

This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: TITLE, AUTHORS, etc

Code assigned:	2012.022aP			(to be completed by ICTV officers)			
Short title: create three new species in the genus <i>Carlavirus</i> , family <i>Betaflexiviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)							
Modules attached (modules 1 and 9 are required)		1 ⊠ 6 □	2 × 7 □	3	4 □ 9 ⊠	5 🗌	
Author(s) with e-mail address	ss(es) of the pr	oposer:					
John Hammond; john.hammor	John Hammond; john.hammond@ars.usda.gov						
List the ICTV study group(s) that have seen this proposal:							
A list of study groups and contact http://www.ictvonline.org/subcom in doubt, contact the appropriate chair (fungal, invertebrate, plant, vertebrate viruses)	mittees.asp . If subcommittee	(Flexiv	irus study	group)			
ICTV-EC or Study Group comments and response of the proposer:							
Date first submitted to ICTV: Date of this revision (if differe	ent to above):						

MODULE 2: NEW SPECIES

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	<i>201.</i>	2.022aP	(assigned by ICTV off	icers)	
To create	e 3 ne	ew species within:			
Subfan	nus: nily:	Carlavirus Betaflexiviridae	•	I in all that apply. If the higher taxon has yet to be created (in a later module, below) write (new)" after its proposed name.	
	rder:	Tymovirales	If no genus is specified, enter"unassigned" in the genus box.		
And nam	ne the	new species:		GenBank sequence accession number(s) of reference isolate:	
Phlox vir	rus S			EF492068 (NC_009383)	
Phlox virus B			EU162589 (NC_009991)		
Phlox vir	rus M			EF507476	

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
 - o If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
 - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Phlox virus S

Phlox virus S (PhlVS) was initially isolated from *Phlox stolonifera* (*Polemoniaceae*), and is readily mechanically transmissible, having an experimental host range including systemic infection of *Phlox drummondii* (*Polemoniaceae*), *Nicotiana benthamiana*, *N. clevelandii*, *N. edwardsonii*, *N. megalosiphon*, and *N. debneyi* (*Solanaceae*), and (local infection only) three species in the *Scrophulariaceae* (*Verbascum phoeniceum*, *Antirrhinum majus*, and *Digitalis purpurea*), *Nicotiana glutinosa*, and *Polemonium caeruleum* (*Polemoniaceae*).

The full genome of PhIVS has been sequenced (EF492068; NC_009383), and consists of 8590 nt not including the poly(A) tail. The genome organization (Polymerase, Triple gene block, Coat protein, and Nucleic acid binding protein) is typical of the genus Carlavirus. Full genome analysis using the PASC tool at NCBI

(http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=overview) reveals a 51.5-53.56% match to four isolates of *Chrysanthemum virus B* (CVB) by BLAST-based alignment, and 61.03-62.79% to *Chrysanthemum virus B* by global alignment, with lower degrees of identity to other species of the genus *Carlavirus*. These include Phlox virus B (51.2% by BLAST-based alignment; 60.6% by global alignment).

Pairwise comparisons of the polymerase and coat protein amino acid sequences reveal identities of 54% or less for the polymerase, and 62% or less for the coat protein. These values are lower than the species demarcation value of <80% identity. Nucleotide sequence comparisons revealed maximum identities of 69% (CVB) or 73% (PhlVB) over only portions (less than 2.5 Kb of the c.6 Kb polymerase gene) of the equivalent polymerase sequences of other carlaviruses; and 68% (*Helenium virus S*, HelVS), 67% (CVB), 71% (*Cowpea mild mottle virus*, CpMMV), and 71% (*Daphne virus S*, DVS) over the core region of the coat protein nucleotide sequence. The overall nucleotide identities of the complete polymerase and coat protein genes are clearly lower than the 72% identity established for demarcation between carlavirus species.

A Neighbor-joining phylogenetic tree of all available carlavirus CP sequences groups PhlVS with HelVS, PhlVB, CVB, and DVS, at equivalent distance to other distinct carlavirus sequences; a similarly constructed tree of polymerase sequences groups PhlVS, PhlVB, CVB, and DVS, consistent with each being distinct species (see Appendix).

Serological cross-reactivity was detected with antisera prepared against *Potato virus S* (PVS) and *Kalanchoe latent virus* (KLV); no serological reactivity to PhlVS was detected with antisera against *Blueberry scorch virus* (BlScV), *Carnation latent virus* (CLV), CVB, *Garlic common latent virus* (GCLV), *Lily symptomless virus* (LSV), *Potato virus M* (PVM), or *Shallot latent virus* (ShLV). Comparison of aligned sequences revealed that PhlVS, PVS, and KLV share a conserved five residue sequence (PKPDP) close to the coat protein N-terminus, which differs from CVB (PKPAP) by a single residue, and which is not present in other carlavirus coat protein sequences examined. A polyclonal antiserum raised against purified PhlVS has been used to specifically detect PhlVS in infected plants by ELISA.

Electron microscopy reveals particles of typical carlavirus morphology, of mean length c. 640nm.

Phlox virus B

Phlox virus B (PhlVB) was initially detected in *Phlox divaricata*, and is mechanically transmitted with some difficulty to a very limited host range, including *Phlox drummondii* (*Polemoniaceae*) and *Digitalis purpurea* (*Scrophulariaceae*).

The full genome of PhlVB has been sequenced (EU162589; NC_009991), and consists of 9058 nt not including the poly(A) tail. The genome organization (Polymerase, Triple gene block, Coat protein, and Nucleic acid binding protein) is typical of the genus Carlavirus. Full genome analysis using the PASC tool at NCBI

(http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=overview) reveals a 51.2% match to PhlVS, 48.9% to DVS, 48.33-48.69% match to five isolates of CVB, and lower matches to other carlaviruses by BLAST-based alignments; and 60.58% to PhlVS, 58.96-60.29% to five isolates of CVB, 59.11% to DVS, and lower matches to other carlaviruses by global alignment.

Pairwise comparisons of the polymerase and coat protein amino acid sequences reveal identities of 55% or less for the polymerase, and 67% or less for the coat protein. These values are lower than the species demarcation value of <80% identity. Nucleotide sequence comparisons revealed maximum identities of 67-77% (CVB) or 73% (PhlVS) over only portions (less than 2.5 Kb of the c.6 Kb polymerase gene) of the equivalent polymerase sequences of other carlaviruses; and 69-72% (DVS), 67-70% (CVB), 71% (PhlVM), and 70%

(PhlVS) over the core region of the coat protein nucleotide sequence. The overall nucleotide identities of the complete polymerase and coat protein genes are clearly lower than the 72% identity established for demarcation between carlavirus species.

A Neighbor-joining phylogenetic tree of all available carlavirus CP sequences groups PhlVB with CVB, PhlVS, HelVS, and DVS, at equivalent distance to other distinct carlavirus sequences; a similarly constructed tree of polymerase sequences groups PhlVB, PhlVS, CVB, and DVS, consistent with each being distinct species (see Appendix).

No serological reactivity to PhIVB was detected with antisera against PhIVS, PVS, KLV, BIScV, CLV, CVB, GCLV, LSV, PVM, or ShLV.

Electron microscopy reveals particles of typical carlavirus morphology.

Phlox virus M

Phlox virus M (PhlVM) was initially detected in an annual phlox hybrid, and has been mechanically transmitted only to *Phlox drummondii*.

To date the partial Triple gene block 2 (TGB2), TGB3, Coat protein, Nucleic acid binding protein, and 3' non-coding region of one isolate (EF507476), and the TGB3, Coat protein, Nucleic acid binding protein, and 3' non-coding region of a second isolate (FJ159381) have been sequenced.

Analysis using the PASC tool at NCBI

(http://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi?textpage=overview) reveals highest identity of the partial genome to *Potato virus M* (PVM), *Narcissus common latent virus* (NCLV), and *Aconitum latent virus* (AcLV) by BLAST-based alignments; and to PVM, NCLV, and *Hop latent virus* (HpLV) by global alignment.

Pairwise (BLAST) comparisons of the coat protein amino acid sequence reveals identities of 80-83% (PVM), 72-77% (NCLV), 73% (AcLV), 64-70% (HpLV) or less; and nucleotide identities of 72-75% (PVM), 70-71% (NCLV), 71% (AcLV) and 70% (HpLV) over aligned (incomplete) regions. Whereas these values fall close to the 80% (amino acid) and 72% (nucleotide) demarcation values for carlavirus species, the most closely related species (PVM) has a much wider host range, being readily transmissible to tomato. Pairwise alignment of PhlVM and PVM using ALIGN (Myers and Miller, CABIOS, 1999, 4:11-17) reveals 71.7% amino acid identity and 67.2% nucleotide identity over the full CP sequence, consistent with PhlVM being a distinct species; there is minimal amino acid identity within the CP N-terminal domain (see Appendix).

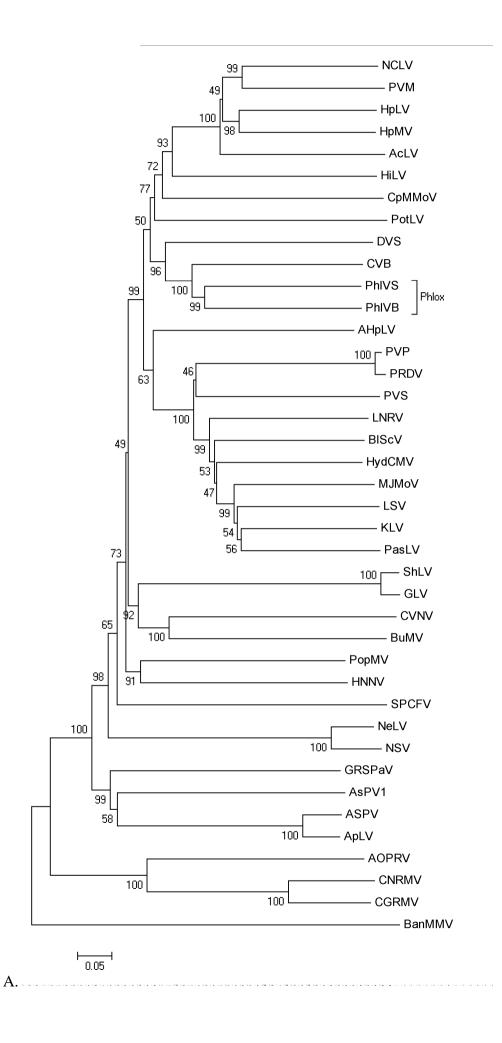
A Neighbor-joining phylogenetic tree of all available carlavirus CP sequences groups PhlVM most closely with AcLV, PVM, and NCLV, at equivalent distance as other distinct species (see Appendix).

Electron microscopy reveals particles of typical carlavirus morphology.

MODULE 9: APPENDIX: supporting material

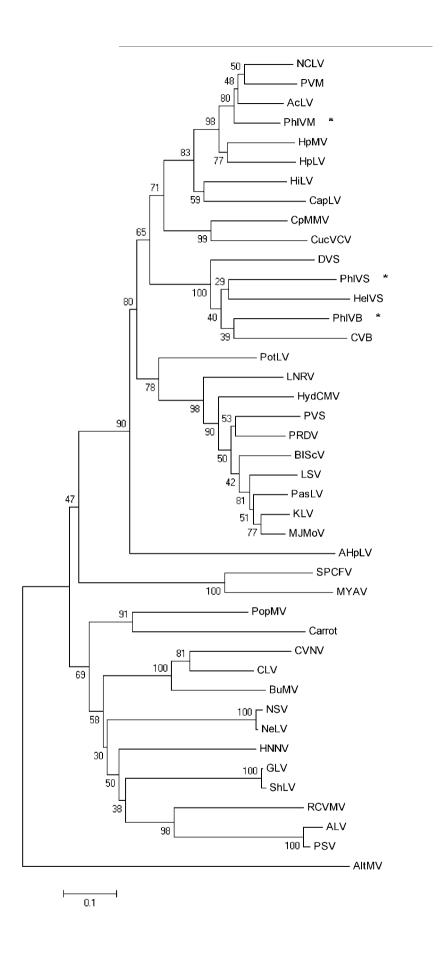
A) Neighbor-joining tree of Carlavirus polymerase amino acid sequences; the position of PhlVS and PhlVB RdRp in the tree are indicated by a bracket.

Virus abbreviations and accession numbers from which sequences were utilized: AcLV = Aconitum latent virus, NC002795; AHpLV = American hop latent virus, NC017859; AOPRV = African oil palm ringspot virus (unassigned), NC017859; ApLV = Apricot latent virus (Foveavirus), NC014821; ASPV = Apple stem pitting virus (Foveavirus), NC003462; AsPV1 = Asian prunus virus 1 (proposed Foveavirus), FJ824737; BanMMV = Banana mild mosaic virus (unassigned); BlScV = Blueberry scorch virus, NC003499; BuMV = Butterbur mosaic virus, NC013527; CGRMV = Cherry green ring mottle virus (unassigned), NC001946; CNRMV = Cherry necrotic rusty mottle virus (unassigned), NC002468; CpMMV = Cowpea mild mottle virus, NC014730; CVB = Chrysanthemum virus B, NC009087; CVNV = Coleus vein necrosis virus, NC009764; DVS = Daphne virus S, NC008020; GLV = Garlic latent virus, NC003557; GRSPaV = Grapevine rupestris stem pitting-associated virus (Foveavirus), NC_001948; HiLV = Hippeastrum latent virus, NC011540; HNNV = Helleborus net necrosis virus, NC012038; HpLV = Hop latent virus, NC002552; HpMV = Hop mosaic virus, NC010538; HydCMV = Hydrangea chlorotic mottle virus, NC012869; KLV = Kalanchoe latent virus, NC013006; LNRV = Ligustrum necrotic ringspot virus, NC010305; LSV = Lily symptomless virus, NC005138; MJMoV = Mirabilis jalapa mottle virus, NC016080; NCLV = Narcissus common latent virus, NC008266; NeLV = Nerine latent virus, JQ395043; NSV = Narcissus symptomless virus, NC008552; PasLV = Passiflora latent virus, NC008292; PhlVB = Phlox virus B, NC009991; PhlVS = Phlox virus S, NC009383; PopMV = Poplar mosaic virus, NC005343: PotLV = Potato latent virus, NC011525: PRDV = Potato rough dwarf virus, NC009759; PVM = Potato virus M, NC001361; PVP = Potato virus P, EU338239; PVS = Potato virus S, NC007289; ShLV = Shallot latent virus, JF320811; SPCFV = Sweet potato chlorotic fleck virus, NC006550.



B) Neighbor-joining tree of Carlavirus coat protein amino acid sequences; the positions of PhIVS, PhIVB, and PhIVM CP in the tree are indicated by aaterisks (*).

Virus abbreviations and accession numbers from which sequences were utilized: AcLV = Aconitum latent virus, NC002795; AHpLV = American hop latent virus, NC017859; ALV = Alfalfa latent virus, AY037925; AltMV = Alternanthera mosaic virus (Potexvirus, outgroup), NC007731; BIScV = Blueberry scorch virus, NC003499; BuMV = Butterbur mosaic virus, NC013527; CapLV = Caper latent virus, HQ588148; Carrot = carrot carlavirus, EU881919; CLV = Carnation latent virus, X52627; CpMMV = Cowpea mild mottle virus, NC014730; CucVCV = Cucumber vein-clearing virus, JN591720; CVB = Chrysanthemum virus B, NC009087; CVNV = Coleus vein necrosis virus, NC009764; DVS = Daphne virus S. NC008020; GLV = Garlic latent virus, NC003557; HelVS = Helenium virus S, D10454; HiLV = Hippeastrum latent virus, NC011540; HNNV = Helleborus net necrosis virus, NC012038; HpLV = Hop latent virus, NC002552; HpMV = Hop mosaic virus, NC010538; HydCMV = Hydrangea chlorotic mottle virus, NC012869; KLV = Kalanchoe latent virus, NC013006; LNRV = Ligustrum necrotic ringspot virus, NC010305; LSV = Lily symptomless virus, NC005138; MJMoV = Mirabilis jalapa mottle virus, NC016080; MYAV = Melon yellowing associated virus, AB510477; NCLV = Narcissus common latent virus, NC008266; NeLV = Nerine latent virus, JQ395043; NSV = Narcissus symptomless virus, NC008552; PasLV = Passiflora latent virus, NC008292; PhlVB = Phlox virus B, NC009991; PhlVM = Phlox virus M, EF507476; PhlVS = Phlox virus S, NC009383; PopMV = Poplar mosaic virus, NC005343; PotLV = Potato latent virus, NC011525; PRDV = Potato rough dwarf virus, NC009759; PSV = Pea streak virus, AF354652; PVM = Potato virus M, NC001361; PVS = Potato virus S, NC007289; RCVMV = Red clover vein mosaic virus, NC012210; ShLV = Shallot latent virus, JF320811; SPCFV = Sweet potato chlorotic fleck virus, NC006550.



C) Phlox virus S host range:

Species	Local/Systemic	Symptoms
Phlox stolonifera	+/+	Mosaic*
Nicotiana benthamiana	+/+	CLL/Chl or bronze mos.
N. clevelandii	+/+	CLL/Chl or bronze mos
N. edwardsonii	+/+	CLL/Chl or bronze mos
N. megalosiphon	+/+	CLL/Chl or bronze mos
N. debneyi	+/+	CLL/Chl or bronze mos
N. glutinosa	+/-	CLL
N. tabacum	-/-	
N. sylvestris	-/-	
Verbascum phoeniceum	+/-	Symptomless
Antirrhinum majus	+/-	CLL
Polemonium caeruleum	+/-	Symptomless
Digitalis purpurea	+/-	Symptomless
Aquilegia hybrid	-/-	
Consolida ambigua	-/-	
Phlox drummondii	+/+	No clear symptoms

^{*}Note that initial infection was mixed with *Alternanthera mosaic virus* and *Tobacco ringspot virus*.

D) Phlox virus B host range

Species	Local/Systemic	Symptoms
Phlox divaricata	+/+	Mosaic
Digitalis purpurea	+/+	Mild mottle

E) Phlox virus M host range

Species

•			•		J	
Annual Phlox hy	brid		+/+		Mosaic	
Phlox drummond	lii		+/+		Mosaic	
F) Pairwise comsequences	F) Pairwise comparison of Phlox virus M (PhlVM) and Potato virus M (PVM) CP amino acid sequences					
ALIGN calculation version 2.0	tes a globa	al alignm	ent of two	sequences		
Please cite: 1	Myers and I	Miller, C	ABIOS (198	9) 4:11-17		
PhlVM_CP PVM_CP scoring matri: 71.7% identity				12/-2	302 aa vs. 304 aa	
PhlVM_CP		DAQKKEQLK				50 NMTEEEATLEQR
PVM_CP		VAKEAGTSQ	AAKGNRPLPT	'AAEFEGDDNS	: GDASVRDAEAI 40	: :::.::: NEEASLERR 50
PhlVM_CP PVM_CP	:::::::	:::::	::::::::	::::::::	::::::::	110 DELSKIMPRAIS .::::::: EALSRIKPIAVS 110
PhlVM_CP PVM_CP	:::::::	:::::::	LGVPTEQVQK :::::::::::::::::::::::::::::::::::	.:::::::	DASSSAYLDPI	170 RGSFEWPGGAIT :::::: ::: RGSFEWPRGAIT 170
PhlVM_CP PVM_CP	::::::::	. : : : : : : :	::::::::	YMLTHNAPPS	DWAAMGFQYE	230 DRFAAFDCFDYV ::::::::: DRFAAFDCFDYV 230
PhlVM_CP PVM_CP	:.:::::	:::::::	::::::	:.:::::	:.::.	290 TGGKNGPELTRD ::::::::: TGGMNGPELTRD 290
PhlVM_CP PVM_CP	300 YRKSNNQ .:::: FSKSNNK 300					

Local/Systemic

Symptoms

G) Pairwise comparison of Phlox virus M (PhlVM) and Potato virus M (PVM) CP nucleotide sequences

ALIGN calcula version 2.0u PhlVM-IC_CP PVM-Ru_CP scoring matri 67.2% identit	Please of x: DNA,	cite: Mye:	rs and Mil	ler, CABIOS /-4	909 nt v 915 nt		
	7 ECC 7 C			30			מ מ מ חחיי
PhlVM-IC_CP	::::	: ::	::: ::: :	:: :: ::	: :: :: :	: : :::	:
PVM-Ru_CP	A'I'GGGA(JATTCAACGA 10	AAGAAAGCTG. 20	30	40	50	GAA-AG
D. 1474 TO OD	60	70	80	90		100	110
PhlVM-IC_CP				CGTACTAAC			
PVM-Ru_CP		AGCGCGACC	ACTGCCGACT		TGAGGGGAAG	GACACATCO	
		120	130	140	150	160	170
PhlVM-IC_CP	ATTTAT(ACCGAAGAAGA :::::::			
PVM-Ru_CP 1		TGGGCGTGC'	rgcagatg		AATGTCATTG	GAGCGGAGG	
				200			
PhlVM-IC_CP				CGTCGTAGCGC :: ::::			
PVM-Ru_CP				CGGAGGGGCGC 210		ACAAACCCA 230	GGGTT
		240	250	260	270	280	290
PhlVM-IC_CP							
PVM-Ru_CP		TGGCAGGCC	AAGGTTGCAG	:: :: : CTAGCTGAAAA 270	TATGCGCCCT	GATCCCACG	
	210						
PhlVM-IC CP	CTACAA:			320 TTGAGCAAAAT			
_				: ::: :: CTCAGCCGGAI			
	300	310	320	330	340	350	
		360		380			410
PhlVM-IC_CP				CGCATCTACGI :::::::::::			
PVM-Ru_CP	TATGGC0	CACATCTGA 370	GGATATGATG 380	CGCATATATGT 390	GAACCTGGAG 400	GGGCTAGGG 410	GTGCC
		420	430	440	450	460	470
PhlVM-IC_CP				ATACAAGCGGT			
PVM-Ru_CP				ATTCAGGCTGT 450			
	-		ŕ			•	

PhlVM-IC_CP PVM-Ru_CP	
PhlVM-IC_CP PVM-Ru_CP	540 550 560 570 580 590 TGCGGTCTTGGCTGTACTTAAACGTGATGCTGAGACGCTTCGTAGGGTGTGTAGACTGTA :::::::::::::::::::::::::::::::::::
PhlVM-IC_CP PVM-Ru_CP	
PhlVM-IC_CP PVM-Ru_CP	660 670 680 690 700 710 TATGGGTTTCCAATATGAGGATAGATTTGCCGCTTTCGACTGCTTCGACTATGTGGTAAA ::::::::::::::::::::::::::::
PhlVM-IC_CP PVM-Ru_CP	720 730 740 750 760 770 TGCAGCAGCAGTGCAACCTCTTGAAGGTCTCATCCGTAGGCCAACCCCACGGGAGCAGAT : : : : : : : : : : : : : : : : : : :
_	780 790 800 810 820 830 TGCGCACAATACGCATAAGGATCTAGCGCTGCGTGCAGCCAACCGCAATCAGGCTTTTGG ::::::::::::::::::::::::::::::
PhlVM-IC_CP PVM-Ru_CP	840 850 860 870 880 CAACACT-TCAACTGAGATTACGGGGGGGAAAGAATGGTCCTGAGCTCACTAGAGATTA ::::::::::::::::::::::::::::::
PhlVM-IC_CP	890 900 TAGAAAGTCGAACAATCAATGA

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.