I

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UBZ25883 Fusarium mangiferae mitovirus 4                          225 NGKLGIVEDPESKRRVIAMVDYHSQLALRKIHNGLLKLL-SNLECDRTFTQD 275
TeRV-2 ★                                                          186 LGKLSIVKDPECKRRVIAMLDYNSQLLLRPIHDALLRSL-TKLPCDRTYTQD 236
UPW42092 Beijing sediment mito like virus 9                       270 LGKTSIIKDPELKRRVIAMLDYNSQMLLRPIHDDLLKLL-SKLPQDRTYTQD 320
QIR30232 Plasmopara viticola lesion associated mitovirus 9        187 LGKLSIIKDPELKRRVIAMLDYNSQLLLRPIHDDLLKNL-SKLSQDRTFTQN 237
USR10271 Fusarium graminearum mitovirus 1                         237 LGKLSIHDPELKRRVIAMLDYNSQLLLRPIHDNLLKLL-NKFSQDRTYTQN 287
YP 009553599 Leptosphaeria biglobosa mitovirus 1                  234 LGKLSVVHDPELKERVIAMVDYTTQFTLKRPHNQLLKLL-SKFDCDRTFTQD 284
NP 660179 Ophiostoma mitovirus 4                                  226 LGKLSIVHDPELKERVIAMVDYTTQFALRPIHNILLNNL-SKLPCDRTFTQD 276
TeRV-1 ★                                                          226 IGKLSVVHDPELKERVIAMVDYTTQFILRPIHDDLLSLL-KKLPCDRTFTQS 276
TpRV-1 ★                                                          226 IGKLSVVHDPELKERVIAMVDYTTQFILRPIHDDLLSLL-KKLPCDRTFTQS 276
CpRV-1 ★                                                          275 LGKLSLKWEPG-KIRVFAMVDSFSQWVLKPLHLAIFDWLRGIKTCDGTFDQV 325
TeRV-3 ★                                                          249 IGMLSIKEEPG-KLRVFAMLDVVTQWLLHPLHKAIFKFV-SIFKYDATFDQQ 298
UYL95348 Chuzhou Mitov tick virus 1                               244 IGKLAAKEEPG-KVRIFAMVDAVTQWLLKPLHQAIFKLL-GIFSFDGTFDQI 293
YP 010798868 Ophiostoma mitovirus 1a                              245 IGKLAAKEEPG-KVRIFAMVDAVTQWLLKPLHEAIFKLL-NIFAFDGTFDQI 294
                                                                      *   .    :*  *   *:::**:*  :*  *:  :*  ::   :      *  *:  *
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II

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UBZ25883 Fusarium mangiferae mitovirus 4                          289 HSLDLSSATDRFPVKLQARLLGEIYRDYRFAQNWLELLLNR 329
TeRV-2 ★                                                          249 WSLDLSAATDRFPIKLQTKVISSIFGDRAFAEAWEKILIDR 289
UPW42092 Beijing sediment mito like virus 9                       333 WSLDLTAATDRFPIDLQEKVISTIYKNREFASAWRNILVDR 373
QIR30232 Plasmopara viticola lesion associated mitovirus 9        250 WSLDLSSATDRFPIDLQVKVISAMYNNRDFANAWKSILIER 290
USR10271 Fusarium graminearum mitovirus 1                         301 WSLDLSAATDRFPISLQVKLLSAIYNDRVFAETWQKILVNR 341
YP 009553599 Leptosphaeria biglobosa mitovirus 1                  297 HSLDLSAATDRFPIDLQEKLLSYIYDDKDFAKSWRNLLVDR 337
NP 660179 Ophiostoma mitovirus 4                                  290 HSLDLSAATDRFPIFLQQKLISLIFNDYEFGKNWRNLLVDR 330
TeRV-1 ★                                                          289 YSLDLSSATDRFPIQLQQKLIALIYKDYEFARNWKDLLVDR 329
TpRV-1 ★                                                          289 YSLDLSSATDRFPIQLQQKLIALIYKDYEFARNWKDLLVDR 329
CpRV-1 ★                                                          344 FSYDLSAATDRLPLRIQEIILNNMIQGKQFGTHWATFLTDR 384
TeRV-3 ★                                                          314 WSFDLSAATDRLPIELQEQVLATFV-NPTFASAWRTILVER 353
UYL95348 Chuzhou Mitov tick virus 1                               311 YSFDLSAATDRLPLSIQISILKHLV-SVPFSEAWGRLLVGR 350
YP 010798868 Ophiostoma mitovirus 1a                              312 YSFDLSAATDRLPLSIQILILKHLV-SVPFSEAWGRLLTGR 351
                                                                      *  **::****:*: :*  ::  :  :  *.  *   :*  *
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III   IV   V   VI

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UBZ25883 Fusarium mangiferae mitovirus 4               340 RYSVGQPMGAYSSWAAFTLTHHLVVAWSA 368   374 GFNQYIILGDDIVI 387   404 MGVDISLPKTHVS 416   420 YEFAKR 425
TeRV-2 ★                                               299 RYSVGQPMGAYSSWAAFTVSHHLVVAWCA 327   334 DFNDYIILGDDIVI 347   362 LGVDISLNKTHTS 374   378 YEFAKR 383
UPW42092 Beijing sediment mito like virus 9            384 KYYSVGQPMGAYSSWAFTLTHHLVVAWA 412   419 NFKDYIILGDDIVI 432   447 LGVNISSQKTHVS 459   263 YEFAKR 268
QIR30232 Plasmopara viticola lesion associated mitovirus 9  300 TYRVGQPMGAYSSWGAFTLSHHLVVAWCA 328   335 DFKDYIILGDDIVI 348   363 LGVDISLNKTHIS 375   279 YEFAKR 284
USR10271 Fusarium graminearum mitovirus 1              351 CYSVGQPMGAYSSWAAFTITHHLVVQWAA 379   386 NFKKYIILGDDIVI 399   414 LGVDISLNKTHVS 426   230 YEFAKR 235
YP 009553599 Leptosphaeria biglobosa mitovirus 1       347 RYSVGQPMGAYSSWAAFTLTHHLVVQWAA 375   387 HFNQYIILGDDIVI 395   410 LGVDISQPKTHVS 422   426 YEFAKR 431
NP 660179 Ophiostoma mitovirus 4                       340 RYSVGQPMGAYTSWAAFTLTHHLVVHWAA 368   375 NFKDYIILGDDIVI 388   403 WGVDISLSKTHVS 415   419 YEFAKR 424
TeRV-1 ★                                               339 RYSVGQPMGAYTSWAAFTLTHHLVVAWAA 367   374 TFHDYIILGDDIVI 387   402 FGVEISVPKTHVS 414   418 YEFAKR 423
TpRV-1 ★                                               339 RYSVGQPMGAYTSWAAFTLTHHLVVAWAA 367   374 TFHDYIILGDDIVI 387   402 FGVEISVPKTHVS 414   418 YEFAKR 423
CpRV-1 ★                                               402 RYITGQPMGAYSSWGMLALTHHILVQLAA 430   439 FFDLYIVLGDDIII 425   468 LKVKINLSKSIVS 480   485 SEYAKK 490
TeRV-3 ★                                               370 QYAVGQPMGAYSSWGMLFDNYIILGDDVVI 398   405 VFDNYIILGDDVVI 418   434 LFVDINLSKGLIS 446   451 LEFAKR 456
UYL95348 Chuzhou Mitov tick virus 1                    363 QYQVGQPMGALSSWGMLALTHHLIVQYAA 392   401 FFKDYIVLGDDIVI 414   430 LKVKINPTKGIMS 442   446 LEFAKR 451
YP 010798868 Ophiostoma mitovirus 1a                   365 QYQVGQPMGALSSWGMLALTHHLVVQYSA 393   402 LFKDYIVLGDDIVI 415   431 LKVKINPTKGIMS 443   448 LEFAKR 453
                                                           *  .****** :**.  :  ::**::*  .*        *.  **:****::*        *.*.  *  *        *:**:
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Fig. S1 Multiple sequence alignment of the RdRp amino acid sequences from the mitoviruses investigated in this study (Indicated by the red stars), and other selected viruses from Mitoviridae. The alignment was generated using Clustal Omega 1.2.1 with default parameters. Conserved residues are denoted by an asterisk (*) and are highlighted in dark blue. Colons (:) and dots (.) signify conserved and semiconserved residues, respectively, and are coloured light blue and grey. There were 6 conserved motifs in total, and these are numbered from I to VI.
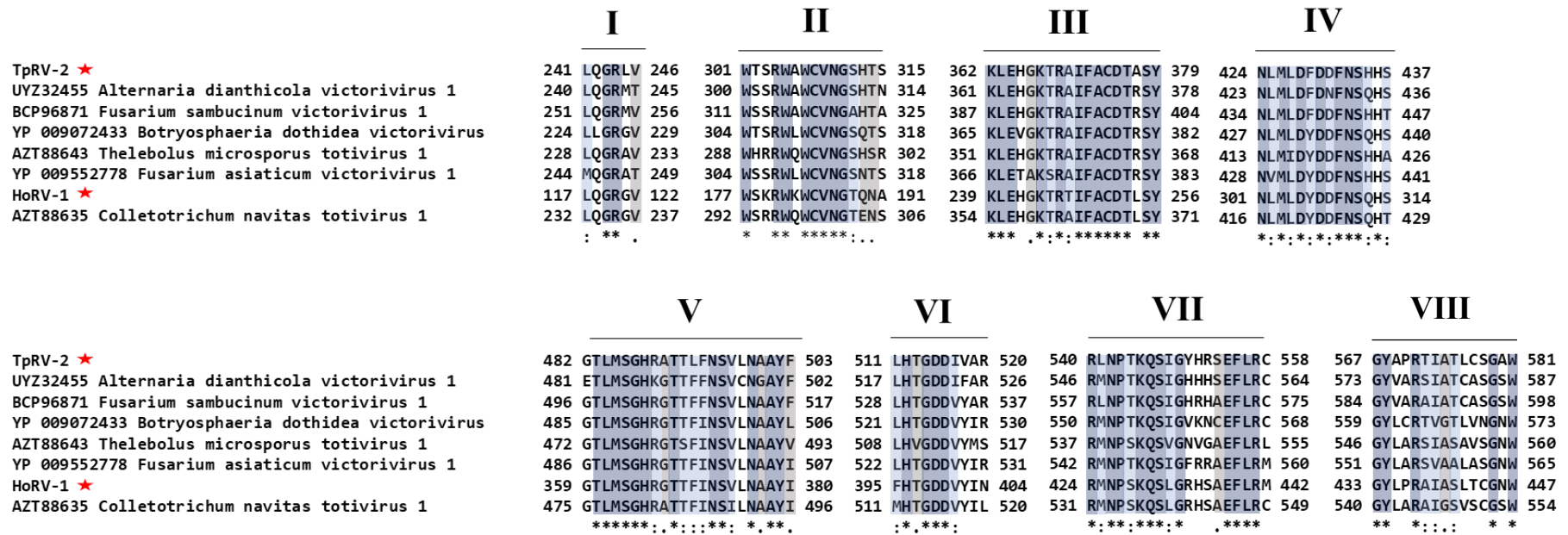
		I		**II**		**III**		**IV**	
TpRV-2 ★	241	LQGRLV	246 301	WTSRWAWCVNGSHTS	315 362	KLEHGKTRAIFACDTASY	379 424	NLMLDFDDFNSHHS	437
UYZ32455 Alternaria dianthicola victorivirus 1	240	LQGRMT	245 300	WSSRWAWCVNGSHTN	314 361	KLEHGKTRAIFACDTRSY	378 423	NLMLDFDNFNSQHS	436
BCP96871 Fusarium sambucinum victorivirus 1	251	LQGRMV	256 311	WSSRWAWCVNGAHTA	325 387	KLEHGKTRAIFACDTRSY	404 434	NLMLDFDDFNSHHT	447
YP 009072433 Botryosphaeria dothidea victorivirus	224	LLGRGV	229 304	WTSRWLWCVNGSQTS	318 365	KLEVGKTRAIFACDTRSY	382 427	NLMLDYDDFNSQHS	440
AZT88643 Thelebolus microsporus totivirus 1	228	LQGRAV	233 288	WHRRWQWCVNGSHSR	302 351	KLEHGKTRAIFACDTRSY	368 413	NLMIDYDDFNSHHA	426
YP 009552778 Fusarium asiaticum victorivirus 1	244	MQGRAT	249 304	WSSRWLWCVNGSNTS	318 366	KLETAKSRAIFACDTRSY	383 428	NVMLDYDDFNSHHS	441
HoRV-1 ★	117	LQGRGV	122 177	WSKRWKWCVNGTQNA	191 239	KLEHGKTRTIFACDTLSY	256 301	NLMLDYDDFNSQHS	314
AZT88635 Colletotrichum navitas totivirus 1	232	LQGRGV	237 292	WSRRWQWCVNGTENS	306 354	KLEHGKTRAIFACDTLSY	371 416	NLMLDYDDFNSQHT	429
		: ** .		* ** *****:..		*** .*:*:******* **		*:*:*:*:***:*:	

		V		**VI**		**VII**		**VIII**	
TpRV-2 ★	482	GTLMSGHRATTLFNSVLNAAYF	503 511	LHTGDDIVAR	520 540	RLNPTKQSIGYHRSEFLRC	558 567	GYAPRTIATLCSGAW	581
UYZ32455 Alternaria dianthicola victorivirus 1	481	ETLMSGHKGTTFFNSVCNGAYF	502 517	LHTGDDIFAR	526 546	RMNPTKQSIGHHHSEFLRC	564 573	GYVARSIATCASGSW	587
BCP96871 Fusarium sambucinum victorivirus 1	496	GTLMSGHRGTTFFNSVLNAAYF	517 528	LHTGDDVYAR	537 557	RLNPTKQSIGHRHAEFLRC	575 584	GYVARAIATCASGSW	598
YP 009072433 Botryosphaeria dothidea victorivirus	485	GTLMSGHRGTTFINSVLNAAYL	506 521	LHTGDDVYIR	530 550	RMNPTKQSIGVKNCEFLRC	568 559	GYLCRTVGTLVNGNW	573
AZT88643 Thelebolus microsporus totivirus 1	472	GTLMSGHRGTSFINSVLNAAYV	493 508	LHVGDDVYMS	517 537	RMNPSKQSVGNVGAEFLRL	555 546	GYLARSIASAVSGNW	560
YP 009552778 Fusarium asiaticum victorivirus 1	486	GTLMSGHRGTTFINSVLNAAYI	507 522	LHTGDDVYIR	531 542	RMNPTKQSIGFRRAEFLRM	560 551	GYLARSVAALASGNW	565
HoRV-1 ★	359	GTLMSGHRGTTFINSVLNAAYI	380 395	FHTGDDVYIN	404 424	RMNPSKQSLGRHSAEFLRM	442 433	GYLPRAIASLTCGNW	447
AZT88635 Colletotrichum navitas totivirus 1	475	GTLMSGHRATTFINSILNAAYI	496 511	MHTGDDVYIL	520 531	RMNPSKQSLGRHSAEFLRC	549 540	GYLARAIGSVSCGSW	554
		******:.*::.**: *.**.		:*.***:		*:**:***:* .****		** *:::. * *	

Fig. S2 Multiple sequence alignment of the RdRp amino acid Sequences from the totiviruses investigated in this study (Indicated by the red stars), and other selected viruses from the genus Victorivirus. The alignment was generated using Clustal Omega 1.2.1 with default parameters. Conserved residues are denoted by an asterisk (*) and are highlighted in dark blue. Colons (:) and dots (.) signify conserved and semi-conserved residues, respectively, and are coloured light blue and grey. There were 8 conserved motifs in total, and these are numbered from I to VIII.