

Early Evolution of the Mitogen-Activated Protein Kinase Family in the Plant Kingdom

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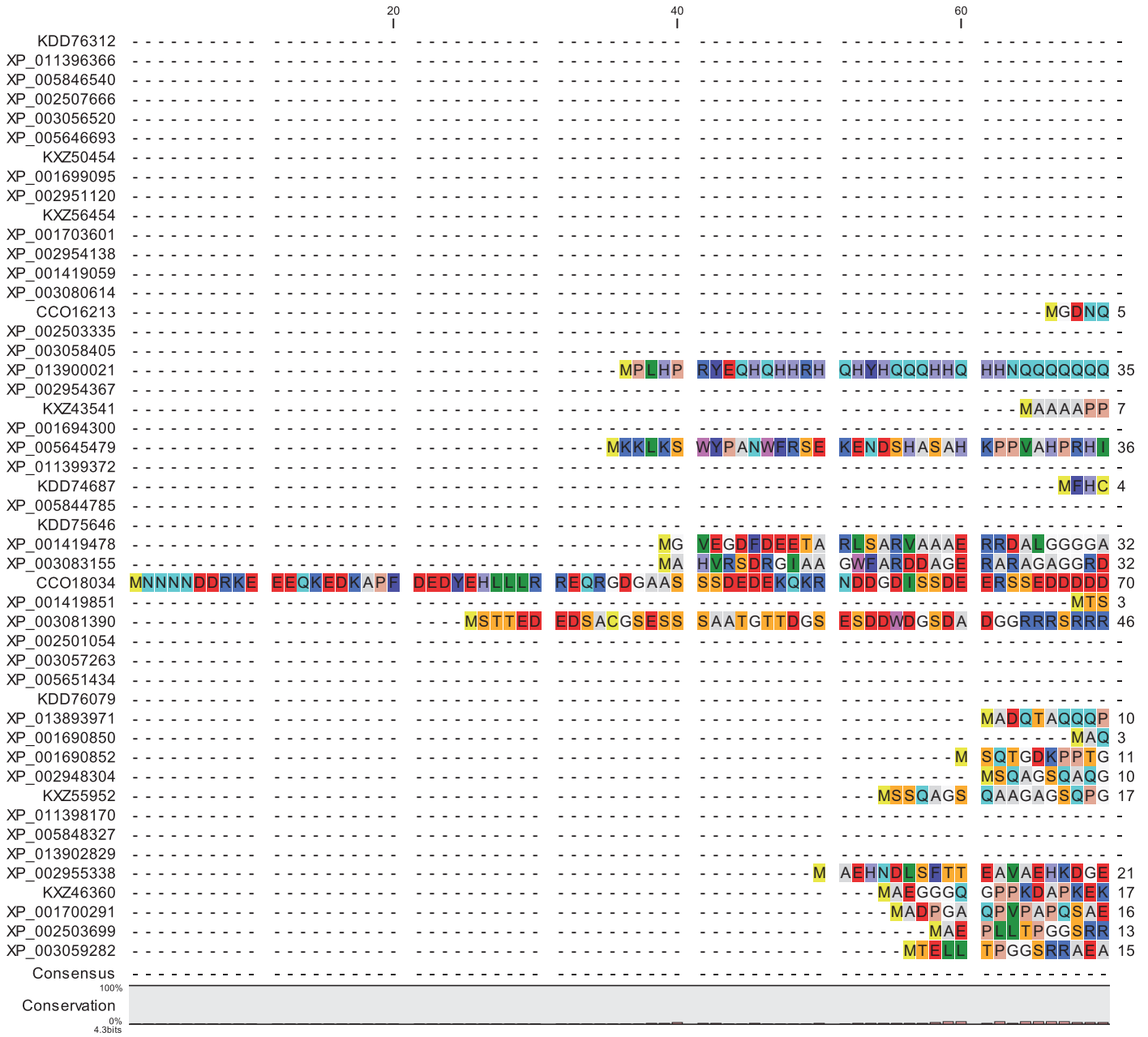
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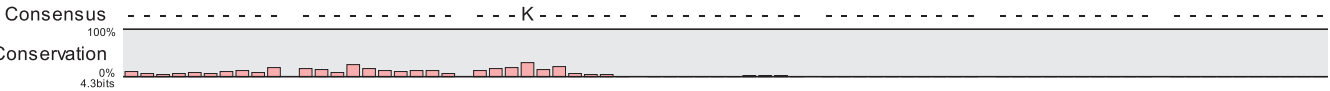
Supplementary Materials



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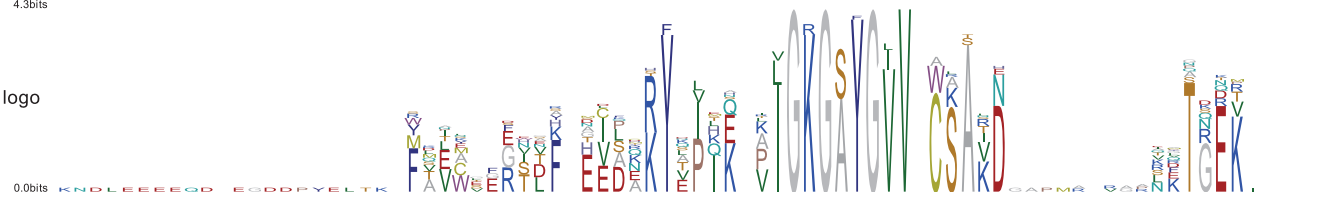
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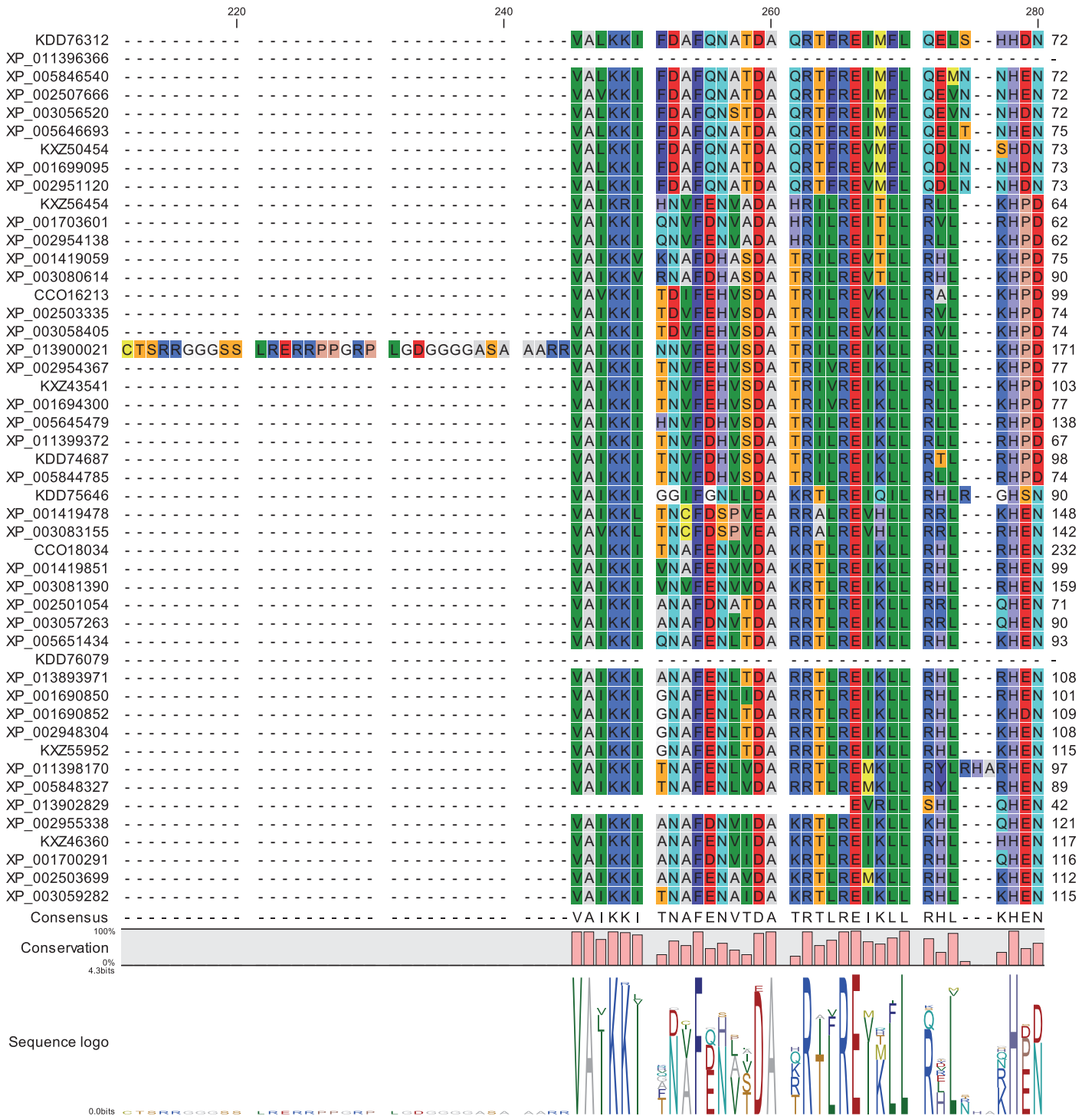


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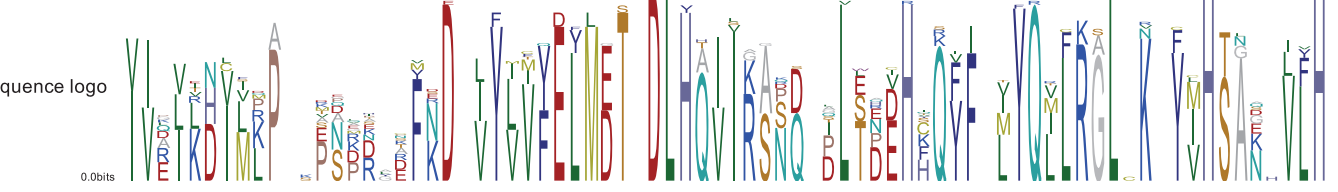
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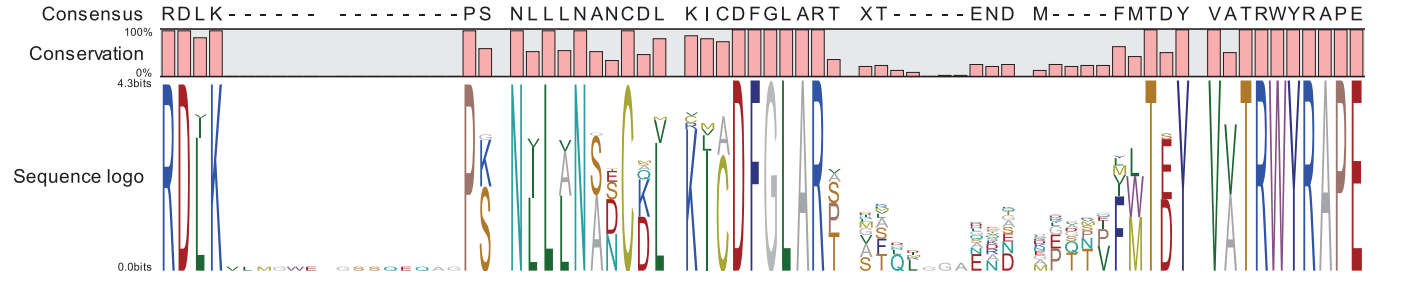


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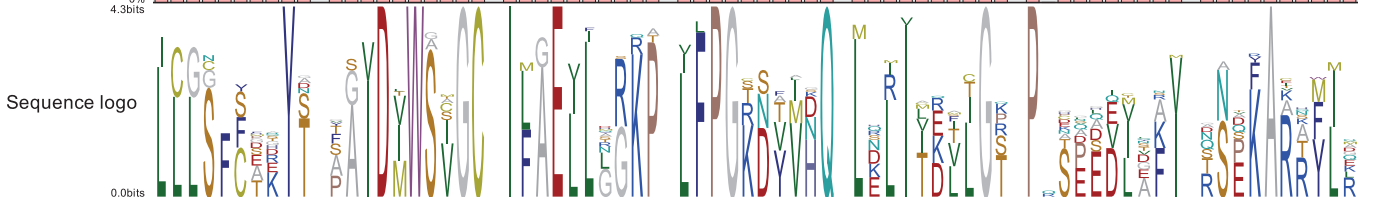


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XP_001690850	RDLK			PS	NVLLN	NASCDL	KICDFG	LART	GT---	EKQ	N---	FMTEY	VVTRWYRAPE	214
XP_001690852	RDLK			PS	NVLLN	NASCDL	KICDFG	LART	GS---	ERN		FMTEY	VVTRWYRAPE	221
XP_002948304	RDLK			PS	NVLLN	NASCDL	KICDFG	LART	SA---	EQN	N---	FMTEY	VVTRWYRAPE	221
KXZ55952	RDLK			PS	NVLLN	NASCDL	KICDFG	LART	GS---	ERN		FMTEY	VVTRWYRAPE	227
XP_011398170	RDLK			PS	NVLLN	NASCDL	KICDFG	LART	ST---	ESN	N---	FMTEY	VVTRWYRAPE	210
XP_005848327	RDLK			PS	NVLLN	NATCDL	KICDFG	LART	ST---	ESN	A---	FMTEY	VVTRWYRAPE	201
XP_013902829	RDLK			PS	NVLLN	NANCDL	RICDFG	LART	GN---	QGA	QEGVGM	LTEY	VVTRWYRAPE	160
XP_002955338	RDLK			PS	NVLLN	NANCDL	KICDFG	LART	ST---	SNE	KE---	FMTEY	VVTRWYRAPE	235
KXZ46360	RDLK			PS	NVLLN	NANCDL	KICDFG	LART	ST---	SNE	KE---	FMTEY	VVTRWYRAPE	231
XP_001700291	RDLK			PS	NVLLN	NANCDL	KICDFG	LART	ST---	SNE	KE---	FMTEY	VVTRWYRAPE	230
XP_002503699	RDLK			PS	NVLLN	NANCDL	KICDFG	LART	GR---	EET		FMTEY	VVTRWYRAPE	225
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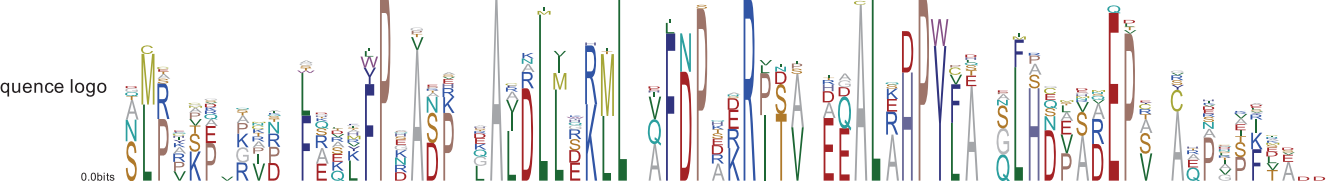
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XP_005846540	LLLS-SHRYIT	YGVDMVACGC	LLGELLNGKP	LFPGTSTMNQ	LDRLELTGR	P-SPOQDS	QSPFAATMLE	268
XP_002507666	LLLS-SSTRYIT	FGVDMVSSGC	LLGELLGKGP	LFPGTSTMNQ	LDRLELTGK	P-ANEDVAV	QSPFAATMLA	255
XP_003056520	LLLS-STKYIT	FGVDMVSSGC	LLGELLFRGQP	VFPGTSTMNQ	LDRLELTGK	P-TEEDLA	QSPFAATMLE	255
XP_005646693	LLLS-SPKYIT	FGVDMVSSAGC	LLGELLTGKP	LFPGSSTMNQ	LDRLELTGQ	P-SQQVDAL	ASPF AATMLE	258
KXZ50454	LLLS-STKYIT	FGVDMVSSGC	LLGELLGKGP	LFPGSSTMNQ	LDRLELFCGR	P-SPSDAA	DSPFAATMMD	256
XP_001699095	LLLS-STKYIT	FGVDMVSSGC	LLGELLMGKP	VFPGTSTMNQ	LDRLELFCGR	P-SPSDVEAL	DSPFAATMME	256
XP_002951120	LLLS-STKYIT	FGVDMVSSGC	LLGELLGKGP	VFPGTSTMNQ	LDRLELFCGR	P-APADVEA	DSPFAATMMD	256
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XP_001703601	LCGC-FYGRYS	SAVDMVSSIGC	IFAEVLLGKP	LFPGRDAVHQ	LQLVTDLLGK	P-PPHVDA	GNVKARAFLN	252
XP_002954138	LCGC-FYGRYT	SAVDMVSSIGC	IFAEVLLGKP	LFPGRDAVHQ	LQLVTDLLGK	P-SQAVIEAL	TNAKARKVLQ	249
XP_001419059	LCGS-FFTKYT	PAIDVWSIGC	IFAEVLRGKP	LFPGKNVVKQ	LELTDLLGT	P-TPLQAKV	RNEKARRFLQ	261
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CCO16213	LCGS-FFTKYT	PAIDVWSIGC	IFAEVLRGKP	LFPGKNVVKQ	LELTDLLGT	P-TPLQAKV	RNEKARRFLG	285
XP_002594335	LCGS-FFTKYT	PAIDVWSIGC	IFAEVLRGKP	LFPGKNVVKQ	LELTDLLGT	P-HPDVNRV	RNEKARRFLG	260
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XP_002954367	LCGS-FFAKYS	PAIDVWSIGC	IFAEVLLGKP	LFPGRNVVHQ	LELTDLLGT	P-SGEVIAKV	RNEKARRFLV	262
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XP_001694300	LCGS-FFAKYS	PAIDVWSIGC	IFAEVLLGKP	LFPGRNVVHQ	LELTDLLGT	P-SPEVIAKV	RNEKARRFLV	261
XP_005645479	LCGS-FFAKYS	TAIDVWSIGC	IFAEVLLGKP	LFPGRNVVHQ	LELTDLLGT	P-SAEVIAKV	RNEKARRFLM	323
XP_011399372	LCGS-FFTKYT	PAIDVWSIGC	IFAEVLRGKP	LFPGRNVVHQ	LELTDLLGT	P-AQEVIAKV	RNEKARRFLV	252
KDD74687	LCGS-FFAKYS	PAIDVWSIGC	IFAEVLLGKP	LFPGRNVVHQ	LELTDLLGT	P-SPEVIAKV	RNEKARRFLV	283
XP_005844785	LCGS-FFAKYS	PAIDVWSIGC	IFAEVLLGKP	LFPGRNVVHQ	LELTDLLGT	P-SPEVIAKV	RNEKARRFLM	259
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XP_003083155	LLLS-SEEYA	ASIDVWSIGC	IFAEVLRGKP	LFPGKDFHQ	MRLLETLS	P-EEADMDFI	SSPYARKYA	324
CCO18034	LLLS-CAEYIT	VAIDVWSIGC	IFAEVLRGKP	LFPGKDYHQ	LNLTKVIGT	P-DEQDLYFV	TSDKARRYLR	417
XP_001419851	LLLS-CAEYIT	SAIDVWSIGC	IFAEVLRGKP	LFPGKDYHQ	LNLTKVIGT	PQNDSELDI	NNEKARRYLR	284
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XP_001690852	LLLS-CEHYIT	SAIDVWSIGC	IFAEVLRGKP	LFPGKDYDQ	LKLKMLGS	P-SEEDLGF	TAPKARTYLR	289
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KXZ55952	LLLS-CEHYIT	AAIDVWSIGC	IFAEVLRGKP	LFPGKDYDQ	LKLKMLGS	P-SEEDLGF	TAPKARTYLR	295
XP_011398170	LLLS-CDSYS	AGIDVWSIGC	IFAEVLRGKP	LFPGKDYDQ	LKLKMLGS	P-SDEELGF	TAPKARTYLR	278
XP_005848327	LLLS-CDSYD	AGIDVWSIGC	IFAEVLRGKP	LFPGKDYDQ	LKLKMLGS	P-SDEELGF	TAPKARTYLR	269
XP_013902829	LLLS-CPDYIT	AAIDVWSIGC	IFAEVLRGKP	LFPGKDYVHQ	LNLTKVIGS	P-GEEDLQFV	SSEKARRYLR	228
XP_002955338	LLLS-CSGYIT	TAIDVWSIGC	IFAEVLRGKP	LFPGKDYVHQ	LSLTKVIGS	P-SEEGEF	TSEKARRYLR	303
KXZ46360	LLLS-CSGYIT	TAIDVWSIGC	IFAEVLRGKP	LFPGKDYVHQ	LSLTKVIGS	P-TEEELGF	TSEKARRYLR	299
XP_001700291	LLLS-CSGYIT	TAIDVWSIGC	IFAEVLRGKP	LFPGKDYVHQ	LSLTKVIGS	P-TEEELGF	TSEKARRYLR	298
XP_002503699	LLLS-CSEYIT	SAIDVWSIGC	IFAEVLRGKP	LFPGKDYVHQ	LNLTKVIGS	P-SEEEEMFI	TSDKARRYLR	293
XP_003059282	LLLS-CAEYIT	AAIDVWSIGC	IFAEVLRGKP	LFPGKDYVHQ	LNLTKVIGS	P-SESEMAFI	SSDKARRYLR	296

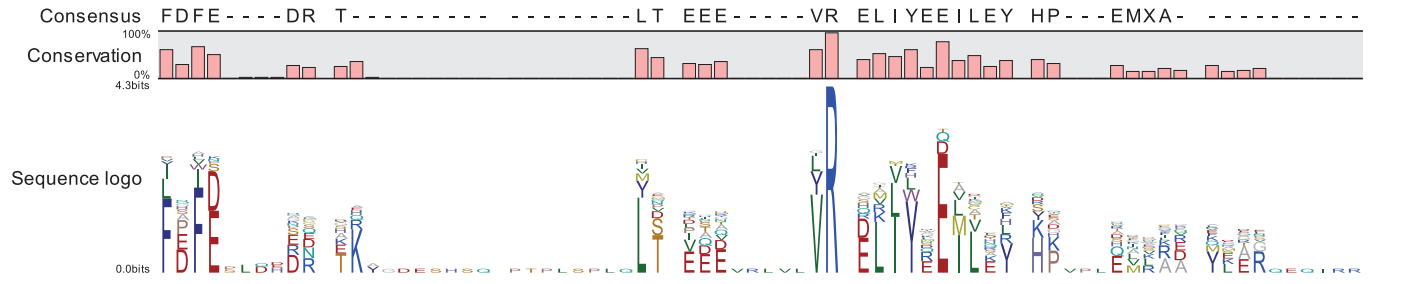
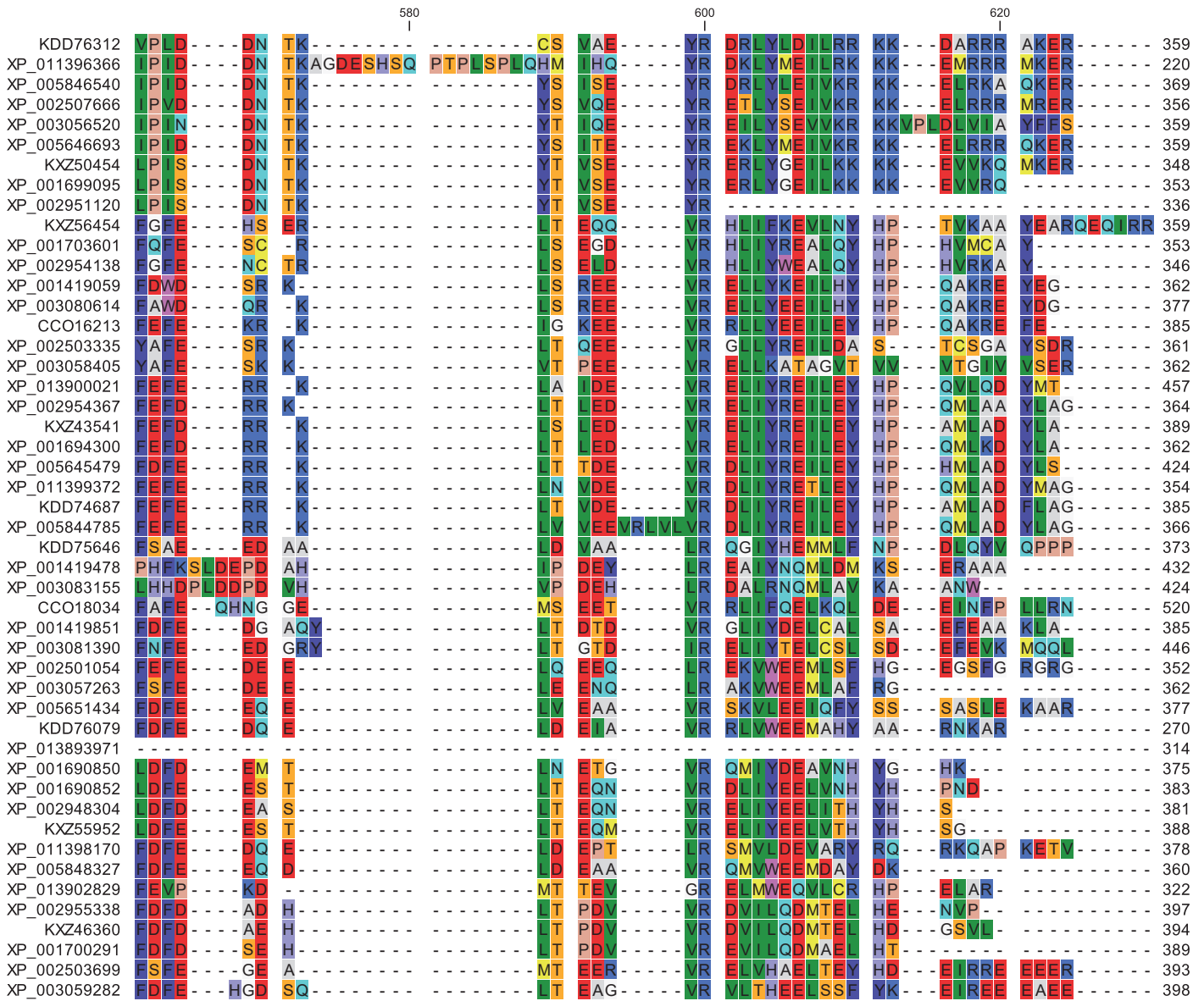
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KDD76312	SMRAPE - APR	LEVAFPDAPP	DARDLSKLL	AFNPDRRISA	EEALRHPYCA	QFRGAEDPEV	A-PCALA --- 323
XP_011396366	SMRAPD - TPR	LEAAFPSASP	EADLLEKLL	QFNPEKRISA	EEALRHPYCL	PFHNLVDEPV	A-PNTIT --- 168
XP_005846540	SMRAPP - TPR	LEATFPSASP	EADLQRL	HFNPDKRISP	EEALRHPYCA	QFHNPHDEPV	A-PATIT --- 333
XP_002507666	SLPPPK - PPK	ATELFPRASH	QAADLQKLL	QFNPEKRITA	EEALRHPYLA	QFHNPADEPY	C-DHRL --- 320
XP_003056520	SLPCTS - SNT	TRELPNTSE	HAFDLHKLL	DINPKRITA	DAAALRHPYS	QFHNPADEPS	C-NHVIS --- 320
XP_005646693	ALPCGD - APA	HLRFPSASP	EAADLSRLL	QFNPAKRITA	EEALRHPYVA	QFHSSADEPS	A-PGILT --- 323
KXZ50454	SCSVYG - GRR	GAELLPNASP	EADLLEKLL	-----SA	EEALRHPYVA	QFHNPADEPV	C-HRILV --- 312
XP_001699095	SCSVYQ - ARR	QDVFPHASP	EADLLEKLL	VFNPHKRLTA	EQAALRHPYVA	QFHNPADEPV	C-NRILV --- 321
XP_002951120	SCTVTA - SRR	ADTFPNASP	EAADLRKLL	VFNPHKRLTA	EQAALRHPYVA	QFHNMADEPV	C-NRILV --- 321
KXZ56454	DPEKR - PKN	LATVFPKADP	RADLLEKLL	ALDPKDRPTP	AAAALRHPYFA	ELPSAQQDR	V-PVPE --- 317
XP_001703601	ALPEKR - PRQ	LSSKFPAADP	LADLLEKLL	AFDPADRPTA	EEALRHPYFA	GPSANQVFR	GQPSVRAADD --- 321
XP_002954138	NMPHKE - PPK	LSSKFPNADP	LADLLEKLL	AFDPADRPTA	EEALRHPYFA	GPEVROETV	-PVAA --- 313
XP_001419059	NMRKIP - AVP	SEKFPGVDP	KALNLSKLL	AFDPDRPTA	AQAALRHPYFD	GADSRPEPA	RRPKKDE --- 328
XP_003080614	QMRKIP - ATP	LSEKFPVDS	KALNLEKLL	AFDPDRPTA	AQAALRHPYFE	GADVSREPS	RHPLPKSV --- 343
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XP_002505335	NMRVKP - RTP	FASRFPGAAP	AAALLEKLL	AFDPERPTA	EEALRHPYFA	GSDPSREPA	AEIVSRTE --- 327
XP_003058405	NMRVKP - KIP	SQRFPKAAAP	GALALDKLL	AFDPERPTA	EEALRHPYFA	NADPAREPA	AEIVSRDE --- 327
XP_013900021	NMRKKA - GVP	FEERFPRADP	RARALLRLL	AFDPADRPTA	EEALRHPYFS	NHSPAREPS	AAAISKLA --- 423
XP_002954367	NMCKP - GIP	FEHLFPRADR	GARLLRLL	AFDPAERPTA	EEALRHPYFT	GHCAREPV	AQPSKMA --- 329
KXZ43541	GMRPKP - GIP	FEQHFRADR	GARLLRLL	AFDPAERPTA	EEALRHPYFA	GHCAREPV	AAPISKMA --- 355
XP_001694300	NMRPKP - GIP	FEQHFRADR	GARLLRLL	AFDPAERPTA	EEALRHPYFA	GHCAREPV	AQPSKLA --- 328
XP_005645479	NMRKKP - GVN	LEQYFPRADR	GARLLKRM	AFDPAERPSS	EEALRHPYFA	GQPGREPS	AQPSKLS --- 390
XP_011399372	NMRKKP - GTS	FEAYFPKADK	GALKRLL	AFDPAERPTS	EEALRHPYFH	GSDPSREPS	AQPSKLA --- 319
KDD74687	NMRKKP - GVP	FEQYFPKADK	GARLLHRL	AFDPSERPTA	EEALRHPYFQ	GSDPSREPS	AQPSKLA --- 350
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KDD75646	SMPFMP - RAN	LQSFPGANP	LADMELKLL	IFDSSARLDI	RGAALRHPYLA	SLHEPADEPE	C-RQPF --- 337
XP_001419478	SLPRKP - KVD	FALVYPDANP	LADLERL	VFDPQRISV	DDAALRHPYLA	SLHDASMEPT	YTPAAD --- 396
XP_003083155	ALPRKP - KID	FASYPRANV	LAVLLERL	VFNPHRRISV	DEAALRHPYLA	SLHDVSAEPR	YAADPSD --- 390
CCO18034	QLPYSK - PMD	FKRLYPEANP	LACDLEKML	IFNPEKRINV	EECALRHPYLA	SLHDVNDPEV	A-NAPFT --- 482
XP_001419851	SLPVT - RCS	FQKLFNASP	KAVDLDKML	VLDPARRITV	EECALRHPYLE	SLHDEVDPEV	A-ESPFT --- 349
XP_003081390	SLPVT - RCN	FRKLFNASP	KAVDLDKML	VLDPARRITV	EECALRHPYLE	SLHDEVDPEV	A-ETPFT --- 409
XP_002501054	TLPHSP - QVR	WERMFPKGNP	QAVDLDKML	QFDPKKRITV	EQAALRHPYLT	ALHDPMEPEPA	SEPAPFE --- 317
XP_003057263	SLPYS - PAR	FDTMVDPANP	DAVDLYKML	EFNPKKRITV	EQAALRHPYLA	NLHDPSEPEPA	SEPAPFE --- 336
XP_005651434	ALPYAQ - RCP	FRVMFPDASH	LADLMEKML	QFNPERRISV	EQAALRHPYLA	QMHDPASELS	A-PDTFD --- 342
KDD76079	SLPAAP - PAD	LRALEPEASAP	QAVDLDKML	QFDPRRRISV	AGAALRHPYLA	SLHDPAEPT	A-KATFC --- 239
XP_013893971	ALPAVE - GNT	SASGPPPRP	-----	-----	-----WPA	G	----- 314
XP_001690850	ALPPSE - KIN	FRKLFDPADP	LADLMEKML	QFDPKRKIDV	LQAALRHPYLA	QLHEEAAEPA	A-AGEFV --- 347
XP_001690852	ALPPAE - KVN	FRKLFPEADP	LADLMEKML	QFDPKRKIDV	HQAALRHPYLA	QLHDEAAEPA	A-PGEFV --- 354
XP_002948304	ALPPSE - KVN	FRKLFPEADP	LADLMEKML	QFDPKRKIDV	HQAALRHPYLA	QLHDEAAEPC	A-PGEFV --- 354
KXZ55952	ALPPSE - KVN	FRKLFPEADP	LADLMEKML	QFDPKRKIDV	HQAALRHPYLA	QLHDEAAEPC	A-PGEFV --- 360
XP_011398170	GAAVQ - KPD	FRASFPDASK	QAVDLMERML	QFDPRRRISV	DDAALRHPYLA	ALHDEAAEPA	A-RGAFK --- 343
XP_005848327	ALAQVE - RTD	AKLFPGASP	LAVDLGRML	VFDPRRRITV	QQAALRHPYLA	QLHDEAAEPA	A-AGVFK --- 334
XP_013902829	SLPRCP - RAD	FQELWPDADP	QAVDLSRML	VFDPSRRISV	DDAALRHPYLA	ALHDEAAEPA	A-DAPFT --- 293
XP_002955338	SLPRSV - RVD	FAQLWPHANK	QAVDLDKML	VFDPTKRITV	EQAALRHPYLA	SLHDVDEPV	C-ATPFT --- 368
KXZ46360	SLPRSE - RVD	FAQLWPNVNK	PALDLDKML	VFDPTKRITV	EQAALRHPYLA	SLHDVDEPV	C-PNPFT --- 364
XP_001700291	SLPRSE - RVD	FQQLWPHANK	TAVDLDKML	VFDPTKRITV	EQAALRHPYLA	SLHDVDEPV	C-PTPFT --- 363
XP_002503699	SLPRT - RVD	FQKLYPEAEP	QAVDLDKML	VFDPASRITV	EEALRHPYLA	SLHDVDEPS	A-SGPF --- 358
XP_003059282	SLPVS - RVD	FAKLYPDADP	SADVLDKML	AFDPSNRITV	EEALRHPYLA	SLHDVDEPS	A-SEPF --- 361

Consensus SLPXKP - RVD FEQLFPDADP LALDLEKLL AFDPAKRITX EEALRHPYLA XLHDPADPEV A-PGPFT ---

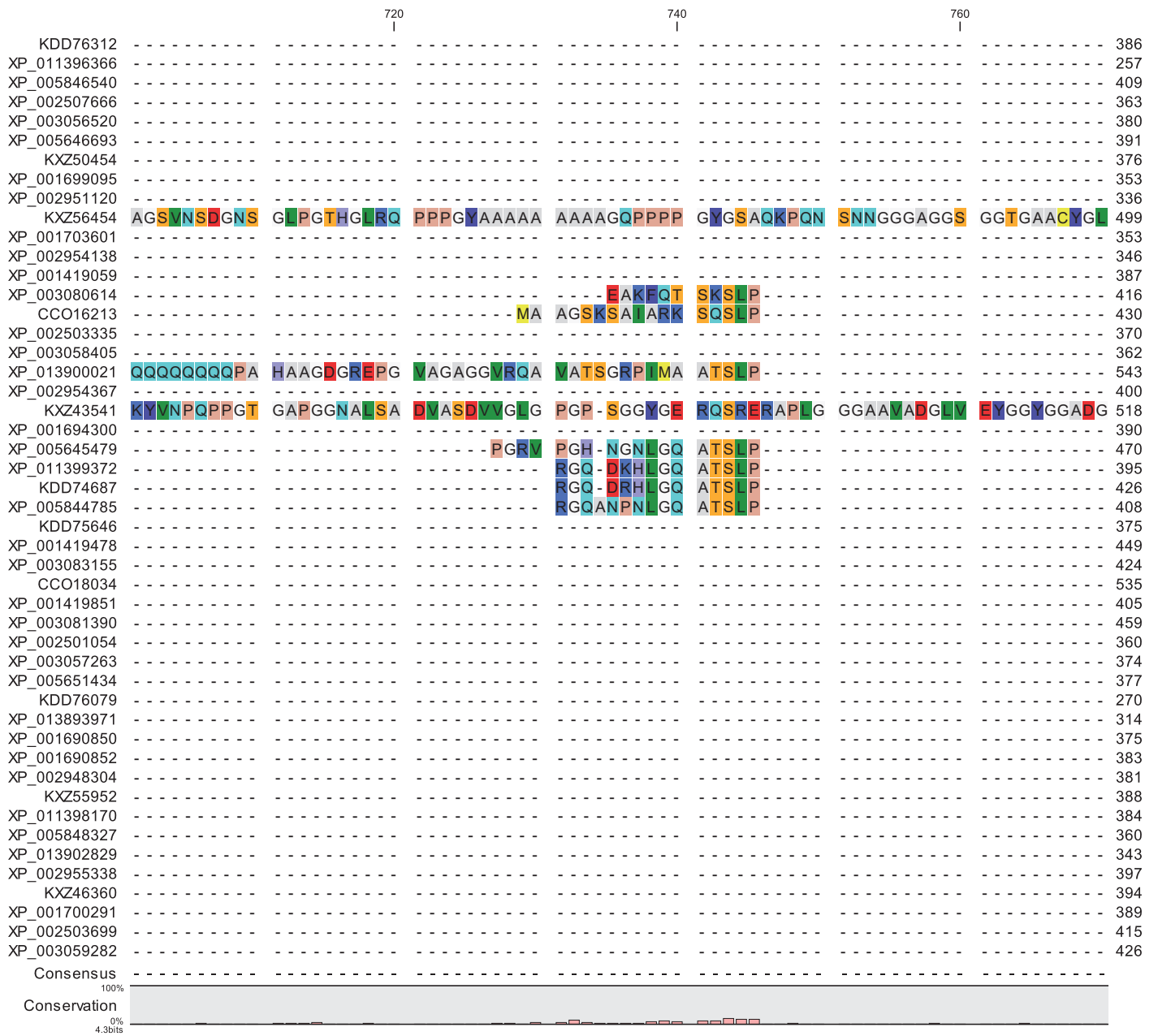






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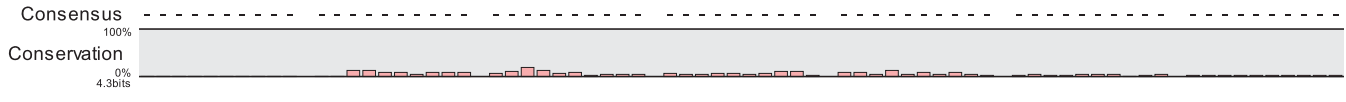




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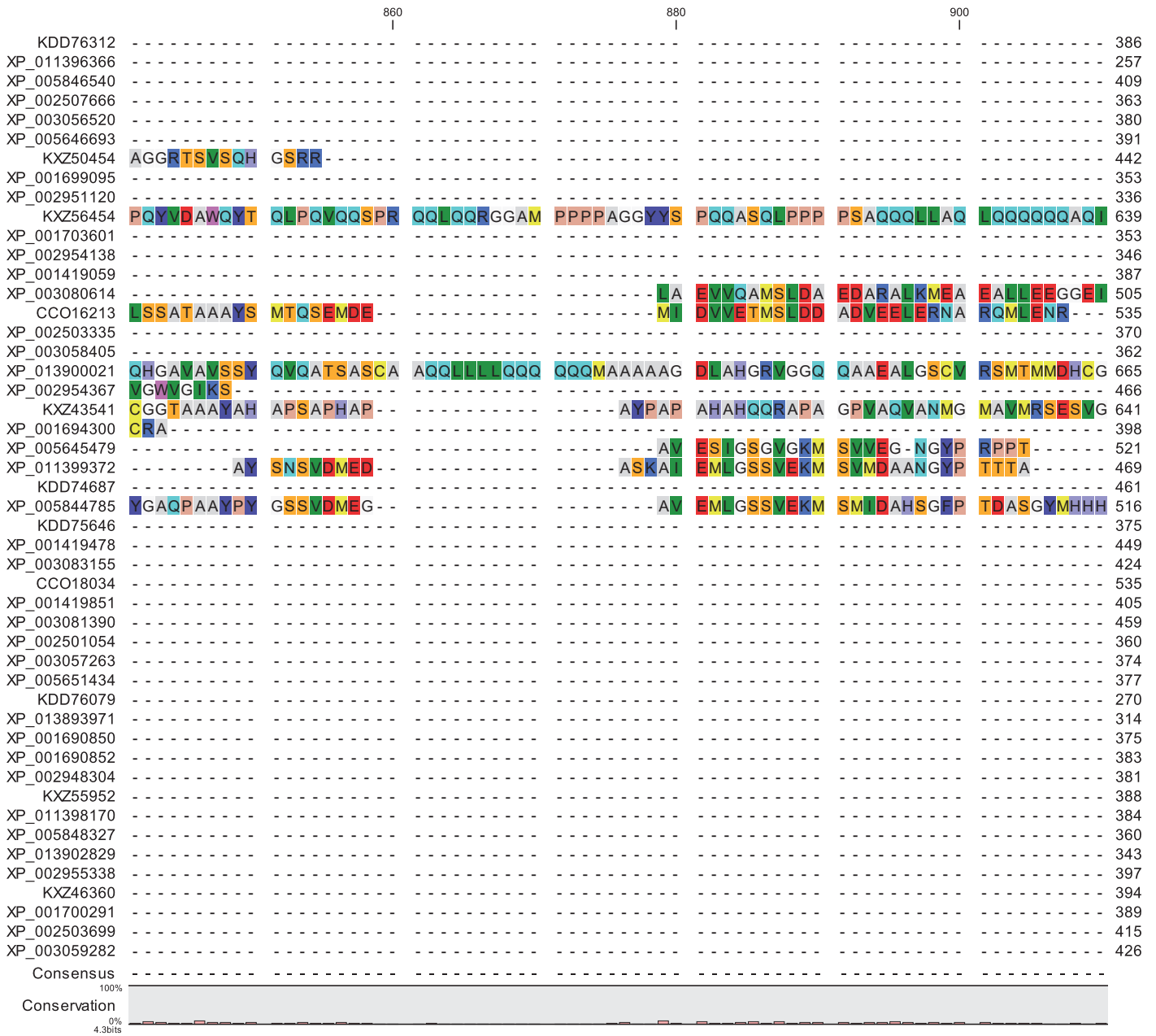


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XP_005646693	-----	-----	-----	-----	391				
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XP_001419059	-----	-----	-----	-----	-----	-----	387		
XP_003080614	-----	KHAGVEYR	DKAAQFAGGL	DALSRSDSVK	PGGSPGLARM	DSDDVEYEEL	QTDDVDRFD	473	
CCO16213	-----	REKMNAYV	KNAEELYGER	VKRQAAEQVY	REKVNSEFS	EGFSLNGSPS	QDDISFEKEK	488	
XP_002503335	-----	-----	-----	-----	-----	-----	370		
XP_003058405	-----	-----	-----	-----	-----	-----	362		
XP_013900021	-----	RERAFEFQ	AEAAKYS	-----	HLLPANA	AAAAAAAAAAAA	AAAAQQQQQL	HQLQQQQQQG	595
XP_002954367	-----	GEGERGK	GEKGGWGGK	RAGGRFTTG	EWRAERLKR	GGDKGGRVHE	GRKSGEEDG	458	
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XP_001694300	-----	-----	GAA	-----	-----	-----	-----	395	
XP_005645479	-----	RERYKDFQ	TEAQRVY	-----	PQWDRQ	GARA	-----	496	
XP_011399372	-----	RERVDFQ	SEAAKYM	S	RGHHMSGVY	GATVGAAP	-----	430	
KDD74687	-----	RERVREFQ	SEAAKYY	H	RGHHMSGVY	GAMVGSVA	-----	461	
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XP_003059282	-----	-----	-----	-----	-----	-----	-----	426	



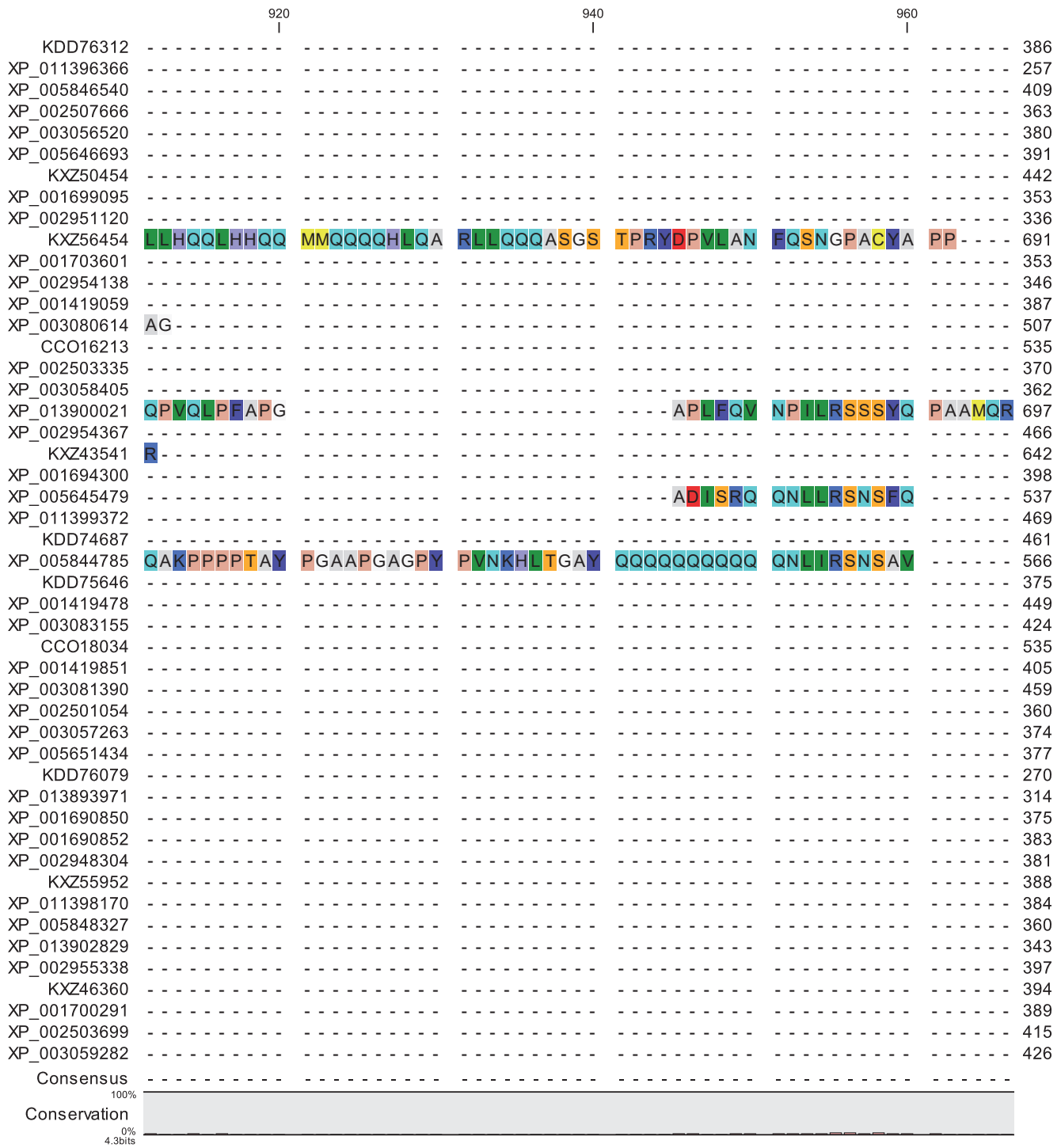
Sequence logo





Sequence logo





Sequence logo



Figure S1
Multiple alignment of MAPK protein sequences from 13 algal species. 48 sequences identified in 13 species (Table 2) were aligned by the MUSCLE alignment method. Amino acids are coloured according to their biochemical properties to visualise conservation and similarities. Consensus and degree of conservation are indicated below each block.

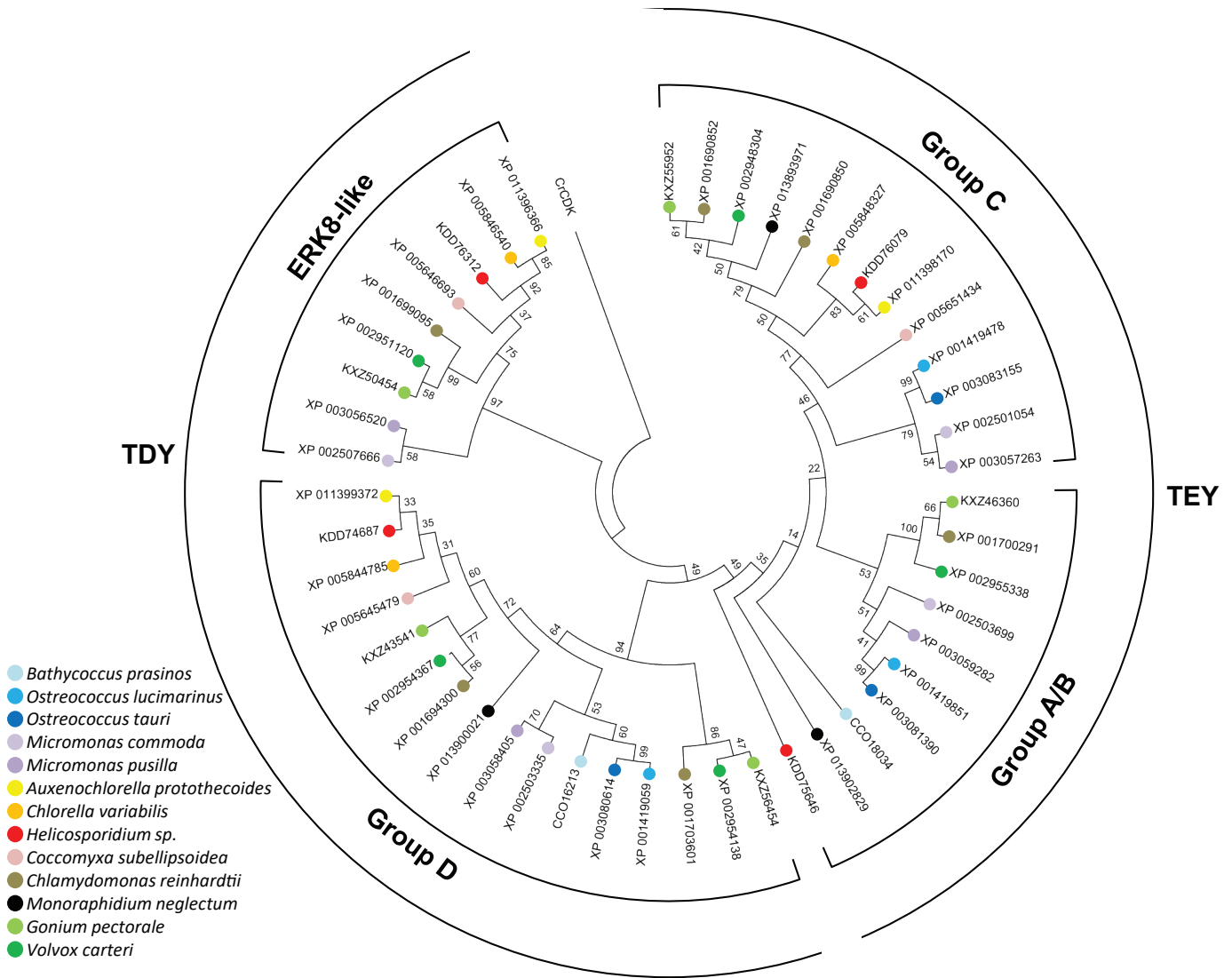


Figure S2

Phylogenetic relationships of MAPK protein sequences in 13 algal species. Full-length amino acid sequences of the 48 identified MAPKs (Table 2) were aligned by the MUSCLE alignment method and the phylogenetic tree was constructed by the Maximum-likelihood method using the MEGA6 software package. A cyclin-dependent kinase (CDKG1) of *Chlamydomonas reinhardtii* was used as outgroup. Species are indicated by the protein IDs in the tree with coloured dots according to the key on the left. Phylogenetic grouping and the type of the conserved phosphorylation site (TxY) are indicated at the inner and outer perimeters, respectively.

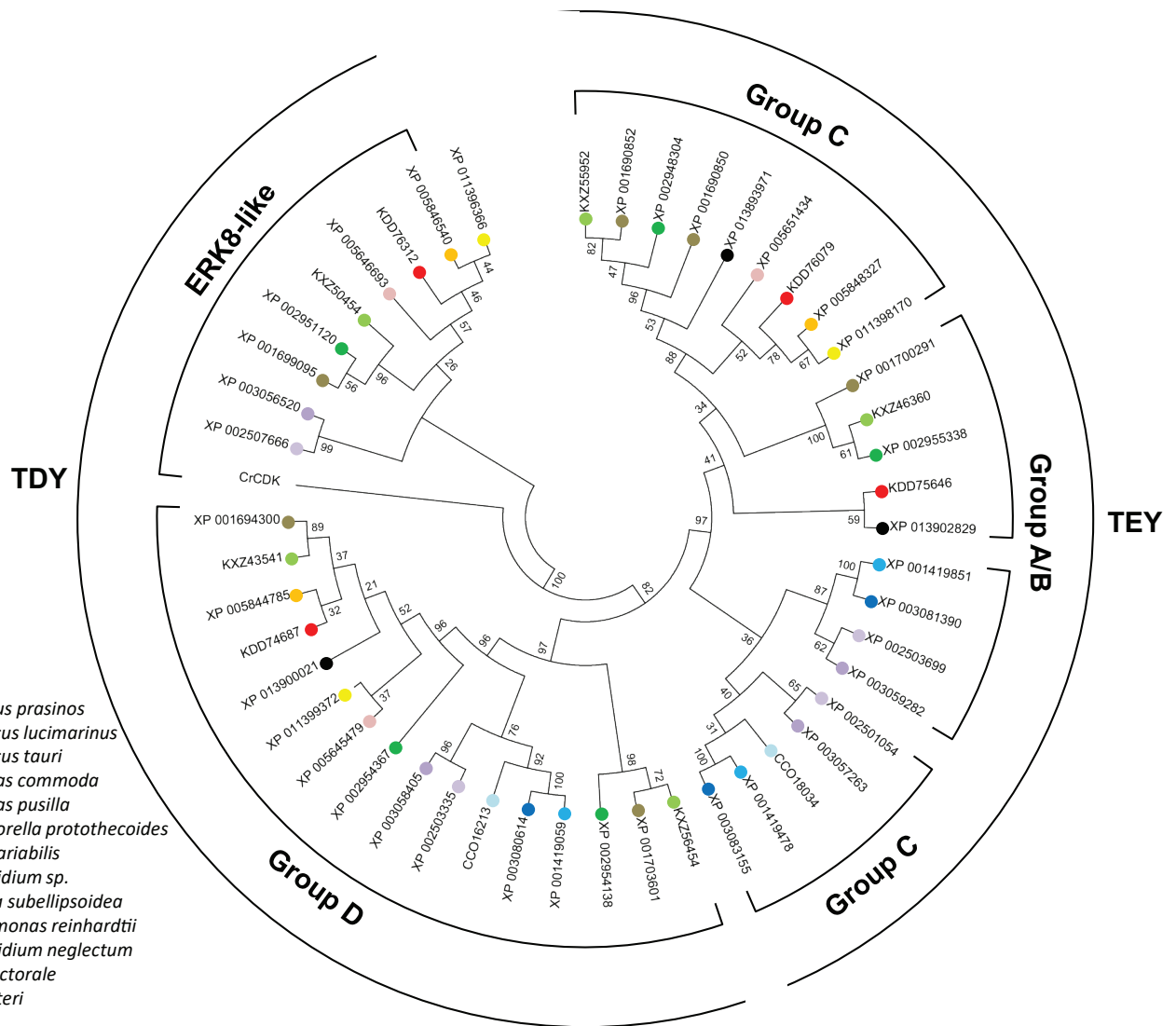


Figure S3

Phylogenetic relationships of MAPK nucleotide sequences in 13 algal species. Full-length nucleotide sequences of the 48 identified MAPKs (Table 2) were aligned by the MUSCLE alignment method and the phylogenetic tree was constructed by the Maximum-likelihood method using the MEGA6 software package. A cyclin-dependent kinase (CDKG1) of *Chlamydomonas reinhardtii* was used as outgroup. Species are indicated by the protein IDs in the tree with coloured dots according to the key on the left. Phylogenetic grouping and the type of the conserved phosphorylation site (TxY) are indicated at the inner and outer perimeters, respectively.

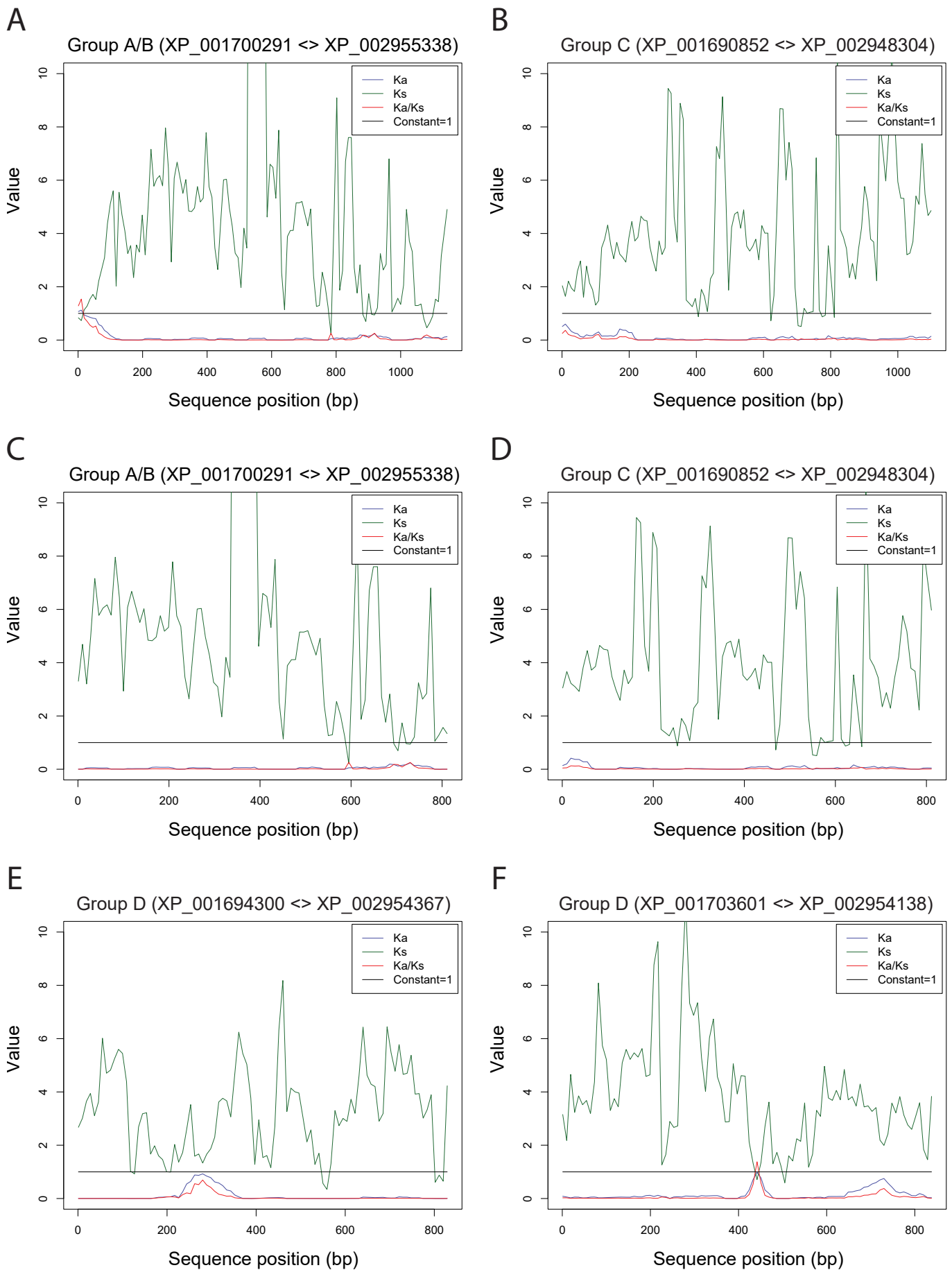


Figure S4

Sliding window analysis of sequence divergence between MAPKs in *C. reinhardtii* and *V. carteri*.

Full-length (A-B) and kinase domain encoding (C-F) nucleotide sequences of the indicated orthologous MAPKs were used at 51-bp window size and 9-bp step size settings for sliding window analysis. The Ka/Ks plots are indicated by red lines.

Table S1

Ka/Ks values of the 48 full-length MAPK sequences. Ka/Ks values were calculated for all pairwise combinations of the 48 algal MAPK nucleotide sequences by generating 1175 pairwise alignments using the MUSCLE method of the MEGA6 software package. AXT format files were subsequently prepared using a self-made script. Ka/Ks values were calculated with the KaKs_Calculator2.0 software using the Model Average (MA) method. Ka/Ks values below 1.0 indicate purifying selection.

Table S2

Ka/Ks values of the 48 MAPK kinase domain sequences. Ka/Ks values were calculated for all pairwise combinations of the 48 algal MAPK kinase domain encoding nucleotide sequences by generating 1175 pairwise alignments using the MUSCLE method of the MEGA6 software package. AXT format files were subsequently prepared using a self-made script. Ka/Ks values were calculated with the KaKs_Calculator2.0 software using the Model Average (MA) method. Ka/Ks values below 1.0 indicate purifying selection.