Supplementary Materials

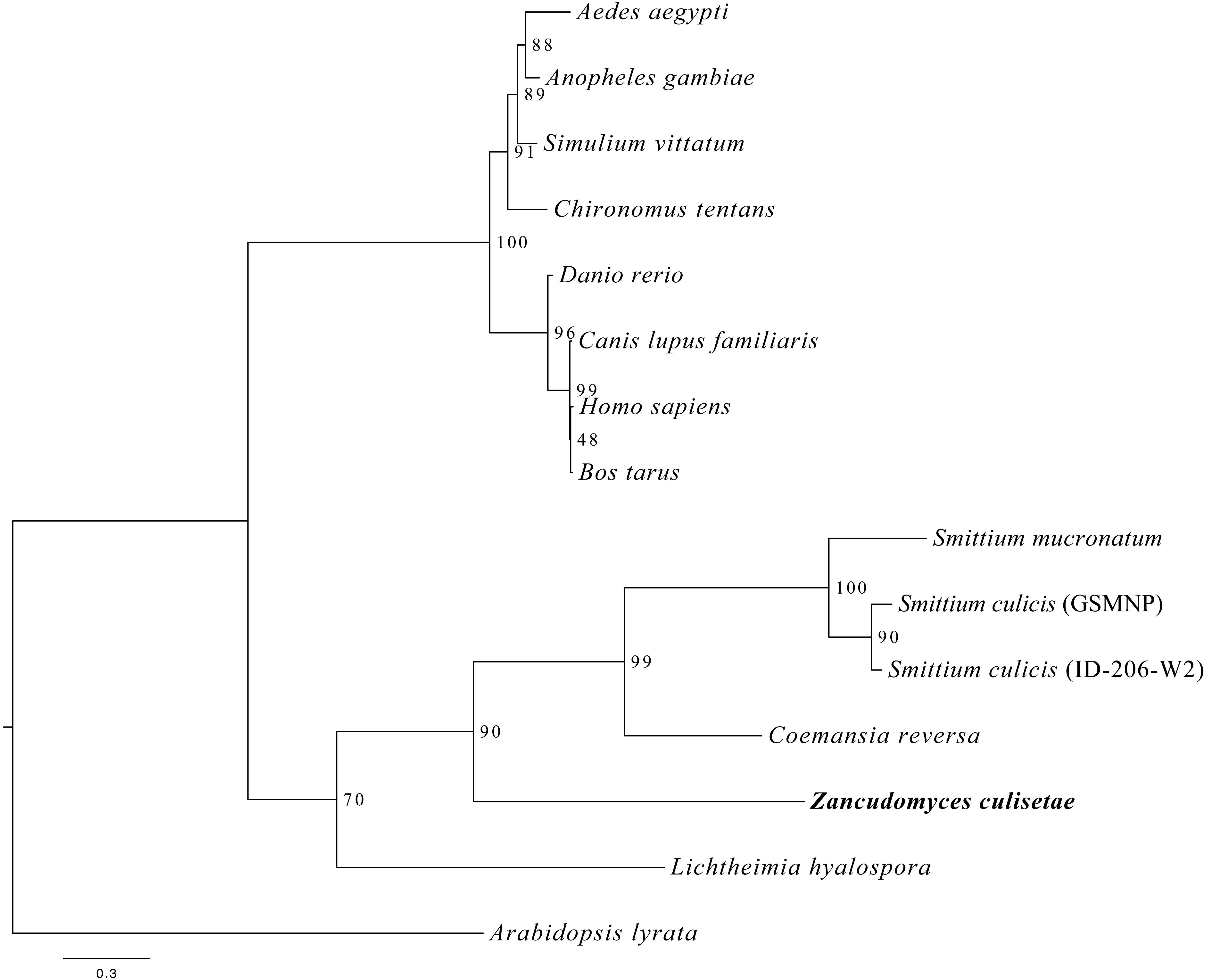
Supplementary Fig. 1. Phylogenetic tree derived from maximum likelihood analysis using the upstream flanking S_TKc gene and its homologs in both animal and fungi. Maximum likelihood bootstrap proportions are shown at nodes. *Zancudomyces culisetae* is in bold, indicating its fungal origin.

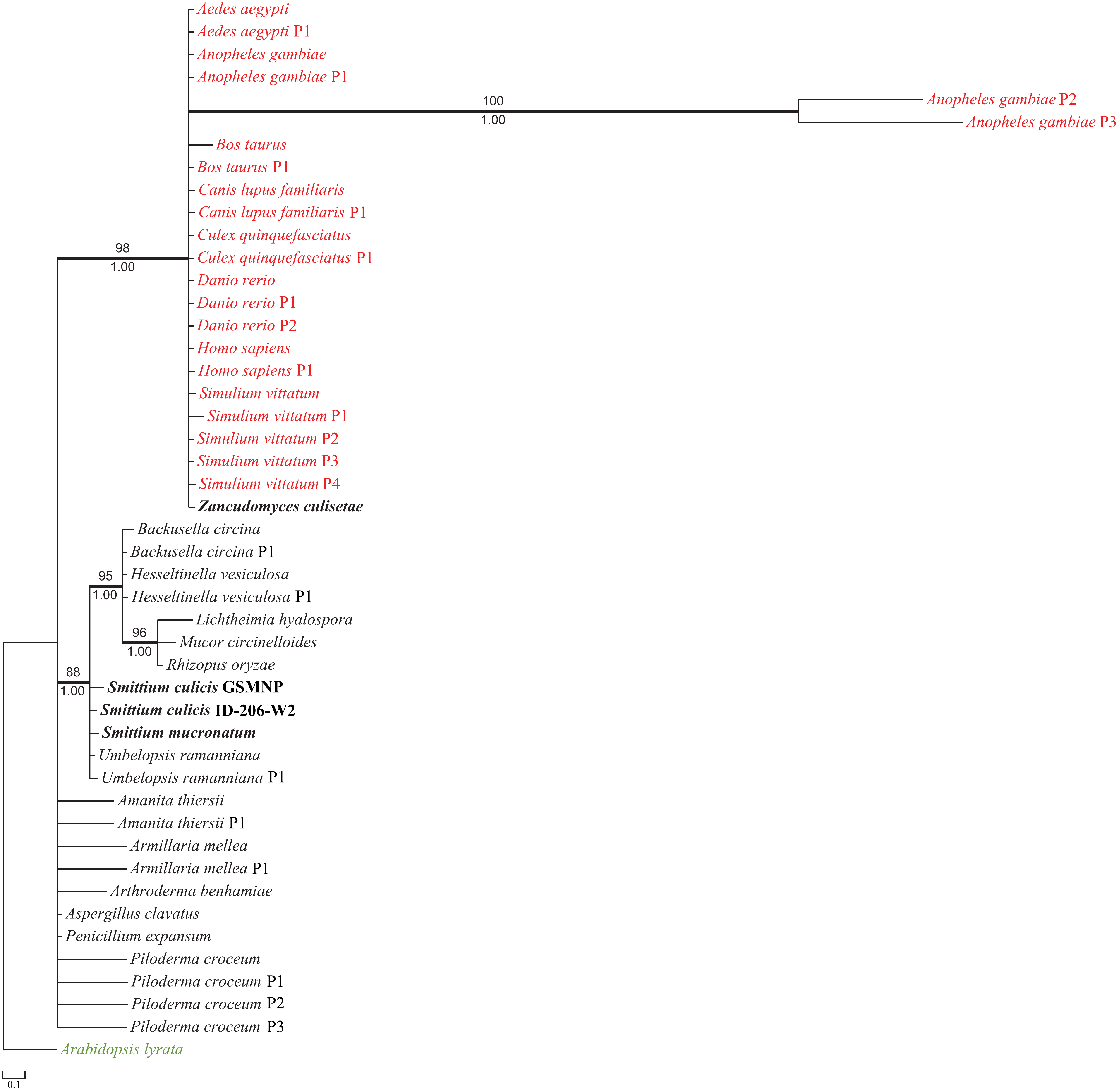
Supplementary Fig. 2. Phylogenetic tree derived from Bayesian analysis using all copies of polyubiquitin amino acid sequences through whole genomes, including both orthologs and paralogs (labeled as “P”). Annotations as in Fig. 2a.

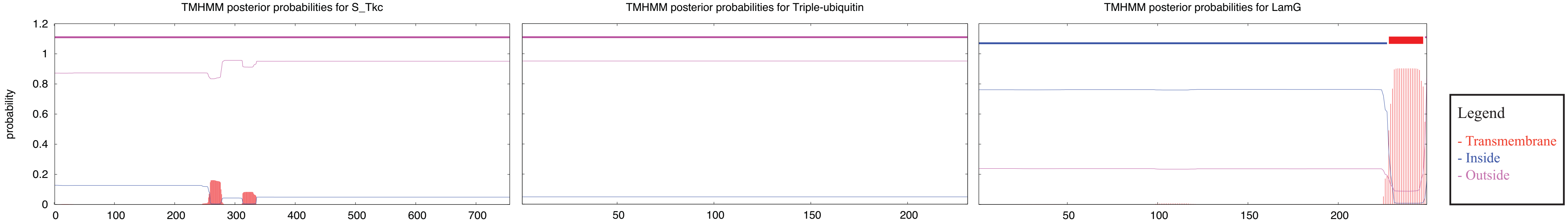
Supplementary Fig. 3. Transmembrane helix prediction for the triple-ubiquitin gene and its flanking genes from the *Zancudomyces culisetae* genome. The posterior probability for transmembrane helix (red), inside (blue), or outside (pink) membrane is plot along the amino acid positions. (There is one transmembrane helix as predicted at one end of the LamG protein)

Supplementary Table 1. BLAT mapping results between five HGT candidates from *Z. culisetae* and Harpellales insect host genomes.

Supplementary Table 2. All homologs detected from the examined eukaryotic genomes using *Z. culisetae*’s triple-ubiquitin gene as a query (E-value < $1E^{-100}$).


Aedes aegypti
88
Anopheles gambiae
89
Simulium vittatum
91
Chironomus tentans
100
Danio rerio
96
Canis lupus familiaris
99
Homo sapiens
48
Bos tarus
Smittium mucronatum
100
Smittium culicis (GSMNP)
90
Smittium culicis (ID-206-W2)
99
Coemansia reversa
90
Zancudomyces culisetae
70
Lichtheimia hyalospora
Arabidopsis lyrata
0.3


Aedes aegypti
Aedes aegypti P1
Anopheles gambiae
Anopheles gambiae P1
100
1.00
Anopheles gambiae P2
Anopheles gambiae P3
Bos taurus
Bos taurus P1
Canis lupus familiaris
Canis lupus familiaris P1
Culex quinquefasciatus
98
1.00
Culex quinquefasciatus P1
Danio rerio
Danio rerio P1
Danio rerio P2
Homo sapiens
Homo sapiens P1
Simulium vittatum
Simulium vittatum P1
Simulium vittatum P2
Simulium vittatum P3
Simulium vittatum P4
Zancudomyces culisetae
Backusella circina
Backusella circina P1
95
1.00
Hesseltinella vesiculosa
Hesseltinella vesiculosa P1
Lichtheimia hyalospora
96
1.00
Mucor circinelloides
Rhizopus oryzae
88
1.00
Smittium culicis GSMNP
Smittium culicis ID-206-W2
Smittium mucronatum
Umbelopsis ramanniana
Umbelopsis ramanniana P1
Amanita thiersii
Amanita thiersii P1
Armillaria mellea
Armillaria mellea P1
Arthroderma benhamiae
Aspergillus clavatus
Penicillium expansum
Piloderma croceum
Piloderma croceum P1
Piloderma croceum P2
Piloderma croceum P3
Arabidopsis lyrata
0.1


TMHMM posterior probabilities for S_Tkc
TMHMM posterior probabilities for Triple-ubiquitin
TMHMM posterior probabilities for LamG
probability
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
700
50
100
150
200
50
100
150
200
Legend
- Transmembrane
- Inside
- Outside

Supplementary Table 1. BLAT mapping results between five HGT candidates from *Z. culisetae* and Harpellales insect host genomes (triple-ubiquitin gene is identified as "g5282").

Q name	Q size	Q start	Q end	T name	T size	T start	T end	Matches	Mis-matches	Rep-matches	N's	Q gap count	Q gap bases	T gap count	T gap bases	Strand	Block count	Block sizes	qStarts	tStarts
g5282	696	30	107	Culex_scaffolds_50	1344033	597445	597978	69	4	0	0	1	4	1	460	+	2	47,26,	30,81,	597445,597952,
g5282	696	441	488	Blackfly_scaffolds_42610	3459	2574	2621	43	4	0	0	0	0	0	0	-	1	47,	208,	2574,
g2050	1986	118	177	Aedes_scaffolds_80	2899062	2888586	2888642	44	0	0	0	1	15	2	12	+	4	9,11,15,9,	118,127,138,168,	2888586,2888601,2888618,2888633,
g2050	1986	188	410	Aedes_scaffolds_730	589389	366551	366931	120	1	0	0	5	101	10	259	-	11	7,10,12,12,11,15,13,11,8,12,10,	1576,1583,1596,1644,1656,1716,1739,1752,1768,1776,1788,	366551,366563,366581,366649,366700,366751,366767,366853,366874,366887,366921,
g5024	1117	557	1043	Aedes_scaffolds_730	589389	366752	366951	81	1	0	0	3	404	6	117	-	7	10,13,12,12,12,15,8,	74,84,427,439,451,535,552,	366752,366767,366785,366802,366887,366921,366943,
g5507	1143	141	231	Chironomus_scaffolds_113	274809	153754	153859	83	4	0	0	2	3	4	18	+	6	9,12,12,42,5,7,	141,151,165,177,219,224,	153754,153766,153778,153796,153841,153852,

g5507	1143	462	559	Blackfly_scaffolds_121909	23588	7401	7528	81	0	0	0	3	16	8	46	+	10	21,6,4,8,8,5,5,7,6,11,	462,483,490,502,510,518,523,528,535,548,	7401,7424,7430,7443,7459,7469,7476,7491,7500,7517,
g5507	1143	488	559	Blackfly_scaffolds_121909	23588	7391	7474	56	1	0	0	2	14	2	26	+	5	31,6,4,7,9,	488,519,526,543,550,	7391,7424,7430,7434,7465,
g5507	1143	126	223	Anopheles_chromosome_5	41963435	23596654	23596741	77	4	0	0	2	16	3	6	+	4	25,12,28,16,	126,151,171,207,	23596654,23596681,23596695,23596725,
g5507	1143	129	183	Anopheles_chromosome_1	61545105	24188883	24189211	51	0	0	0	1	3	2	277	+	4	6,23,9,13,	129,138,161,170,	24188883,24188889,24189093,24189198,
g5507	1143	104	174	Aedes_scaffolds_93	2767225	1301935	1301987	50	1	0	0	2	19	1	1	+	3	24,12,15,	104,134,159,	1301935,1301959,1301972,
g5507	1143	90	149	Culex_scaffolds_220	618684	46854	46907	50	3	0	0	1	6	0	0	-	2	12,41,	994,1012,	46854,46866,
g5507	1143	135	182	Anopheles_chromosome_6	24393108	8850622	8850671	45	2	0	0	0	0	2	2	-	3	11,31,5,	961,972,1003,	8850622,8850634,8850666,
g5507	1143	101	174	Aedes_scaffolds_694	626384	188088	188144	53	0	0	0	2	20	2	3	-	4	15,5,6,27,	969,997,1002,1015,	188088,188104,188111,188117,
g5507	1143	90	131	Aedes_scaffolds_67	2980391	178778	178819	37	4	0	0	0	0	0	0	-	1	41,	1012,	178778,
g8181	1500	141	231	Chironomus_scaffolds_113	274809	153754	153859	83	4	0	0	2	3	4	18	+	6	9,12,12,42,5,7,	141,151,165,177,219,224,	153754,153766,153778,153796,153841,153852,

g8181	1500	438	481	Blackfly_scaffolds_66111	6672	5174	5216	41	1	0	0	1	1	0	0	+	2	32,10,	438,471,	5174,5206,
g8181	1500	190	525	Blackfly_scaffolds_121909	23588	7443	7590	111	3	0	0	4	221	6	33	+	9	8,21,4,19,6,22,5,11,18,	190,212,233,440,459,465,489,496,507,	7443,7457,7479,7483,7506,7518,7550,7555,7572,
g8181	1500	126	223	Anopheles_chromosome_5	41963435	23596654	23596741	77	4	0	0	2	16	3	6	+	4	25,12,28,16,	126,151,171,207,	23596654,23596681,23596695,23596725,
g8181	1500	398	454	Anopheles_chromosome_5	41963435	23596601	23596660	54	2	0	0	0	0	1	3	+	2	5,51,	398,403,	23596601,23596609,
g8181	1500	392	451	Anopheles_chromosome_1	61545105	42783707	42783766	57	2	0	0	0	0	0	0	+	1	59,	392,	42783707,
g8181	1500	424	487	Anopheles_chromosome_1	61545105	42783703	42783770	58	5	0	0	0	0	1	4	+	2	56,7,	424,480,	42783703,42783763,
g8181	1500	104	174	Aedes_scaffolds_93	2767225	1301935	1301987	50	1	0	0	2	19	1	1	+	3	24,12,15,	104,134,159,	1301935,1301959,1301972,
g8181	1500	396	471	Aedes_scaffolds_528	843722	16313	16386	69	4	0	0	1	2	0	0	+	2	35,38,	396,433,	16313,16348,
g8181	1500	392	463	Aedes_scaffolds_528	843722	16273	16344	65	6	0	0	0	0	0	0	+	1	71,	392,	16273,
g8181	1500	167	224	Aedes_scaffolds_528	843722	16363	16421	51	1	0	0	2	5	4	6	+	6	4,7,4,16,4,17,	167,171,179,183,203,207,	16363,16368,16375,16380,16399,16404,
g8181	1500	90	131	Culex_scaffolds_220	618684	46866	46907	38	3	0	0	0	0	0	0	-	1	41,	1369,	46866,
g8181	1500	404	461	Culex_scaffolds_188	728313	179236	179313	53	0	0	0	1	4	3	24	-	5	6,8,23,5,11,	1039,1045,1053,1080,1085,	179236,179244,179260,179283,179302,

g8181	1500	126	440	Anopheles_chromosome_6	24393108	6806368	6806462	86	2	0	0	3	226	3	6	-	6	46,7,4,13,12,6,	1060,1322,1329,1337,1350,1368,	6806368,6806414,6806422,6806429,6806444,6806456,
g8181	1500	135	182	Anopheles_chromosome_6	24393108	8850622	8850671	45	2	0	0	0	0	2	2	-	3	11,31,5,	1318,1329,1360,	8850622,8850634,8850666,
g8181	1500	302	450	Anopheles_chromosome_6	24393108	18746695	18746937	58	1	0	0	2	89	3	183	-	4	28,13,7,11,	1050,1079,1180,1187,	18746695,18746726,18746917,18746926,
g8181	1500	179	227	Anopheles_chromosome_6	24393108	6806365	6806414	44	0	0	0	1	4	3	5	-	4	20,4,16,4,	1273,1293,1301,1317,	6806365,6806386,6806393,6806410,
g8181	1500	142	425	Anopheles_chromosome_5	41963435	30603952	30604011	49	1	0	0	1	233	2	9	-	3	13,16,21,	1075,1088,1337,	30603952,30603973,30603990,
g8181	1500	147	440	Anopheles_chromosome_2	53200684	40760594	40760749	104	4	0	0	3	185	3	47	-	4	48,16,28,16,	1060,1277,1301,1337,	40760594,40760685,40760703,40760733,
g8181	1500	430	478	Anopheles_chromosome_1	61545105	17036174	17036229	44	1	0	0	1	3	3	10	-	4	6,7,27,5,	1022,1028,1038,1065,	17036174,17036187,17036195,17036224,
g8181	1500	394	442	Anopheles_chromosome_1	61545105	17036187	17036229	39	0	0	0	1	9	2	3	-	3	7,27,5,	1058,1074,1101,	17036187,17036195,17036224,

g8181	1500	101	174	Aedes_scaffolds_694	626384	188088	188144	53	0	0	0	2	20	2	3	-	4	15,5,6,27,	1326,1354,1359,1372,	188088,188104,188111,188117,
g8181	1500	306	345	Aedes_scaffolds_508	889804	519823	519859	35	1	0	0	1	3	0	0	-	2	6,30,	1155,1164,	519823,519829,

Legend:

1. Q name - Query sequence name
2. Q size - Query sequence size
3. Q start - Alignment start position in query
4. Q end - Alignment end position in query
5. T name - Target sequence name
6. T size - Target sequence size
7. T start - Alignment start position in target
8. T end - Alignment end position in target
9. Matches - Number of bases that match that aren't repeats
10. Mismatches - Number of bases that don't match
11. Repmatches - Number of bases that match but are part of repeats
12. N's - Number of 'N' bases
13. Q gap count - Number of inserts in query
14. Q gap bases - Number of bases inserted in query
15. T gap count - Number of inserts in target
16. T gap bases - Number of bases inserted in target
17. Strand - '+' or '-' for query strand. For translated alignments, second '+'or '-' is for genomic
18. Block count - Number of blocks in the alignment (a block contains no gaps)
19. Block sizes - Comma-separated list of sizes of each block
20. qStarts - Comma-separated list of starting positions of each block in query
21. tStarts - Comma-separated list of starting positions of each block in target

Supplementary Table 2. All homologs detected from the examined eukaryotic genomes using *Z. culisetae*’s triple-ubiquitin gene as a query (E-value < $1E^{-100}$).

Strains	**Copy No. of Polyubiquitin gene**	**Ortholog (O) or Paralog (P)**	**Location**	**Repeat No.**	**Upstream**	**Downstream**	**E-value**
Aspergillus clavatus	1	O	Scaffold_1099423829800	4	GAL	RRM_ETP1	$3E^{-153}$
Arthroderma benhamiae	1	O	Supercontig_42	3	GAL	Pkc-like	$4E^{-153}$
Penicillium expansum	1	O	Scaffold_2	4	GAL	RRM_ETP1	$6E^{-153}$
Amanita thiersii	2	O	Scaffold_1	5	WD40	PIN_SF	$3E^{-153}$
		P1	Scaffold_27	4	FAM72	SET	$2E^{-154}$
Armillaria mellea	2	O	Scaffold_NODE_108303	5	DUF2012	WD40	$2E^{-153}$
		P1	Scaffold_NODE_100039	5	FAM72	SET	$1E^{-148}$
Piloderma croceum	4	O	Scaffold_00016	5	WD40	PIN_SF	$2E^{-153}$
		P1	Scaffold_00043	3	N/A	cytidine_deaminase-like	$2E^{-156}$
		P2	Scaffold_00043	3	N/A	N/A	$9E^{-156}$
		P3	Scaffold_29	4	Pkc-like	N/A	$2E^{-154}$
Backusella circina	2	O	Scaffold_257	4	ALDH_SF	Suf	$7E^{-149}$
		P1	Scaffold_75	5	P-loop_NTPase	N/A	$5E^{-149}$
Hesseltinella vesiculosa	2	O	Scaffold_6	4	N/A	Suf	$3E^{-150}$

		P1	Scaffold_14	3	P-loop_NTPase	N/A	$2E^{-151}$
Mucor circinelloides	1	O	Scaffold_13	5	N/A	Suf	$7E^{-150}$
Lichtheimia hyalospora	1	O	Scaffold_4	4	P-loop_NTPase	Kinesin	$5E^{-152}$
Rhizopus oryzae	1	O	Scaffold_3.9	3	N/A	Suf	$4E^{-153}$
Umbelopsis ramanniana	2	O	Scaffold_63	5	AdoMet_Mtases	Adaptin_N	$8E^{-152}$
		P1	Scaffold_16	3	Lysozyme-like	Zf-AN1	$2E^{-117}$
Zancudomyces culisetae	1	O	Scaffold_1672	3	Lamg	S_Tkc	$1E^{-165}$
Smittium culicis GSMNP	1	O	Scaffold_5123	3.5	N/A	N/A	$4E^{-154}$
Smittium culicis ID206W2	1	O	Scaffold_922	2.5	N/A	N/A	$2E^{-107}$
Smittium mucronatum	1	O	Scaffold_2577	1.5	N/A	N/A	$1E^{-64}$
Anopheles gambiae	4	O	Chr_2R	14	Rnase_Zc3h12a+tetra-UBQ+triple-UBQ	PDCD2_C	$5E^{-151}$
		P1	Chr_2R	3.5	NT_Pol-beta-like	FAST_2	$1E^{-160}$
		P2	Chr_2R	3	Rnase_Zc3h12a+tetra-UBQ	UBQx14	$1E^{-112}$
		P3	Chr_2R	4	Rnase_Zc3h12a	triple-UBQ	$4E^{-107}$
Aedes aegypti	2	O	Supercontig_1.99	8	PDCD2_C+UBQ+di-UBQ	Rnase_Zc3h12a	$2E^{-156}$
		P1	Supercontig_1.17	4	NT_Pol-beta-like	RAP+FAST_2	$9E^{-161}$

Culex quinquefasciatus	2	O	Supercontig_3.50	14	PDCD2_C+di-UBQ	Rnase_Zc3h12a	$5E^{-151}$
		P1	Supercontig_3.448	7	NT_Pol-beta-like	RAP	$2E^{-157}$
Simulium vittatum	5	O	Scf7180000737758	7	N/A	N/A	$1E^{-157}$
		P1	Scf7180000782555	3	N/A	RAP	$3E^{-161}$
		P2	Scf7180000712356	6	N/A	N/A	$6E^{-159}$
		P3	Scf7180000776578	6.5	NT_Pol-beta-like	N/A	$4E^{-158}$
		P4	Scf71800007	4	N/A	N/A	$1E^{-138}$
Homo sapiens	2	O	Chr_NC_000012.12	9	P-loop_NTPase	CD36	$9E^{-155}$
		P1	Chr_NC_000017.11	3	N/A	N/A	$1E^{-161}$
Bos taurus	2	O	Chr_AC_000174.1	4	P-loop_NTPase	CD36	$1E^{-154}$
		P1	Chr_AC_000176.1	4	ER	ANK	$3E^{-159}$
Canis lupus familiaris	2	O	Chr_NC_006608.3	5	P-loop_NTPase	CD36	$5E^{-159}$
		P1	Chr_NC_006587.3	3	GFA	ANK	$3E^{-160}$
Danio rerio	3	O	Chr_NC_007121.6	7	Sulfotransfer_1	CD36	$1E^{-156}$
		P1	Chr_NC_007116.6	8	Chemokine_CXC	P2X_receptor	$5E^{-156}$
		P2	Chr_NC_007116.6	6	GFA	Chemokine_CXC	$1E^{-150}$