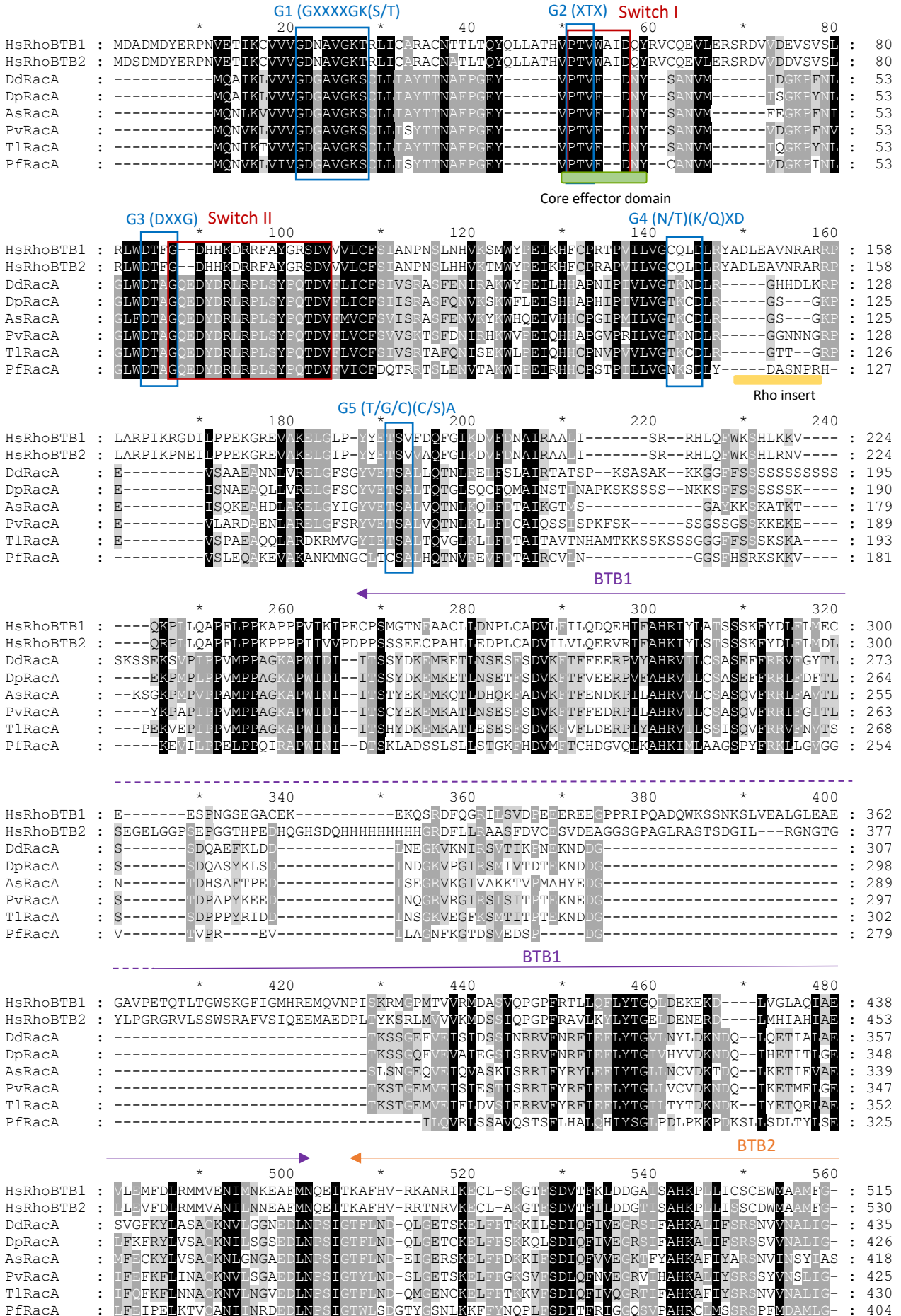


SUPPLEMENTARY MATERIALS



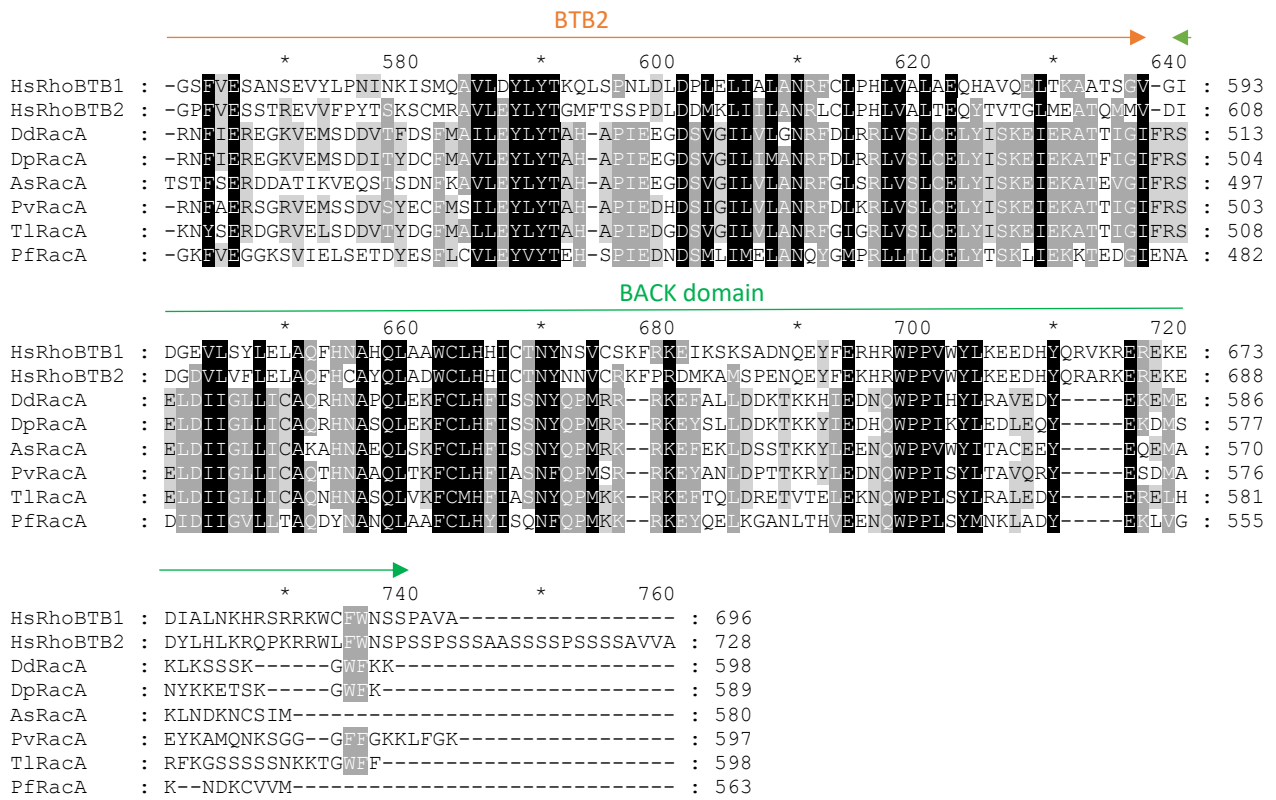


Figure S1. Sequence alignment of RhoBTB 1 and 2 and RacA subfamily proteins. The amino acid sequences were aligned using the MUSCLE algorithm [62]. Multiple alignments were visualized and edited using the GeneDoc software 2.7. The amino acid residues were shaded using the conserved residue shading mode set to level 4 with default settings. Similar residues conserved in all proteins have a dark shading. The residues with 100%, 80%, and 60% conservation are shaded as white letters on the black background, white letters on the dark-grey background, and black letters on the light-grey background, respectively. Specific regions are indicated as follows: the G boxes are framed in blue (G1, G2, G3, G4, and G5 with the respective consensus motifs), the switch I and switch II regions are framed in red, a purple line designates the first BTB domain, an orange line the second BTB domain, and a green line the C-terminus back domain. The core effector domain and the Rho insert are indicated by green and orange bars, respectively.

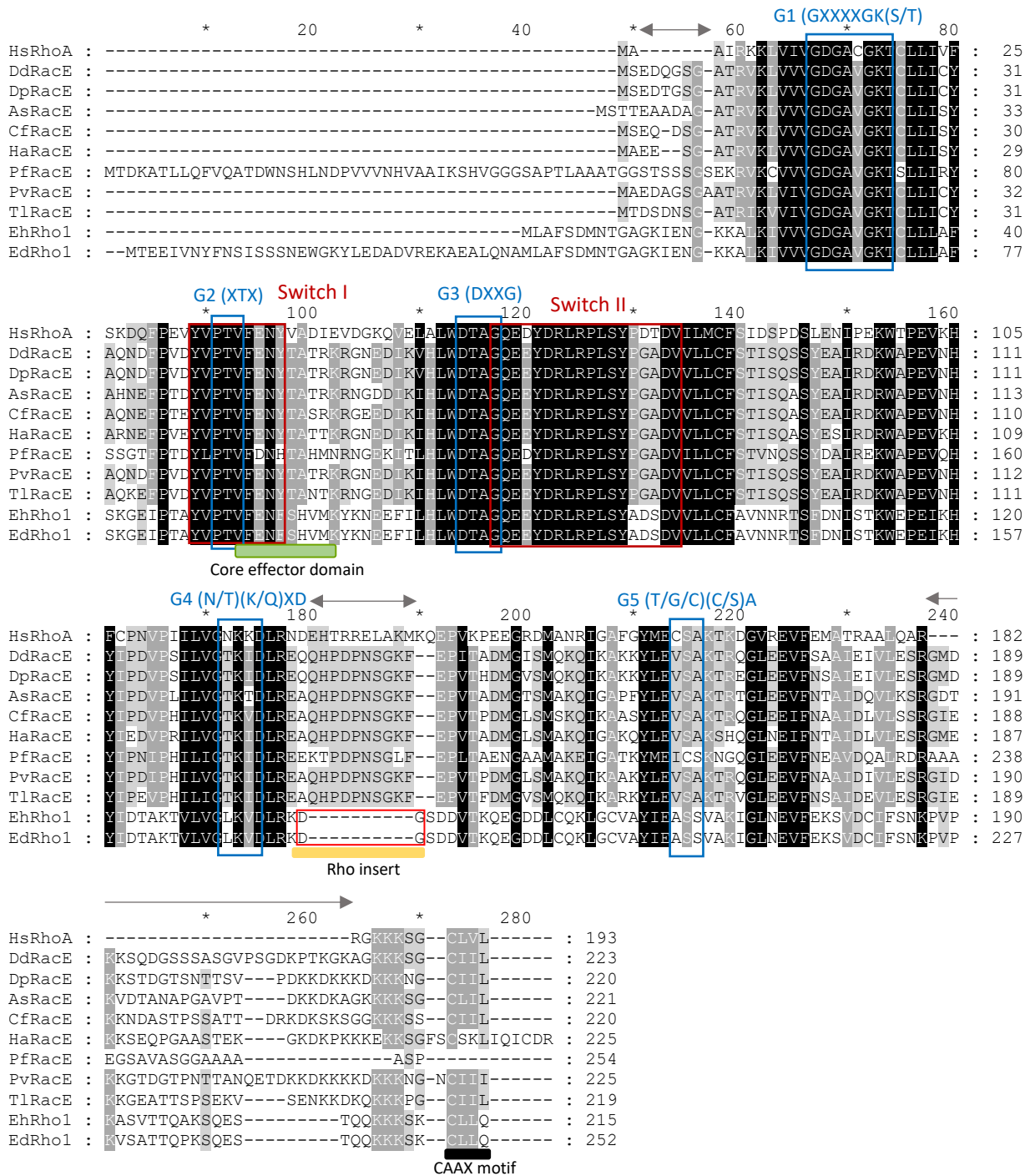


Figure S2. Sequence alignment of RhoA and RacE subfamily proteins. The amino acid sequences were aligned using the MUSCLE algorithm [62]. Conserved sequences are shaded using the GeneDoc software 2.7 (see Figure S1). Specific regions are indicated as follows: the G boxes are framed in blue (G1, G2, G3, G4, and G5 with the respective consensus motifs), and the switch I and switch II regions are framed in red. The core effector domain, the Rho insert, and the conserved CAAX motif at the C-terminus are indicated by green, orange and black bars, respectively. The grey lines at the N- and C-terminus represent the important deletions in human or entamoeban proteins.

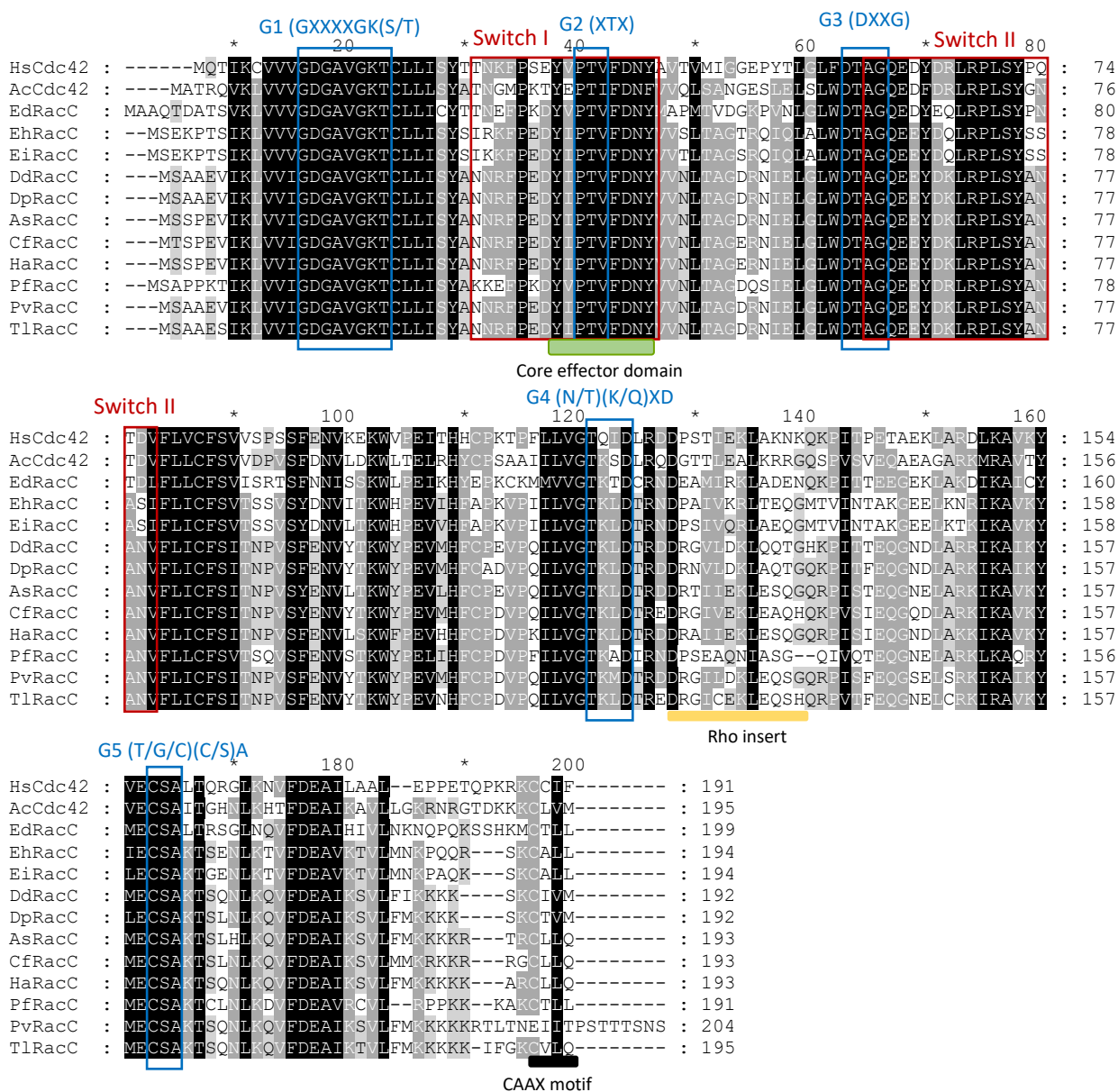


Figure S3. Sequence alignment of Cdc42 and RacC subfamily proteins. The amino acid sequences were aligned using the MUSCLE algorithm [62]. Conserved sequences are shaded using the GeneDoc software 2.7 (see Figure S1). The amino acid residues are shaded as in Figure S1. Specific regions are indicated as follows: the G boxes are framed in blue (G1, G2, G3, G4, and G5 with the respective consensus motifs), and the switch I and switch II regions are framed in red. The core effector domain, the Rho insert, and the conserved CAAX motif at the C-terminus are indicated by green, orange and black bars, respectively.

Table S1. Rho GTPases in Amoebozoa.

Amoebozoa						
	Phylum	Organism	Rho GTPases			
Conosa	Archamoebae	<i>Entamoeba histolytica</i>	EhCdc42	EhRacG	XP_653308.1	XP_652457.1
			EhRacA	EhRacH	XP_649502.1	XP_648766.1
			EhRacB	EhRho1	XP_656301.1	XP_654700.1
			EhRacC	XP_653885.1	XP_656258.1	XP_656370.1
			EhRacD	XP_653812.1	XP_652660.1	
		<i>Entamoeba dispar</i>	EdRacA-like	EdRacG-like	XP_001739099.1	XP_001738433.1
			EdRacB-like	EdRacH-like	XP_001737112.1	XP_001734489.1
			EdRacC-like	EdRho1-like	XP_001737038.1	XP_001739566.1
			EdRacD-like	XP_001736788.1	XP_001736684.1	XP_001741992.1
		<i>Entamoeba invadens</i>	EiCdc42-like	EiRacD-like	XP_004185779.1	XP_004258224.1
			EiRacA-like	EiRacG-like	XP_004258338.1	XP_004185920.1
			EiRacB-like	EiRacH-like	XP_004183588.1	XP_004184178.1
			EiRacC-like	XP_004261490.1	XP_004261195.1	XP_004254590.1
	Dictyostelia	<i>Dictyostelium discoideum</i>	DdRac1A	DdRacF1	DdRacG	DdRacM
			DdRac1B	DdRacF2	DdRacH	DdRacN
			DdRac1C	DdRacC	DdRacI	DdRacO
			DdRacA	DdRacD	DdRacJ	DdRacP
			DdRacB	DdRacE	DdRacL	DdRacQ
		<i>Dictyostelium purpureum</i>	DpRac1A-like	DpRacD-like	DpRacL-like	DpRacU-like
			DpRac1B-like	DpRacE-like	DpRacP-like	DpRacV-like
			DpRacB-like	DpRacG-like	DpRacR-like	DpRacW-like
			DpRacA-like	DpRacH-like	DpRacS-like	
			DpRacC-like	DpRacJ-like	DpRacT-like	
		<i>Acytostelium subglobosum</i>	AsRac1A-like	AsRacC-like	AsRacH-like	
			AsRac1B-like	AsRacD-like	AsRacL-like	
			AsRacA-like	AsRacE-like	AsRacP-like	
			AsRacB-like	AsRacG-like		
		<i>Cavenderia fasciculata</i>	CfRac1A-like	CfRacC-like	CfRacH-like	
			CfRac1B-like	CfRacD-like	CfRacL-like	
			CfRacB-like	CfRacE-like	CfRacP-like	
		<i>Heterostelium album</i>	HaRac1B-like	HaRacC-like	HaRacH-like	
			HaRac1C-like	HaRacD-like	HaRacL-like	
			HaRacB-like	HaRacE-like	HaRacP-like	
			HaRacF2-like	HaRacG-like		
		<i>Polysphondylium violaceum</i>	PvRac1A-like	PvRacC-like	PvRacL-like	
			PvRac1B-like	PvRacD-like	PvRacP-like	
			PvRacA-like	PvRacE-like		
			PvRacB-like	PvRacH-like		
		<i>Tieghemostelium lacteum</i>	TIRac1A-like	TIRacD-like	TIRacL-like	
			TIRacA-like	TIRacE-like	TIRacP-like	
			TIRacB-like	TIRacG-like		
			TIRacC-like	TIRacH-like		
Lobosa	Variosea	<i>Planoprotostelium fungivorum</i>	PfRac1-like	PfRacA-like	PfRacE-like	
			PfRac1B-like	PfRacC-like	PfRacH-like	
Lobosa	Discosea	<i>Acanthamoeba castellanii</i>	AcCdc42-like	AcRac1B-like	AcRac3-like	
			AcRac1	AcRac2-like		

Rho GTPase genes from *Dictyostelium discoideum* and *Entamoeba histolytica* were identified based on publicly available information and additional analysis at the National Center for Biotechnology Information database (NCBI). Amino acid sets of Rho GTPases from both organisms were used as queries for a sequence similarity search in the NCBI database with the *blastp* algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Predicted protein homologs from other Amoebozoa with fully annotated genomes that showed the highest percent identity are listed. Proteins previously reported in the literature were named according to already published data. Names of unpublished but predicted

proteins from other Amoebozoa were chosen based on homology and designated by adding "-like" to their names. Accession numbers of all sequences reported in the table can be seen in the Supplementary File 2. (Accession numbers and amino acid sequences of selected species).