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:	***2012.007aV***	(to be completed by ICTV officers)

Short title: In the genus *Alphavirus*, create a species named *Madariaga virus* comprising some of the virus strains currently classified in the species *Eastern equine encephalitis virus*

(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)	**1** ☒	**2** ☒	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☒	

Author(s) with e-mail address(es) of the proposer:

Nicole C. Arrigo (corresponding author and chair of the ICTV Togaviridae Study Group; ncarrigo@gmail.com)

Scott C. Weaver (co-corresponding author and member of the ICTV Togaviridae Study Group; sweaver@utmb.edu)

List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	Togaviridae Study Group Nicole C. Arrigo, Chair ncarrigo@gmail.com

ICTV-EC or Study Group comments and response of the proposer:

Date first submitted to ICTV: June 2012
Date of this revision (if different 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.

Code	***2012.007aV***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Alphavirus***
Subfamily:	
Family:	***Togaviridae***
Order:	***Unassigned***

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Madariaga virus	**GU001936**

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

- Explain how the proposed species differ(s) from all existing species.
  - 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

PROPOSAL OVERVIEW: The proposal to create a new species within the *Alphavirus* genus is based on genetic, pathogenic, epidemiologic and ecologic distinctions between currently-classified Eastern equine encephalitis virus (EEEV) strains of North American origin (United States, Canada and Mexico) and the Western Caribbean islands (Jamaica and Cuba) (NA EEEV, lineage I) and strains of Central/South American origin, including the continental countries and Eastern Caribbean islands (Trinidad & Tobago and Guyana) (SA EEEV, lineages II-IV). EEEV strains isolated from Central/South America and the Eastern Caribbean and belonging to genetic lineages II-IV are proposed for reassignment to a new species, *Madariaga virus* (MADV). The proposed name is based on the geographic origin of the earliest representative virus strain, isolated from General Madariaga Partido, Buenos Aires Province, Argentina, in 1930 (1, 2).

Background on current classification of EEEV and species demarcation within the *Alphavirus* genus:

The current ICTV definition of a virus species is a "polythetic class of viruses that constitute a replicating lineage and occupy a particular ecological niche (3, 4)." This definition acknowledges the contemporary use of genomics to characterize taxonomic relationships; however, it also specifies the use of multiple characteristics to define a virus species. Some of these polythetic criteria may include, but are not limited to, genetic and phylogenetic relationships, geographic distribution, differences in ecology and transmission cycles, pathogenicity, morphology, replication patterns and antigenicity.

Eastern equine encephalitis virus (EEEV) is presently the only species comprising the EEE serocomplex in the *Alphavirus* genus, family *Togaviridae* (Module 9: Appendix, Figure 1). Taxa belonging to the *Alphavirus* genus, and the EEEV species, were originally classified based solely on antigenic properties (5). Two antigenic varieties of EEEV were recognized, North American and South American (6), and later refined to 4 antigenic subtypes that correspond to 4 phylogenetically distinct lineages; one circulating in North America and the Western Caribbean (NA EEEV) and 3 circulating in Central/South America and the Eastern Caribbean (SA EEEV) (Module 9: Appendix, Figures 2 and 3) (7).

Although species demarcation criteria are not officially defined for members of the *Alphavirus* genus, genetic and phylogenetic analysis of the E1 gene of all representative members demonstrated that different species within the same antigenic complex generally show up to 21% nucleotide and 8% amino acid sequence divergence (8). Based on this guideline, genetic divergence data along with evidence for differences in ecological niche and pathogenicity have led to multiple proposals for reconsideration of *Alphavirus* taxonomy, many of which have subsequently been accepted by the ICTV. For example, *Tonate virus* was designated a species unique from *Mucambo virus* within subtype III of the VEE complex based on 16% nucleotide and 7% amino acid sequence divergence, as well as antigenic differences and the use of different reservoir hosts. The distinction of *Mayaro* and *Una virus* species was also supported by recent molecular epidemiological studies, despite their previous conspecific designation based on antigenic relationships (9).

Overview of justification for reclassification of EEEV of North and Central/South American origin:

Recent genetic and evolutionary analyses (10) of the complete structural polyprotein open reading frame of a broad representation of NA and SA EEEV strains provide support for their separation as distinct species based on the genetic divergence observed between alphavirus species in the same antigenic complex. Furthermore, differences in their geographic, epidemiologic, ecologic, pathogenic, genetic, phylogenetic, and evolutionary characteristics fulfill the polythetic criteria to recognize EEEV strains of North and Central/South American origin as distinct alphavirus species. This revision would provide a more medically and scientifically accurate representation of viruses comprising the EEE complex, which has important public health and biosafety implications.

Summary of evidence highlighting differences between NA and SA EEEV and supporting reclassification of EEEV strains from Central/South America as a new alphavirus species:

1. *Genetic and evolutionary patterns*:
    a. Pairwise comparison of structural genome ORF shows nucleotide and amino acid sequence divergence of at least 22.5-23.5% and 8.2-11.2%, respectively (Module 9: Appendix, Table 1), between NA EEEV (lineage I) and SA EEEV (lineages II-IV) (10).
    b. Lineages of NA EEEV and SA EEEV differed equally from their closest alphavirus relative, Venezuealan equine encephalitis virus (VEEV), with 42-43% nucleotide and amino acid sequence divergence from VEEV enzootic subtype I (Module 9: Appendix, Table 1). This equal divergence from VEEV supports SA EEEV's distinction from NA EEEV, as well as other alphaviruses.
    c. NA EEEV forms a single, highly conserved, monophyletic lineage dominated by a temporal pattern of evolution throughout its geographic range. Alternatively, SA EEEV comprises 3 genetically diverse lineages, two of which consist of highly conserved geographic groupings that completely lack temporal associations, and the third consisting of a single virus strain (Module 9: Appendix, Figure 2).
2. *Geographic distribution* (Module 9: Appendix, Figure 3):
    a. EEEV strains isolated from North America (United States, Canada and Mexico) and the Western Caribbean islands (Jamaica and Cuba) comprise genetic lineage I
    b. EEEV strains isolated from Central/South America, including the continental countries and Eastern Caribbean islands (Trinidad & Tobago and Guyana) comprise genetic lineages II-IV

3. *Ecological niche*:
    a. NA EEEV:
        i. The enzootic transmission cycle of NA EEEV primarily involves the ornithophilic mosquito vector, *Culiseta melanura*, and passerine birds in hardwood swamp habitats (Reviewed in 11, 12).
    b. SA EEEV:
        i. Ecological associations and isolations of SA EEEV from *Culex (Melanoconion)* spp. in the Spissipes section suggest that they are the primary enzootic, and potentially epizootic, vectors (13-16) in tropical forest foci in Central/South America.
        ii. Vertebrate usage by SA EEEV is not well described, with serological associations including wild birds, ground-dwelling rodents, marsupials and reptiles (16-22).
4. *Epidemiology*:
    a. Transmission of NA EEEV can result in sporadic outbreaks of severe disease in humans, equids and other domestic animals, including game birds, swine, and dogs that are considered dead-end hosts (12, 24-26). Symptomatic disease is typically severe, leading to case fatality rates between 30-90% for humans and 80% in equids (12).
    b. SA EEEV can result in equine disease with comparable case fatality rates; however, there is historically little to no association with human disease, despite evidence of human exposure in areas of endemic and epizootic activity (27-30). Since its discovery in Argentina in 1930, there have been only 2 documented cases of human neurologic disease associated with SA EEEV (31, 32).
5. *Pathogenicity*:
    a. Differences in mechanisms of pathogenesis, and potentially in tissue tropism, between NA and SA EEEV likely contribute to the overall attenuation of SA EEEV in humans and many other domestic and wild animals. This pattern is in contrast to the severity of disease resulting from NA EEEV infection in humans and most other naturally and experimentally infected animals.
        i. NA EEEV primarily affects the CNS of naturally infected humans and equids, and other domestic and wild animals, resulting in severe and prolonged neurologic sequelae (33-35). Laboratory infection of rodents (35-39), non-human primates (35, 40, 41), and birds (15, 42-43) also demonstrate severe neurologic disease with high mortality rates.
        ii. SA EEEV strains are not typically associated with human neurologic disease; however, they do affect the CNS of naturally infected equids and some experimentally infected animals. In laboratory mice, lineages II-III induce high rates of mortality upon subcutaneous inoculation (44, 45), and one lineage III strain caused fatal disease in guinea pigs exposed to aerosols (37). However, lineage IV is highly attenuated in mice (44, 45), and nearly 100% survival was observed with all SA EEEV strains used in experimental infections of common marmosets (40), adult cotton rats (42), and house sparrow (42).
        iii. SA EEEV replicates more efficiently in lymphoid tissues and other extra-neural tissues than NA EEEV (44, 45).
        iv. SA EEEV induces higher levels of interferon and is more sensitive to interferon than NA EEEV (44, 45).

Practical significance of distinguishing NA and SA EEEV as distinct species:

Perceptions of EEEV are primarily influenced by North American strain characteristics, namely the avian-mosquito transmission cycle, geographic range, highly pathogenic nature resulting in severe human and equine encephalitis and the highly conserved, temporally associated evolutionary pattern. However, the distinct characteristics of SA EEEV are not reflected by this depiction. Considering the goal of classification as a means to enhance the understanding of a virus taxon from multiple perspectives, we recommend designating NA and SA EEEV as separate virus species given their distinct geographic, epidemiologic, ecologic, pathogenic, genetic, phylogenetic, and evolutionary characteristics. This revision, based on polythetic criteria, would provide a more medically and scientifically accurate representation of the viruses comprising the EEE complex, thus facilitating discussions of appropriate biosafety measures and public health efforts.

Because NA EEEV lineage I strains are considered the prototypes, we propose a reassignment of all SA EEEV lineages II-IV strains to a new species called *Madariaga virus* (MADV), based on the location of the earliest strain isolated in 1930 from General Madariaga Partido, Buenos Aires Province, Argentina (1, 2). Madariaga virus would be comprised of three antigenically and genetically distinct subtypes/ lineages, proposed to be designated as MADV subtypes II, III, and IV, which correspond to their former designation as EEEV II, III, and IV. Maintaining the antigenic subtype numbering for MADV and not designating a MADV subtype I will reduce confusion with previous EEEV classification. This numbering also allows for the potential classification of MADV isolates into a newly designated MADV subtype I.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

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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.

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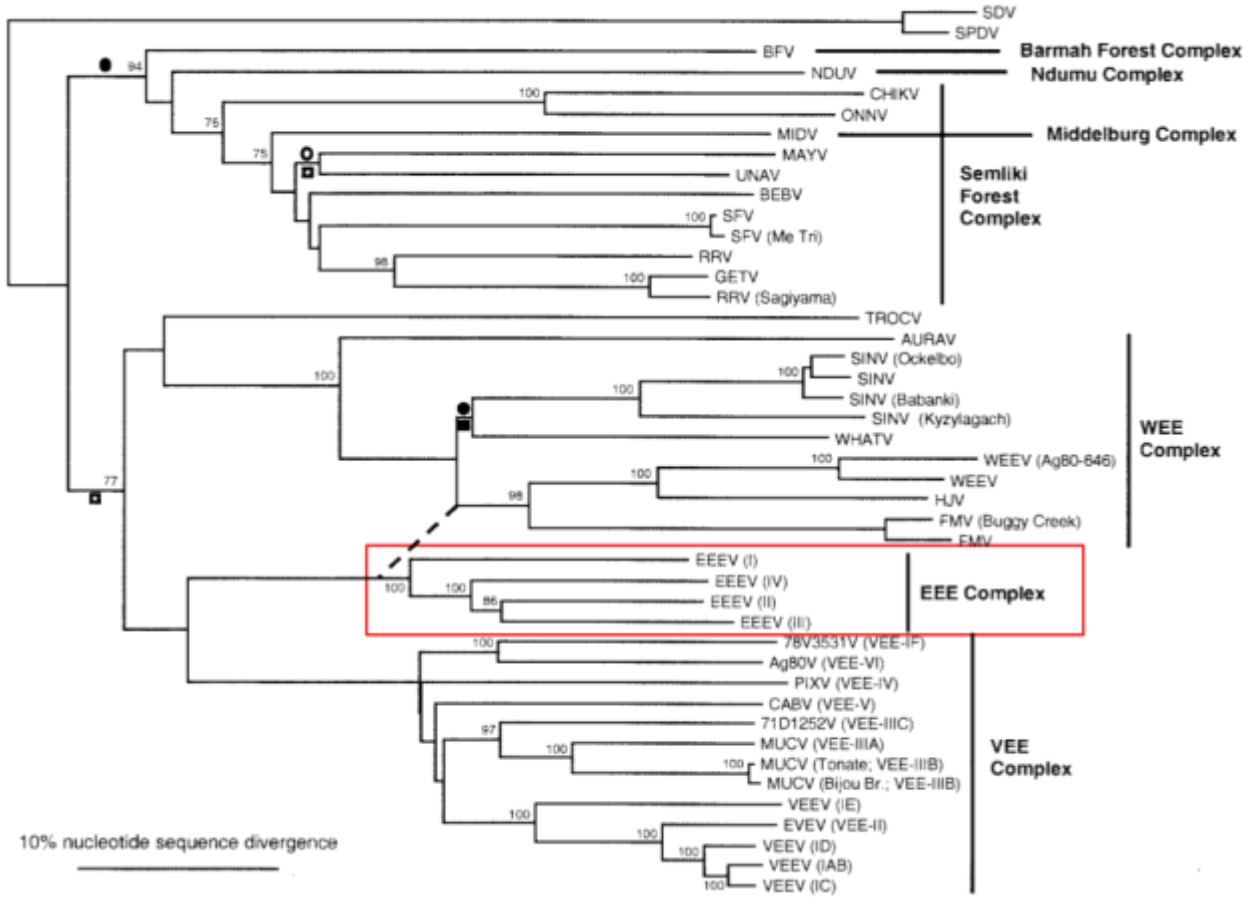

Figure 1. Phylogenetic tree of all *Alphavirus* species generated from partial E1 envelope gylocoprotein gene sequences using neighbor-joining distance method. Scale bar represents a genetic distance of 10% nucleotide divergence. Red box identifies the EEE serocomplex and the 4 distinct genetic lineages comprising the current taxonomic organization of the EEEV species. EEEV lineage I includes only NA EEEV strains and lineages II-IV includes only SA EEEV strains.

Adapted from Powers AM, Brault AC, Shirako Y, Strauss EG, Kang W, Strauss JH, Weaver SC. Evolutionary Relationships and Systematics of the Alphaviruses. Journal of Virology, Nov. 2001, p. 10118–10131 Vol. 75, No. 21, DOI: 10.1128/JVI.75.21.10118–10131.2001

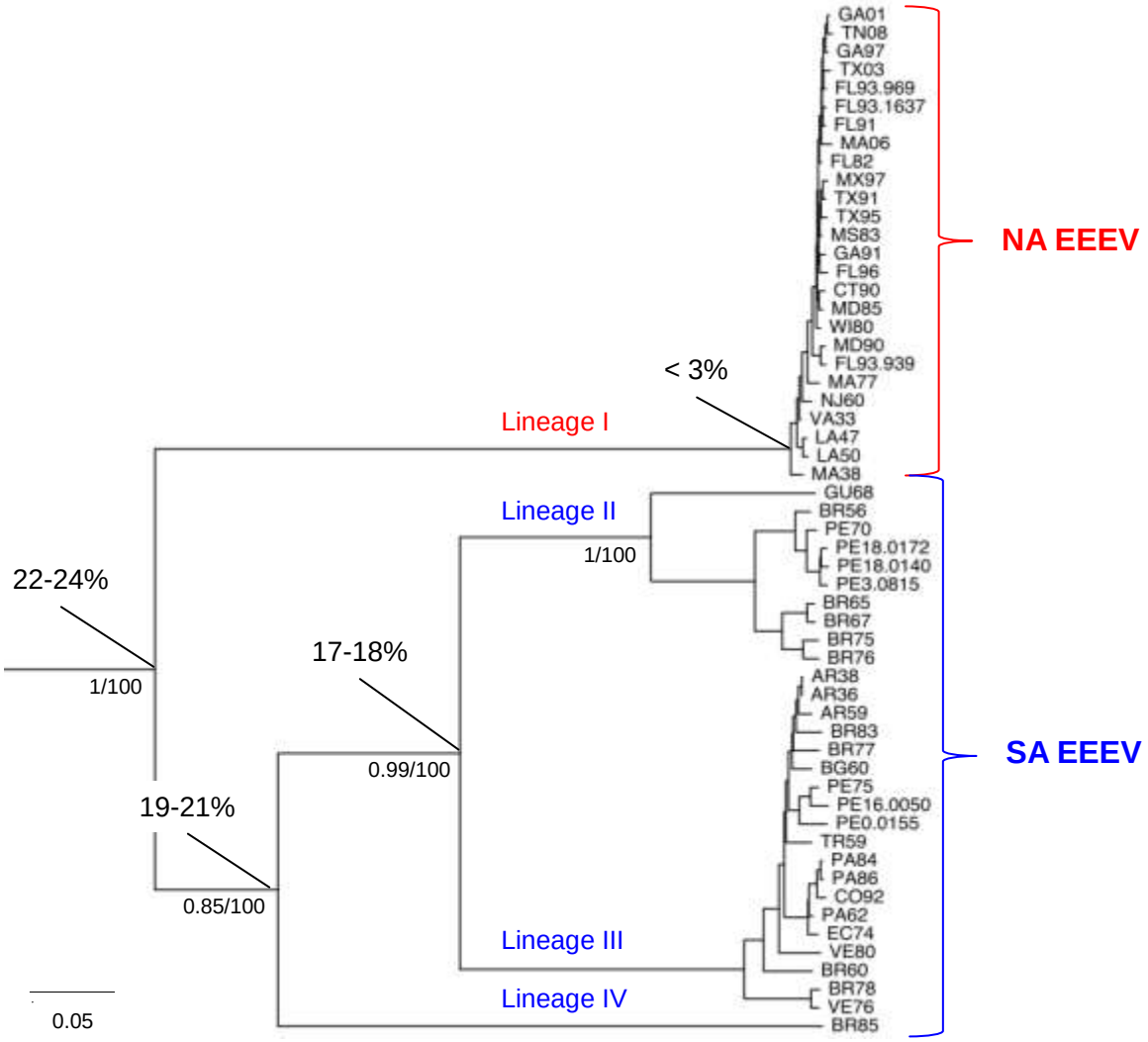

Figure 2. Phylogenetic tree of representative Eastern equine encephalitis virus strains generated from the complete structural polyprotein open reading frame. The four major EEEV lineages are noted, with NA EEEV comprising lineage I and SA EEEV comprising lineages II-IV. Bayesian posterior probability (PP) values and maximum parsimony (MP) bootstrap values are noted for all major nodes of lineage divergence (PP/MP values). Percentage at each of the major node indicates percent genetic divergence between lineages. Scale bar represents a genetic distance of 10% nucleotide divergence.

Adapted from Arrigo NC, Adams AP, Weaver SC. Evolutionary Patterns of Eastern Equine Encephalitis Virus in North versus South America Suggest Ecological Differences and Taxonomic Revision. Journal of Virology, Jan. 2010, p. 1014–1025 Vol. 84, No. 2, doi:10.1128/JVI.01586-09.

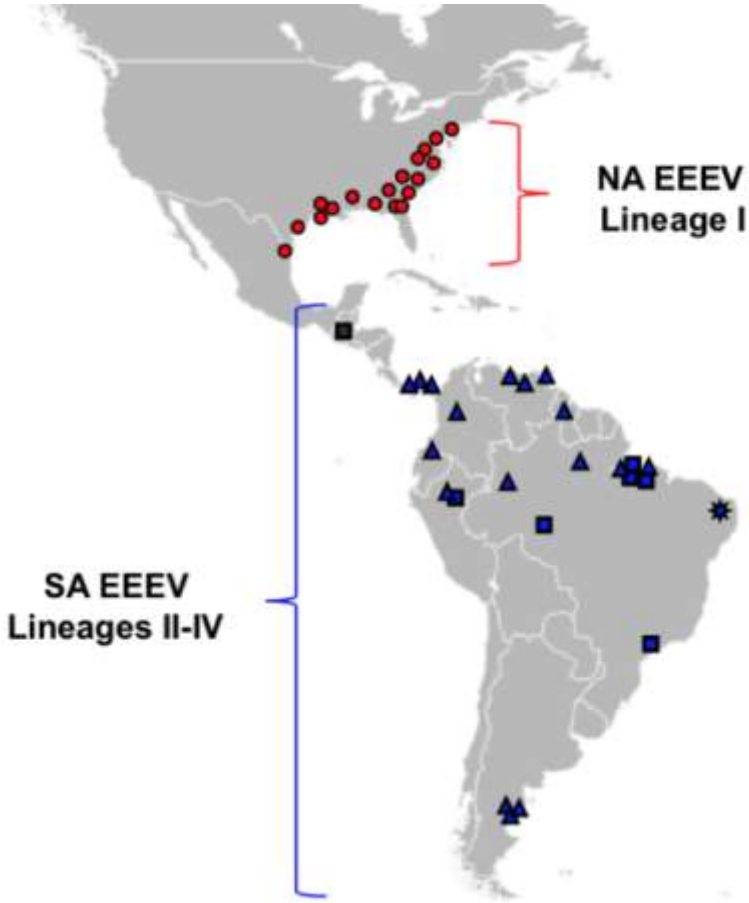

Figure 3. Map showing the geographic distribution of the Eastern equine encephalitis virus (as presently classified). Symbols represent locations of isolation of representative virus strains in each genomic lineage.

Adapted from Arrigo NC, Adams AP, Weaver SC. Evolutionary Patterns of Eastern Equine Encephalitis Virus in North versus South America Suggest Ecological Differences and Taxonomic Revision. Journal of Virology, Jan. 2010, p. 1014–1025 Vol. 84, No. 2, doi:10.1128/JVI.01586-09.

Table 1. Nucleotide and amino acid sequence divergence among EEEV and VEEV[a]

Percentage sequence divergence from[b]:					
	NA EEEV Lineage I	SA EEEV Lineage II	SA EEEV Lineage III	SA EEEV Lineage IV	VEEV[c]
NA EEEV Lineage I	---	22.8 - 23.9	22.5 - 23.5	22.7 - 23.0	41.1 - 42.2
SA EEEV Lineage II	8.9 - 10.5	---	16.5 - 18.0	20.7 - 21.2	41.6 - 42.5
SA EEEV Lineage III	8.2 - 9.7	3.3 - 4.6	---	19.3 - 19.9	41.6 - 43.2
SA EEEV Lineage IV	10.2 - 11.2	7.8 - 8.9	6.9 - 7.6	---	41.3 - 42.4
VEEV[c]	42.1 - 43.0	41.6 - 42.8	41.3 - 42.2	41.3 - 42.2	---

[a] Upper diagonal indicates nucleotide sequence divergence; lower diagonal indicates amino acid sequence divergence.

[b] All members of each EEEV lineage were compared and are represented by ranges of percent sequence divergence.

[c] VEEV includes representatives of subtypes IAB, IC, ID, and IE.

Adapted from Arrigo NC, Adams AP, Weaver SC. Evolutionary Patterns of Eastern Equine Encephalitis Virus in North versus South America Suggest Ecological Differences and Taxonomic Revision. Journal of Virology, Jan. 2010, p. 1014–1025 Vol. 84, No. 2, doi:10.1128/JVI.01586-09.

Table 2. All known taxa and their associated Genbank accession numbers proposed for removal from current EEEV species classification and reassignment to newly proposed alphavirus species, *Madariaga virus (MADV)*

	Genome region for which nucleotide sequences are published in Genbank								
Strain Name	**NSP1**	**NSP3**	**NSP4**	**Partial NSP gene cds**	**E1**	**E2**	**3'UTR**	**Structural polyprotein gene cds**	**Full-length Genome**
ArgLL			S47282; U01640		S4728 7	U01600	U01560	GU001915	
ArgB			U01641			U01602	U01561	GU001916	
BeAn5122			U01646			U01604	U01565	AF159559	
GML207963			U01645			U01605	U01566		
ArgM			U01642			U01601	U01562	GU001917	
Tr24443			U01651			U01611	U01575	GU001918	
Tr25714			U01643			U01603		GU001919	
BeAr18205			AF421500			AF421501	AF421502	GU001920	
GML900188			U01648			U01610	U01568	GU001922	
BeAr81828			AF421503			AF421504	AF421505	GU001923	
BeAr126650			AF421506			AF421507	AF421508	GU001924	
68U230			AF421509			AF421510	AF421511		
68U231								GU001925	
77U1104			U01653			U01612	U01571	GU001926	
75V1496			U01649			U01606	U01567	GU001927	
BeAr300851			AF421515	EF034076		AF421516	AF421517	GU001928	
75U40			AF421512			AF421513	AF421514	GU001929	
El Delerio			U01654			U01614	U01574	GU001930	
250714			AF421530			AF421531	AF421532		
76V25343			U01647			U01607	U01564	GU001931	
77U1			AF421518			AF421519	AF421520	GU001932	
BeAr348998			AF421521			AF421522	AF421523	GU001933	
IVICPan57151			U01655			U01613	U01573	GU001934	
Pan66058-60			U01656			U01615	U01572		
BeAn416361			AF421527			AF421528	AF421529	GU001935	
414556			AF421524			AF421525	AF421526		
GML903836				EF034079				GU001936	
GML903866			U01650			U01608	U01569		
BeAr436087				EF034077				AF159561	EF151503
MARU435731			U01644			U01609	U01570	AF159560	
C49			AF421533			AF421534	AF421535	GU001937	
PE-0.0155									DQ241304
PE-3.0815									DQ241303
PE-18.0172								GU001940	
PE-16.0050		DQ280395				DQ307816		GU001938	

PE-18.0140		DQ280409				DQ307830		GU001939	
PE-2.0010		DQ280417				DQ307838			
PE-4.0661		DQ280416				DQ307837			
PE-4.0807		DQ280415				DQ307836			
PE-4.0808		DQ280414				DQ307835			
PE-10.0146		DQ280413				DQ307834			
PE-10.0170		DQ280412				DQ307833			
PE-11.0207		DQ280411				DQ307832			
PE-15.0058		DQ280410				DQ307831			
PE-18.0169		DQ280408				DQ307829			
PE-18.1150		DQ280407				DQ307828			
PE-22.0263		DQ280406				DQ307827			
PE-22.0285		DQ280405				DQ307826			
PE-22.0526		DQ280404				DQ307825			
PE-22.0534		DQ280403				DQ307824			
PE-22.0678		DQ280402				DQ307823			
PE-24.0111		DQ280401				DQ307822			
PE-24.0132		DQ280400				DQ307821			
PE-22.0552		DQ280399				DQ307820			
PE-22.0110		DQ280398				DQ307819			
PE-17.0547		DQ280397				DQ307818			
PE-16.0140		DQ280396				DQ307817			
PE-11.0352		DQ280394				DQ307815			
PE-11.0331		DQ280393				DQ307814			
PE-11.0042		DQ280392				DQ307813			
PE-5.0519		DQ280391				DQ307812			
PE-5.0183		DQ280390				DQ307811			
PE-5.0151		DQ280389				DQ307810			
PE-4.0775		DQ280388				DQ307809			
PE-3.0869		DQ280387				DQ307808			
PE-3.0803		DQ280386				DQ307807			
PE-3.0391		DQ280385				DQ307806			
PE-3.0041		DQ280384				DQ307805			
PE-1.0999		DQ280383				DQ307804			
PE-1.0643		DQ280382				DQ307803			
IP3499/09	HM020403								
IP5032-08	GU112449								
IP1314-05	GU112448								
IP8657-06	GU112447								
IP2608-06	GU112446								
IP7686-08	GU112445								
IP4085-09	GU112444								
IP4084-09	GU112443								
IP6009-09	GU112442								

IP5691-09	GU112441								
IP4240-09	GU112440								
IP4239-09	GU112439								
IP3727-09	GU112438								
IP3726-09	GU112437								
IP3725-09	GU112436								
IP3724-09	GU112435								
IP3723-09	GU112434								
IP3501-09	GU112433								
IP3500-09	GU112432								
IP/8657-06	EU257811								
IP/1314-05	EU257810								
IP/2608-06	EU257809								
IP/1096-05	EU257808								