

Supplementary Information

The Diverse Calpain Family in Trypanosomatidae: Functional Proteins Devoid of Proteolytic Activity?

Table 1. Calpain sequences retrieved from *A. deanei* genome.

Se- quence ID	Assemble ID	Domain ar- chitecture	Predicted molec- ular mass (kDa)	Conserved cata- lytic triad (C,H,N)	Note
EPY232 92.1	GCA_000 442575.2	DUF1935	14.54	-----	Identical Proteins
CAD221 3457.1	GCA_903 995115.1				
EPY281 59.1	GCA_000 442575.2	DUF1935	12.92	-----	Identical Proteins
CAD221 9314.1	GCA_903 995115.1				
CAD221 9315.1	GCA_903 995115.1				
EPY330 88.1	GCA_000 442575.2	DUF1935	15.25	-----	Identical Proteins
CAD221 5177.1	GCA_903 995115.1				
EPY344 98.1	GCA_000 442575.2	DUF1935	16.09	-----	Identical Proteins
EPY430 70.1					
EPY438 02.1	GCA_903 995115.1	DUF1935	20.03	-----	Identical Proteins
CAD221 5188.1	GCA_903 995115.1				
EPY377 00.1	GCA_000 442575.2				
CAD221 5183.1	GCA_903 995115.1	DUF1935	14.39	-----	Identical Proteins
EPY388 60.1	GCA_000 442575.2				
EPY397 96.1	GCA_903 995115.1	DUF1935	14.06	-----	Identical Proteins
EPY414 74.1	GCA_000 442575.2				
CAD221 5168.1	GCA_903 995115.1				
EPY420 18.1	GCA_000 442575.2	DUF1935	15.82	-----	-----
EPY383 40.1	GCA_000 442575.2	fragmented CysPc	23.97	-----	Short amino acid sequence from the catalytic domain
EPY386 63.1	GCA_000 442575.2	fragmented CysPc, CBSW	32.88	-----	Short amino acid sequence from the catalytic domain
EPY177 91.1	GCA_000 442575.2	CysPc	80.68	-----	Identical Proteins
CAD221 5189.1	GCA_903 995115.1				
EPY310 64.1	GCA_000 442575.2	CysPc	61.9	present	Identical Proteins

CAD221 7327.1	GCA_903 995115.1				
EPY330 36.1	GCA_000 442575.2	CysPc	53.67	-----	-----
EPY197 82.1	GCA_000 442575.2				
CAD221 4161.1	GCA_903 995115.1	CysPc	104.13	-----	Identical Proteins
EPY355 01.1	GCA_000 442575.2				
CAD221 5192.1	GCA_903 995115.1	CysPc	41.89	-----	Identical Proteins
CAD221 5193.1	GCA_903 995115.1	CysPc	56.70	-----	-----
EPY409 13.1	GCA_000 442575.2				
CAD221 5397.1	GCA_903 995115.1	CysPc	30.53	-----	Identical Proteins
CAD221 5575.1	GCA_903 995115.1	CysPc	75.57	-----	-----
EPY175 50.1	GCA_000 442575.2				
CAD222 2307.1	GCA_903 995115.1	CysPc	54.99	-----	Identical Proteins
EPY400 95.1	GCA_000 442575.2	CysPc	66.74	present	-----
EPY169 71.1	GCA_000 442575.2	2xCysPc	50.11	-----	-----
EPY354 73.1	GCA_000 442575.2	DUF1935, CysPc	81.01	present	-----
EPY345 08.1	GCA_000 442575.2				
EPY379 30.1	GCA_000 442575.2	DUF1935, CysPc	95.30	-----	Identical Proteins
CAD221 4023.1	GCA_903 995115.1				
EPY274 35.1	GCA_000 442575.2				
CAD221 4024.1	GCA_903 995115.1	DUF1935, CysPc	95.86	-----	Identical Proteins
EPY246 69.1	GCA_000 442575.2				
CAD221 4025.1	GCA_903 995115.1	DUF1935, CysPc	82.47	-----	Identical Proteins
EPY286 34.1	GCA_000 442575.2				
CAD221 4026.1	GCA_903 995115.1	DUF1935, CysPc	128.39	-----	Identical Proteins
EPY393 28.1	GCA_000 442575.2				
CAD221 4027.1	GCA_903 995115.1	DUF1935, CysPc	88.95	-----	Identical Proteins
CAD221 5190.1	GCA_903 995115.1	DUF1935, CysPc	77.06	-----	-----
EPY265 98.1	GCA_000 442575.2				
EPY438 76.1	GCA_000 442575.2	DUF1935, CysPc	77.53	-----	Identical Proteins
CAD221 5191.1	GCA_903 995115.1				

CAD221	GCA_903	DUF1935,	77.01	-----	-----
5195.1	995115.1	CysPc			
EPY264	GCA_000	DUF1935,	77.03	-----	-----
55.1	442575.2	CysPc			
EPY276	GCA_000				
08.1	442575.2	DUF1935,	94.24	present	Identical Proteins
CAD222	GCA_903	CysPc			
0197.1	995115.1				
EPY298	GCA_000	DUF1935,	80.85	-----	-----
41.1	442575.2	CysPc			
EPY313	GCA_000	DUF1935,	46.69	-----	-----
84.1	442575.2	CysPc			
EPY321	GCA_000				
39.1	442575.2	DUF1935,	80.20	present	Identical Proteins
CAD221	GCA_903	CysPc			
4018.1	995115.1				
EPY351	GCA_000	DUF1935,	75,67	-----	-----
55.1	442575.2	CysPc			
EPY359	GCA_000	DUF1935,	80.33	present	-----
37.1	442575.2	CysPc			
EPY367	GCA_000	DUF1935,	62.07	-----	-----
66.1	442575.2	CysPc			
EPY374	GCA_000	DUF1935,	85.88	-----	-----
55.1	442575.2	CysPc			
EPY403	GCA_000	DUF1935,	77.54	-----	-----
46.1	442575.2	CysPc			
EPY418	GCA_000	DUF1935,	83.53	-----	-----
99.1	442575.2	CysPc			
EPY210	GCA_000				
44.1	442575.2	CysPc, CBSW	73.48	-----	Identical Proteins
CAD221	GCA_903				
8077.1	995115.1				
EPY417	GCA_000				
80.1	442575.2	CysPc, CBSW	123.76	present	Identical Proteins
CAD221	GCA_903				
6952.1	995115.1				
CAD222	GCA_903	CysPc, CBSW	121.94	-----	-----
0901.1	995115.1				
CAD221	GCA_903	CysPc, CBSW	130.64	-----	-----
3168.1	995115.1				
EPY204	GCA_000	CysPc, CBSW,	217.36	-----	-----
06.1	442575.2	CysPc			
EPY222	GCA_000	CysPc, CBSW,	174.58	-----	-----
62.1	442575.2	CysPc			
EPY325	GCA_000	CysPc, CBSW,	98.51	-----	-----
74.1	442575.2	CysPc			
CAD221	GCA_903	CysPc, CBSW,	97.73	-----	-----
3171.1	995115.1	CysPc			
CAD221	GCA_903	CysPc, CBSW,	354.59	-----	-----
5402.1	995115.1	CysPc			
EPY227	GCA_000				
07.1	442575.2	RPT1, CysPc,	236.37	-----	Identical Proteins
CAD221	GCA_903	CBSW			
5572.1	995115.1				
CAD221	GCA_903	CysPc, CBSW,	522.62	-----	-----
5393.1	995115.1	CysPc, RPT1			
EPY280	GCA_000				
67.1	442575.2	KISC, 3xARM,	103.81	-----	Identical Proteins; Kinesin motor do- main associated with CBSW
CAD222	GCA_903	CBSW			
1641.1	995115.1				

CAD221 5401.1	GCA_903 995115.1	RPT1, CysPc	189.04	-----	-----
EPY204 04.1	GCA_000 442575.2	2xRPT1, CysPc	161.06	-----	-----
CAD221 5396.1	GCA_903 995115.1	RPT1, RPT2, CysPc	301.59	-----	-----
EPY215 60.1	GCA_000 442575.2	2xRPT1	24.58	-----	RPT sequences annotated as calpain-like proteins
EPY270 93.1	GCA_000 442575.2	2xRPT1	157.43	-----	Identical Proteins; RPT sequences annotated as calpain-like proteins
CAD221 5574.1	GCA_903 995115.1				
EPY162 12.1	GCA_000 442575.2	2xRPT1	11.23	-----	Identical Proteins; RPT sequences annotated as calpain-like proteins
CAD221 5398.1	GCA_903 995115.1				
EPY204 05.1	GCA_000 442575.2	2xRPT1	58.08	-----	RPT sequences annotated as calpain-like proteins
EPY222 64.1	GCA_000 442575.2	2xRPT1	125.40	-----	RPT sequences annotated as calpain-like proteins
EPY245 73.1	GCA_000 442575.2	2xRPT1	239.87	-----	RPT sequences annotated as calpain-like proteins
EPY259 74.1	GCA_000 442575.2	2xRPT1	189.50	-----	Identical Proteins; RPT sequences annotated as calpain-like proteins
CAD221 5400.1	GCA_903 995115.1				
EPY297 04.1	GCA_000 442575.2	RPT1, RPT2, RPT1	101.27	-----	RPT sequences annotated as calpain-like proteins

The ID of the calpain sequences was retrieved from two different assemblies of the *Angomonas deanei* genome (Genebank ID 14191). The sequences were ordered based on their domain architecture. The predicted molecular mass was calculated in http://www.bioinformatics.org/sms/prot_mw.html. Sequences annotated as “calpain-like proteins” but with less than 100 amino acids or without any calpain associated domain were not included. CBSW—calpain-type alpha-beta-sandwich domain, CysPc—proteolytic core domain, KISC—kinesin domain, DUF1935—conserved N-terminal calpain-related domain from trypanosomatids, RPT—repeated domain found in de-ubiquitinating proteins, 2× and 3× indicate the number of times that a domain appears in the sequence. Fragmented CysPc stands for short amino acid sequences from the catalytic domain.

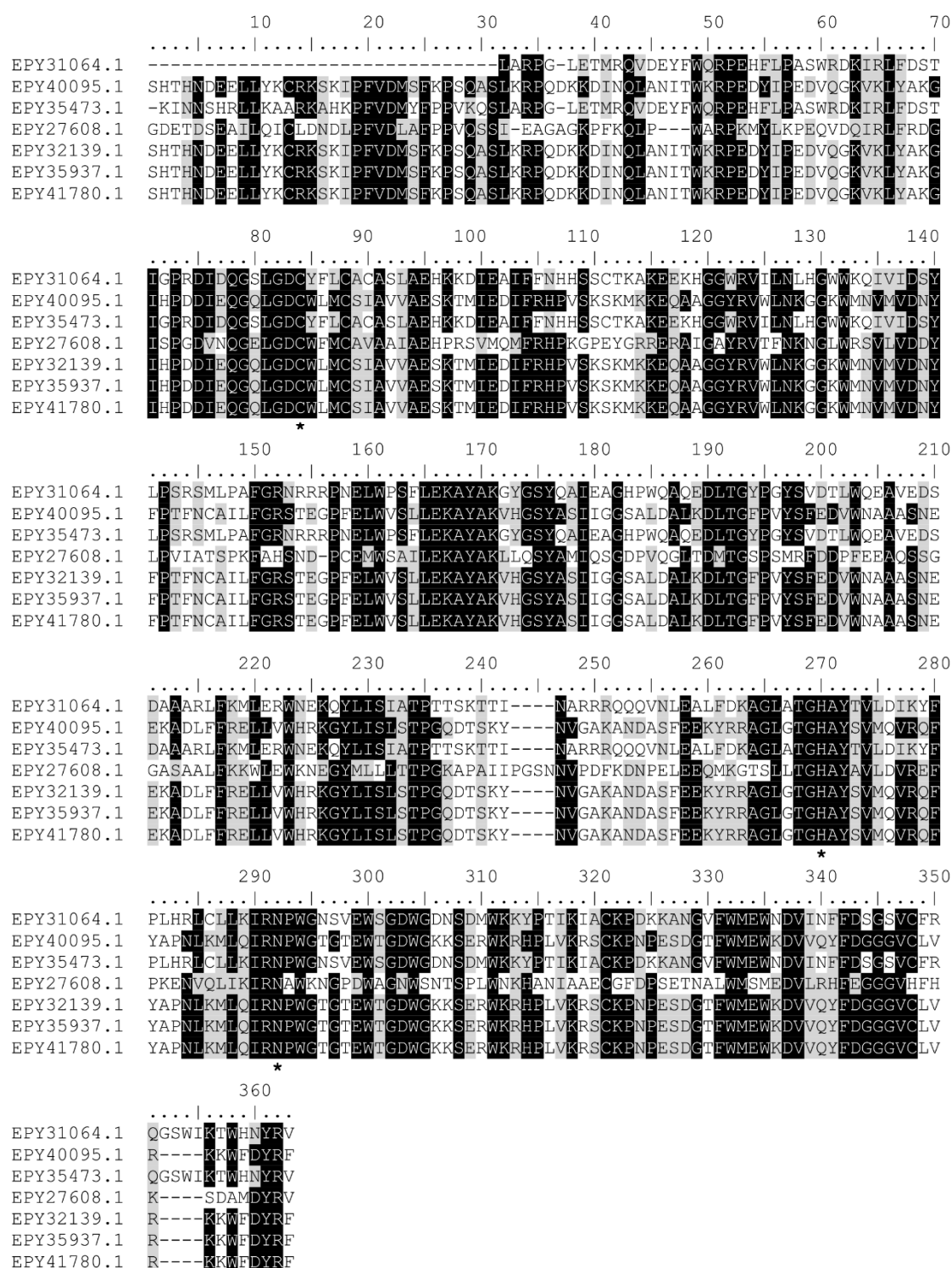


Figure S1. Multiple sequence alignment of the cysteine peptidase core (CysPc) domain of *Angomonas deanei* with the conserved catalytic triad (C,H,N). The sequence alignment was performed using ClustalW. GeneDB ID of the *A. deanei* calpain sequences are on the left. Identical and similar residues are represented in black and grey, respectively. The amino acids responsible for the proteolytic activity of conventional calpains are indicated by a *.