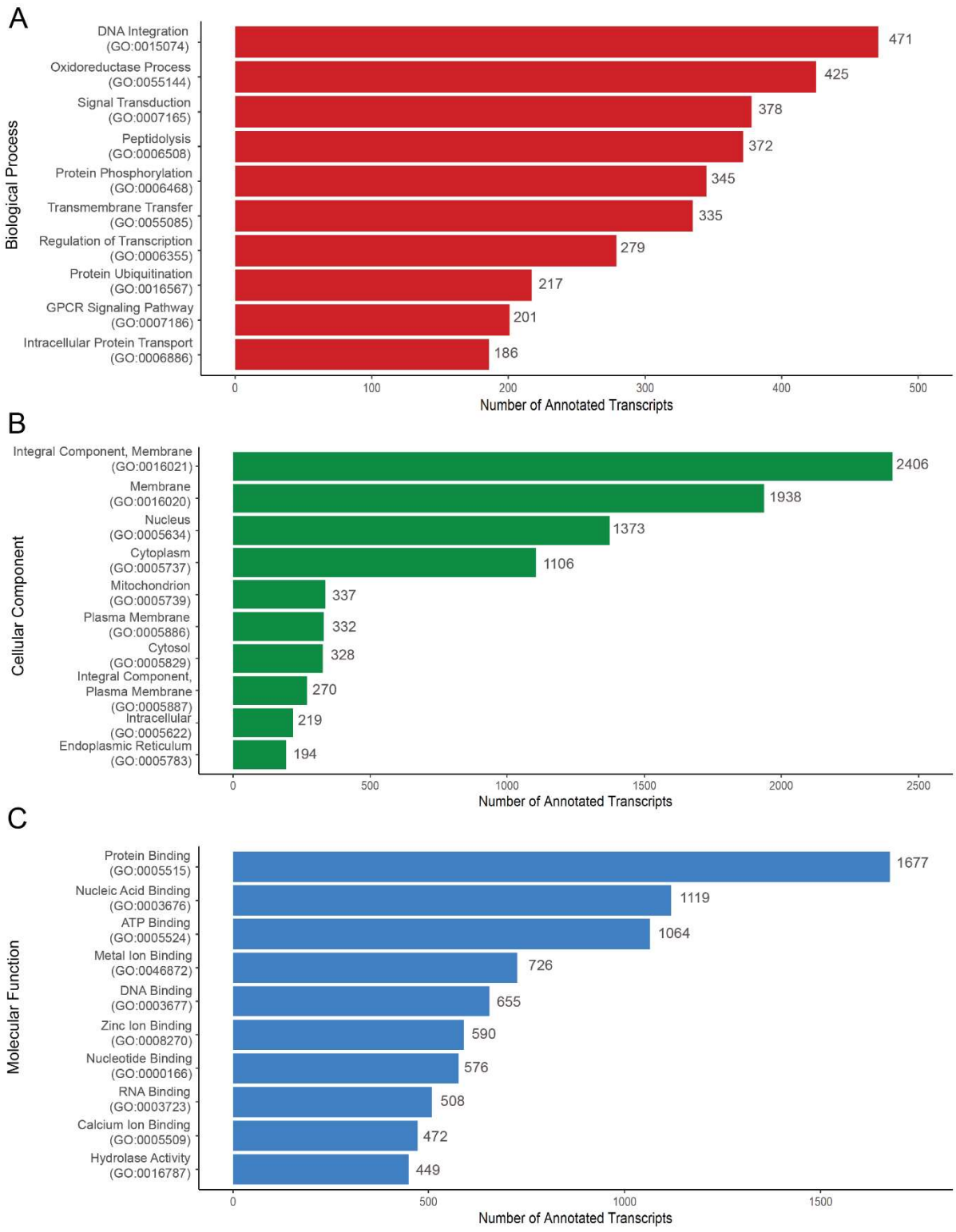


Figure S4. Top ten most frequent Gene Ontology terms in A) Biological Process, B) Cellular Component, and C) Molecular Function domains for the *de novo Plexaura kükenthali* transcriptome.

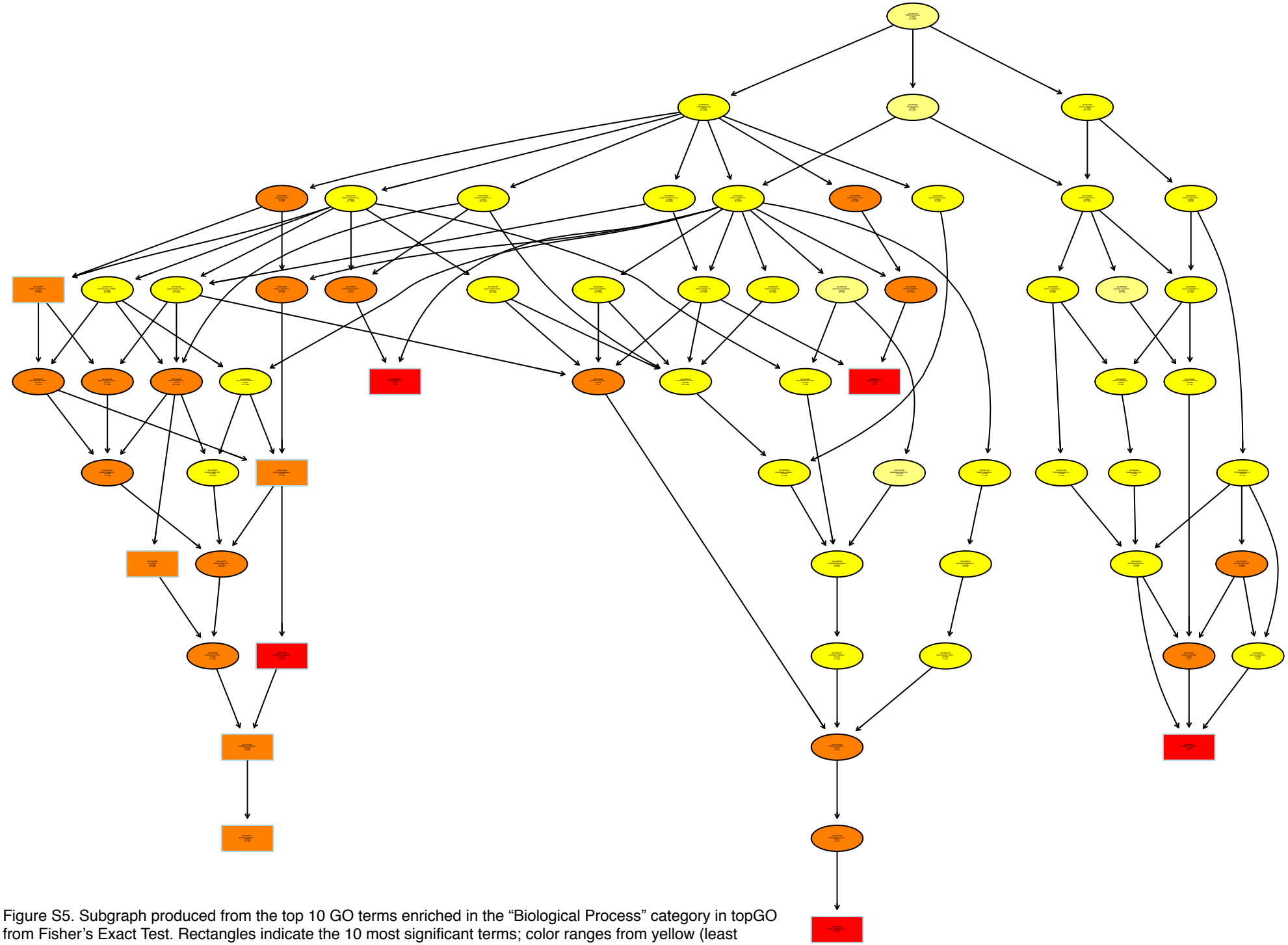


Figure S5. Subgraph produced from the top 10 GO terms enriched in the “Biological Process” category in topGO from Fisher’s Exact Test. Rectangles indicate the 10 most significant terms; color ranges from yellow (least significant) to red (most significant). The first two lines show the GO identifier and a trimmed GO name. The third line shows the number of significant genes and the total number of genes annotated for the respective GO term.

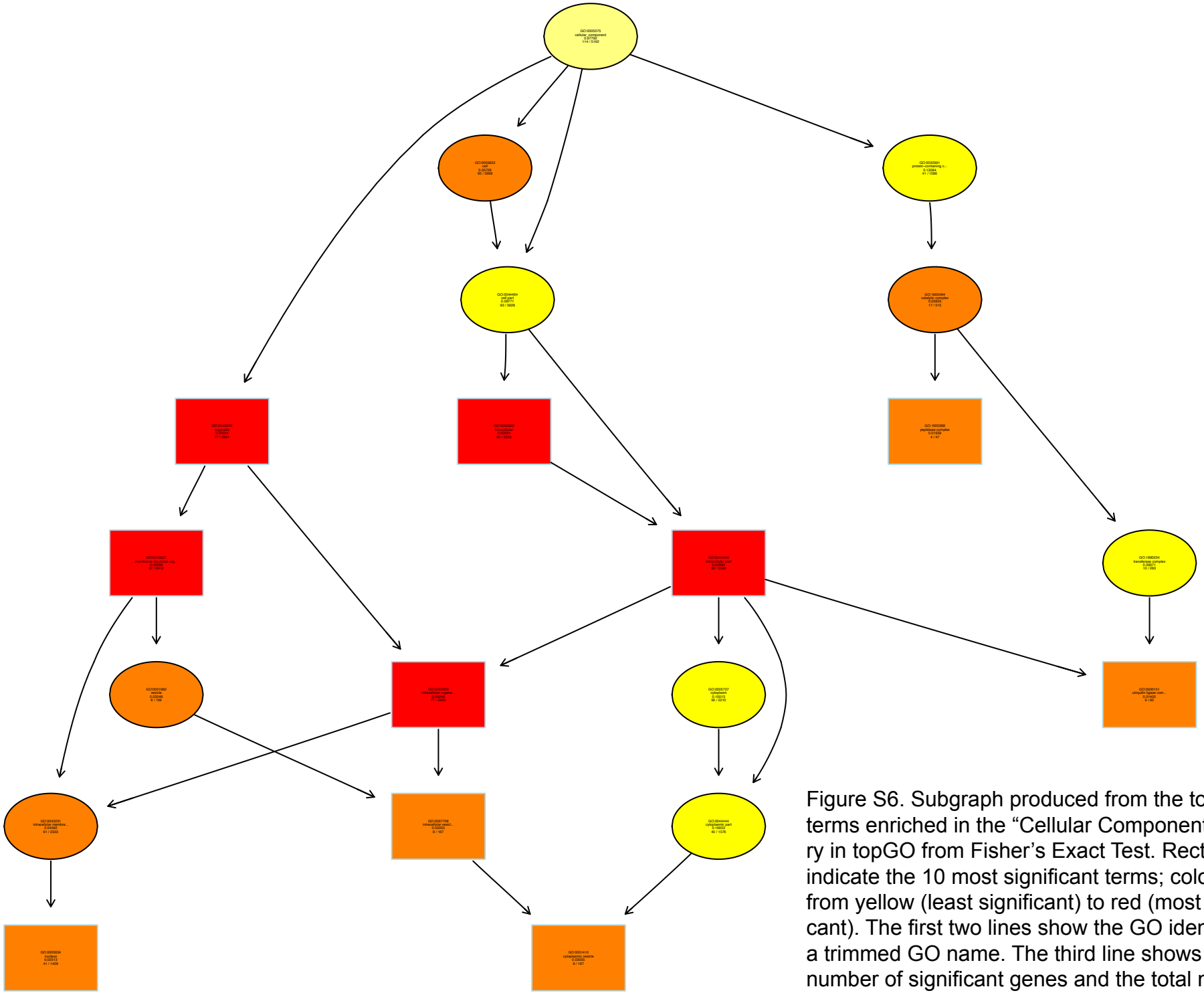


Figure S6. Subgraph produced from the top 10 GO terms enriched in the “Cellular Component” category in topGO from Fisher’s Exact Test. Rectangles indicate the 10 most significant terms; color ranges from yellow (least significant) to red (most significant). The first two lines show the GO identifier and a trimmed GO name. The third line shows the number of significant genes and the total number of genes annotated for the respective GO term.

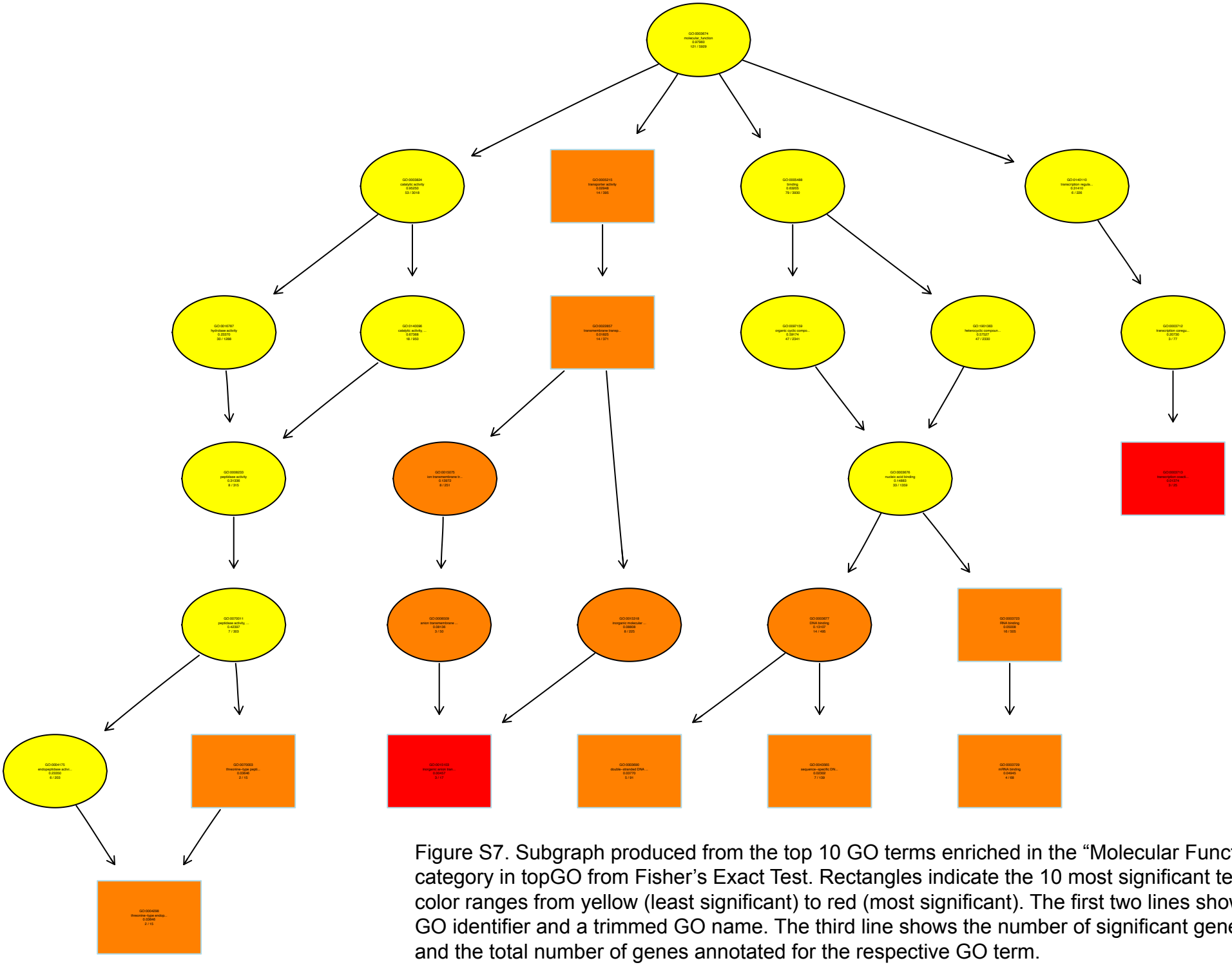


Figure S7. Subgraph produced from the top 10 GO terms enriched in the “Molecular Function” category in topGO from Fisher’s Exact Test. Rectangles indicate the 10 most significant terms; color ranges from yellow (least significant) to red (most significant). The first two lines show the GO identifier and a trimmed GO name. The third line shows the number of significant genes and the total number of genes annotated for the respective GO term.

Figure S8. Images of colony H-F3, a putative hybrid between *P. homomalla* and *P. kükenthali.* While morphologically this colony appears as a typical *P. homomalla* individual, molecular evidence suggests it may be of hybrid origin with *P. kükenthali* as the maternal parent.

Table S4. Summary of R_{ST} values calculated using a) full and b) curtailed symbiont datasets. Values below diagonal are R_{ST} values above diagonal are corrected p-values using the Bonferroni method.

a.

			P. homomalla		*P. kükenthali*	
		St. John	P. Rico	Florida	St. John	P. Rico
P. homomalla	St. John		0.00004	0.00004	0.00004	0.00004
	P. Rico	0.2286		0.00004	0.00004	0.00004
	Florida	0.34132	0.45372		0.00004	0.00004
P. kükenthali	St. John	0.15267	0.4394	0.47829		0.03000
	P. Rico	0.22919	0.51247	0.51474	0.09799	

b.

			P. homomalla		*P. kükenthali*	
		St. John	P. Rico	Florida	St. John	P. Rico
P. homomalla	St. John		0.00004	0.0004	0.0004	0.0004
	P. Rico	0.25847		0.0004	0.0004	0.0004
	Florida	0.34132	0.49205		0.0004	0.0004
P. kükenthali	St. John	0.14654	0.4739	0.47991		0.0060
	P. Rico	0.22919	0.53838	0.51474	0.08906	

Table S5. Summary of Φ_{PT} values calculated using a) full and b) curtailed symbiont datasets. Values below diagonal are Φ_{PT} values above diagonal are corrected p-values using the Bonferroni method.

a.

			P. homomalla		*P. kükenthali*	
		St. John	P. Rico	Florida	St. John	P. Rico
P. homomalla	St. John		0.015	0.015	0.015	0.015
	P. Rico	0.229		0.015	0.015	0.015
	Florida	0.341	0.454		0.015	0.015
P. kükenthali	St. John	0.152	0.440	0.479		0.015
	P. Rico	0.229	0.512	0.515	0.095	

b.

			P. homomalla		*P. kükenthali*	
		St. John	P. Rico	Florida	St. John	P. Rico
P. homomalla	St. John		0.045	0.015	0.030	0.030
	P. Rico	0.237		0.015	0.015	0.015
	Florida	0.286	0.376		0.015	0.015
P. kükenthali	St. John	0.155	0.465	0.433		0.015
	P. Rico	0.189	0.480	0.429	0.081	

Table S6. Microsatellite allele frequencies for symbionts harbored by *P. homomalla* and *P. kükenthali.* Allele sizes in bold are unique to one of the two species and *n* refers to the number of unique alleles.

Locus	*P. homomalla*			*P. kükenthali*	
B7Sym15	Florida	St. John	P. Rico	St. John	P. Rico
229		0.4375	0.875		
231	1				
233			0.125		
247		0.3125		0.393	
249		0.0625		0.214	
251		0.1875		0.250	0.500
253				0.143	0.250
255					0.250
n	1	4	2	4	3
B7Sym34	Florida	St. John	P. Rico	St. John	P. Rico
247	0.182				
251	0.364			0.037	0.059
255	0.182			0.074	0.059
259	0.273	0.313	0.214	0.037	0.118
263		0.313	0.071	0.407	0.176
267		0.250	0.714	0.222	0.176
271		0.125		0.222	0.118
275					0.235
283					0.059
n	4	4	3	6	8
B7Sym36	Florida	St. John	P. Rico	St. John	P. Rico
163	1.000	0.500	1.000		1.000
167				0.120	
169		0.500		0.880	
n	1	2	1	2	1
B7Sym4	Florida	St. John	P. Rico	St. John	P. Rico
138	1	0.688	0.813	0.115	
140		0.313		0.538	0.235
142			0.188	0.192	0.059
144				0.115	0.353
146				0.038	0.294
148					0.059
n	1	2	2	5	5
GV2_100	Florida	St. John	P. Rico	St. John	P. Rico
190				0.074	0.188
193				0.111	0.063
196		0.125		0.296	0.063
199		0.250		0.407	0.438
202		0.063		0.111	0.188
205		0.063			0.063
208		0.438	0.071		
211	0.455		0.143		
214	0.364		0.500		
217	0.182	0.063	0.143		
NA			0.143		
n	3	6	5	5	6

Table S7. Summary of symbiont genotypes harbored by *P. homomalla* and *P. kükenthali* in each region by species using the curtailed data set.

	P. homomalla			*P. kükenthali*		*Total*
Region	Florida	St. John	Puerto Rico	St. John	Puerto Rico	
No. Genotypes	5	14	6	19	14	58
No. Genotypes Shared	4	2	2	3	1	12
No. Samples	10	15	11	21	14	71