

Table S1: Purification procedure of 26S proteasome isoform from *T. bernacchii* red blood cells (RBCs)

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	U/ml blood	Purification fold	Yield (%)
Extract	645120	99.4	6490	586473	1	100
DEAE	303206	18.8	16128	-	2.5	47
Phenyl peak 1	30321	1.4	21658	-	3.3	4.7
Phenyl peak 2	23953	2.9	8260	-	1.3	3.7
Superdex 200 peak 1	758	0.02	37900	-	5.8	0.1

The proteasome activity was measured using LLVY as substrate and expressed assuming $\epsilon=1 \text{ mM}^{-1} \text{ cm}^{-1}$

Table S2: Structural analysis of the ten models obtained for the seven proteasome subunits.

Analyses have been performed with PROCHECK, PROSA web and Hbplus. Columns concerning the PROCHECK analysis report, as absolute numbers and percentage, the amino acids falling in the Ramachandran plot regions (most favoured, additional favoured, generously allowed, disallowed). PROSA web results report the Z-score obtained, which give a whole measure of the quality of the model in comparison to the value obtained for the template chain (last line for each table). The Hbplus column reports the number of H-bonds observed for each model. This is not a measure of model quality, but it is reported for a comparison among models and to the template chain.

Subunit alpha 4

PROCHECK								PROSA web	Hbplus	
	Number of amino acids in regions:				Percentage					
	Most favoured		Generously		Most favoured		Generously		Z-score	
	Additional	Disallowed	Additional	Disallowed	Additional	Disallowed	Additional	Disallowed		Hbonds
Model 1	214	10	0	0	95.5	4.5	0	0	-7.12	214
Model 2	215	9	0	0	96	4	0	0	-7.07	208
Model 3	215	9	0	0	96	4	0	0	-7.04	214
Model 4	215	9	0	0	96	4	0	0	-7.24	208
Model 5	214	10	0	0	95.5	4.5	0	0	-7.1	209
Model 6	211	13	0	0	94.2	5.8	0	0	-7.14	201
Model 7	215	9	0	0	96	4	0	0	-7.11	213
Model 8	215	9	0	0	96	4	0	0	-7.12	207
Model 9	215	9	0	0	96	4	0	0	-7.11	208
Model 10	215	9	0	0	96	4	0	0	-7.14	201
Template: 3UNB_B 248aa									-7.12	204

Subunit alpha 5

	PROCHECK								PROSA web	Hbplus
	Number of amino acids in regions:				Percentage					
	<i>Most favoured</i>		<i>Generously</i>		<i>Most favoured</i>		<i>Generously</i>		Z-score	
	<i>Additional</i>	<i>Disallowed</i>	<i>Additional</i>	<i>Disallowed</i>	<i>Additional</i>	<i>Disallowed</i>	<i>Additional</i>	<i>Disallowed</i>		Hbonds
Model 1	199	7	0	0	96.6	3.4	0	0	-7.44	179
Model 2	197	9	0	0	95.6	4.4	0	0	-7.61	178
Model 3	200	5	1	0	97.1	2.4	0.5	0	-7.52	181
Model 4	199	7	0	0	96.6	3.4	0	0	-7.51	168
Model 5	198	7	1	0	96.1	3.4	0.5	0	-7.37	190
Model 6	196	10	0	0	95.1	4.9	0	0	-7.57	183
Model 7	198	7	1	0	96.6	3.4	0.5	0	-7.4	185
Model 8	198	8	0	0	96.6	3.9	0	0	-7.41	180
Model 9	198	8	0	0	96.6	3.9	0	0	-7.58	179
Model 10	198	8	0	0	96.6	3.9	0	0	-7.46	179
Template: 3UNB_D 233aa									-7.24	176

Subunit alpha 7

	PROCHECK								PROSA web	Hbplus
	Number of amino acids in regions:				Percentage					
	<i>Most favoured</i>		<i>Generously</i>		<i>Most favoured</i>		<i>Generously</i>		Z-score	
	<i>Additional</i>		<i>Disallow</i>		<i>Additional</i>		<i>Disallow</i>			Hbonds
Model 1	207	6	3	0	95.8	2.8	1.4	0	-7.32	173
Model 2	206	7	3	0	95.4	3.2	1.4	0	-7.33	166
Model 3	206	6	3	1	95.4	2.8	1.4	0.5	-7.28	175
Model 4	206	7	3	0	95.4	3.2	1.4	0	-7.4	165
Model 5	206	7	3	0	95.4	3.2	1.4	0	-7.37	168
Model 6	207	5	3	1	95.8	2.3	1.4	0.5	-7.39	168
Model 7	208	5	3	0	96.3	2.3	1.4	0	-7.43	179
Model 8	203	10	3	0	94	4.6	1.4	0	-7.37	183
Model 9	209	4	3	0	96.8	1.9	1.4	0	-7.38	162
Model 10	208	5	3	0	96.3	2.3	1.4	0	-7.27	168
Template: 3UNB_C 239aa									-7.97	157

Subunit beta 1

	PROCHECK								PROSA web	Hbplus
	Number of amino acids in regions:				Percentage					
	<i>Most favoured</i>		<i>Generously</i>		<i>Most favoured</i>		<i>Generously</i>		Z-score	
	<i>Additional</i>		<i>Disallow</i>		<i>Additional</i>		<i>Disallow</i>		Hbonds	
Model 1	175	9	0	1	94.6	4.9	0	0.5	-7.85	159
Model 2	176	8	0	1	95.1	4.3	0	0.5	-7.96	169
Model 3	174	10	0	1	94.1	5.4	0	0.5	-7.98	166
Model 4	174	10	0	1	94.1	5.4	0	0.5	-7.97	159
Model 5	174	10	0	1	94.1	5.4	0	0.5	-7.98	166
Model 6	174	10	0	1	94.1	5.4	0	0.5	-7.92	154
Model 7	173	11	0	1	93.5	5.9	0	0.5	-7.97	161
Model 8	175	9	0	1	94.6	4.9	0	0.5	-7.88	169
Model 9	174	10	0	1	94.1	5.4	0	0.5	-7.89	170
Model 10	173	11	0	1	93.5	5.9	0	0.5	-7.96	158
Template: 3UNB_L 213 aa									-7.78	177

Subunit beta 2

	PROCHECK								PROSA web	Hbplus
	Number of amino acids in regions:				Percentage					
	<i>Most favoured</i>		<i>Generously</i>		<i>Most favoured</i>		<i>Generously</i>		Z-score	
	<i>Additional</i>	<i>Disallowed</i>	<i>Additional</i>	<i>Disallowed</i>	<i>Additional</i>	<i>Disallowed</i>	<i>Additional</i>	<i>Disallowed</i>		Hbonds
Model 1	168	10	0	0	94.4	5.6	0	0	-6.41	146
Model 2	166	11	1	0	93.3	6.2	0.6	0	-6.58	154
Model 3	167	10	1	0	93.8	5.6	0.6	0	-6.51	160
Model 4	168	10	0	0	94.4	5.6	0	0	-6.38	152
Model 5	168	10	0	0	94.4	5.6	0	0	-6.44	153
Model 6	170	7	1	0	95.5	3.9	0.6	0	-6.38	158
Model 7	167	11	0	0	93.8	6.2	0	0	-6.50	154
Model 8	167	11	0	0	93.8	6.2	0	0	-6.52	155
Model 9	167	10	1	0	93.8	5.6	0.6	0	-6.42	156
Model 10	168	9	1	0	94.4	5.1	0.6	0	-6.50	155
Template: 3UNB_J 196 aa									-5.98	151

Subunit beta 3

	PROCHECK								PROSA web	Hbplus
	Number of amino acids in regions:				Percentage					
	<i>Most favoured</i>		<i>Generously</i>		<i>Most favoured</i>		<i>Generously</i>		Z-score	
	<i>Additional</i>		<i>Disallow</i>		<i>Additional</i>		<i>Disallow</i>		Hbonds	
Model 1	168	7	1	2	94.4	3.9	0,6	1.1	-8.49	152
Model 2	168	7	1	2	94.4	3.9	0,6	1.1	-8.35	152
Model 3	168	7	1	2	94.4	3.9	0,6	1.1	-8.44	149
Model 4	169	6	1	2	94.9	3.4	0,6	1.1	-8.46	151
Model 5	168	7	1	2	94.4	3.9	0,6	1.1	-8.50	149
Model 6	168	7	1	2	94.4	3.9	0,6	1.1	-8.55	151
Model 7	169	6	1	2	94.9	3.4	0,6	1.1	-8.42	155
Model 8	168	7	1	2	94.4	3.9	0,6	1.1	-8.38	149
Model 9	170	6	1	1	95.5	3.4	0,6	0.6	-8.41	160
Model 10	170	6	1	1	95.5	3.4	0,6	0.6	-8.51	159
Template: 3UNB_I 204 aa									-8.26	153

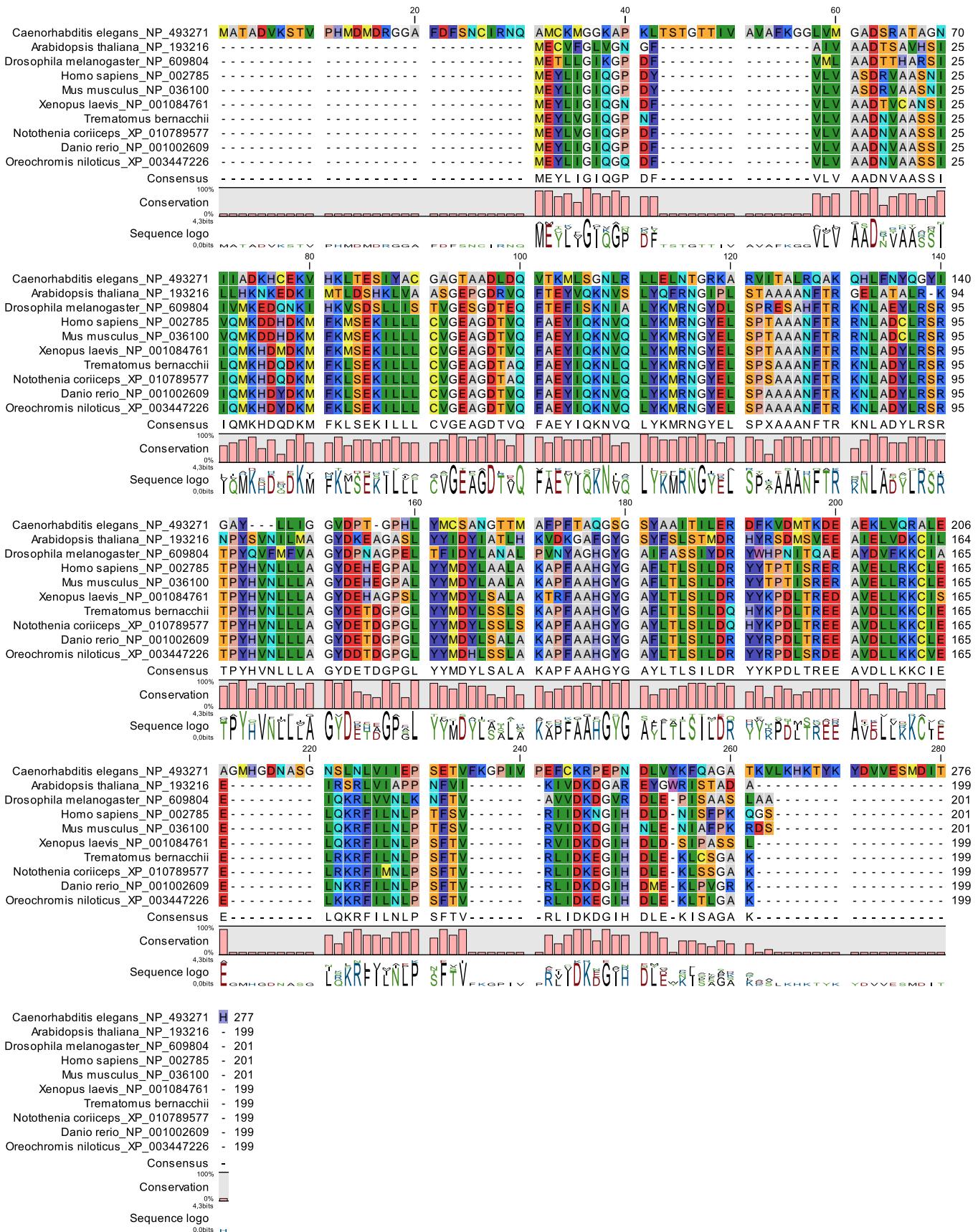
Subunit beta 5

	PROCHECK								PROSA web	Hbplus
	Number of amino acids in regions:				Percentage					
	<i>Most favoured</i>		<i>Generously</i>		<i>Most favoured</i>		<i>Generously</i>		Z-score	
	<i>Additional</i>		<i>Disallowed</i>		<i>Additional</i>		<i>Disallowed</i>			Hbonds
Model 1	168	10	0	0	94.4	5.6	0	0	-6.18	167
Model 2	169	9	0	0	94.9	5.1	0	0	-6.11	173
Model 3	169	9	0	0	94.9	5.1	0	0	-6.22	169
Model 4	170	8	0	0	95.5	4.5	0	0	-6.23	172
Model 5	170	8	0	0	95.5	4.5	0	0	-6.42	165
Model 6	172	6	0	0	96.6	3.4	0	0	-6.45	176
Model 7	172	6	0	0	96.6	3.4	0	0	-6.21	189
Model 8	172	7	0	0	96.1	3.9	0	0	-6.27	165
Model 9	169	9	0	0	94.9	5.1	0	0	-6.16	174
Model 10	170	8	0	0	95.5	4.5	0	0	-6.22	166
Template: 3UNB_K 205 aa									-6.32	172



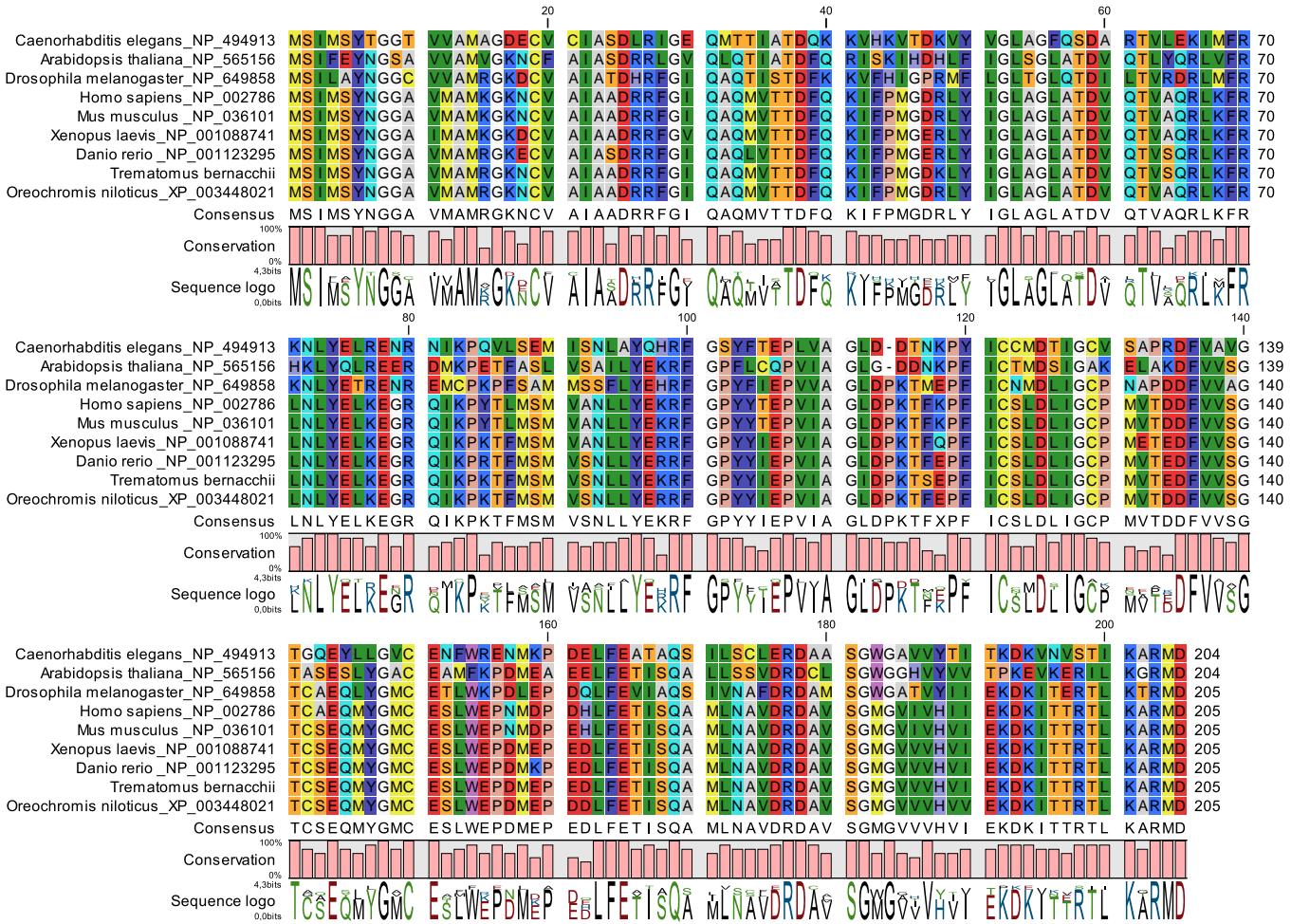
	1	2	3	4	5	6	7	8	9	10
Drosophila melanogaster_NP_652031		15,02	21,25	21,26	21,57	21,09	19,84	21,83	21,83	21,83
Caenorhabditis elegans_NP_498806	41		35,25	36,82	37,98	36,43	37,21	37,21	38,76	38,37
Arabidopsis thaliana_NP_191641	51	92		42,80	43,03	43,27	44,40	44,40	43,15	43,57
Xenopus laevis_NP_001080435	54	95	104		78,33	77,18	79,17	80,42	78,75	78,33
Mus musculus_NP_035315	55	98	105	188		93,36	81,67	82,50	81,67	80,83
Homo sapiens_NP_002784	54	94	106	186	225		79,67	80,91	80,50	80,50
Danio rerio_NP_001003889	50	96	107	190	196	192		90,72	88,61	88,19
Oreochromis niloticus_XP_003454565	55	96	107	193	198	195	215		91,98	91,56
Trematomus bernacchii	55	100	104	189	196	194	210	218		98,73
Notothenia coriiceps_XP_010781254	55	99	105	188	194	194	209	217	234	
	55									
10										

Figure S1. MUSCLE alignment of proteasome subunit beta 1 amino acid sequences, with species names and accession numbers. The consensus sequence, the conservation histogram and the sequence logo are shown at the bottom of the alignment. The Table in the last page contains the pairwise comparison of the sequences, with the number of identities (below the diagonal) and percent identity (above the diagonal).



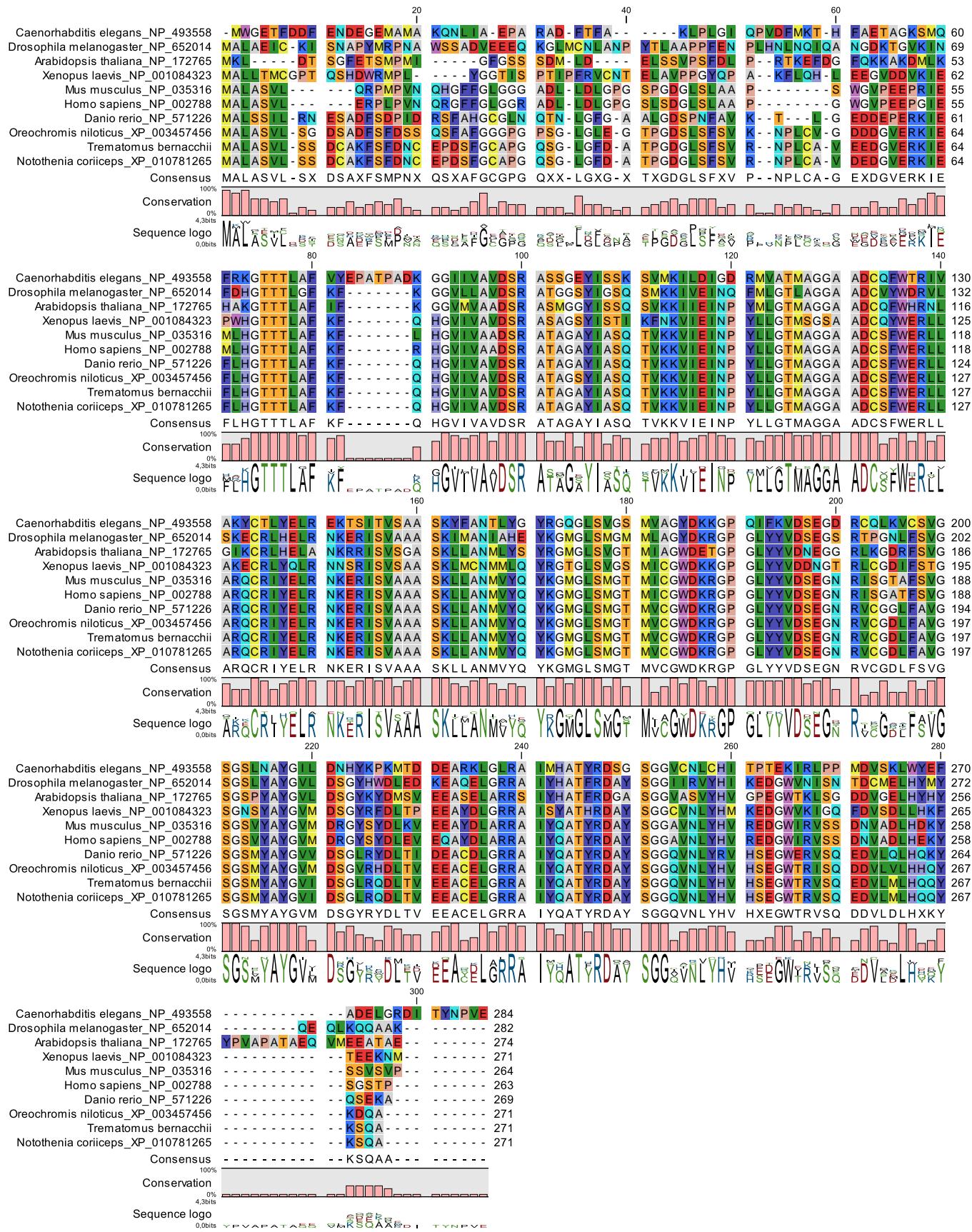
	1	2	3	4	5	6	7	8	9	10
Caenorhabditis elegans_NP_493271		14,23	14,95	14,95	14,59	16,73	19,22	19,93	18,86	19,22
Arabidopsis thaliana_NP_193216	2	40		41,09	43,56	43,07	47,00	44,50	45,00	45,50
Drosophila melanogaster_NP_609804	3	42	83		49,75	50,25	55,72	52,24	53,73	53,23
Homo sapiens_NP_002785	4	42	88	100		96,52	81,59	77,11	77,11	81,09
Mus musculus_NP_036100	5	41	87	101	194		81,59	77,11	76,62	81,59
Xenopus laevis_NP_001084761	6	47	94	112	164	164		81,41	82,41	85,43
Trematomus bernacchii	7	54	89	105	155	155	162		97,49	90,45
Notothenia coriiceps_XP_010789577	8	56	90	108	155	154	164	194		90,45
Danio rerio_NP_001002609	9	53	91	107	163	164	170	180	180	
Oreochromis niloticus_XP_003447226	10	54	90	104	158	158	165	178	180	185

Figure S2. MUSCLE alignment of proteasome subunit beta 2 amino acid sequences, with species names and accession numbers. The consensus sequence, the conservation histogram and the sequence logo are shown at the bottom of the alignment. The Table in the last page contains the pairwise comparison of the sequences, with the number of identities (below the diagonal) and percent identity (above the diagonal).



	1	2	3	4	5	6	7	8	9
Caenorhabditis elegans_NP_494913									
Arabidopsis thaliana_NP_565156	1		44,61	55,12	51,22	50,73	48,29	50,73	49,76
Drosophila melanogaster_NP_649858	2	91		51,22	56,10	57,56	57,07	57,56	58,54
Homo sapiens_NP_002786	3	113	105		63,90	62,44	63,90	64,88	64,88
Mus musculus_NP_036101	4	105	115	131		98,54	91,22	90,24	91,22
Xenopus laevis_NP_001088741	5	104	118	128	202		92,68	91,71	92,68
Danio rerio_NP_001123295	6	99	117	131	187	190		94,15	93,66
Trematomus bernacchii	7	104	118	133	185	188	193		94,63
Oreochromis niloticus_XP_003448021	8	102	120	133	187	190	192	195	
	9	102	120	136	191	192	194	195	197

Figure S3. MUSCLE alignment of proteasome subunit beta 3 amino acid sequences, with species names and accession numbers. The consensus sequence, the conservation histogram and the sequence logo are shown at the bottom of the alignment. The Table in the last page contains the pairwise comparison of the sequences, with the number of identities (below the diagonal) and percent identity (above the diagonal).

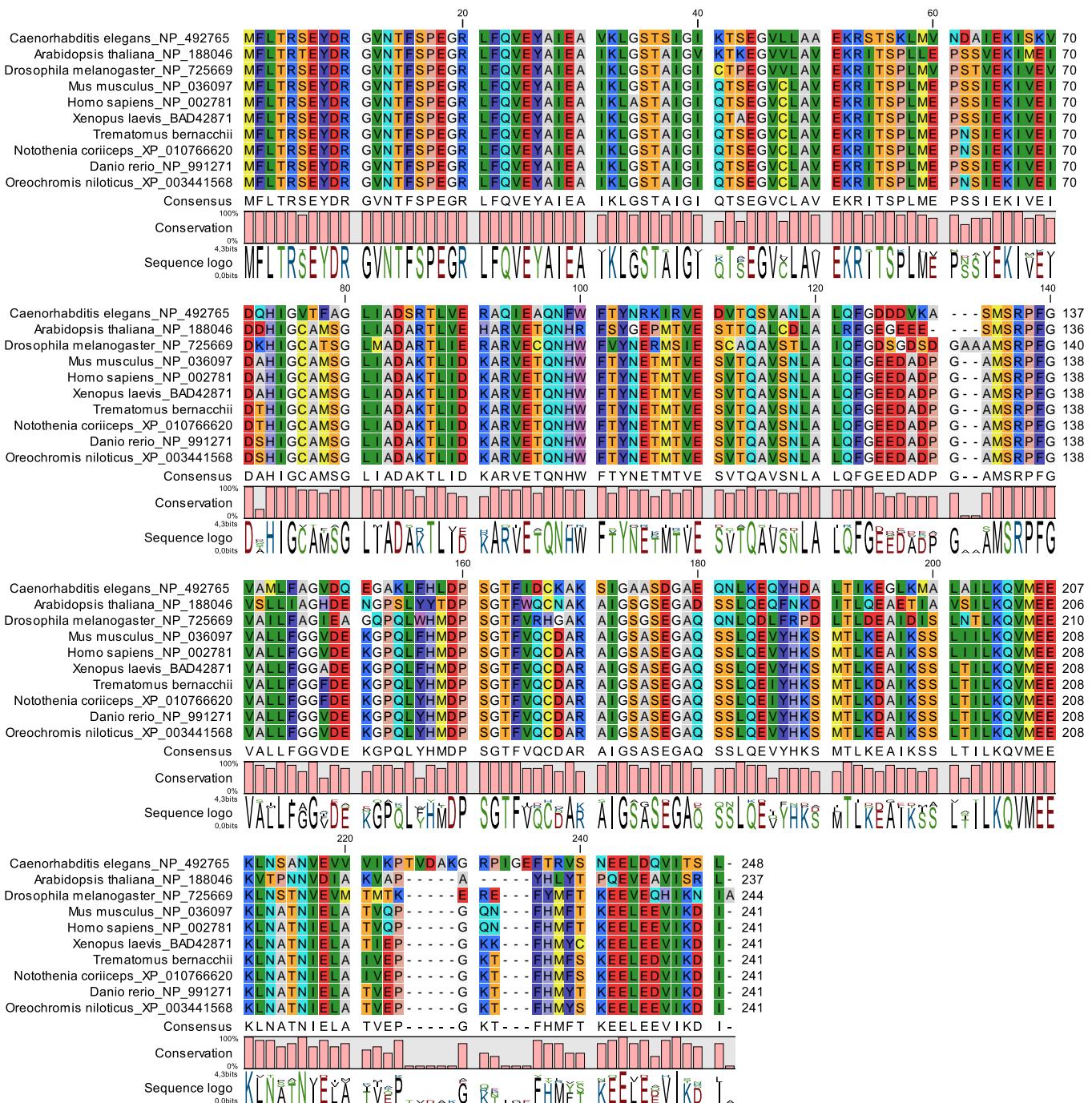


	1	2	3	4	5	6	7	8	9	10
Caenorhabditis elegans_NP_493558										
Drosophila melanogaster_NP_652014	1		37,25	38,61	35,49	35,27	34,93	36,43	36,43	36,43
Arabidopsis thaliana_NP_172765	2	111		47,59	46,29	49,65	49,65	51,42	53,55	52,13
Xenopus laevis_NP_001084323	3	117	138		45,96	50,87	51,92	49,30	50,35	49,30
Mus musculus_NP_035316	4	104	131	131		52,90	52,54	55,43	55,80	53,62
Homo sapiens_NP_002788	5	103	140	146	146		92,80	69,74	71,17	70,44
Danio rerio_NP_571226	6	102	140	149	145	245		69,63	70,33	68,86
Oreochromis niloticus_XP_003457456	7	106	145	141	153	189	188		80,51	80,88
Trematomus bernacchii	8	106	151	144	154	195	192	219		88,93
Notothenia coriiceps_XP_010781265	9	106	147	141	148	193	188	220	241	
	10	106	147	141	147	193	188	219	242	270

Figure S4. MUSCLE alignment of proteasome subunit beta 5 amino acid sequences, with species names and accession numbers. The consensus sequence, the conservation histogram and the sequence logo are shown at the bottom of the alignment. The Table in the last page contains the pairwise comparison of the sequences, with the number of identities (below the diagonal) and percent identity (above the diagonal).

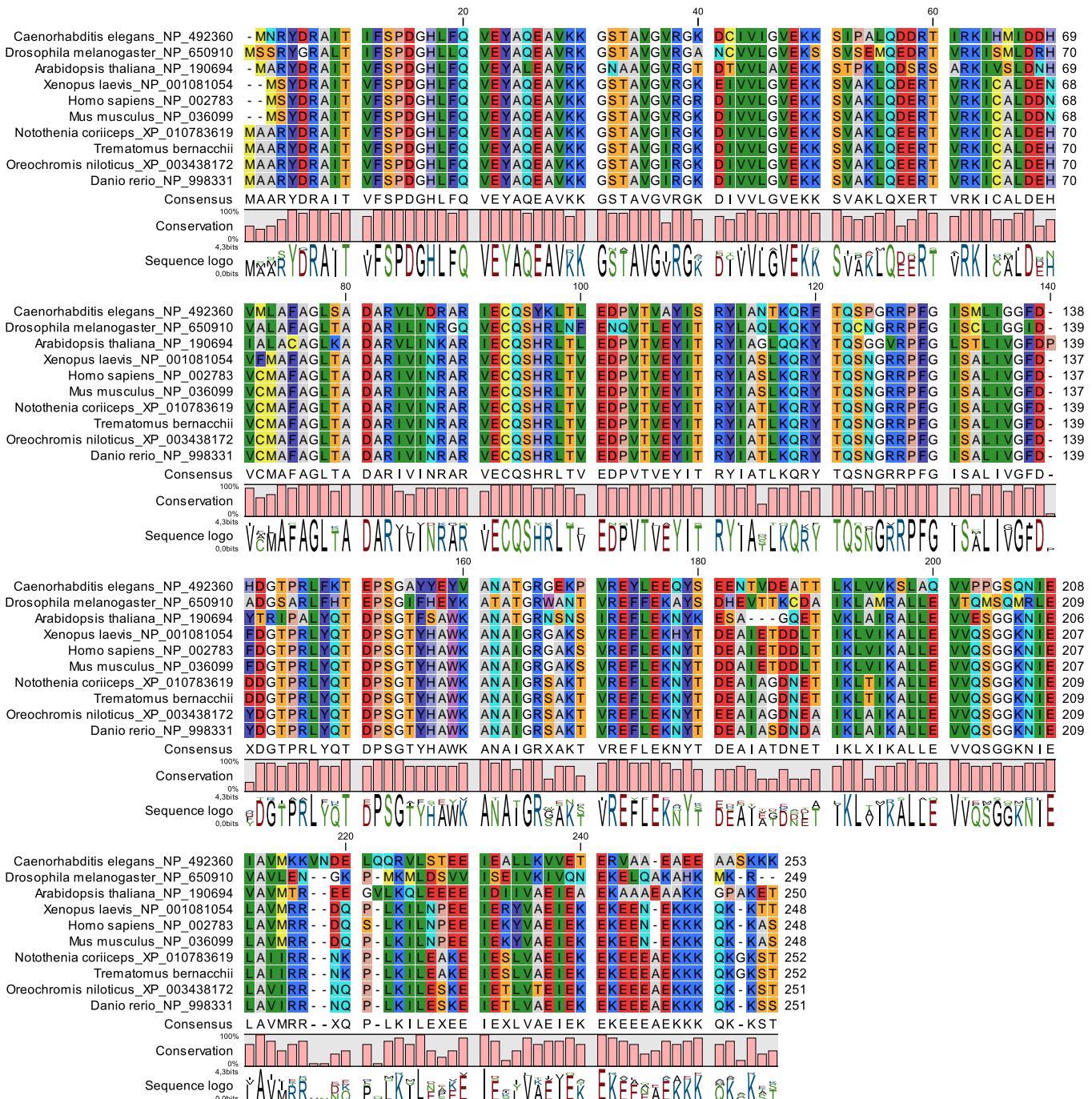
	1	2	3	4	5	6	7	8	9	10	
Drosophila melanogaster_NP_651843	1		44,88	50,78	50,76	51,89	52,27	52,65	52,65	52,27	51,89
Arabidopsis thaliana_NP_188850	2	114		55,34	56,49	56,11	56,11	55,73	55,73	56,11	56,49
Caenorhabditis elegans_NP_491520	3	130	140		62,84	63,22	63,60	62,84	63,22	62,84	62,45
Xenopus laevis_NP_001089811	4	134	148	164		96,55	96,93	93,87	93,49	95,40	95,40
Mus musculus_NP_036096	5	137	147	165	252		98,85	93,87	93,49	95,79	95,79
Homo sapiens_NP_002780	6	138	147	166	253	258		93,10	92,72	95,79	96,17
Notothenia coriiceps_XP_010770288	7	139	146	164	245	245	243		99,62	96,93	95,79
Trematomus bernacchii	8	139	146	165	244	244	242	260		96,55	95,40
Oreochromis niloticus_XP_003450834	9	138	147	164	249	250	250	253	252		98,85
Danio rerio_NP_999862	10	137	148	163	249	250	251	250	249	258	

Figure S5. MUSCLE alignment of proteasome subunit alpha 4 amino acid sequences, with species names and accession numbers. The consensus sequence, the conservation histogram and the sequence logo are shown at the bottom of the alignment. The Table in the last page contains the pairwise comparison of the sequences, with the number of identities (below the diagonal) and percent identity (above the diagonal).



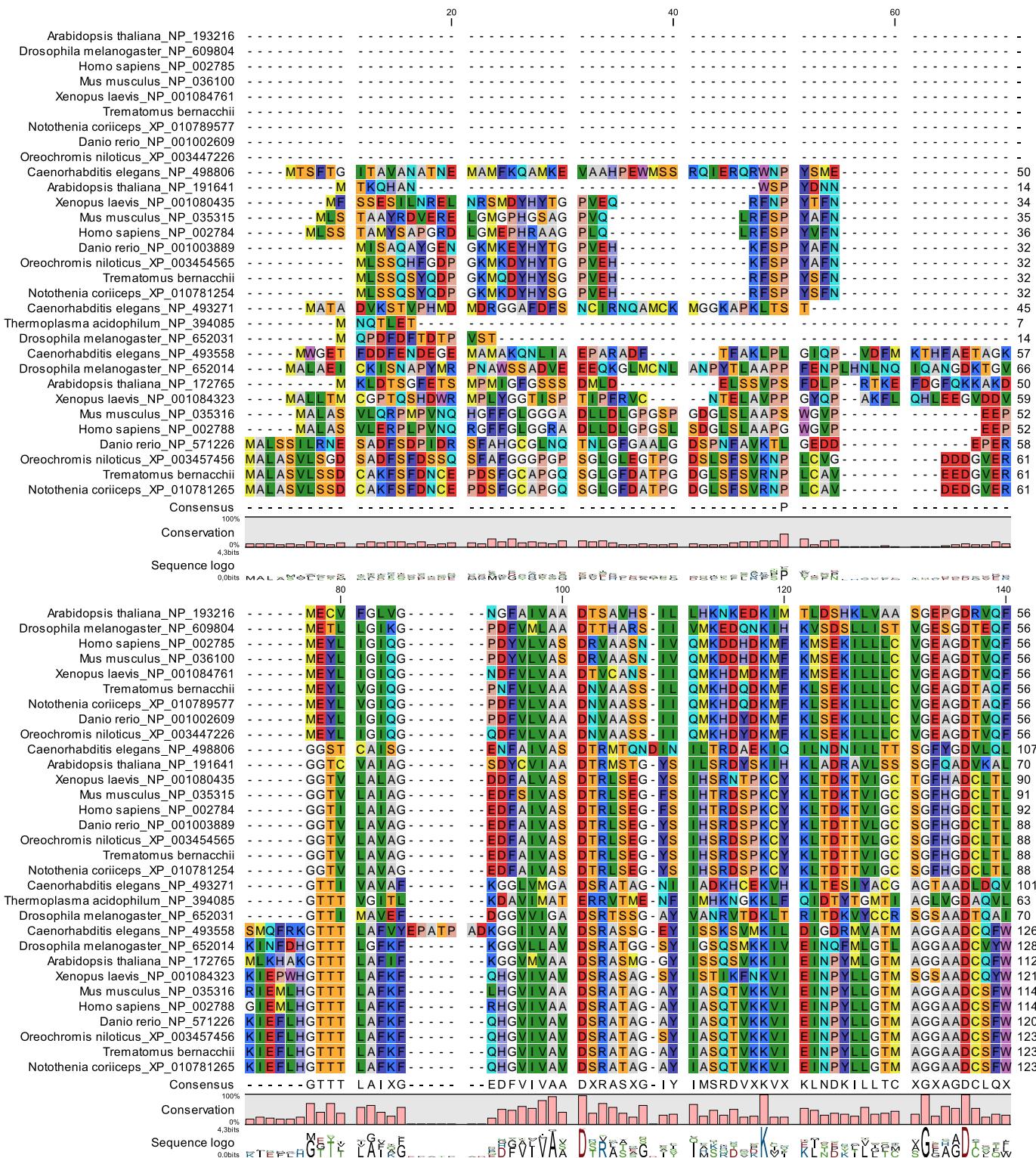
	1	2	3	4	5	6	7	8	9	10
Caenorhabditis elegans_NP_492765										
Arabidopsis thaliana_NP_188046	1	52,82	55,95	60,64	60,24	60,24	59,84	59,84	59,84	60,64
Drosophila melanogaster_NP_725669	2	131		61,07	68,05	67,63	67,63	67,22	67,22	68,46
Mus musculus_NP_036097	3	141	149		70,08	69,67	69,26	68,44	68,44	69,26
Homo sapiens_NP_002781	4	151	164	171		99,59	96,27	94,61	94,61	96,27
Xenopus laevis_BAD42871	5	150	163	170	240		95,85	94,19	94,19	95,85
Trematomus bernacchii	6	150	163	169	232	231		94,61	94,61	96,27
Notothenia coriiceps_XP_010766620	7	149	162	167	228	227	228		100,00	97,10
Danio rerio_NP_991271	8	149	162	167	228	227	228	241		97,10
Oreochromis niloticus_XP_003441568	9	149	165	169	232	231	232	234	234	98,34
	10	151	164	168	232	231	233	234	234	237

Figure S6. MUSCLE alignment of proteasome subunit alpha 5 amino acid sequences, with species names and accession numbers. The consensus sequence, the conservation histogram and the sequence logo are shown at the bottom of the alignment. The Table in the last page contains the pairwise comparison of the sequences, with the number of identities (below the diagonal) and percent identity (above the diagonal).



	1	2	3	4	5	6	7	8	9	10
1		50,20	54,51	59,68	60,08	60,08	57,65	57,65	58,04	57,65
2	128		51,57	58,17	57,77	58,17	59,52	59,52	60,56	61,35
3	139	131		65,61	66,01	66,01	65,75	65,75	66,54	65,75
4	151	146	166		96,37	96,77	87,30	87,30	87,25	87,65
5	152	145	167	239		98,79	86,90	86,90	86,85	88,05
6	152	146	167	240	245		87,30	87,30	87,25	88,45
7	147	150	167	220	219	220		100,00	96,03	95,63
8	147	150	167	220	219	220	252		96,03	95,63
9	148	152	169	219	218	219	242	242		98,01
10	147	154	167	220	221	222	241	241	246	

Figure S7. MUSCLE alignment of proteasome alpha 7 subunit amino acid sequences, with species names and accession numbers. The consensus sequence, the conservation histogram and the sequence logo are shown at the bottom of the alignment. The Table in the last page contains the pairwise comparison of the sequences, with the number of identities (below the diagonal) and percent identity (above the diagonal).



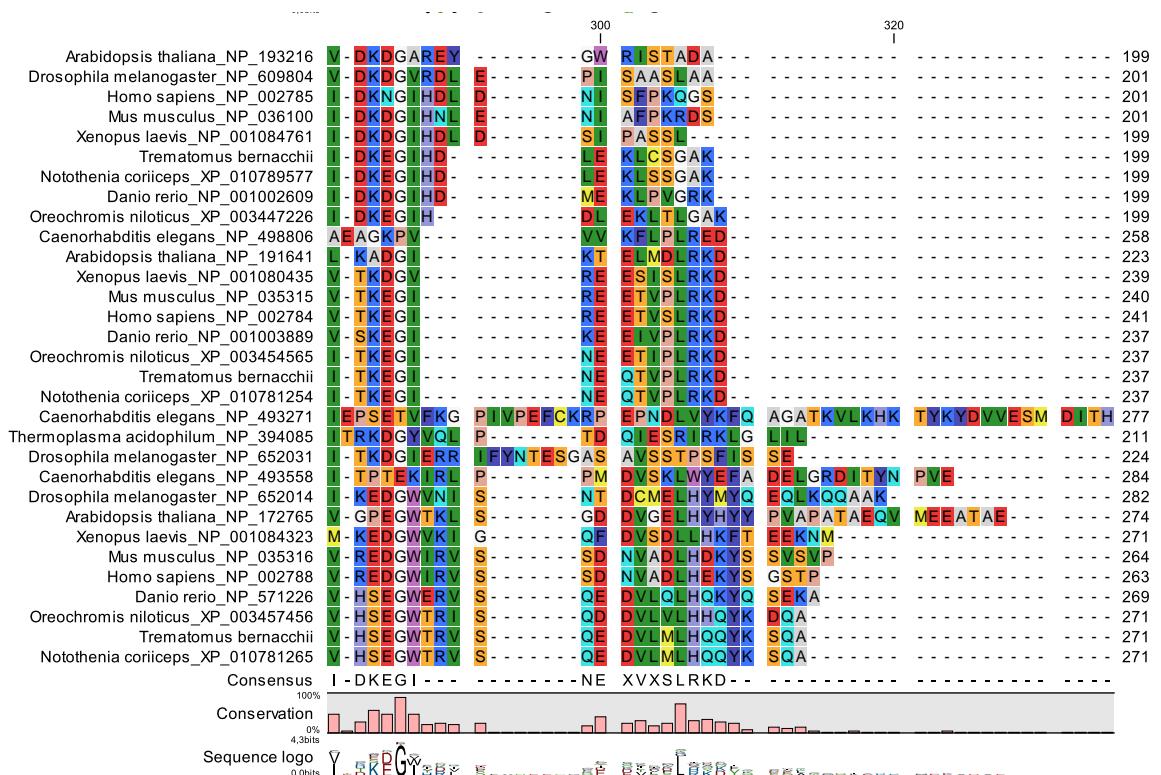


Figure S8. MUSCLE alignment of the catalytic proteasome subunits, with species names and accession numbers, utilized for the phylogenetic analysis.

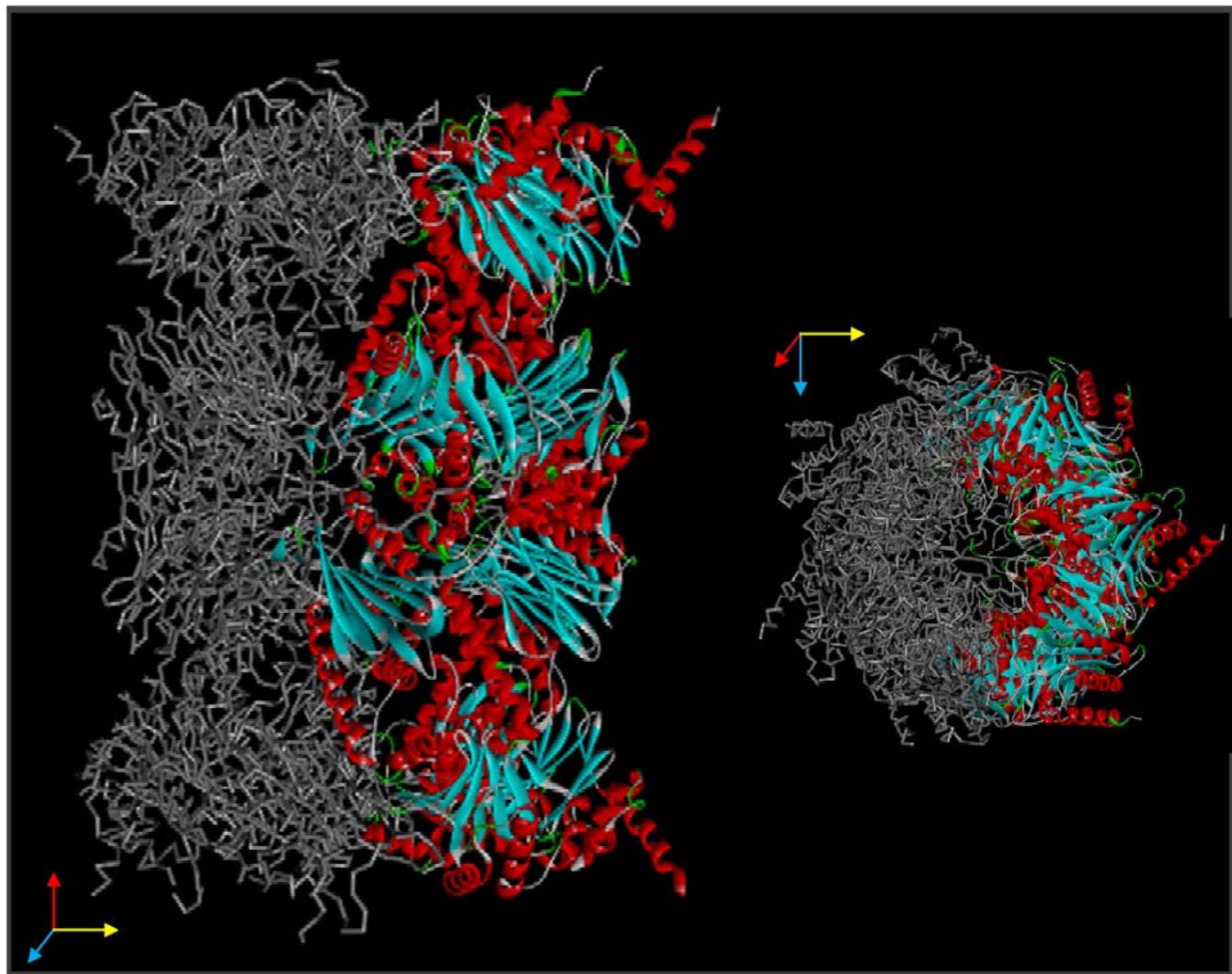


Figure S9. Theoretical assembly of the seven modelled chains of *T. bernacchii* proteasome (in red, alpha helices; in cyan, beta strands). The assembly is based on the reference structure of mouse whole proteasome (PDB code: 3UNB). The simple backbone (in grey) of the corresponding mouse chains is shown for the remaining chains (not modelled).