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## Assessment of mitogen-activated protein kinases as therapeutic targets for the treatment of babesiosis and theileriosis

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Abstract: The Piroplasmida order comprises parasitic protozoa including the Theileria and Babesia species that are transmitted by vector ticks and can cause severe diseases in domestic and wild animals. Because of limited therapies and available drug resistance, the discovery of new, effective, and safer drugs for veterinary use is important. Mitogen-activated protein kinases (MAPK) are a group of serine-threonine protein kinases found in diverse species, including animals and protozoa that conduct vital cellular functions. Therefore, they have been at the centre of drug design studies for many years. Computer-aided structure-based drug design is a fast and effective way in drug discovery efforts to identify candidate compounds. In this study, we conducted comparative sequence analysis of MAPK proteins from the Theileria (T. annulata, T. parva., T. orientalis, and T. equi) and Babesia species (B. bigemina, B. microti, and B. bovis). Three-dimensional protein structures from relevant species (T. annulata and B. bovis) were modelled and compounds were screened for interaction. Results showed that the inhibitors designed for human use could also be potent against Prioplasmida MAPKs. Furthermore, the structural differences between Prioplasmida and mammalian MAPKs could be a way for researchers to better instigate selective drug design.

Key words: Mitogen-activated protein kinase, computer-aided drug design, theileriosis, babesiosis

### 1. Introduction

The Theileria and Babesia species (Order Piroplasmida) are unicellular protozoan parasites belonging to the phylum Apicomplexa that exhibit characteristic apical complex and apicoplast organelles. Apicomplexans are responsible for serious diseases such as toxoplasmosis (Toxoplasma gondii) and malaria in humans (Plasmodium falciparum); furthermore, theileriosis and babesiosis, mainly found in in domestic and farm animals, account for many deaths, cause a decline in the quality of life, decrease efficiency, and cause economic losses. Theileria parva and T. annulata cause East Coast fever and Tropical theileriosis, respectively, and occur in a wide range of geographical regions, including Africa, southern Europe, and Asia. The Babesia species is spread across southern Europe, Africa, America, Asia, and Australia and infects buffaloes, deer, sheep, cattle, and also humans [1-3]. Due to its resistance to buparvaquone, a conventionally used drug to treat theileriosis, and because of limited therapies in bovine babesiosis because of drug residues in by-products (milk and meat) and toxicity, it is necessary to discover safer and more effective and medications [4-8].

The MAPK signalling cascade is essential and regulates numerous cellular functions in eukaryotes such as proliferation, differentiation, development, inflammation, cell death, and stress response. They are phosphorylated by upstream MAP4Ks, MAP3Ks, and MAPKKs and turn on specific MAPKs terminated by the phosphorylation of nuclear factors and other kinases as cytosolic targets [9]. The MAPK family is classified under the cell cycle associated kinase (CMGC) group, along with 7 other families, including cyclin-dependent kinases (CDK), glycogen synthase kinases (GSK), and Cdk-like kinases (CLK) in humans [10].

Piroplasms have the smallest protein kinome size (there are only 42 protein kinases in T. annulata) compared to other organisms in Apicomplexa [11,12]. MAPK family proteins are presented in low numbers in all apicomplexans and found in only 2 members: Theileria and Babesia [11,13]. The absence of the entire STE group of serine/threonine protein kinases, joined to the MAPK signalling cascade in most eukaryotes, point to a unique signalling mechanism for apicomplexans [11]. There are many studies indicating the various roles of the MAPKs of protozoan parasites, some of which include ERK1

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and ERK2 of *Giardia* spp. in encystation [14], MAPK2s of *P. falciparum* and *P. berghei* in the asexual cell cycle [15], and male gametocide formation [16], respectively. Moreover, various cellular functions in the *Leishmania* and *Trypanosoma* species have been reported, such as drug resistance, survival, proliferation, flagellum length control, and differentiation [17–20]. Along with other protein kinases, the MAPKs of protozoans are therefore attractive drug targets for the treatment of animal diseases [21,22].

Like other closely related apicomplexans, piroplasms have MAPKs like ERK7/8 (MAPK-15), designated as MAPK1 (MAPK2 in *T. gondii*), which carry a T(D) Y motif conserved in the activation loop [11,13,22]. However, the other MAPK, called MAPK2 (MAPK3 in *T. gondii*), is classified as an atypical MAPK with a TGH motif in the activation loop of *T. gondii* and piroplasms (TSH in *P. falciparum*) [13,21,22]. Their unique structural and functional features, compared to their mammalian counterparts and their evolutionary distance, make apicomplexan MAPKs attractive, druggable proteins, and this paves the way for selective drug design [13,21,23].

The main aim of this study is to repurpose the validated MAPK inhibitors, which were previously designed to combat human diseases on *Theileria* and *Babesia* MAP kinase 1 and 2 by computational structural biology approaches. This is the first report consisting of genome-wide screening of MAPK genes of piroplasms in detail with structural modelling, molecular dynamics analysis, and docking on *Theileria* and *Babesia* MAPK1 and MAPK2 proteins.

## 2. Material and methods

## 2.1 Sequence retrieval and analysis

Mitogen-activated protein kinase 2 sequences from T. annulata (Ankara str.), T. orientalis (Shintoku str.), T. parva (Muguga str.), and T. equi (WA str.) were retrieved from the NCBI RefSeq database. Sequences belonging to MAPK1s from both the Theileria and Babesia species [B. bigemina, B. microti (RI str.), and B. bovis (T2Bo str.)] and the MAPK2 of the Babesia species were predicted by DELTA-BLAST from NCBI (blast.ncbi.nlm.nih.gov) and BLASTP from the Ensembl Protist genome database (http://protists.ensembl. org) using P. falciparum 3D7 MAPK1 (XP\_001348468.1) and MAPK2 (XP\_001347818.1) as reference protein sequences. Resultant sequences were further confirmed by the Conserved Domain Database (CDD) from NCBI [24] and Pfam (protein families database) 32.0 [25] for an exact MAP kinase domain explication. Homologous sequences of MAPK1 and MAPK2 from the host species Homo sapiens and Bos taurus and the other apicomplexans (T. gondii and Cryptosporidium parvum) were identified by BLASTP search against RefSeq database using an E-value threshold of  $1 \times 10^{-10}$ .

MAPK1 MAPK2 sequences from and the Theileria and Babesia species and H. sapiens MAPK15 (NP 620590.2), MAPK11 (NP\_002742) and MAPK14 (NP 001306.1), B. taurus MAPK15 (NP 001039575.1), MAPK11 (NP 001073804.1) and MAPK14 (NP\_001095644.1), T. gondii ERK7 (XP\_018636795.1) and MAPK3 (XP 002369585.1), and C. parvum MAPK2 (XP\_001388246.1) sequences were analysed for conserved residues and divergences by applying multiple sequence analysis using the T-COFFEE multiple sequence alignment program [26]. Multiple sequence alignments were visualized on an ESPript 3.0 server [27]. Pairwise sequence alignments were conducted using the EMBOSS-water tool [28]. Phylogenetic relationships of MAPKs among apicomplexans were assigned via MEGA7 software [29] using the neighbor-joining method employing 500 bootstrap replications. Motifs within MAPK sequences were predicted and analysed by a MEME (Multiple Em for Motif Elicitation) server in MEME suite 5.1.0 [30]. Predictions of disordered regions were conducted by applying a false positive rate of 5% using a PrDOS server [31].

## 2.2. Comparative modelling and validation

A comparative modelling of the MAPK1 and MAPK2 structures from T. annulata and B. bovis was conducted with a Modeller 9.16 program [32] using template PDB files identified by BLASTP search against the PDB database, pairwise sequence alignment, and MAPK amino acid sequences. After the modelling process, potential energy was minimized by applying 2000 and 1000 steps of steepest descent and conjugate gradient algorithm, respectively, using the AMBERff14SB force field from Chimera 1.10.2 program [33]. Minimized structures were validated by ERRAT [34], ProSA [35], ProQ [36], and RAMPAGE [37] servers to attain 3D-model quality. Superimpositions and RMSD calculations based on C-alpha traces between models and templates were done in PyMOL 2.2.0 (PyMOL Molecular Graphics System, Ver. 2.0 (Schrödinger, NY, USA). Druggability assessment of protein cavities was predicted by a DoGSiteScorer server [38].

## 2.3. Molecular dynamics simulations and docking

The molecular dynamics of the MAPK1 and MAPK2 proteins of *T. annulata* and *B. bovis* were assessed by all-atom molecular dynamics simulations by using the NAMD 2.9 program [39] and the CHARMM force field. Structures were prepared within a water box and analysed after the MD simulations in the VMD 1.9.3 program [40] for RMSD and RMSF values. Initially, the first 2500 steps of the total 5000 steps of energy minimisation were conducted, wherein, at first, the protein structures were fixed; following this, the whole system was allowed free movement. A simulated annealing was applied for the system so that the temperature would be fixed at 300K in

NVT conditions. The system was then equilibrated in an NPT (1-atm pressure and 300 K temperature) ensemble. Lastly, a final 50 ns of production md simulation was done in same NPT conditions with 2fs timesteps. MAPK inhibitors were searched for and downloaded in the proper file formats from the IUPHAR/BPS Guide Pharmacology (http://www.guidetopharmacology. to org). Inhibitor and protein structures were prepared by LigPrep (Schrödinger, NY, USA) and ProteinPrepWizard, respectively, in Maestro (Schrödinger, NY, USA) by applying the OPLS3 force field. ATP binding pockets of MAPK1 and MAPK2 were selected as the target site, and receptor grids were defined by the Receptor Grid Generation module from Glide (Schrödinger, NY, USA). In total, 38 MAPK inhibitors were docked to the active site of proteins by applying a 2-tier procedure, wherein, as a first step, 30% of the best ligands that were selected by Glide SP docking were analysed further by a Glide XP docking step (Schrödinger, NY, USA). The top 5 scoring ligands were further assessed for their MM-GBSA binding energies in Prime (Schrödinger, NY, USA).

## 3. Results and discussion

## 3.1. Sequence retrieval and analysis

Evolutionary analyses of MAPKs from apicomplexans including *T. annulata*, *T. parva*, and *B bovis* were reported by Talevich et al. [11,12]. MAPK sequences from other *Theileria* and *Babesia* species were identified in detail by DELTA-BLAST searches from both NCBI RefSeq and

the Ensembl Protist genome databases (Table 1). Based on homology, the *Theileria* and *Babesia* species contain 2 types of mitogen-activated protein kinases like *P. falciparum*, to which MAPK1 is evolutionary related with the MAPK15-like (ERK7/8) (CDD number: cd07852) protein; MAPK2 constitutes the "Serine/Threonine Kinase, Mitogen-Activated Protein Kinase domain" (CDD identity cd07834) (Table 1) [11,21,22].

According to the sequence analysis, MAPK1s from both species have an N-terminal kinase domain with a mean sequence length of 342. However, in the MAPK2 kinase domain located at the C-terminus, the sequence varies in length (Figures 1-2). When comparing sequences of piroplasm MAPKs with B. taurus and H. sapiens MAP kinases, MAPK1 shows homology with the mammalian MAPK15 (ERK7/8), and p38 MAPK (like MAPK11, MAPK14) proteins present a higher identity match with the piroplasm MAPK2. In fact, based on local alignments of MAPK1s from *B. bovis* and *T. annulata* in humans and cattle, MAPK15 showed about 38% and 48% identity between MAPK specific domains, respectively. In contrast, a 32% identity was shown between mammalian MAPK11 and MAPK2s of both B. bovis and T. annulata based on conserved kinase domains. Percentage identities of MAPK1 and MAPK2 vary between species of Theileria and Babesia. The closest sequences were between T. annulata and T. parva, whose identities were calculated at about 70% and 86.5% for MAPK1 and MAPK2, respectively. Phylogenetic trees also indicate a close

 Table 1. Mitogen-activated protein kinases from Theileria and Babesia genomes.

Organism/strain	NCBI protein Name	Assigned as	NCBI Accession	Length	CDD Kinase Domain
Theileria annulata/	Protein kinase	MAPK1	XP_954104	709	7-354
Ankara	MAPK2	MAPK2	XP_954376	642	193-624
Theileria orientalis/	Protein kinase	MAPK1	XP_009689510	789	7-365
Shintoku	MAPK2	MAPK2	XP_009689160	620	158-602
Theileria parva/ Muguga	Serine/threonine protein kinase	MAPK1	XP_766199	677	7-351
	MAPK2	MAPK2	XP_765886	642	193-624
Theileria equi/	Protein kinase domain containing protein	MAPK 1	XP_004833198	598	5-342
WA	MAPK2	MAPK2	XP_004829898	581	146-563
Delesie bierrie	МАРК	MAPK1	XP_012766705	502	5-342
Babesia bigemina	МАРК	MAPK2	XP_012767953	600	173-582
Babesia microti/	Extracellular signal-regulated kinase 2	MAPK1	XP_021338713	583	5-341
RI	МАРК	MAPK2	XP_012648003	556	106-537
Delessie lessie/ T2De	МАРК	MAPK1	XP_001610969	506	5-342
Babesia bovis/ T2Bo	МАРК	MAPK2	XP_001610481	584	159-566

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	1 	10	20	30	4 <u>0</u>	50	6 Q		9 Q
T.annulata	MEV.S MEV.N	. EHIDDHILSRY	KIIQKIGKGAYG	IVWKAVKRD	INEVVALKKIF	DAFRNSTDA DAFRNSTDA	QRTYREIMFL QRTYREIMFL	QKLKKCPNIVKLR QKLKKCPNIVKLM	
T.parva		. EHIDDHILSRY	KITOKICKCAYO		INEVVALKKIF			OKLKKCPNIVKLR	
T.equi	M.N	DHIDDHVLAKF	KILOKLGKGAY	IVWKAVDLR	TNEVIALKKIF			<b>OSLKKCONIVELK</b>	
B.bigemina	м. s	DHIDEHILSKF	HILOKLGKGAYO	IVWKVVNKE	T Q E V V A L K K I F			HYLRKCNNIVEIK	
B.bovis	<mark>M</mark> . <mark>S</mark>	. DHIDEHILSKF	<b>H</b> ILQKL <mark>G</mark> KGAYO	IVWKVANKE	T <mark>QEVV</mark> ALKKIF	DAFRN <mark>S</mark> TDA		H Y L R K A H N I I E L K	
B.microti		<mark>N</mark> S <mark>IEDHIL</mark> K <mark>RF</mark>	K <mark>ILNKL</mark> G <mark>K</mark> GAYG	IV <mark>WKAI</mark> DKT	T <mark>GETV</mark> A <mark>l</mark> kkif	<mark>d a f r n</mark> s t d s		TELRGRPGIIGLK	
T.gondii	<mark>M</mark> . <mark>S</mark>			I V W K S T D R R	I <mark>NETV</mark> ALKKIF	D A F <mark>Q N</mark> A T D A		QELAGHENIVRLK	
P.falciparum	MPK.EDCKTEKS	GIDSIDENVLKKY	DILKKVGKGAYG	VVFKGRCKKI	NKNIVAVKKIF	GAFONCTDA		Y E L N G H D N I I K L M	
B.taurus H.sapiens		. AEVDRHVAQRY . TVVDPRIVRRY	LLKRKLGKGAYG	TVWKAVDRR:	I GEVVAIKKIF	DAFRDRTDA DAFRDRTDA		QEFGDHPNIVRL QEFGDHPNIISLI	
consensus>70								lnI!.l.	
concentrate / c							2		
	90	100	110	120	130	140	150	160 1	70
T.annulata		FEYVETDLHAVI		•	•	•	•	DFGL <mark>ARSV</mark> APNNN	•
T.orientalis		FEYVETDLHAVI						DFGL <mark>ARSV</mark> APNNN	
T.parva	YPADNNRDVYLV	<mark>FEYVE</mark> TDLHAVI	<b>R</b> .SNILEEVHKF	<b>YILYOLLKA</b>	IHFI <mark>HT</mark> GDLL <mark></mark> H			D F G L <mark>A R S V</mark> A P N N N	
T.equi	LPANNN <mark>RDVYL</mark> V	<mark>/FEYME</mark> TDL <mark>HA</mark> VI	R.SN <mark>ILEDV</mark> HKF	Y <mark>ILYQ</mark> IIKA:	IHYI <mark>HS</mark> GELL <mark></mark> H	RD <mark>L</mark> KPSN <mark>I</mark> L	LSSKCHVKLA	DFGL <mark>ARSV</mark> AHDEE	с.т
B.bigemina	YAAKNDRDLYI	FEYVDTDLHAVI	R.INILEEVHKE	(YILYQIIKA)	IHFIHSGDLLH	RD <mark>L</mark> KPSN <mark>V</mark> L	LNAKCNIKLA	DFGL <mark>ARSI</mark> AHDEI	
B.bovis		F <mark>EYID</mark> TDL <mark>HS</mark> VI		Y <mark>IIYQ</mark> LLKA	INFIHSGDLLH	RD <mark>L</mark> KPSN <mark>V</mark> L		DFGL <mark>S</mark> R <mark>SV</mark> AYDET	
B.microti		F <mark>EHME</mark> TDL <mark>HT</mark> VI						DFGL <mark>ARSL</mark> KTF	
T.gondii B.falgiparum	IKAKNDNDIYL	FDYMETDLHAVI						DFGL <mark>ARSV</mark> AHSES DFGL <mark>ARSI</mark> STHVN	
B.taurus		FESMDTDLNAVI						DFGL <mark>ARPL</mark> SG	
H.sapiens								DFGL <mark>ARSL</mark> GD	
consensus>70	A.N#rDvYlv	F#y.#TDLhaVI	rniLe#vHk.	yI.%Qll.a	fiHsGdllH	RD1KPSN!L	lnC.!Kla	DFGLaRsv	
		-		-					
		180	190	200	210	220	230	240	
T.annulata	DKCLS	KDNHTGTG	TVMTDYVATRWY	RAPEILVGST	KYTKGVDMWA	· ·	IGKPMEPGSS	INOLAKVITETG	MP
	AKDLNQSISKTI	VKDKGNTETEKD	IVMTDYVATRWY	RAPEILVGSI		IGCIFGEML			MP
T.parva		.KDNTTG		RAPEILVGS		IGCIFAEML			MP
T.equi		DSA		RAPEILVGS		IGCIFA <mark>EM</mark> L		TIN <mark>QL</mark> SKVVAFTG	ΙP
B.bigemina		DEA		RAPE <mark>ILVG</mark> S <mark>I</mark>		IGCILA <mark>EL</mark> L			MP
B.bovis		DEA	<b>PVLTDYVATRWY</b>	RAPEILVGSI	KYTKGVDMW <mark>A</mark>	IGCILAELL			MP
B.microti			PLLTDYVATRWY PVLTDYVATRWY	RAPEILIGSN	KYTKAVDMWA RYTKGVDMWS				ΙP
T.gondii									
D falcinarum									R P K P
		NKV	<b>P</b> ILTDYVATRWY	RAPE <mark>ILLG</mark> S <mark>I</mark>	HYT <mark>ED</mark> VDMW <mark>S</mark>	LGCIMG <mark>EL</mark> L	C <mark>GKPLFTGN</mark> S'	T <mark>MNQL</mark> EKIIQV <mark>IG</mark>	KP
P.falciparum B.taurus H.sapiens			P <mark>ILTD</mark> YVATRWY HALTEYVATRWY	RAPE <mark>ILLG</mark> S <mark>I</mark> RAPE <mark>VLLS</mark> SS	THYT <mark>ED</mark> VDMWS WYTPGVDMWS	LGCI <mark>MG<mark>el</mark>l LGCILGEML</mark>	C <mark>G K P L F T</mark> G N S' R G R P L F P G T S'	T <mark>MNQLEKIIQVIG</mark> TLHQLELILEAIP	K P P P
B.taurus H.sapiens		NKV LPEVPEG LPEGPED	P <mark>ILTD</mark> YVATRWY HALTEYVATRWY QAVTEYVATRWY	RAPE <mark>ILLG</mark> S RAPE <mark>VLLS</mark> S RAPE <mark>VLLS</mark> S	THYT <mark>ED</mark> VDMWS WYTPGVDMWS IRYTLGVDMWS	LGCIMGELL LGCILGEML LGCILGEML	C <mark>G K P L F T G N S</mark> ' R <mark>G R P L F P G T S'</mark> R <mark>G R P L F P G T</mark> S'	T <mark>MNQLEKIIQVIG</mark> TLHQLELILEAIP	K P P P P P
B.taurus H.sapiens		NKV LPEVPEG LPEGPED	P <mark>ILTD</mark> YVATRWY HALTEYVATRWY QAVTEYVATRWY	RAPE <mark>ILLG</mark> S RAPE <mark>VLLS</mark> S RAPE <mark>VLLS</mark> S	THYT <mark>ED</mark> VDMWS WYTPGVDMWS IRYTLGVDMWS	LGCIMGELL LGCILGEML LGCILGEML	C <mark>G K P L F T G N S</mark> ' R <mark>G R P L F P G T S'</mark> R <mark>G R P L F P G T</mark> S'	T <mark>MNQLEKIIQVIG</mark> TLHQLELILEAIP T <mark>LHQLELIL</mark> ET <mark>IP</mark>	K P P P P P
B.taurus H.sapiens consensus>70	50 260	NKV LPEVPEG LPEGPED 	PILTDYVATRWY HALTEYVATRWY QavtEyvAtrwy .vlt#yvAtrwy 280	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPE!L.gSt 290	THYTEDVDMWS WYTPGVDMWS HRYTLGVDMWS . YTkgVDMW. 300	LGCIMGELL LGCILGEML LGCILGEML .GCIE\$L 310	C <mark>GKPLFTGNS</mark> R <mark>GRPLFPGTS</mark> R <mark>GRPLFPGTS</mark> .g.PlFpG.S <sup>4</sup> 320	IMNOLEKIIQVIG TLHOLELILEAIP ILHOLELILEAIP T.nQl.k!tg 330	KP PP PP .P
B.taurus H.sapiens <i>consensus</i> >70 2. T.annulata	50 260 SEEDMESLSSPF		PILTDYVATRWY HALTEYVATRWY Qavteyvatrwy .vlt#yvatrwy 280 RKNIKEYFP.NT	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPE!L.gSt 290 SEECLDLSK	HYTEDVDMWS WYTPGVDMWS HYTLGVDMWS .YTkgVDMW. 300 LLOENPTKRII	LGCIMGELL LGCILGEML LGCILGEML .GCIE\$L 310 NTVEALSHP	CGKPLFTGNS RGRPLFPGTS RGRPLFPGTS .g.PlFpG.S 320 YLSNFHK.NTI	IMNÖLEKIIQVIG TLHÖLELILEAIP ILHÖLELILETIP T.nql.k!tg 330 PLPRLSRAISIPV	KP PP PP .P
B.taurus H.sapiens <i>consensus&gt;70</i> 2 T.annulata T.orientalis	50 260 SEEDMESLSSPF SESDMDSLGSPF	LNKV LPEVPEG LPEGPED 270 TKVMISSLNTIR TKVMITSLGNIE	PILTDYVATRWY HALTEYVATRWY OAVTEYVATRWY .vlt#yvATRWY 280 RKNIKEYFP.NT KKPMREYFP.KA	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPE!L.gSt 290 SEECLDLLSK EEEALDLLSS	HYTEDVDMWS WYTPGVDMWS HYTLGVDMWS : YTkgVDMW. 300 LLOFNPTKRI LLOFNPTKRI	LGCIMGELL LGCILGEML .GCILGEMJ .GCIE\$L 310 NTVEALSHP TVNALNHP	CGKPLFTGNS RGRPLFPGTS RGRPLFPGTS .g.PlFPG.S 320 YLSNFHK.NT1 YLAAFHK.SN	TMNÖLEKIIQVIG TLHÖLELILEAIP TLHÖLELILET T.nQl.k!tg 330 PLPRLSRATSIPV ILPSLTRATSIPV	KP PP PP .P CD
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva	50 260 SEEDMESTSSPF SEEDMDSIGSPF SEEDMDSISSPF	NKV LPEVPEG LPEGPED 	PILTDYVATRWY HALTEYVATRWY QAVTEVVATRWY .vlt#yvATRWY 280 RKNIKEYFP.NT KKPMREYFP.KA KKNIKEYFP.NT	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPEVLLSS RAPE!L.gSt 290 SEECLDLLSE EEEALDLLSE CEEGLDLLTS	HYTEDVDMWS SWYTPGVDMWS IRYTLGVDMWS : YTkgVDMWS : JOFNPTKRI LLOFNPTKRI LLOFNPTKRI	LGCIMGBLL LGCILGBML LGCILGBML .GCIE\$L 310 VIVEALSHP VIVALSHP VIVALAHP	CGKPLFTGNS RGRPLFPGTS RGRPLFPGTS 320 YLSNFHK.NTI YLAAFHK.SN YLSNFHK.NTI	THNOLEKIIQVIG TLHQLELILEAIP TLHQLELILETIP T.nQl.k!tg 330 PLPRISRAISIPV PLPRISRAISIPV PLPRLARAISIPV PLPRLARAISIPV	KP PP .P CD CD
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi	50 260 SEGDMESISSPF SESDMDSIGSPF SEDMDSISSPF SSDDLDALGSPF		PILTDYVATRWY HALTEYVATRWY Qavteyvatrwy .vlt#yvatrwy 280 RKNIKEYFP.NT KKPMREYFP.KA KKNIKEYFP.NT RKPLHEYFP.NT	$\begin{array}{c} \textbf{RAPEILLSS}\\ \textbf{RAPEVLSS}\\ \textbf{RAPEVLSS}\\ \textbf{RAPE!L.gSt}\\ \textbf{290}\\ \textbf{SEECLDLLSK}\\ \textbf{EEEALDLLSK}\\ \textbf{CEEGLDLNT}\\ \textbf{TQEALDLTK}\\ \end{array}$	HYTEDVDMWS WYTPGVDMWS HYTLGVDMWS .YTkgVDMW. LLOFNPTKRI LLOFNPTKRI LLOFNPTKRI	LGCIMGBLL LGCILGBML .GCI.GBML .GCI.GBML .GCI.E\$L .TVEALSHP .TVEALSHP .TVALNEP .TVALNEP .TVALNEP	CGKPLFTGNS RGRPLFPGTS RGRPLFPGTS .g.PlFPG.S YLSNFHK.NTI YLAAFHK.SNI YLSNFHK.NTI YLSNFHKSNTI	THNOLEKIIQVIG TLHQLELILEAIP TLHQLELILETIP T.nQl.k!tg 930 PLPRTSRATSIPV ILPSLTRAISIPV PLPRLARAISIPV PLPLARAITIPI	KP PP PP .P CD CD CD
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina	50 260 SEEDMESTSSPF SEEDMESTSSPF SEEDMDSLSSPF SEEDMDSLSSPF SEEDMESTGSPF	L	PILTDYVATRWY HALTEYVATRWY ORVEYVATRWY VIT#YVATRWY 280 RKNIKEYFP.NT KKPMREYFP.NT RKPILEYFP.NT RKPLHEYFP.MY QRSIKUYLP.NA	RAPEILSS RAPEVILSS RAPEVILSS RAPE'L .gst 290 SEECLDIIS EEEALDIIS CEEGLDINF TQEALDIINF PDDAIDIVR	HYTEDVDMWS WYTPGVDMWS HYTLGVDMWS .YTkgVDMW. LLOFNPTKRI LLOFNPTKRI LLOFNPTKRI	LGCIMGBLL LGCILGBML .GCI.GBML .GCI.GBML .GCI.E\$L .TVEALSHP .TVEALSHP .TVALNEP .TVALNEP .TVALNEP	CGKPLFTGNS RGRPLFPGTS RGRPLFPGTS .g.PlFPG.S YLSNFHK.NTI YLAAFHK.SNI YLSNFHK.NTI YLSNFHKSNTI	THNOLEKIIQVIG TLHQLELILEAIP TLHQLELILETIP T.nQl.k!tg 930 PLPRTSRATSIPV ILPSLTRAISIPV PLPRLARAISIPV PLPLARAITIPI	KP PP PP .P CD CD CD
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.boyts	50 260 SEEDMESLSSPF SESDMSLSSPF SEEDMSLSSPF SEDMESLSPF SEDMESLSSPF SEDMESLSSPF SEDMESLSSPF	L PEVPEG L PEGPED TKVMISSLNTIR TKVMISSLNTIR TKVMISSL.TIR AKMMYSINNIT TAMMMSSLTKIP TTLMMGSLASVQ	PILTDYVATRWY HALTEYVATRWY QAVTEYVATRWY 280 RKNIKEYFP.NT KKPMREYFP.NT RKNIKEYFP.NT RKPLHEYFP.NY QRSIKDYLP.NA NKPVKEYFP.NA	RAPEILLGST RAPEVILSSE RAPEVILSSE RAPE!L.gSt 290 SEECLDILSK CEEGLDILTS TQEALDILTS PDDAIDLVVS	EYTEDVDAWS WYTPGVDAWS INYTGVDAWS INYTGVDAWS ILOFNPTKRII ILOFNPTKRII ILOFNPTKRII ILOFNPTKRII ILOFNPKRII ILOFNPKRII	LGCIMGELL LGCILGEML LGCILGEML SGCI.E\$L 310 NTVEALSHP NTVDALAHP STIDALNHP STIDALNHP STIDALNHP TLALNHP	CGKPLFTGNS RGRPLFPGTS .g.PlfpGTS .g.PlfpG.S YLSNFHK.NTI YLSNFHK.NTI YLSNFHK.NTI YLSNFHK.NTI YLSNFHK.SND YRSFHKSND	TMNÖLEKIIQVIG TLHQLELILEAIP TLHQLELILEAIP T.nQl.k!tg PLPRISRAISIPV FLPRISRAISIPV PLPRIARAISIPV PLPRIARAISIPV HLPILYKAITIPI SLAALPKAVKIPV SLPKIKAVKIPV	KP PP PP CD CD CD CD CD CD
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti	50 2 60 SE 50 ME SI SS PF SE 50 MS I S SP F SE 50 MS I S SP F SE 50 LD AL SS SP F SD 10 D E SI G SP F SD 10 D E SI G SP F SD 10 D E SI G SP F	L	PILTDYVATRWY HALTEYVATRWY QAVDEYVATRWY .vlt#yVATRWY RKNIKEYFP.NT KKPMREYFP.NT KKPKEYFP.NT RKPLHEYFP.NA QRSIKDYLP.NA NKPVKEYFP.NA KKEFFHFTKQV	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPE!L.gSt 290 SEECLDLIS CEEGLDLIS CEEGLDLIS PDDAIDLYR PDDAIDLYR PDDAIDLYR	HYTEDVDAWS HYTEVDAWS LUCFNPTKRI LUCFNPTKRI LUCFNPKRI LUCFNPKRI LUCFNPKRI LUCFNPKRI LUCFNPKRI LUCFNPKRI	LGCILGEML LGCILGEML LGCILGEML .GCI.E\$L NTVEALSHP TVNALNHP NTVDALAH STIDALNHP STLLAINHP	CCFPLFTGNS RGPPLFPGTS RGPPLFPGTS .g.PlFPG.S YLSNFHK.NT YLAAFHK.SN YLAAFHK.SN YLSOFHKSNCJ YURSFHKSNCJ YVRSFHKSNCJ YVVSFHKSNCJ	THNOLEKIIQVIG TLHOLELILEAIP TLHOLELILEAIP T.NQI.k!tg PLPRTSRATSIPV ILPSLTRAISIPV PLPRLARAISIPV HLFLIXAISIPV SLAALPKAVKIPV SIPVMYKAVKIPI SLAALPKAVKIPV	KP PP PP CD CD CD CD CD CD CD
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.boyts	5 0 2 6 0 S E E D ME S I S S P F S E S D M S I G S P F S E E D M S I G S P F S E D M S I G S P F S D D D A I G S P F S D E D I K S I G S P F S D E D I K S I G S P F S D E D U A V K S P F N K K D E D I R S P T	L PEVPEG .L PEGPED 	PILTDYVATRWY HALTEYVATRWY QAVTEYVATRWY 280 RKNIKEYFP.NT KKPIKEYFP.NT RKPLEYFP.NT RKPLEYFP.NA KMEFEHFFTKQV VKNFKDAFP.NA	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPE'LLSS RAPE'LLSS RAPE'LGS SEECLDLLSS CEGLDLLSS CEGLDLLSS TQEALDLLSS PDDAVDLTSS PDDAVDVP PQNAKDLTSS SNESLDLLSS	BYTPGVDMWS WYTPGVDMWS TYTLGVDMWS JOO LLQFNPTKRII LLQFNPTKRII LLQFNPKRRI LLQFNPKKRII LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI	LGCILGEML LGCILGEML GCILGEML .GCI.SEL TVEALSHP TVEALSHP TVDALAHP TUDALAHP TLEALNHP TLEALNHP JLEALNHP SAEKGLEHP SAEKGLEHP	CGKPLFTGNS RGRPLFPGTS .g.PlfpGTS .g.PlfpGTS .y. YLSNFHK.NTI YLSAFHK.NTI YLSQFHKSNKI YLSQFHKSNKI YUSSFHKSND YVSFNMGKYI YVSFNMGKYI YVSFNMGKYI	TMNÖLEKIIQVIG TLHQLELILEAIP MIHQLELILEAIP T.nQl.k!tg PLPRISRAISIPV PLPRISRAISIPV HLPILYKAITIPI SLAALPKAVKIPV SLAALPKAVKIPV SLFVMYKAVKIPU KLTTLPGPVHIPT EP.VCGKIIAIPI 2.PCGKIIAIPI	KP PP PP .P CD CD CD CD CD CD CD CD DD ND
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.biovis B.microti T.gondii P.falciparum B.taurus	50 2 60 SE 50 ME SIS 5 PF SE 50 ME SIS 5 PF S 50 10 10 ALG 5 PF S 50 10 10 ALG 5 PF S 50 20 1K SIG 5 PF S 10 1 K SIG 5 PF	L	PILTDYVATRWY HALTEYVATRWY QAVDEYVATRWY .vlt#YVATRWY RKNIKEYFP.NT KKPLHEYFP.NT RKPLHEYFP.MV QRSIKDYLP.NA NKPVKEYFP.NA KMEFEHFFTKQV VKNFKDAFP.NA KKNLKDICY.KA RQTLDALLPPDT	RAPEILLSS RAPEVLLSS RAPEVLSS RAPEVLSS SECLDINS SECLDLNS CEEGLDLNS TQEALDLLS PDDAIDVR PDDAIDVR PDDAIDVR PDDAIDVR SPEALDLKS SNESLDLLS	HYTEDVDMWS WYTEGVDMWS YTLGVDMWS YTLGVDMWS LOFNPTKRI LOFNPKRI LOFNPKRI LOFNPKRI LOFNPKRI LEFNPKRI LEFNPKRI LOFNPKRI LLOFNPKRI LLOFNPNKRI	LGCILGEML LGCILGEML .GCI.GEML .GCI.EEML .GCI.E\$L TVNALNHP TVNALNHP TVDALAHP TVDALAHP TIDALNHP TILALNHP TILALNHP JALALNHP AEXGLEHP AEXGLEHP AEXGLEHP	CCFPLFTGNS RGRPLFPGTS .g.PlFpG.S YLSNFHK.NT YLSNFHK.NT YLSNFHK.NT YLSNFHK.NT YLSPHKSND YVRSFHKSND YVRSFHKSND YVRSFHSPD YVRCFHSFD YVRCFHSFD	TMNÖLE KIIQVIG TLHQLELILEAIP TLHQLELILETIP T.nQl.k!tg PLPRLSRATSIPV ILPSLTRAISIPV PLPRLARAISIPV HIPILYKAITIPI SIAALPKAIVKIPV SIPVMYKAVKIPI FP.VGKIIAIPI E.P.VGKIIAIPI E.P.VGKHIITIPI	KP PP PP CD CD CD CD CD CD CD CD CD CD CD CD CD
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva B.bigemina B.boyis B.microti T.gondii P.falciparum B.taurus H.sapiens	5 Q 2 6 Q S E E DME S L S S P F S E S DMI S L G S P F S S D D L D A L G S P F S D D L E S L G S P F S D D D E S L G S P F S D D D E S L G S P F S D D D E S L G S P F S P D D L A V S P P N K K D E D T R S P F S K D L L A L G S G C S E D L L A L G S G C	270 IL. PEVPEG L. PEGPED TKVMISSLNTER TKVMISSLNTER TKVMISSL.TTR TKMISSL.TKP TILMGSLASVQ SSMMFANVKHIK ATMMESLPLGSRP RASVLHLGSRP	PILTDYVATRWY HALTEYVATRWY QAVTEYVATRWY 280 RKNIKEYFP.NT KKPIKEYFP.NT KKPIKEYFP.NT RKPLHEYFP.WV QRSIKDYLP.NA NKPVKEYFP.NA KMEFEHFFTKQV VKNFKDAFP.NA KMLKDICY.KA RQTLDALLPPDT RQTLDALLPPDT	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPEVLLSS RAPE'L.GST SEECLDLLSS CEEGLDLLSS CEEGLDLLSS PDDAIDLYS PDDAIDLYS PDDAVDLVV PQDAXDLYS SPEALDLLS SPEALDLLSS	BYTPGVDMWS WYTPGVDMWS TYTLGVDMWS LLQFNPTKRI LLQFNPTKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI	LGCILGEMU LGCILGEMU SGCI.GEMU SGCI.ESU TVEALSHP TVNALNHP STIDALNHP STIDALNHP STIDALNHP STIDALNHP STIDALNHP SAENGLEHP SAENALKHK SAQALQHP	CCKPLFTGNS RGRPLFPGTS RGRPLFPGTS 320 YLSNFHK.NT YLAAFHK.NT YLSNFHK.NT YLSNFHKSND YVSFHKSND YVVSFHKSND YVSFHKSND YVSFHKSND YVSFHKSD YVSFHKSD YVQFHCPSD	TMNÖLEKIIQVIG TLHQLELILEAIP TLHQLELILETIP T.nQl.k!tg PLPRISRAISIPV ULPSLTRAISIPV VLPSLTRAISIPV VLPSLTRAISIPV SLAALPKAVKIPV SLAALPKAVKIPV SLAALPKAVKIPV SLAALPKAVKIPV SLFVMYKAVKIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.W.TLGGDVRLPV SW.AREADVRPRA	
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.biovis B.microti T.gondii P.falciparum B.taurus	5 Q 2 6 Q S E E DME S L S S P F S E S DMI S L G S P F S S D D L D A L G S P F S D D L E S L G S P F S D D D E S L G S P F S D D D E S L G S P F S D D D E S L G S P F S P D D L A V S P P N K K D E D T R S P F S K D L L A L G S G C S E D L L A L G S G C	270 IL. PEVPEG L. PEGPED TKVMISSLNTER TKVMISSLNTER TKVMISSL.TTR TKMISSL.TKP TILMGSLASVQ SSMMFANVKHIK ATMMESLPLGSRP RASVLHLGSRP	PILTDYVATRWY HALTEYVATRWY QAVTEYVATRWY 280 RKNIKEYFP.NT KKPIKEYFP.NT KKPIKEYFP.NT RKPLHEYFP.WV QRSIKDYLP.NA NKPVKEYFP.NA KMEFEHFFTKQV VKNFKDAFP.NA KMLKDICY.KA RQTLDALLPPDT RQTLDALLPPDT	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPEVLLSS RAPE'L.GST SEECLDLLSS CEEGLDLLSS CEEGLDLLSS PDDAIDLYS PDDAIDLYS PDDAVDLVV PQDAXDLYS SPEALDLLS SPEALDLLSS	BYTPGVDMWS WYTPGVDMWS TYTLGVDMWS LLQFNPTKRI LLQFNPTKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI	LGCILGEMU LGCILGEMU SGCI.GEMU SGCI.ESU TVEALSHP TVNALNHP STIDALNHP STIDALNHP STIDALNHP STIDALNHP STIDALNHP SAENGLEHP SAENALKHK SAQALQHP	CCKPLFTGNS RGRPLFPGTS RGRPLFPGTS 320 YLSNFHK.NT YLAAFHK.NT YLSNFHK.NT YLSNFHKSND YVSFHKSND YVVSFHKSND YVSFHKSND YVSFHKSND YVSFHKSD YVSFHKSD YVQFHCPSD	TMNÖLE KIIQVIG TLHQLELILEAIP TLHQLELILETIP T.nQl.k!tg PLPRLSRATSIPV ILPSLTRAISIPV PLPRLARAISIPV HIPILYKAITIPI SIAALPKAIVKIPV SIPVMYKAVKIPI FP.VGKIIAIPI E.P.VGKIIAIPI E.P.VGKHIITIPI	
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva B.bigemina B.boyis B.microti T.gondii P.falciparum B.taurus H.sapiens	5 Q 2 6 Q S E E DME S L S S P F S E S DMI S L G S P F S S D D L D A L G S P F S D D L E S L G S P F S D D D E S L G S P F S D D D E S L G S P F S D D D E S L G S P F S P D D L A V S P P N K K D E D T R S P F S K D L L A L G S G C S E D L L A L G S G C	270 IL. PEVPEG L. PEGPED TKVMISSLNTER TKVMISSLNTER TKVMISSL.TTR TKMISSL.TKP TILMGSLASVQ SSMMFANVKHIK ATMMESLPLGSRP RASVLHLGSRP	PILTDYVATRWY HALTEYVATRWY QAVTEYVATRWY 280 RKNIKEYFP.NT KKPIKEYFP.NT KKPIKEYFP.NT RKPLHEYFP.WV QRSIKDYLP.NA NKPVKEYFP.NA KMEFEHFFTKQV VKNFKDAFP.NA KMLKDICY.KA RQTLDALLPPDT RQTLDALLPPDT	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPEVLLSS RAPE'L.GST SEECLDLLSS CEEGLDLLSS CEEGLDLLSS PDDAIDLYS PDDAIDLYS PDDAVDLVV PQDAXDLYS SPEALDLLS SPEALDLLSS	BYTPGVDMWS WYTPGVDMWS TYTLGVDMWS LLQFNPTKRI LLQFNPTKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI LLQFNPKKRI	LGCILGEMU LGCILGEMU SGCI.GEMU SGCI.ESU TVEALSHP TVNALNHP STIDALNHP STIDALNHP STIDALNHP STIDALNHP STIDALNHP SAENGLEHP SAENALKHK SAQALQHP	CCKPLFTGNS RGRPLFPGTS RGRPLFPGTS 320 YLSNFHK.NT YLAAFHK.NT YLSNFHK.NT YLSNFHKSND YVSFHKSND YVVSFHKSND YVSFHKSND YVSFHKSND YVSFHKSD YVSFHKSD YVQFHCPSD	TMNÖLEKIIQVIG TLHQLELILEAIP TLHQLELILETIP T.nQl.k!tg PLPRISRAISIPV ULPSLTRAISIPV VLPSLTRAISIPV VLPSLTRAISIPV SLAALPKAVKIPV SLAALPKAVKIPV SLAALPKAVKIPV SLAALPKAVKIPV SLFVMYKAVKIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.W.TLGGDVRLPV SW.AREADVRPRA	
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva B.bigemina B.boyis B.microti T.gondii P.falciparum B.taurus H.sapiens	50 260 SEEDMESISSPF SESDMISISSPF SEDDISISSPF SDDIDIGISSPF SDDIESIGSPF SDDIESIGSPF SDDIESIGSPF SDDIAIGSG SPEDVAVKSPF NKKDIEDIRSPF SKEDLIAIGSGC s.eD.e.l.Spf	L	PILTDYVATRWY HALTEYVATRWY Qavteyvatrwy .vlt#yvatrwy RKNIKEYFP.NT KKPMREYFP.NT RKPLHEYFP.NT RKPLHEYFP.MV QRSIKUYLP.NA NKPVKEYFP.NA KMEFEHFFTKQV VKNFKLAFP.NA KKNLKDICY.KA RQTLDALLPPDT e.p.n.	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPE'L.SS RAPE'L.SS RECLDLLS EEEALDLLS CEEGLDLLS PDDAIDLTN PDDAIDLVR PDDAVDLVVF PQNAKDLYVK PPDAIDLS SPEALDLLS SPEALDLLS .e#alDLL.	EYTEDVDMWS WYTPGVDMWS SVYTKGVDMWS SVYTKGVDMWS LLOFNPTKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLVFAPHKRI LLVFAPHKRI LLVFAPHKRI	LGCILGEMU LGCILGEMU SGCI.GEMU SGCI.ESU TVEALSHP TVNALNHP STIDALNHP STIDALNHP STIDALNHP STIDALNHP STIDALNHP SAENGLEHP SAENALKHK SAQALQHP	CCKPLFTGNS RGRPLFPGTS RGRPLFPGTS 320 YLSNFHK.NT YLAAFHK.NT YLSNFHK.NT YLSNFHKSND YVSFHKSND YVVSFHKSND YVVSFHKSND YVSFHKSND YVSFHKSND YVSFHKSND YVSFHKSND YVSFHKSND YVSFHKSND	TMNÖLE KIIQVIG TLHQLE LILEAIP T.HQLELILETIP T.nQl.k!tg PLPRISRAISIPV FLPRISTANISIPV PLPRIARISIPV PLPRIARISIPV SIPVMYKAVKIPI SILAALPKAVKIPV SIPVMYKAVKIPI KITTLPGPVHIPT E.PTCRHIITIPI E.PTCRHIITIPI E.W.TIGGDVRPRA l!.ipv	
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70	50 260 SEEDMESTSSPF SESDMSIGSPF SEDDMSIGSPF SEDDMSIGSPF SEDDMESIGSPF SDDDESIGSPF SDDDTKSIDSOF SESDMESIGSPF SCOMENTION SESDET	270 .LPEVPEG .LPEGPED .LPEGPED  TKVMISSLNTIR TKVMISSL.TIR TKVMISSL.TIR TKMMYSINNIT TAMMSSLTKIP TILMMGSLASVQ SSMMFANVKHIK AATMMESLPLGK AEKIISSFVDLK NISVLQHLGSRP ASVLHQLGSRP m.sl	PILTDYVATRWY HALTEYVATRWY QAVTEYVATRWY 280 RKNIKEYFP.NT KKPMREYFP.NT KKPIKEYFP.NY QRSIKDYLP.NA NKPVKEYFP.NA KMEFEHFFTKQNA KKNIKAFENFFTKQNA KKNLKDICY.KA RQTLDALLPPDT e.pn.	RAPEILLGS RAPEVLLSS RAPEVLLSS RAPEVLLSS RAPE'L.GST SEECLDLLSS EEEALDLLSS CEEGLDLINF TQEALDLITS PDDAVDLVVF PQDAVDLVVF PQDAVDLVVF SPEALDLES SPEALDLLSS SPEALDLLSS SPEALDLLSS	EYTEDVDMWS WYTPGVDMWS TYTLGVDMWS TYTLGVDMWS TYTLGVDMWS LLOFNPTKRI LLOFNPTKRI LLOFNPKKRI LLOFNPKKRI LLOFNPKKRI LLOFNPKKRI LLOFNPKKRI LLOFNPKKRI LLOFNPKKRI LLVFAPHKRL LLVFAPHKRI LLVFAPHKRI	LGCILGEMG GCILGEML GCILGEML GCILGEML .GCI.ESL TVEALSHP TVDALSHP TVDALSHP TVDALSHP STDALSHP STDALSHP STDALSHP STDALSHP SAESGLEHP SAESGLEHP SAESGLEHP daLSHP	CCKPLFTGNS RGRPLFPGTS RGRPLFPGTS J20 YLSNFHK.NTI YLAAFHK.NTI YLSNFHK.NTI YLSNFHK.NTI YLSNFHK.SNDI YUSFHKSNDI YVVSFNMGKYJ YVVSFNMGKYJ YVVSFNMGKYJ YVEFHKSIDI YVEFHSIIDI YVEFHSIIDI YVQFHCPSDI YVQFHCPSDI Y.Q.FHCPSDI Y.Q.FHCPSDI YFh	TMNÖLEKIIQVIG TLHQLELILEAIP T.HQLELILEAIP T.HQLELILETT T.nQl.k!tg PLPRLSRAISIPV FLPRLSRAISIPV FLPRLARAISIPV FLPRLARAISIPV FLPRLARAISIPV SLAALPKAVKIPV SIPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI S.IPVMYKAVKIPI	KPPP CCCCCCDCCDD CCCCCDD NDD EE #
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70 T.annulata	50 260 SESDMDSISSPF SEDMDSISSPF SEDMDSISSPF SEDDIDALGSPF SEDDISIGSPF SDEDISIGSPF SDEDISIGSPF SDEDISIGSPF SDEDISIGSPF SEEDVDAVKSPF NKKDIEDIRSPF SKEDLALGSGC SEEDILALGSGC S. ED. E. 1. Spf 340 35 NVKYDLINYRHI		PILTDYVATRWY HALTEYVATRWY (QAVTEYVATRWY .vlt#YVATRWY RKNIKEYFP.NT KKPMREYFP.NT KKPLHEYFP.NT RKPLHEYFP.MV QRSIKUYLP.NA NKPVKEYFP.NA KMEFEHFFTKQV VKNFKDAFP.NA KMEFEHFFTKQV VKNFKDAFP.NA SKULKDICY.KA RQTLDALLPPDT e.pnn. 370 NSLD.NTINTVN	RAPEILLS RAPEVLS RAPEVLS SECLDIS SECLDIS SECLDIS CEEGLDIN TQEALDIS PDDAIDIN R PDDAIDIN R PDDAUIV SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SAQ NVN.SGNTVG	<pre>HYTEDVDMWS: WYTEGVDMWS HYTEGVDMWS LUENPTKRI LUENPTKRI LUENPTKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI S390 TVSMVSNSVN.</pre>	LGCILGEML LGCILGEML .GCI.GEML .GCI.GEML .GCI.E\$L TVNALNHP TVNALNHP TVDALAHP TVDALAHP TLALNHP TLALNHP TLALNHP AEXGLEHP AEXGLEHP AEXGLEHP AAQALQHP daLnHP	CCFPLFTGNS RGRPLFPGTS .g.PlFpG.S YLSNFHK.NT YLSNFHK.NT YLSNFHK.NT YLSNFHK.NT YLSNFHK.NT YURSFHKSND YVRSFHKSND YVRSFHSSDJ YVRFHSSDJ YVRCFHSPDJ YVRCFHSSDJ Y	TMNÖLE KIIQVIG TLHQLE LILEAIP T.HQLE LILETIP T.nQl.k!tg PLPRLSRATSIPV LLPSLTRAISIPV PLPRLARAISIPV SIPVHYKAVKIPI SIAALPKAIVKIPV SIPVMYKAVKIPI SIAALPKAIVKIPI SIAALPKAITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI S.PTCRHIITIPI	KRR. CCCCCCRDRQH. CCCCCCRDRQH.
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva B.bigemina B.boyis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70 T.annulata T.orientalis	50 260 SEEDMESISSPF SESDMDSISSPF SEDDDISSPF SEDDISSPF SDDIESIGSPF SDDIESIGSPF SDDIESIGSPF SDDIESIGSPF SDEDISSP SREDLAIGSGC S.EDLAIGSGC S.ED.EAIGSGC 340 35 NVKYDLINYRHL NIKYDLINYRHL		PILTDYVATRWY HALTEYVATRWY QavteyvAtrwy .vlt#yvAtrwy RKNIKEYFP.NT KKPMREYFP.NT KKPIKEYFP.NT RKPLHEYFP.NY QRSIKDYLP.NA NKPVKEYFP.NA KMEFEHFFTKQV VKNFKDAFP.NA KKNIKDICY.KA RQTLDALLPPDT e.p.n. 370 NSLD.NTINTVN GQKG.NNGTSTS	RAPEILLGST RAPEVLLSST RAPEVLLSST RAPE'L.GST SEECLDLLSST EEEALDLLSS CEEGLDLLSS PDDAVDLVVF PDDAVDLVVF PDDAVDLVVF PDDAVDLVVF SPEALDLLSS SPEALDLLSS SPEALDLLSS SPEALDLLSS SPEALDLLSS SPEALDLLSS SPEALDLLSS SPEALDLLSS SPEALDLLSS SPEALDLSS SPEALDLSS SPEALDLSS SPEALDLSS SPEALDLSS SPEALDLSS SPEALDLSS SPEALDLSS SPEALDLSS SPEALDLSS SPEALDLSS	EYTEDVDMWS WYTPGVDMWS SVYTKGVDMWS SVYTKGVDMWS LLOFNPTKRII LLOFNPTKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLOFNPKRII LLYFAPHKRII LLYFAPHKRI LLYFAPHKRI S390 TVSMVSNSVN.	LGCILGEMGELL GCILGEML GCI.GEML GCI.GEML TVEALSHP TVEALSHP TVDALAHP TTDALNHP TLALNHP TLALNHP TLALNHP TLALNHP TLALNHP TLALNHP ABNALSHH AAQALQHP daLnHP 	CCKPLFTGNS RGRPLFPGTS RGRPLFPGTS 320 YLSNFHK.NTI YLAAFHK.NTI YLSNFHK.NTI YLSNFHK.NTI YLSYFHKSND YVSFNMGKYJ YVVSFNMGKYJ YVRSFHSYDJ YVRSFHSYDJ YVRFHCPASJ YVQRFHCPASJ YVQRFHCPASJ YVQRFHCPASJ YVQRFHCPASJ YVQRFHCPASJ YVQRFHCPASJ	TMNÖLE KIIQVIG           TLHQLE LILEAIP           TLHQLE LILEAIP           TLHQLE LILEAIP           T.NQ1.k!tg           330           PLPRISRAISIPV           TLPRLSTANSIPV           PLPRISRAISIPV           TLPLYKAITIPI           SLAALPKAVKIPV           SLAALPKAVKIPV           SLAALPKAVKIPV           SLANPKAVKIPY           SLANPKAVKIPY           SLPVMYKAVKIPI           SLPCRHITIPI           SP.CGRHITIPI           SW.JEGDYHRPA           SUN AREADYRPA          l!.ipv           400          SIGSTHSNUV	KRP. CCCCCCNDNQH.
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.equi B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70 T.annulata T.orientalis T.parva	50 260 SEEDMESTSSPF SEDMESTSSPF SEDMDSLSSPF SEDMESLSSPF SEDDDALSSPF SDEDTESLSSPF SDEDTESLSSPF SDEDTESLSSPF SPEDVDAVKSPF NKKDEDTRSPF SKEDLALGSGC SEDTLALGSGC SEDTLALGSGC S.O.O.1.SPf 340 35 NVKYDLINYRHL NVKYDLINYRHL	270 TKVMISSINTR TKVMISSINTR TKVMISSINTR TKVMISSI.TIR AKMMVYSINNIT TIMMGSLASVQ SSMMFANVKHIK AATMMESIPUGK AATMMESIPUGK AEKIISSFVDLK NISVLQHLGSRP m.sl Q 360 IXKFIQSN.SNINI IYKFIQUSN.NTNI	PILTDYVATRWY HALTEYVATRWY (AVDEYVATRWY .vlT#YVATRWY 2800 RKNIKEYFP.NT KKPMREYFP.NT KKPLHEYFP.NT RKPLHEYFP.NA QRSIKUYLP.NA NKPVKEYFP.NA KKNIKDICY.NA RQFLDALLPPDT 	RAPE VILS S RAPE VILS S RAPE VILS S RAPE VILS S 290 SEECIDIIS EE CIDIIS CEEGIDIITS CEEGIDIITS CEEGIDIITS PDDAIDITS SPEALDIIS SPEALDIIS SPEALDIIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS SPEALDIS TOTO SSSA.TSNTSH VN.SOINTY	<pre>HYTEDVDMWS; HYTEQVDMWS; HYTLGVDMWS; HYTLGVDMWS; HYTLGVDMWS; HYTLGVDMWS; HYTLGVDMWS; HYTLGVDMWS; HYTLGVDMKS; HYTLGVNPKKRI; HUGFNPKKRI; HUGFNPKKRI; HUGFNPKKRI; LUFAPHKRI; LU</pre>	LGCILGEML LGCILGEML .GCI.GEML .GCI.GEML .GCI.E\$L .TVNALMHP .TVDALAHP .TVDALAHP .TVDALAHP .TDALMHP .TLALMHP .AEXGLEHP .AEXGLEHP daLMHP daLMHP 	CCKPLFTGNS RGRPLFPGTS RGRPLFPGTS RGRPLFPGTS YLSNFHK.NT YLSNFHK.NT YLSAFHK.SN YLSAFHK.SNT YVRSFHKSND YVRSFHKSND YVRSFHKSND YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFHSSNI YVRSFNS VFSSNI YVRSFNS N	Immõle KIIQVIG           ILHQLE LILEAIP           330           PIPRISRATSIPV           ILPSITRAISIPV           ILPSITRAISIPV           SIQUERLARAISIPV           SIQUERLARAISIPV           SILPSITRAISIPV           SIQUERLARAISIPV           SIQUERLARAISIPV           SIQUERLARAVKIPV           SIQUERLARAVKIPV           SIQUERLYKAVKIPI           SILTIPOVIE           SIQUENT           SICRHIITIPI           SUTCRHIITIPI           S	
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B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70 T.annulata T.orientalis T.parva T.equi B.bigemina B.boyis	50 2 60 SEEDMESTSSPF SEDMESTSSPF SEDMESTSSPF SEDMESTSSPF SEDEDTSTSPF SDEDTSTSPF SDEDTSTSPF SDEDTSTSPF SKEDTATSSPF SKEDTATSSF SKEDTATSSF SKEDTATSSF 340 35 NVKYDLINYRHL NVKYDLTNYRHL NVKYDVANYRR		PILTDYVATRWY HALTEYVATRWY QAVTEYVATRWY .vlt#YVATRWY KKNIKEYFP.NT KKPMREYFP.NT KKPLHEYFP.NT RKPLHEYFP.MV QRSIKUYLP.NA NKPVKEYFP.NA KKNIKDLFP.NA KKNLKDICY.KA RQTLDALLPPDT 	RAPEVILSS RAPEVLSS RAPEVLSS RAPEVLSS RAPEVLSS SECLDISS CECLDISS CECLDISS CECLDISS CECLDISS CECLDISS CECLDISS CECLDISS PDAIDUR PDAIDUR PDAIDUR SPEALDISS SPEALDISS SPEALDISS SPEALDISS SPEALDISS SSS.TSNTSH TVN.SGNTVG SSS.TSNTSH STPT.FGYK HEHSTTMMDV.K	<pre>HYTEDVDMWS: WYTEGVDMWS: YTEGVDMWS: YTEGVDMWS: LOFNPTKRI LOFNPTKRI LOFNPTKRI LOFNPTKRI LOFNPKRI LOFNPKRI LOFNPKRI LOFNPKRI LOFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI LUFNPKRI EDVWW. </pre>	LGCILGEMGELL GCILGEML LGCILGEML .GCI.E\$L 310 TVEALSHP TVNALNHP TVDALAHP TVDALAHP TDALNHP TLALNHP TLALNHP TLALNHP TLALNHP ALNHP ALNHP ALNHP ALNHP ALNHP 	CCKPLFTGNS CCKPLFQGTS AGRPLFPGTS AGRPLFPGTS AGRPLFPGTS AGRPLFPGTS ACCASE ACCASE AVSENT ACCASE	Immodule KIIQVIG           TLHQLE LILEAIP           TLHQLE LILEAIP           TLHQLE LILEAIP           T.NQI.k!tg           330           PLPRLARAISIPV           PLPRLARAISIPV           SIDVINKAVKINI           SIDVMYKAVKIPI           SIDVMYKAVKIPI           SIDVMYKAVKIPI           SUTCRHITIPI           SV.TCGKIIAIPI           SW.TLGOVRLY           W.TLGOVRLY           SW.TLGOVRLY           SIGSTHSNU           TTDKSAD           YTSKSVN	
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva B.bigemina B.boyis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti	50 260 SEEDMESISSPF SEDMISISSPF SEDDIDALGSPF SEDDIDALGSPF SDDDDESIGSPF SDDDESIGSPF SDDDESIGSPF SDDDESIGSPF SDDDESIGSPF SBEDIALGSGC s.eD.e.l.Spf 340 35 NVKYDLINYRHL NVKYDLINYRHL NVKYDLINYRHL NVKYDVANYRL NVKYDVANYRL		PILTDYVATRWY HALTEYVATRWY Qavreyvatrwy .vlt#yvatrwy RKNIKEYFP.NT KKPMREYFP.NT KKPLHEYFP.NT RKPLHEYFP.NA VKDYKEYFP.NA KKNIKDICY.KA RQTLDALLPPDT e.pnn. 370 NSLD.NTINTVN GQKG.NNGTSTS NCNT.NTVNTGN SSHGRNNDDSNN QRAHKDDASALA SGKPPLDGFPHL IADTHEDGPTKS	RAPEILLGST RAPEVLLSST RAPEVLLSST RAPEVLLSST RAPE'L.GST SEECLDLLSS EEEALDLLSS CEEGLDLLSS PDDAIDLUSS SPDALDLUSS SPEALDLLSS SPEALDLLSS SPEALDLLSS SSA.TSNTSH TVN.SGNTVG SSSA.TSNTSH TVN.TGNIV. STPT.FGYX .HEHK	<pre>""""""""""""""""""""""""""""""""""""</pre>	LGCILGEMGELL LGCILGEML LGCILGEML .GCIE\$L .GCIE\$L .GCIE\$L .TVEALSHP TVEALSHP TVDALAHP TTDALAHP TIDALAHP TIDALNHP TLEALNHP TLEALNHP  TGN       	CCKPLFTGNS RGRPLFPGTS RGRPLFPGTS 320 YLSNFHK.NT YLAAPHK.SN YLSNFHK.NT YLSNFHK.NT YLSOFHKSND YVSFNMGKYJ YVSFNMGKYJ YVSFNMGKYJ YVSFNMGKYJ YVSFNGFSS YVSFNMGKYJ YVSFNGFSS YVSFNG YVSFNGSS SAVKEPTSPS AVKEPTSPS SVNDTRSVY	EMNÖLE KIIQVIG TLHQLE LILEAIP T.HQLE LILEAIP T.HQLE LILET T.NQ1. k!tg 330 PLPRISRAISIPV TLPSLTRAISIPV HEPILYKAITIPI SLAALPKAVKIPV SIPVMYKAVKIPI KITILPGPVHIPT EP.VCGKIIAIPI EW.TIGGDVRLPV W.TIGGDVRLPV M.AREADYRPRA l!ipv 400 TGNTVNLV SIGSTHSNU TQGN TKDEGST YTTDKSSD	КРРР CCCCCCCNDNQH. CCCCCCCNDNQH.
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti T.gondii	50 260 SEEDMESTSSPF SEDMESTSSPF SEDMDSLSSPF SEDDDALGSPF SEDDDETSLGSPF SDEDTESLGSPF SDEDTESLGSPF SDEDTESLGSPF SDEDTESLGSPF SPEDVDAVKSPF NKKDEDTRSPF SKEDLALGSGC SEEDTLALGSGCC SEEDTLALGSGCC SEEDTLALGSGCC SEEDTLALGSGCC SEEDTLALGSGCC SEEDTLALGSGCC SEEDTLALGSGCCC SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCS SEEDTLALGSGCCSS SEEDTLA		PILTDYVATRWY HAITEYVATRWY (avdeyvatrwy) .vlt#yvatrwy (k) vlt#yvatrwy (k) vlt#yvatrwy (k) vlt#yvatrwy (k) vlt#yvatrwy (k) vltweype (k) v	RAPE VILS S RAPE VILS S RAPE VILS S RAPE VILS S RAPE VILS S SEECIDIIS SEECIDIIS CEEGIDIITS CEEGIDIITS CEEGIDIITS SCEEGIDIITS SPEALDIIS SPEALDIIS SPEALDIIS SPEALDIIS SPEALDIIS SPEALDIIS SPEALDIS SPEALDIS STONTY SSSA.TSNTSH . e # alDLI. SSTNMDV.K HLF.KKFRKS	<pre>HYTEDVDMWS: HYTLGVDMWS: HYTLGVDMWS: HYTLGVDMWS: HYTLGVDMWS: HYTLGVDMWS: HYTLGVDMWS: HYTLGVDMWS: HYTLGVDMSH HYTLGVDMSH LLOFNPKKRI LLOFNPKKRI LLOFNPKKRI LLOFNPKKRI LLUFAPDKKRI LLVFAPDKKRI LLYFAPDKKRI LLYFAPDKKRI LLYFAPDKKRI LLYFAPDKKRI LLYFAPDKKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LLYFAPDKRI LYFAPSKI LYFASSI LYFA</pre>	LGCILGEML LGCILGEML .GCI.GEML .GCI.GEML .GCI.E\$L .G	CCKPLFTGNS CCKPLFQGTS AGRPLEPGTS AGRPLEPGTS AGRPLEPGTS AGRPLEPGTS AGRPLEPGTS ACCOMMENT AGREAN ACCOMMENT ACCOMME	ImmOule KIIQVIG           CLHQLE LILEAIP           CLHQLE LILEAIP           J30           PLPRIS RATSIPV           ILPSITRAISIPV           PLPRIS RATSIPV           SIPURIS RATSIPV           SIGSTHSNU           SIGST           SIGST           YTTDKSAD           KKKNV           SISSRAGS.	
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70 T.annulata T.orientalis T.equi B.bigemina B.boyis B.microti T.gondii P.falciparum	50 260 SE DMS SIS SPF SE DMS SIS SPF SE DMS SIS SPF SD DD ALGS PF SD DD ALGS PF SD DD IKS IG SPF SD DD IKS IG SPF SD DI IKS IG SPF NKK DIED IRS F SK DD ILAIG SGC S. ED . E. I. SPF NKK DIED IRS F SK DD ILAIG SGC S. ED . E. I. SPF NKK YD IN YRHI NYK YD VANYRHI NYK YD VANYRRI NYK YD VANYRRI NKY SVEDYRNI NKY SVEDYRNI NKY SVEDYRDK		PILIDYVATRWY HALTEYVATRWY QAVTEYVATRWY 2800 RKNIKEYFP.NT KKPMREYFP.NT KKPKEYFP.NT RKPLHEYFP.NT RKPLHEYFP.NA NKPVKEYFP.NA KKNIKDLFP.NA KKNIKDLFP.NA KKNLKDICY.KA RQTLDALLPPDT epnn. 370 NSLD.NTINTVN GQKG.NNGTSTS NCNT.NTVNTGN SSHGRNNDDSNN QRAHKDDASALA SGARPLOGFPHL IADTHEDGPTKS QRRHTAGSSGR	RAPEYLLSS RAPEYLLSS RAPEYLLSS RAPEYLLSS RAPEYLLSS RAPE'L.GST SEECIDINS CEGLDLNS TQEALDLLS PDDAIDLYS PDDAIDLYS SPEALDLLS SPEALDLLS SPEALDLLS SPEALDLLS SSA.TSNTSH TVN.TGNIV. STTMV.TGNIV. STTMV.SCHYS STHALSS	HYTEDVDMWS           WYTPGVDMWS           300           LLOFNPTKRI           LLOFNPKRI           LLOFNPKKRI           LVFAPDKRI           LVYAPKSKI           NQYVPSTISI	LGCILGEMGELL GCILGEML LGCILGEML .GCI.GEML .GCI.E\$L 100 100 100 100 100 100 100 10	CCKPLFTGNS CCKPLFPGTS RGRPLFPGTS .g.PlFpG.S YLSNFHK.NT YLAAFHK.SN YLSNFHK.NT YLSQFHKSND YVSGFHSFEN YVSGFHSFEN YVSGFHSFEN YVSGFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YVQRFHSFEN YQQRFHSFEN SVPTGTSGS .AVKEFTNFY .SVPTGTSGS .AVKEFTNFY .SVPTGTSGS	EMNÖLE KIIQVIG TLHQLE LILEAIP T.HQLE LILETIP T.NQ1. k!tg PLPRISHAISIPV PLPRISHAISIPV PLPRISHAISIPV PLPRISHAISIPV SIPVMYKAVKIPI KITTLPGPVHIPT E.PTCRHIITIPI E.PTCRHIITIPI E.TCRHIITIPI E.TCRHIITIPI E.TCRHITIPI E	KPPP CCCCCCCNDNQH CCCCCCCNDNQH 
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus H.sapiens consensus>70 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus	50 260 SEEDMESISSPF SEEDMESISSPF SEDDLASISSPF SEDDLASISSPF SEDDLASISSPF SDEDFESISSPF SDEDFESISSPF SDEDFESISSPF SDEDFESISSPF SEDULASSPF NKKDIEDIRSPF SKEDLALGSGC S.ED.E.I.SPF 340 35 NVKYDLINYRHI NKYDLINYRHI NKYDLINYRHI NKYDLINYRHI NKYDLINYRHI NKYDLNYRRI NKYDVANYRL NKYDVANYRL NKYSUNYRRI NKYSUNYRNI NKYSVDNYRNI NKYRVNFYRNR	270 TKVMISSINIT TKVMISSINIT TKVMISSINIT TKVMISSI.TIR TKVMISSI.TIR AKMMVYSINNIT TIMMGSLASVQ SSMMFANVKHIK AATMMESLPLGK AATMMESLPLGK AATMMESLPLGK AEKIISSFVDLK NISVLQHLGSRP NISVLQHLGSRP ASVLHQLGSRP m.sl Q 360 IYKFIQSN.NTNI IYKFIQSN.NTNI IYKFIDERK.NE IYKFIDERK.SI VKEYINSQN.FL VSEVIKKK.HD VSEVIKKK.HD VSEVIKKK.HD VSEVIKKK.HD	PILTDYVATRWY HALTEYVATRWY QAVDEYVATRWY QAVDEYVATRWY QAVDEYVATRWY CREATER REALESS REALE	RAPE VILSS RAPE VILSS RAPE VILSS RAPE VILSS RAPE VILSS SEECIDIISS CEEGIDIISS CEEGIDIISS CEEGIDIISS CEEGIDIISS SPEALDISS SPEALDISS SPEALDISS SPEALDISS SPEALDISS SPEALDISS SSSA.TSNTSH SSTNMDV.K HLF.NKFRSS EEK.KDRYYR	<pre>HYTEDVDMWS; HYTEQVDMWS; HYTLGVDMWS; HYTLGVDMWS; HYTLGVDMWS; LIOFNPKRH; LIOFNPKRH; LIOFNPKRH; LIOFNPKRH; LIOFNPKRH; LIOFNPKRH; LIOFNPKRH; LIOFNPKRH; LIOFNPKRH; LIOFNPKRH; LIVFAPHKR; LVFAPHKR; LVFAPHKKR</pre>	LGCI LGE ML LGCI LGE ML LGCI LGE ML .GCIE\$L 310 TVE ALSHP TVD ALAHP TVD ALAHP TDD ALMHP TDD ALMHP TDD ALMHP TDD ALMHP ALKAK ACALCHP ACALCHP daLnHP	CCFPLFTGNS CCFPLFQGTS AGRPLEPGTS AGRPLEPGTS AGRPLEPGTS AGRPLEPGTS AGRPLEPGTS ACCOMPANENT	EMNÖLE KIIQVIG ELHQLE LILEAIP ALRQLE LILEAIP T.nQl.k!tg 330 PLPRISRATSIPV ILPSITAS FATSIPV ILPSITASISIPV HDFILYKAITIPI SLAALPKAVKIPV SIPVYKAVKIPI KLTTLPGVHIPT EP.VGGKIIAIPI EW.TLGGDVRLPV W.TLGGDVRLPV W.TGGTVNLVI W.TGGTVNLVI 	
B.taurus H.sapiens consensus>70 2 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti T.gondii P.falciparum H.sapiens consensus>70 T.annulata T.orientalis T.parva T.equi B.bigemina B.bovis B.microti T.gondii P.falciparum B.taurus H.sapiens	50 2 60 SEEDMESTSSPF SEDMESTSSPF SEDMUSTSSPF SEDDDLALGSPF SDDLALGSPF SDDLALGSPF SDDLALGSPF SDDDLALGSPF SDDDLALGSGC SEDDLALGSGCC SEDDLALGSCCC SEDDLALGSCCC SEDDLALGSCCC SEDDLALGSCCC SEDDLALGSCCCC SEDDLALGSCCCC SEDDLALGSCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		PILIDYVATRWY HALTEYVATRWY (AVEYVATRWY .vlt#YVATRWY (KNIKEYFP).NT KKPMREYFP.NT KKPLHEYFP.NT RKPLHEYFP.NT RKPLHEYFP.NA VKNFKDAFP.NA NKPVKEYFP.NA KKNIKDICY.KA RQTLDALLPPDT 	RAPEVILSS RAPEVLSS RAPEVLSS RAPEVLSS RAPEVLSS SECLDISS CECLDISS CECLDISS CECLDISS CECLDISS CECLDISS CECLDISS CECLDISS SEALDISS PDDAIDY PDDAIDY SPEALDISS SPEALDISS SPEALDISS SPEALDISS SPEALDISS SPEALDISS SSS. TSNTSH STTM.SSTS STT.FGYS FGYS FSS SES SES SES SES SES SES SES SES SES	<pre>HYTEDVDMWS: WYTEGVDMWS: YTEGVDMWS: YTKGVDMWS: UDENETKRI LLOFNPTKRI LLOFNPTKRI LLOFNPTKRI LLOFNPKKRI LLOFNPKKRI NOV</pre>	LGCILGEMGELL LGCILGEML .GCI.GEML .GCI.GEML .GCI.E\$L .GCI.E\$L .GCI.E\$L .GCI.E\$L .GCI.E\$L .GCI.E\$L .GCI.E\$L .GCM.E\$L	CCKPLFTGNS CCKPLFPGTS RGRPLFPGTS 320 YLSNFHK.NTI YLSAFHK.SN YLSAFHK.NTI YLSAFHKSNDI YVRSFHKSNDI YVRSFHKSNDI YVRSFHSNDI YVRCFHSSNDI YVRCFHSSND YVRCFHS	EMNÖLE KIIQVIG TLHQLE LILEAIP T.HQLE LILETIP T.NQ1. k!tg PLPRISHAISIPV PLPRISHAISIPV PLPRISHAISIPV PLPRISHAISIPV SIPVMYKAVKIPI KITTLPGPVHIPT E.PTCRHIITIPI E.PTCRHIITIPI E.TCRHIITIPI E.TCRHIITIPI E.TCRHITIPI E	KPPP CCCCCCNDNQH. DN

Figure 1. Multiple-sequence analysis of the MAPK1 proteins of piroplasms with *T. gondii* MAPK2, *P. falciparum* MAPK1, *B. taurus* MAPK15, and *H. sapiens* MAPK15 (based on 70% consensus).

relationship between apicomplexan MAPKs (Figures 3A and 3B). Both MAPK1 and MAPK2 have unstructured lengthy regions at their C and N-termini, respectively, and inside the activation loop segment like other MAP kinases from apicomplexans [21,22]. The multiple sequence alignment of the MAPK1s with those of other apicomplexans (*P. falciparum* and *T. gondii*) suggests a consensus on a phosphorylation lip triad located at the 186-TDY-190 position (*T. annulata* numbering), which designates the MAPK1 from the *Theileria* and *Babesia* species as a typical MAPK like that of *P. falciparum* (Figure 1) [22]. On the other hand, MAPK2s from the *Theileria* 

and *Babesia* species belong to atypical MAPKs, which bear a phosphorylation lip sequence in the form of a "TGH" motif, like the atypical MAPK of *T. gondii* (MAPK3) [13]. However, *B. microti* MAPK2 bears a triad motif, in the form of a "TSH", like the atypical *P. falciparum* MAPK2 (Figure 2) [21]. The MEME server was used to show motifs thought sequences (Figures 3C and 3D). Within MAPK1 proteins, there is a consensus across 8 motifs. However, 3 additional motifs were found in apicomplexan MAPK2 sequences, and 2 of them (7 and 8) were located at the C-terminus of the specific MAPK insertion site and not found in their mammalian counterparts. Since they have a

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	170	180	190 200	210	220 230	240
T.annulata	AKYRPLKSTTLK			·	NKFVAIKRIHKVEDDLII	DCKRILREIA
T.orientalis	TKFKPIKSTHLK	KEKSSNKPT.VO <mark>N</mark> GIN	WELGERYKFIDMVGS	G <mark>SYGNVC</mark> RAYDSQE	NKFVAIKRIHKVFDDLII	DCK <mark>RILREI</mark> A
T.parva T.equi	TKYKPLKSSTIK ERYKPNRYSSVHTL			<mark>S Y G H V C R A Y D S Q L S Y G H V C R A Y D L Q E</mark>		DCKRILREIA DCKRILREIA
B.bigemina	TEHRRQ HPAPVPTR	TKGASAKQNLVAGNTV	WELPEKYKVIDIVGS	G <mark>SYGQVCRAF</mark> DIEN	<b>QRYVAIKRIHKVFEDLI</b>	DCKRILREIA
B.microti		KFASNLDRNISK <mark>S</mark> KTT	WNIPPRYEL <mark>K</mark> RLI <mark>G</mark> S(	<mark>S Y G Q V S</mark> K <mark>A Y D S</mark> I E	NRYVAIKRIHKVFDDLVI	DCK <mark>RILREI</mark> A
B.bovis T.gondii	TDERRLRNPPLK TVSGSQQEGQQRKQ				IQ <mark>RYVAIKRIHKVFEDLII</mark> K <mark>RVVAIKKI</mark> LRVFEDLII	DCKRILREIA
C.parvum	TDFRNSKSTSTT	. ASSHSGSSLSHPHNK	WSIPSRYOVRHLIGT		NRLVAIKKIHRVFEDLVI	DCKRILREIA
P.falciparum	CNIVEKKNNKSK	EEKINIKEA <mark>I</mark> IKN	VKVPDNYEIKHLIGR	G <mark>SYGYVY</mark> LAYDKNA	N <mark>K</mark> N <mark>V A I K K V</mark> N R M F E D L I I	DCK <mark>RILRE</mark> IT
H.sapiensMAPK11 H.sapiensMAPK14		QEL <mark>N</mark> KTV QEL <mark>N</mark> KTI	WEVPQRLQGLRPVGS WEVPERYQNLSPVGS		RQK <mark>VAVKKLSRPFQSLI</mark> GLRVAVKKLSRPFQSII	HAR <mark>RTYREL</mark> R HAKRTYRELR
B.taurusMAPK11		QLLNKII	WEVPORLOGLRPVGS	GAYGSVC SAYDTRI	RORVAVKKLSRPFOSLI	
B.taurusMAPK14		QEL <mark>N</mark> KTI	WEVPERYONLSPVGS	G <mark>AYGSVC</mark> A <mark>AF</mark> DTKT		
consensus>70					VA!K.iF#dl!d	dckRilREi.
	250 260	270	280 290	300	310 320	330
T.annulata T.orientalis	ILNRLDHPNVVKILDILV ILNRLDHPNVVKILDILV	. P . DN <mark>LETFDVLYVVL</mark> PP . NL <mark>. ETFDVLYVVL</mark>	EIAASDIKQLVRSPAH EIAASDIKQLVRSPAH		LSGVHYLHSVGIYHRDL LSGVHYLHSVGIFHRDL	
T.parva	ILNRLDHPNVVKILDILV	. P. DNLETFDVLYVVL	EIAASDIKQLVRSPAH		LSGVHILHSVGI YHRDLI	KPANCLINRD
T.equi	ILNRLDHPNVVRILDILV	. P. RNLETFDVLYVVL	EIAASDIKQLVRSPAH	<mark>INENHIR</mark> MLIYNL	L <mark>SGVHYL</mark> HSVGI <mark>Y</mark> HRDLH	K P <mark>A N C L V N R</mark> D
B.bigemina B.mignati	ILNRLDHP <mark>NVVKVLDI</mark> VI ILNRLDHPNVVKLLDIII	. P. SDLENFNVLYVVL . P. SNMETFDELYVVL	EIAASDIKLLVRSPAH EIADSDFKKLLRSSAH		LCGVNYLHAVGIYHRDL LVGVOYLHNMGIYHRDL	KPANCLVNRD
B.microti B.bovis	ILNRLDHPNVVKLLDIII ILNRLDHPNVVKVLDIVI		EIADSDEKKLLRSSA		LCGVYYLHARGIYHRDL	K PANCLVN RD
T.gondii	ILNRLNHDHVVKVLDIVI	. P. KDVEKEDELYVVL	EIADSDFKKLFRTPV	<mark>LTELHIK</mark> TLLYNL	L <mark>V</mark> GVKYV <mark>HSAG</mark> ILHRDLH	
C.parvum	ILSRLNHDHIVKILDICV		EIAD SDFKKLFRTPV		LVGLKYI <mark>HSAG</mark> IYHRDLI	K P <mark>A N C L V N Q</mark> D
P.falciparum H.sapiensMAPK11	ILNRLKSDYIIRLHDLI LLKHLKHENVIGLLDVFT		EIADSDLKKLFKTPI TLMGADLNNIVKC.QA		LLGEKFIHESGIIHRDL LRGLKYTHSAGTIHRDL	K P ANCLLNOD K P <mark>S N V A V N</mark> E D
H.sapiensMAPK14	LLKHMKHENVIGLLDVFT	. PARSLEEFNDVYLVT	HLMGADLNNIVKC.QH			KPSNLAVNED
B.taurusMAPK11	LLKHLKHENVIGLLDVFT		TLMGADLNNIVKC.Q2		L <mark>RGLKYI</mark> HSAGIIHRDLH	
B.taurusMAPK14 consensus>70	LLKHMKHENVIGLLDVFT iL.r\$.h.n!!lDi.v		HLMGADLNNIVKC.QH		LRGLKYIHSADIIHRDL	
consensus, io	340 35			50 460	470 48	
T.annulata	CSVKICDFGLARTTTFPE			N <mark>YS</mark> FA <mark>VDIWSVGCI</mark>	· ·	·
T.orientalis	CS <mark>VKIC</mark> DFGLAR <mark>T</mark> VTYFE	EYVNLTPTF <mark>RQL</mark> TGH <mark>V</mark>	<mark>V</mark> TRWYRAPE <mark>LI</mark> L <mark>LQ</mark> NI	DY <mark>SFAVDMWSV</mark> GC1	F <mark>AELLNMLK</mark> VN <mark>V</mark> GEPSD	R S P L F P G <mark>S</mark> C 🕕
T.parva	<b>CSVKIC</b> DFGLAR <mark>T</mark> TTFPE	EFMDSTR <mark>RQLTGH</mark> V		NY <mark>SFA</mark> VDIWS <mark>V</mark> GCI		
T.equi B.bigemina	CS <mark>IKICDFGLART</mark> TTLSD CS <mark>VKICDFGLART</mark> VTHRR	GYPEDL. TROLIGHY	VTRWYRAPE <mark>LILLO</mark> E VTRWYRAPE <mark>LILLO</mark> E			
B.microti	CSVKICDFGLARALSSKG	CTFNSA KKQLTSHV	VTRWYRAPEIILLOD			
B.bovis	<b>CSVKICDFGLARTIAQPV</b>	TQAPLGS <mark>RQL<mark>T</mark>GH</mark> V	<mark>VTRWYRAPE<mark>LI</mark>L<mark>LQ</mark>DI</mark>	NY <mark>TAA IDV</mark> WS <mark>V</mark> GC 1		
T.gondii C.parvum	CS <mark>VKVC</mark> DFGLAR <mark>T</mark> V CG <mark>VKICDFGLART</mark> VKRPT		VTRWYRAPE <mark>LILLO</mark> E VTRWYRAPE <mark>LILLO</mark> E			
P.falciparum	CSVKICDFGLARTINSDK			NYTNSIDIWSTGC1		
H.sapiensMAPK11	C <mark>ELRIL</mark> DFGLAR <mark>Q</mark> A	D <mark>EEM</mark> TGYV			M <mark>AEL<mark>LQG</mark><mark>.</mark></mark>	. <mark>Ka</mark> lfpg <mark>s</mark> .
H.sapiensMAPK14	CELKILDFGLAR <mark>H</mark> T CELRILDFGLAR <mark>Q</mark> A				MAEL <mark>LTG</mark> <mark>.</mark> MAELLQG	. RTLFPGT. . KALFPGS.
B.taurusMAPK11 B.taurusMAPK14		DEMIGIV				.RTLFPGT.
consensus>70	C.vk!cDFGLAR					
	490 500	5	520	530	540 550	560
T.annulata	CFPLSPDNKNANP		QLNLIFNVLGTPSEE	DINC <mark>IQK</mark> A.D <mark>VRR</mark>	VKFFAKRGFQDLRTKFK	GASLESIDLL
T.orientalis	CFPLSPDNKNSSS		QLNLIFNVLGTPSDE	<mark>DINWIDK</mark> P.D <mark>VQKY</mark>	VRIFAT <mark>R</mark> TFQ <mark>DL</mark> RT <mark>RY</mark> K	GSSLESLDLL
T.parva T.equi	CFPLSPDNKNANP				<mark>VK</mark> FFAK <mark>R</mark> SFQ <mark>DLRTKF</mark> K VRLFGERPSVNLYERFH	
B.bigemina	CFPLSPDNKNSTD			DIAAIAKP.DVRRI		ATPPLAVDLL
B.microti	CFPLSPDHKNDKKYRDAN	VNKNDILECNNQRDM <mark>D</mark>	QLNMIFNVLGTPWED	EVECLE <mark>K</mark> E.Y <mark>VKK</mark> Y	IRMFPPRSGIDLTEKFK	G S <mark>S</mark> P E A <mark>V D</mark> L L
B.bovis	CFPLSPDNKNPSD	K.AKEH <mark>D</mark> KFHTRGNR <mark>D</mark>	QL <mark>NIIFNVIGTPCEE</mark> QLNVIFNILGTP <b>S</b> EE		IRMFHPRKGIDLYKRFK	
T.gondii C.parvum	CFPLSPDNKOOTEDY	RFKIRGNRD	QL <mark>NVIFNILGTP</mark> SEE OLNMIFNVLGSPLDE			AS <mark>S</mark> ADA <mark>IHLL</mark> GASSOSIDLL
P.falciparum	CFPLSPDHNSKKV	RFKIRGNR <mark>D</mark> HEKSNR <mark>D</mark>	QL <mark>NIIFNVIGTPPEE</mark>	D L K C <mark>I</mark> T <mark>K</mark> Q . E <mark>V I K Y</mark>	<mark>IKLF</mark> PT <mark>R</mark> DGI <mark>DL</mark> SK <mark>KY</mark> S	SI <mark>s</mark> keg <mark>idll</mark>
H.sapiensMAPK11	• • • • • • • • • • • • • • • • • • •	DYI				GANPLAIDLL GANPLAVDLL
H.sapiensMAPK14 B.taurusMAPK11		DYI	OLKRIMEVVGTPSPE	VLAKISSE.H	IQSLTQMPKMNFANVFI	GANPLAVDLL
B.taurusMAPK14	<mark></mark>	DHI <mark>D</mark>	QLKLILRLVGTPGAE	LLKK <mark>I</mark> S <mark>S</mark> E . S <mark>ARNY</mark>		GANPLA <mark>VDLL</mark>
consensus>70	cfplspd	n.D	QLn.Ifnv.GtP.e#	di.ky	/ifr#1%.0	gidLL
	570 580	5 9 Q		e o ó	610 62	•
T.annulata		NHPYFKSISK <mark>P</mark> R		.SNFDSIPK	TLPFNDWVNMSESQLRY	SFLREIQRYH
T.orientalis T.parva		NHP <mark>YF</mark> KSINR <mark>P</mark> R NHP <mark>YF</mark> KSISK <mark>P</mark> R	• • • • • • • • • • • • • • • • •	.HNFAHV.PK	ULPFNDWENMSESQLRY TLPFNDWVNMSESQLRY	
T.equi	SKMLTFNPSKRITVKEAL	QHPYFSNITKGK QHE <mark>YF</mark> RDLYN <mark>P</mark> R		.EISEYSEK	RLPFNDWISMSESQLRY	AFLREIQRYH
B.bigemina	Q RML V F N P E K R I K V S E A L	QHE <mark>YF</mark> RDLYN <mark>P</mark> R		.HAEVPSQP	VVPFNDWINMSEGQLRY	AFLR <mark>EI</mark> QRHH
B.microti B.bovis		A H P F F D P I K N D Q N F N I	NISSTNACGDGSYDG	EDGPCAWKK	LHLPFNDSMDMTESELKK	
T.gondii	K R M L V F N P N K R I T I N E C L	AHPFFKEVRIAE		.VETNATEK	RLPFNDWINMSESQLRI RLPFNDWMNMDEPQLRY	
C.parvum	K KMT V FN PN K PT TV D FAL	S <mark>HSLF</mark> KNIRN <mark>E</mark> M		.LEIISHEK	TLPFDDWSSMTERELRY	FELKETORES
P.falciparum H.sapiensMAPK11	E <mark>SMLRF</mark> NAQ <mark>KRITI</mark> DKAL GRMLVLDSDORVSAAEAL	SHPYLKDVRKEN	• • • • • • • • • • • • • • • • • •	LENF.STE.K	ILPFDDWMVLSETQLRY	IFL <mark>KEI</mark> QSFH LTYQEVLSFK
H.sapiensMAPK11 H.sapiensMAPK14	E KMLVLDSDQRVSAAEAL	AHAYFAQYHDPD		.DEPVA.DP	DOS.FESRDLLIDEWKS	LTYDEVISFK
B.taurusMAPK11	GRMLVLDSDQRVSAAEAL	AHAYFSQYHDPD		.DEPE.A.E.P	LILPFDDWMVLSETQLRY (DES.VEAKERTLEEWKE (DQS.FESRDLLIDEWKS (DES.VEAKERTVEEWKE	LTYQEVLSFK
B.taurusMAPK14	E <mark>KML</mark> VLDSD <mark>KRITA</mark> AQAL \$L.fn.dkR!.v.eaL	AHAYFAQYHDPD		.DEPVA.DP	<mark>(</mark> DQ <mark>S.</mark> F <mark>E</mark> SRD <mark>LLI</mark> DE <mark>WK</mark> S pfn#nm.e.#lr.	LTYDOVISFV
consensus>70					vin#nm.e.#1r.	

Figure 2. Multiple-sequence analysis of the MAPK2 proteins of piroplasms with *T. gondii* MAPK3, *P. falciparum* MAPK2, *C. parvum* MAPK2, *B. taurus* MAPK11 and MAPK14, *H. sapiens* MAPK11, and MAPK14 (based on 70% consensus).

role in the intrinsic autophosphorylation of a component of the MAPK insertion site of the mammalian p38-beta MAPK [41], additional motifs (7 and 8) in apicomplexans could be considered to be druggable sites for phylumspecific MAPK inhibitors (Figures 4A and 4B)

# 3.2. Comparative protein modelling and structure validation

Representative structures of the kinase domains of MAPK1 and MAPK2 from *T. annulata* and *B. bovis* were

modelled by a comparative approach using a Modeller 9.16 program based on the atomic coordinates of *C. parvum* MAPK1 (PDB ID: 3OZ6) and *T. gondii* MAPK3 (PDB ID: 3RP9) structures, respectively (Figure 4). MAPK1s from *T. annulata* and *B. bovis* show 56% (Query cover: 49% and E-value: 2e-142) and 52% (Query cover: 67% and E-value: 1e-137) identity matches with the crystal structure of *C. parvum* MAPK1. MAPK2 shows 49% (Query cover: 77% and E-value: 8e-173) and 57% (Query

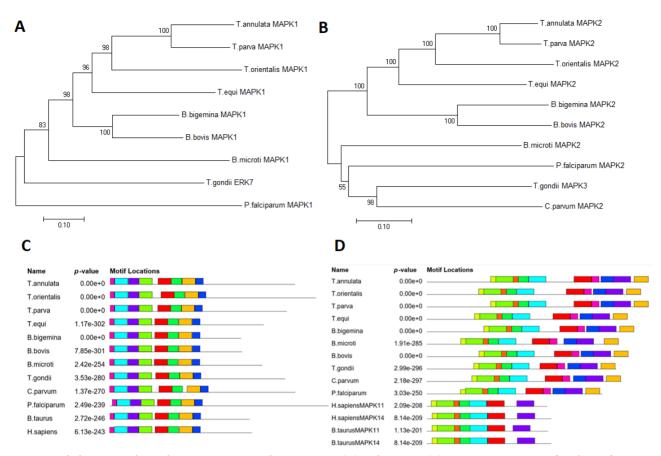


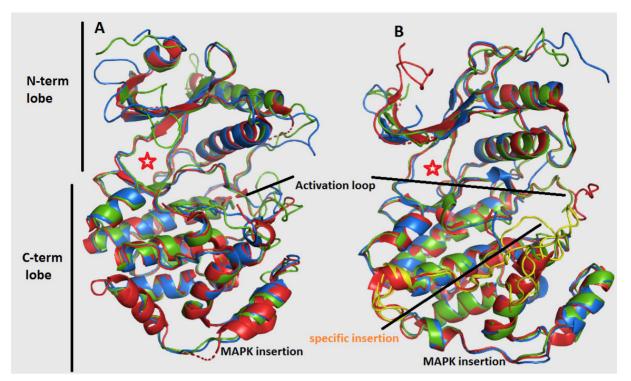
Figure 3. Phylogenetic relationships among apicomplexan MAPK1 (A) and MAPK2 (B) proteins. MEME motif analysis of MAPK1 from apicomplexans with *B. taurus* and *H. sapiens* MAPK15 (C) and MAPK2 with *B. taurus* and *H. sapiens* MAPK11 and MAPK14 (D).

cover: 72% and E-value: 4e-169) identity with the crystal structure of *T. gondii* MAPK3 for both *T. annulata* and *B. bovis*, respectively. Having a good sequence consensus is a crucial step for homology model predictions, and higher identity values make the construction of more accurate models possible. In this case, kinase domains were predicted based on templates, which have a mean identity of 54%, which is suitable for structure-based drug design and prediction of binding modes of small molecules in the active site. All generated models were subjected to geometry optimisation via potential energy minimisation. Resultant protein structures were further validated for 3D structure quality, and all structures were shown to have an adequate grade for further structural studies (Table 2).

Root mean square deviation (RMSD) values that represent structural similarities to template structures were found to be 0.697 Å and 0.684 Å for MAPK1s, 0.714 Å and 0.719 Å for MAPK2s for both *T. annulata* and *B. bovis*, respectively. These values are higher than expected due to the presence of long disordered activation loops in all structures. The ATP binding pocket is the major drugtargeting site, which has been the subject of many structurebased drug design efforts [42]. DoGSiteScorer was used to predict targeting pockets and determine their drugabilities on MAPK1 and MAPK2 proteins. The server analysis results showed that the ATP sites have the highest scoring sites on MAPK structures for drug targeting (Table 2).

## 3.3. Molecular dynamics simulations and docking

Molecular dynamics of the T. annulata and B. bovis MAPK 1 and 2 were analysed by all-atom molecular dynamics simulations under NPT (1-atm pressure and 300 K temperature) conditions for 50 ns. Due to the absence of any molecular dynamics studies on apicomplexan MAPKs, structural features were compared with mammalian MAPK counterparts in this study [42,43]. As seen in Figure 5A, the MAPK1 structures of both B. bovis and T. annulata were found to be more stable than MAPK2 proteins, with average RMSD values of 3.05 Å and 3.78 Å, respectively. The highest RMSD value belongs to the B. bovis MAPK2, which is unstable and shows large fluctuations until the 40-ns mark. The disordered activation loop in MAPK2 is longer than that of MAPK1 proteins. Therefore, fluctuations due to this unstructured activation loop, especially in BbMAPK2, have been clearly



**Figure 4.** Cartoon view of the superimposition of MAPK1 (A) and MAPK2 (B) structures with template proteins of *C. parvum* (3OZ6\_A) and *T. gondii* (3RP9\_A). Red: templates; Green: *T. annulata* MAPK1 and MAPK2; Blue: *B. bovis* MAPK1 and MAPK2; specific insertions in piroplasm MAPK2 proteins are coloured yellow. A red asterisk shows ATP binding pockets used for the molecular docking site.

	TaMAPK1	TaMAPK2	BbMAPK1	BbMAPK2
ProSA (Z-Score)	-7.87	-7.47	-7.66	-7.98
ProQ (LGScore)*	5.336	4.518	6.019	4.009
ERRAT overall score	93.0556	80.5419	87.7246	78.5714
Ramachandran plot scores				
Favoured region	87.2%	86.6%	88.4%	85.6%
Allowed region	12.0%	11.8%	9.3%	13.2%
Outlier region	0.8%	1.6%	2.3%	1.2%
RMSD**	0.697	0.714	0.684	0.719
DoGSiteScorer DrugScore	0.69	0.82	0.83	0.81

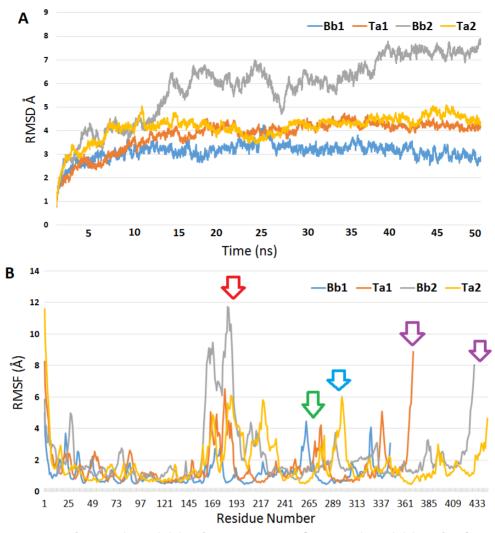
Table 2. Structural validations of MAPK kinase models.

\*LGscore > 1.5 = fairly good model; LGscore > 2.5 = very good model; LGscore > 4 = extremely good model.

 $^{**}\text{RMSD}$  values were calculated for C-alpha traces by superimposing them with template PDB files.

observed on the RMSF graph between 145–193 amino acids (Figure 5B, red arrow).

In all MAPKs analysed in this study, large fluctuations in the activation loop were visible (Figure 5B, red arrow), and similar results have also been found regarding the activation loop thermal mobility in research conducted on the human p38 MAPK [42,43]. Another feature of RMSF analysis is the MAPK-specific insertion mobility in TaMAPK1 and BbMAPK1, which was also seen in the ERK2 and p38 MAPK (Figure 5B, green arrow) [43].



**Figure 5.** Root mean square deviation (RMSD) (A) and root mean square fluctuation (RMSF) (B) graphs of TaMAPK1 (orange), TaMAPK2 (yellow), BbMAPK1 (blue), and BbMAPK2 (grey) structures. Red arrow: activation loop; green arrow: MAPK specific insertion; blue arrow: specific insertion site; purple arrow: C-terminal region.

However, in the case of MAPK2s, the MAPK-specific insertion site did not show distinct fluctuations, unlike their mammalian counterparts, while the apicomplexanspecific insertion site shows a high degree of mobility (Figure 5B, blue arrow). Moreover, the C-tail region, like those of other MAPKs, showed higher fluctuations which are thought to have a role in allosteric regulations in MAP kinases [42-44]. By using the IUPHAR/BPS Guide to Pharmacology database, we identified 38 active MAPK kinase (especially p38 MAPK) inhibitors with the lowest IC50 values and screened MAPK targets via a 2-tier protocol consisting of the selection of the top 30% best compounds by SP docking and further analysis through a Glide XP docking step for the best possible hits (Table 3). Based on screening and molecular docking results, a compound with the PubChem ID 11714580

(named as PF-03715455) was found in all MAPKs' ATP binding sites, 3 of which (except TaMAPK2) had the highest DeltaG-binding energy. Pose views showed that the compound of interest interacted with binding pocket residues and made hydrogen bonds with TaMAPK1 (Ser142, Gly27, and Asp156), BbMAPK1 (Asp98), and BbMAPK2 (Arg23, Ala92, and Asp133) (Figures 6A, 6B, and 6D). Furthermore, with TaMAPK2, the compound formed a pi and a salt-bridge interaction with Tyr6 and Lys24 residues, respectively (Figure 6C). Another pi interaction also occurred between the 3-chloro-4-hydroxyphenyl moiety of the compound and the His100 residue of BbMAPK1.

Compound 11714580 is an investigational, small chemical currently in phase I trials that belongs to the pyrazole class and was designed to combat asthma and

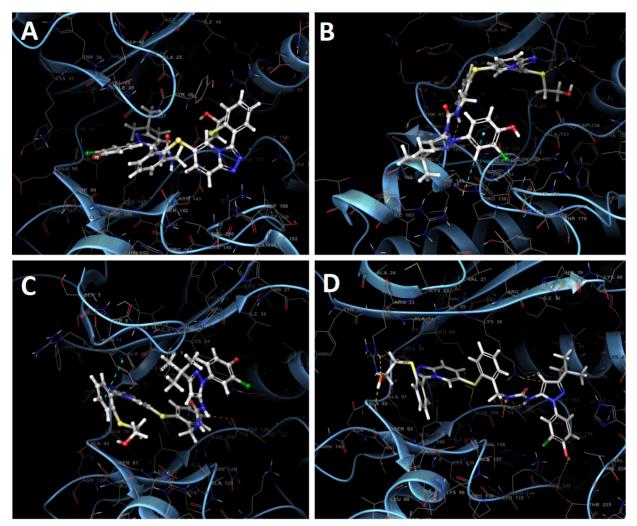
Pubchem ID	Target name	XP GScore	XP HBond	MMGBSA dG Bind
	TaMAPK1			
11714580*		-7.501	-1.372	-49.01
156422		-7.498	-1.212	-49.01
25125014**		-7.211	-1.040	-41.27
176167		-6.866	-0.148	-35.81
644241		-6.533	-0.973	-45.93
	TaMAPK2			
5228		-5.728	-0.485	-29.40
11714580*		-5.410	-0.434	-11.24
25125014**		-5.012	-0.960	-21.74
11314340		-4.896	-0.645	-21.18
10297982		-4.379	-1.300	-27.17
	BbMAPK1			
11314340		-7.077	-1.981	-29.34
11714580*		-6.903	-1.134	-40.97
25125014**		-6.456	-1.440	-35.20
11485656		-5.316	-1.407	-40.28
10297982		-4.706	-0.665	-38.94
	BbMAPK2			
10297982		-6.458	-0.696	-39.52
11714580*		-5.436	-1.587	-60.59
25125014**		-5.298	-3.095	-14.49
176167		-4.893	-1.560	-32.92
103905584		-4.435	-1.126	-26.38

**Table 3.** Molecular docking results of the MAPK inhibitor library on TaMAPK1 and 2 and BbMAPK1 and 2.

\*Pubchem ID\_11714580 and \*\* Pubchem ID\_25125014 compounds were found in all MAPKs.

pulmonary diseases by inhibiting the p38-alpha MAPK kinase in humans. Functional and structural studies have shown that the compound bound to the ATP site of human p38-alfa MAPK with high affinity ( $K_p$ : 0.001 nM) and showed preclinical safety properties for human use [45]. The other compound which interacted with all MAP kinases, with binding scores worse than 11714580, is 25125014 (BS-194), a pyrazolo [1,5-a] pyrimidine derivative designed for cancer chemotherapy. It shows potency and selectivity on human cyclin-dependent protein kinases (1, 2, 7, and 9) and ERK8 (MAPK15) with a low IC50 value (0.33 uM) [46]. These 2 small molecules, which have potency on human MAPKs, have interacted with MAPK 1 and 2 in T. annulata and B. bovis in silico, so further in vitro studies should be done for a more precise inhibition potential unravelling. Furthermore, reports have shown that the inhibitors designed for human MAPKs (especially for p38) act on apicomplexan organisms, including P. falciparum, by blocking replication in human erythrocytes, *Eimeria tenella*, kinetoplastid parasite *L. donovani*, *T. gondii*, and the microsporidian parasite *Encephalitozoon cuniculi* [47–50]. This indicates a great potential for human MAPK inhibitors as promising drugs to fight parasitic diseases and makes a way for an improvement to the current inhibitors with higher efficiency and safety.

Since the first oncogene was designated as a protein kinase in 1978, great efforts in drug discovery have been made on different types of protein kinases. In addition, 13 small chemicals targeting serine/threonine protein kinases were approved by the FDA for use on human diseases [51,52]. Due to the pathophysiological roles of MAP kinases in apicomplexans, they have become molecules of strong interest in drug development [22,47]. In this study, we investigated the interaction potencies of MAPK kinase inhibitors with *T. annulata* and *B. bovis* MAPK 1 and 2 protein structures by using integrated computational



**Figure 6.** Molecular docking views of 11714580 (PubChem ID) within the ATP binding pocket of TaMAPK1 (A), BbMAPK1 (B), TaMAPK2 (C), and BbMAPK2 (D). Yellow and orange dots: H-bonds; cyan dots: pi interactions.

techniques. The results showed that the inhibitors designed for MAPKs of mammalians also have inhibition potency on piroplasm MAPKs. However, there is also an opportunity for selective drug design for piroplasm MAPKs, which show sequential and structural differences due to their evolutionary divergence when compared with their host counterparts.

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## **Conflict of Interest**

The author declares that there is no conflict of interest.

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