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:	***2013.002aI***	(to be completed by ICTV officers)

Short title: Create three new species in the genus *Betaentomopoxvirus*
(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:

Basil Arif – barif@nrcan.gc.ca
Elisabeth Herniou – elisabeth.herniou@univ-tours.fr
Julien Thézé – julien.theze@univ-tours.fr
Madoka Nakai – madoka@cc.tuat.ac.jp
Jun Takatsuka - junsan@ffpri.affrc.go.jp

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)	*Poxviridae* study group. M.A. Skinner, Chair

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

There were a few minor and a few major responses to this proposal, mostly asking for additional materials and argumentation to support the proposal.

1. The bulk of the argumentation presented supports the inclusion of these viruses into the Genus *Betaentompoxvirus* and the committee accepted that argumentation. However, the argumentation for three separate species is lacking.
2. Correct spelling of rosaceana in the species name
3. It would be better to present the characteristics and arguments of each of the three species separately instead of lumping them all together. That way it would be easier to see what the different characteristics are to warrant separate species demarcations.
4. In writing, clearly differentiate the name of the species (all in italics) from the name of the virus (normal case) that you would put in that taxon. Under 1. The natural host, you wrote what should be a virus name all in italics. Only the "species" name should be all in italics, the virus (i.e. that sample in the fridge) is written in normal font. Thus *Adoxophyes honmai entomopoxvirus* is a species name but Adoxophyes honmai entomopoxvirus (etc. for the others) is the virus name. Though there is some contention, some virus names have the species name of the insect from which it was derived (e.g. *Adoxophyes honmai*) in italics, but the word "entomopoxvirus" should be in normal font (e.g. *Adoxophyes honmai* entomopoxvirus).
5. Under 1. Natural host you mention that AHEV is infectious "to a number of Adoxophyes species". Could you be more specific, e.g. give some of the names of the species affected.
6. Under 2. Conservation of gene order. The committee found it difficult to interpret Fig 1 colinearity maps for synteny (gene order) comparisons. Most other proposals which use gene order as a criterion for differentiation of taxa use Mauve alignment which the committee found easier to interpret (The program is available for free at http://gel.ahabs.wisc.edu/mauve).

7. While the committee accepted that all three viruses fall within the subfamily *Entomopoxvirinae* and the genus *Betaentomopoxvirus*, they were not as convinced that each virus deserved its own species. For example, it was not clear what specific demarcation criteria were used to designate viruses as belonging to the same or different species. This needs to be clarified. For some families (not necessarily applicable to poxviruses), species taxa are differentiated by percent homology of their genomes or genes. For example species designations for the *Parvoviridae* are given to viruses having <95% relatedness of the non structural gene DNA sequence. Currently (9th report of the ICTV) the species demarcation criteria within the *Betaentomopoxvirus* genus are listed as "host range" and "virion morphology". I note that all three viruses infect Lepidopteran species, but how tight does the "host range" need to be to be considered a different species. As is known for the baculoviruses, the same or related virus might infect different hosts and thus host range may be insufficient. Virion morphology is also given as a demarcation criterion for species. What aspect of virion morphology differentiates the three viruses as belonging to different species and is that sufficient?
8. More specifically, based on the phylogenetic tree (Fig 3) (which lacks a scale that should be included), both CREV and CBEV appear to be very closely related (97.2% for 49 core poxvirus genes and high for spheroidin). Both viruses infect *Choristoneura* species. What specifically is different between CBEV (classified in the species *Choristoneura bienis entomopoxvirus*) and CREV (proposed as belonging to a new species *Choristoneura rosaceana entomopoxvirus*)? Are CBEV and CREV sufficiently different to belong to different species? This could be addressed at the genome sequence level since the genomes for both are now in Genbank. Arguments similar to those mentioned in Thézé et al 2013 that two species is warranted for these two viruses could be included in the argumentation in this taxon proposal to be more convincing. A minor point, it appears that Fig 3. was taken from Thézé et al 2013 and thus this should be acknowledged in the legend to Fig. 3.
9. It was not clear if the whole Poxviridae study group saw the proposal, or just the Chair, Mike Skinner. The Executive Committee suggests that the revised proposal be reviewed by the Study Group before resubmission.

Though peripheral to the current proposal, currently one entomopoxvirus, *Melanoplus saguinipes* EPV, remains unclassified although some of its biological characteristics are known and the complete genome sequence is available, accession number NC_001993. The ICTV executive committee encourages the authors of this current proposal to also consider including a proposal for classifying this particular virus. This could be part of the revised proposal or a separate proposal. Having a single virus in a species and a single species in a genus are quite acceptable, as long as demarcation criteria are clear.

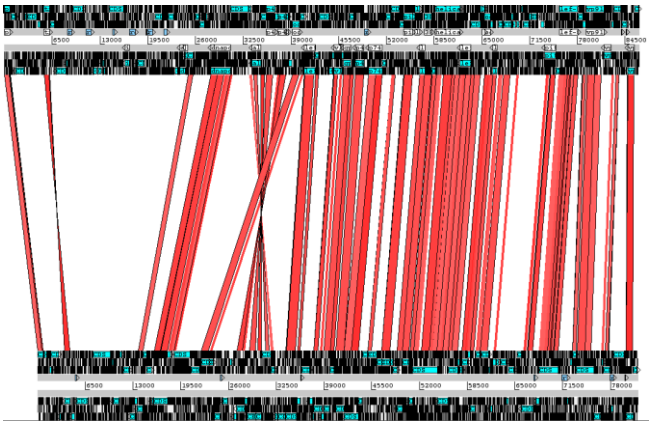
Basil Arif in an email to me on July 03, 2013 suggested as much since the Orthopteran EPVs appear quite distinct from the Lepidopteran EPVs and thus deserve their own Genus. The suggestion that genera could be classified on the basis of the Order of the host is also made in Thézé et al 2013.

Response to Comments on the first submission

Dear Colleagues.

Then you for your comments on the first submission to include three new EPVs in the genus *Betaentomopoxvirus*. I have tried to correct and modify the previous submission to comply with your comments. I did not agree with one or two of your comments as you see in my response below:

1. Spellings and correct writing (italics vs normal) have been done.
2. The insect species infected by AHEV have been outlined.

3. I have included more data to back up the argument that three viruses belong to three new species as you see in the submission.
4. Maps of gene order have been redone to show synteny. I note that the synteny maps in the previous submission have already been published in J. Virol (Thézé *et al.*, 2013). I would like to say that results showing two genomes exhibiting close synteny are not indications that the two genomes belong to the same species. In one of our previous work, we showed very close synteny between two baculoviruses, the Neodiprion lecontei nucleopolyhedrovirus (NeleNPV) and the Neodiprion sertifer nucleopolyhedrovirus (NeseNPV, Figure below. Lauzon *et al*., 2006. J.gen.Virol.87:1477-1489). Yet, these two viruses belong to two different species because they are not cross infective. Similar examples exist with poxviruses and the host range becomes crucial demarcation criterion.
   NeseNPV.
   
   NeleNPV

5. One comment (under 7) on the previous submission indicated that "the species demarcation criteria within the *Betaentomopoxvirus* genus are listed as host range and virion morphology". And goes one to ask "what aspect of virion morphology differentiates the three viruses as belonging to different species and is that sufficient?". If I read this correctly, it indicates that there should be virion morphology differences with in this genus. This is inaccurate. According to the 9th Report, lepidopteran or coleopteran hosts, ovoid, about 350 x 250 nm virion morphology with a sleeve-shaped lateral body and cylindrical core with surface globular subunits 40 nm in diameter.Virion morphology is a demarcation criterion within the sub-family *Entomopoxvirinae* where it places a virus in one of the genera and not from one species to another within one genus. We cannot have 10 species within one genus, each having different morphological characters. This has been known for a long time, even before we changed the classification from A, B, and C to the present name of the genera (Robert R. Grandos. (1981). Entomopoxvirus Infections in Insects. In: Pathogenesis of Invertebrate Microbial Diseases. (E. Davidson, ed.), Allanhead Osmum, Inc,. pp101-126. Bawden, A.L. *et al*., (1999). Complete genomic sequence of the *Amsacta moorei* entomopoxvirus: Analysis and comparison with other poxviruses. Virology 273: 120-139.). Virion morphology is a very important criterion to place a virus in a certain genus. In case of doubt, kindly send this proposal to Dr. Richard Moyer at the U. of Florida.
6. Comment: "How tight is the host range" AHEV is infective to the insects outline in the proposal and the virus is markedly distinguishable from MySEV, the latter being a parasite of noctuids. While CREV replicates productively in the oblique banded leaf roller with all the typical symptoms of EPV infection such as inhibition of moulting causing the insets to become very large in size, glossy and whitish appearance and the

insect virtually becoming a sack filled with occlusion bodies. CREV replicates to a very limited extent in the spruce budworm without causing the symptoms typical of EPV infection. The two insects are widely different, the leaf roller being a pest of agricultural plants while the budworm is a forest pest.

7. I agree very much that genomic criteria should help in classification of EPVs. This has been discussed quite often in the past. However, we have not yet set which criteria should be used to address this problem with EPVs and until this is done, the present demarcation criteria should be used. It is mentioned in the comments the close relatedness CREV and CBEV when one compares the 49 core genes as well as *sph* (97.2%). But this is not unexpected since these core genes are highly conserved in members of *Entomopoxvirinae*. Hence, clear genomic demarcation criteria should be in place to avoid confusion. I have included genomic criteria to distinguish the different viruses within different species

Date first submitted to ICTV:	June 24, 2013
Date of this revision (if different to above):	July 2, 2014

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	***2013.002aI***	(assigned by ICTV officers)

To create 3 new species within:

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.

Genus:	***Betaentomopoxvirus***
Subfamily:	***Entomopoxvirinae***
Family:	***Poxviridae***
Order:	

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Adoxophyes honmai entomopoxvirus	HF679131
Choristoneura rosaceana entomopoxvirus	HF769133
Mythimna separata entomopoxvirus	HF679134

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

Characterization of three new entomopoxvirus isolates, Adoxophyes honmai entomopoxvirus (AHEV), Mythimna separata entomopoxvirus (MySEV) and Choristoneura rosaceana entomopoxvirus (CREV) suggest that all three viruses meet the demarcation criteria for inclusion in the genus *Betaentomopoxvirus:* According to the Ninth report of the International Committee on Taxonomy of Viruses the distinguishing features for this genus are; lepidopteran or orthopteran hosts, ovoid, about 350 x 250 nm virion morphology with a sleeve-shaped lateral body and cylindrical core with surface globular subunits 40 nm in diameter. All the viruses produce the characteristic ovoid occlusion bodies. The genomic distinguishing characteristics of AMEV, AHEV, CBEV, CREV and MySEV are delineated in Table 1.
Below are descriptions of viruses suggested to make up the three proposed species.

Adoxophyes honmai entomopoxvirus

One virus, Adoxophyes honmai entomomopoxvirus (AHEV) is considered to belong to this species. AHEV was first isolated in Japan from the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae).
The distinct properties associated with members of the proposed species *Adoxophyes honmai entomopoxvirus* are:

1. Infective to certain members of the family Tortricidae, specifically,
   Adoxophyes honmai – Smaller tea tortrix
   Adoxophyes orana – Smaller fruit tortrix
   Adoxophyes dubia
   Homona magnanima - Oriental tea tortrix moth
   Archips insulanus
   (Takatsuka *et al*., 2010; Nakai and Kunimi, 1998)
   All the above host species are members of the Family Tortricidae and are pests of agricultural plants. They are found mainly in Japan but also in parts of South East Asia.

2. Morphologically, the virion is typical of viruses in the genus *Betaentomopoxvirus.* AHEV

has a cylindrical core and a lateral body embedded within the spheroidin protein of the ovoid occlusion body (Fig. 1).

3. REN analysis (e.g. HindIII) showing AHEV is markedly different from other species, e.g. MySEV (Fig.2A).

4. Hybridization of the genome of AHEV to different isolates of the same virus but not to the genome of viruses of MySEV (Fig.2B, Takatsuka *et al*., 2010), thus clearly differentiating these two viruses.

5. Genomic DNA of 228,750 bp (to date the smallest genome in the genus *Betaentomopoxvirus*) with about 247 open reading frames and 21% G+C (NC_021247).

6. Phylogenetic studies of 49 poxvirus conserved genes between AHEV and other betaentomopoxviruses (Fig. 3) demonstrate that AHEV is in a separate clade from the viruses in the other species.

Mythimna separata entomopoxvirus

Mythimna separata entomopoxvirus (MySEV) was isolated from a noctuid insect, *Mythimna* (*Pseudaletia*) *separata* (Lepidoptera: Noctuidae) and replicated in two lepidopteran cell lines from *M. separata* and *Bombyx mori* (Hukuhara *et al*., 1990). The host insect has more than one common name such as the Northern armyworm, Oriental armyworm or Rice ear-cutting caterpillar. It is found in China, Japan and South East Asia and feeds on plants in lawns and pastures.

The distinct properties associated with members of the proposed species *Mythimna separata entomopoxvirus* are:

1. Host range. To date, the virus is only infective to *M. separate*, which unlike adoxophyes, it is a noctuid insect.

2. REN analysis (e.g. HindIII) showing MySEV is markedly different from other species, e.g. AHEV (Fig.2A).

3. Hybridization of the AHEV genome to different isolates of AHEV but not to the genome of MySEV (Fig.2B, Takatsuka *et al*., 2010), thus clearly differentiating the two viruses.

3. Genomic DNA of 281,182 bp genome with about 306 open reading frames and 19.7% G+C (Fig. 4. NC_021248).

4. Phylogenetic studies of 49 poxvirus conserved genes between MySEV and other betaentomopoxviruses (Fig 3) demonstrate that MyEV is in a separate clade from the viruses in the other species.

5. Protein clustering - It was conducted to recognize core gene in the sub-family *Entomopoxvirinae*. AMEV, MySEV, CREV, CBEV and AHEV shared 148 conserved genes compared to 104 conserved genes in all EPVs (Fig. 4). Many of the 148 conserved genes particularly those involved in replication, transcription/mRNA modification and envelope protein synthesis are arranged in conserved order among the five EPVs suggesting a strong selective pressure to keep this order intact, thus further corroborating the inclusion of MySEV, CREV, and AHEV in the genus *Betaentomopoxvirus*.

6. Synteny- Alignment of the genomes of AHEV and MySEV shows very little synteny between the two genomes (Fig. 5).

These characteristics clearly distinguish AHEV and MySEV from each other and from othe betaentomopoxviruses and as such warrant establishment of two new species.

Choristoneura rosaceana entomopoxvirus

Choristoneura rosaceana entomopoxvirus (CREV) virus was isolated from the oblique banded leaf roller, *Choristoneura rosaceana* (Lepidpotera: Tortricidae), which is a pest of orchard plants such as apples, plums, etc. but also causes major damage to rosaceous plants such as ornamental shrubs.

The distinct properties associated with members of the proposed species *Choristoneura rosaceana entomopoxvirus* are:
1. Biological features. Typical of members of this genus, the virions are embedded in an ovoid occlusion body composed mainly of spheroidin. The infection in the natural host, *C. rosaceana* results in the larvae becoming huge due to inhibition of moulting with glossy whitish skin and quite milky. There appears to be very limited replication in the Eastern Spruce budworm, *Choristoneura fumiferana* with very limited occlusion body production, if at all and without the symptoms seen when the virus infects *C. rosaceana* (C. Lucarotti, personal communication. christopher.lucarotti@nrcan-rncan.gc.ca ; Perera *et al*., 2010). Only the oblique banded leaf roller can be used for production of virus. By comparison, CBEV replicates productively in the eastern spruce budworm with typical EPV symptoms such as inhibition of moulting and larvae become very large. It is the insect of choice to mass produce CBEV.

2. Genomic DNA of 282,895 bp genome with about 296 open reading frames and 19.5% G+C (NC_021246) and about 25 kbp smaller than that for CBEV (307,691) (Table 1).

3. Phylogenetic studies of 49 poxvirus conserved genes between MySEV and other betaentomopoxviruses (Fig 3) demonstrate that while CREV is closely related to CBEV* it is sufficiently distinct from other betaentompoxviruses.

4. Synteny – Genomic alignments show little or no synteny between the genomes of CREV and AHEV and between CREV and MySEV indicating significant gene rearrangements over evolutionary periods (Fig. 5).
It is also worth mentioning that the core regions of all poxviruses appear to be relatively conserved while the peripheral regions are much less conserved (Fig. 6. McLysaght *et al*., 2003).

*. The genomes of CREV and CBEV are, markedly different in size and gene content (Table 1). The genome of CBEV is nearly 25 kbp larger than CREV where the ITRs of the former are much larger and contain few copies of the N1R/p28 gene as well as genes potentially encoding hypothetical proteins. Another difference is the presence of numerous genes dispersed throughout the two genome and potentially encoding proteins of unknown functions. The two genomes contain 35 genes that are different, which represent about 10% of the total coding capacity of the genomes. In addition, by utilizing dot plots (created with the Gepard program), Thézé *et al*., (2013) compared synteny in the genomes of CREV and CBEV (Fig. 7a). By comparison they did similar plots of two chordopoxvirus genomes belonging to two different species in the genus *Yatapoxvirus* (Fig.7b). It can be seen that there are more deletions, insertions and rearrangements between the genomes of CBEV and CREV than between the genomes of Tanapoxvirus and Yabe monkey tumor virus. The clear differences in the genomic makeup of CBEV and CREV in terms of size, gene content and organization strongly suggest the two viruses should be in two different species. Thézé et al., (2013) argued that genomic differences strongly support a separate species for CREV even though this may not be totally corroborated by phylogenetic relationships and core gene nucleotide distances.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

McLysaght, A., Baldi, P.F. and Gaut, B.S. 2003. Extensive gene gain associated with adaptive evolution of poxviruses. Proc. Natl. Acad. Sci. U. S. A. 100:15655–15660.

Nakai, M. and Kunimi, Y. 1998. Effects of timing of entomopoxvirus administration to the smaller tea tortrix *Adoxophyes* sp. (Lepidoptera: Tortricidae) on the survival of the endoparasetoid, *Ascogaster reticulates* (Hymenoptera: Braconidae). Biol. Control 13:63-69.

Hukuhara, T., Xu, J.H. and Yano, K. 1990. Replication of an entomopoxvirus in two lepidopteran cell lines. J. Invertebr. Pathol. 56: 222-232.

Takatsuka, J., Okuno, S., Ishii, T., Nakai, M. and Kunimi, Y. 2010. Fitness-related traits of entomopoxviruses isolated from *Adoxophyes honmai* (Lepidoptera: Tortricidae) at three localities in Japan. J. Invertebr. Pathol. 105:121-131.

Perera, S., Li, Z., Pvalik, L. and Arif, B. 2010. Entomopoxviruses, p 83–115. *In* Asgari S, Johnson KN (ed), Insect virology. Caister Academic Press, Norfolk, United Kingdom.

Thézé, J., Takatsuka, J., Li, Z., Gallais, J., Doucet, D., Arif, B., Nakai, M., and Herniou, E. A. (2013). New insights into the evolution of *Entomopoxvirinae* from complete genome sequences of four entomopoxviruses infecting *Adoxophyes honmai*, *Choristoneura biennis*, *Choristoneura rosaceana* and *Mythimna separata*. J Virol. 87:7992-8003.

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.

Table 1 – Genomic characteristics of five EPVs

Genome	Size (bp)	No. of ORFs	No. of singletons	ITR size (bp)	GC content (%)	Coding capacity
Amsacta moorei entomopoxvirus	232,392	294	73	9,458	17.8	95.4
Adoxophyes honmai entomopoxvirus	228,750	247	27	5,617	21	89.8
Choristoneura biennis entomopoxvirus"	307,691	334	19	23,817	19.7	91
Choristoneura rosaceana entomopoxvirus	282,895	296	11	13,406	19.5	90.2
Mythimna separata entomopoxvirus	281,182	306	64	7,347	19.7	90.5

Figures

Fig. 1 TEM of AHEV showing the ovoid spheroid composed mainly of spheroidin. Embedded virions are typical of the genus *Betaentomopoxvirus.* The highly refractile bodies are the spindles composed of fusolin (Nakai, M. unpublished).

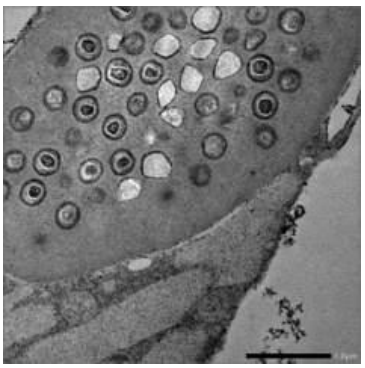


Fig. 2. Hind III digestion of DNAs of three AHEV isolates and MySEV DNA samples. B. Southern blot hybridization of AHEV DNA to the three isolates and to MySEV DNA. (Takatsuka *et al*., 2010)

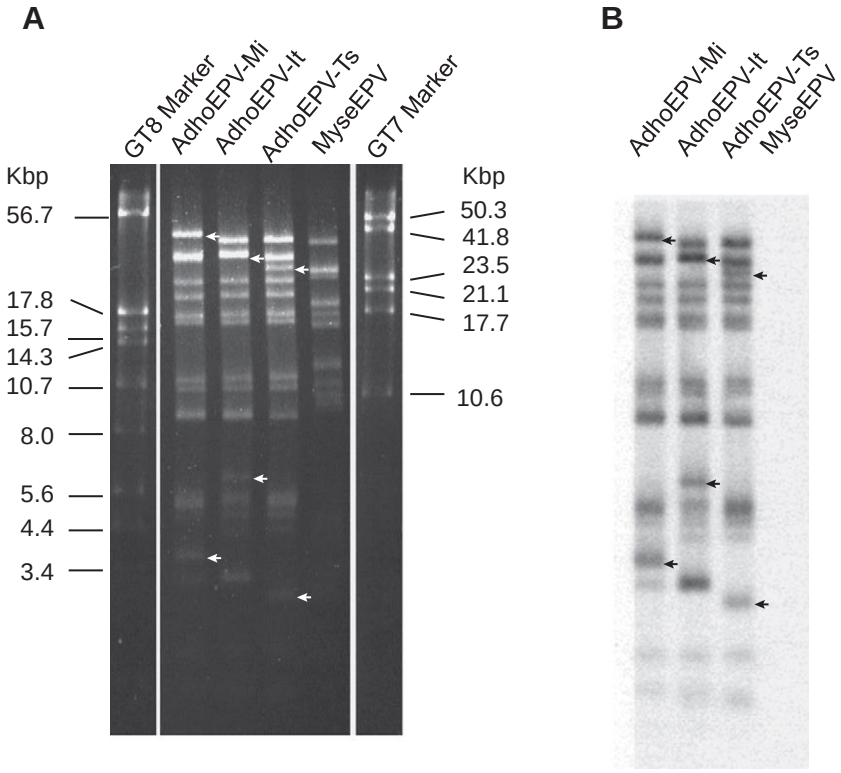


Fig. 3. Phylogenetic tree was derived from alignments of 49 poxvirus conserved core genes. Support for nodes shows maximum likelihood nonparametric bootstraps (100 replicates) (Thézé, J. *et al*., 2013)

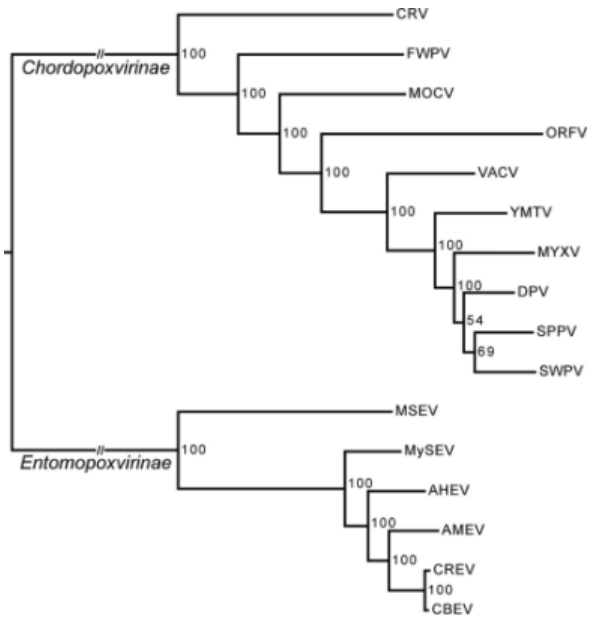


Fig. 4. Localization of 148 conserved genes in the genome of MySEV. Conserved genes in *Poxviridae* are indicated in red; green in *Entomopoxvirinae* and blue in *Betaentomopovirus* genomes (Thézé, J. *et al*., 2013).

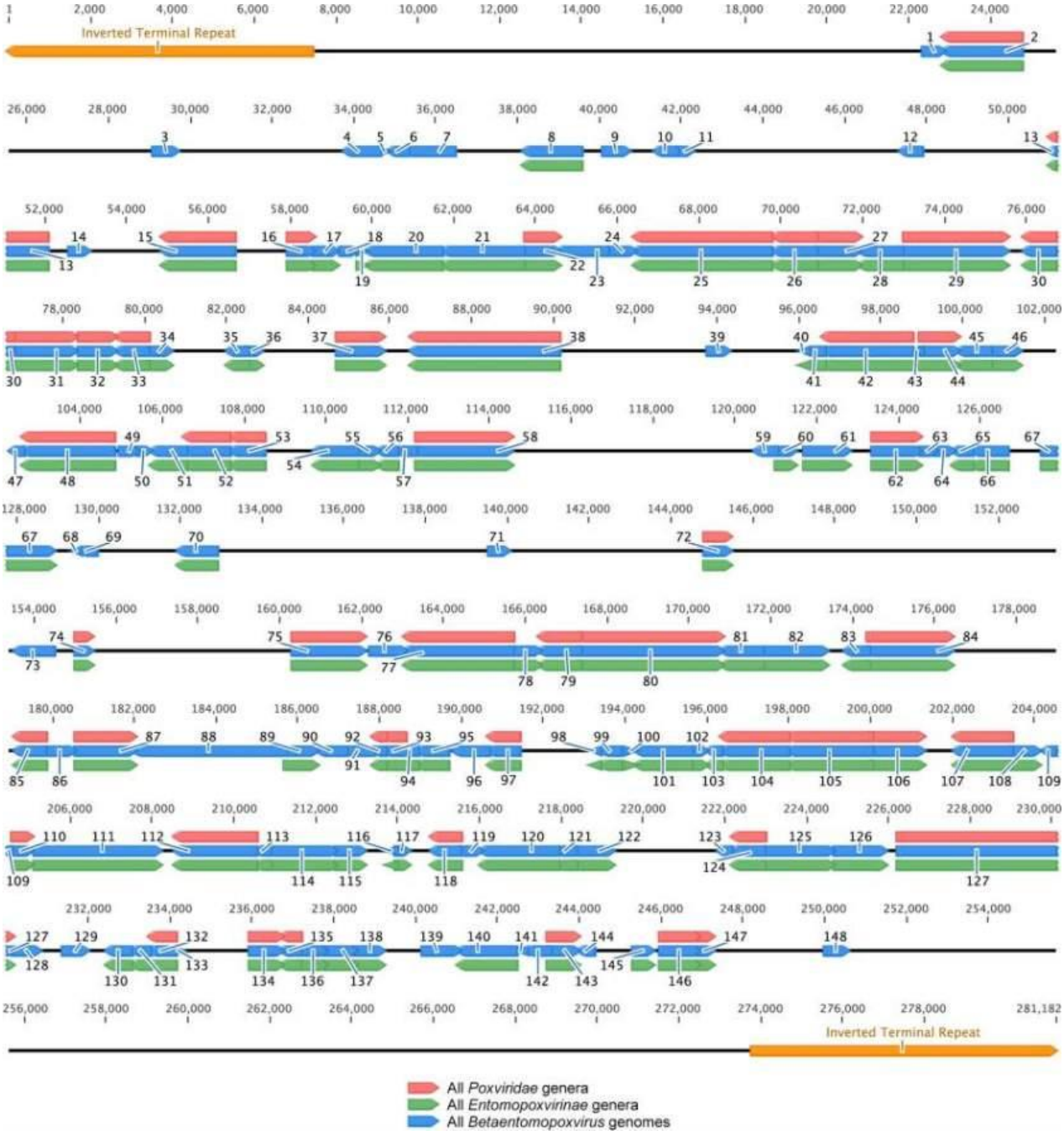


Fig. 5. Genomic Alignments

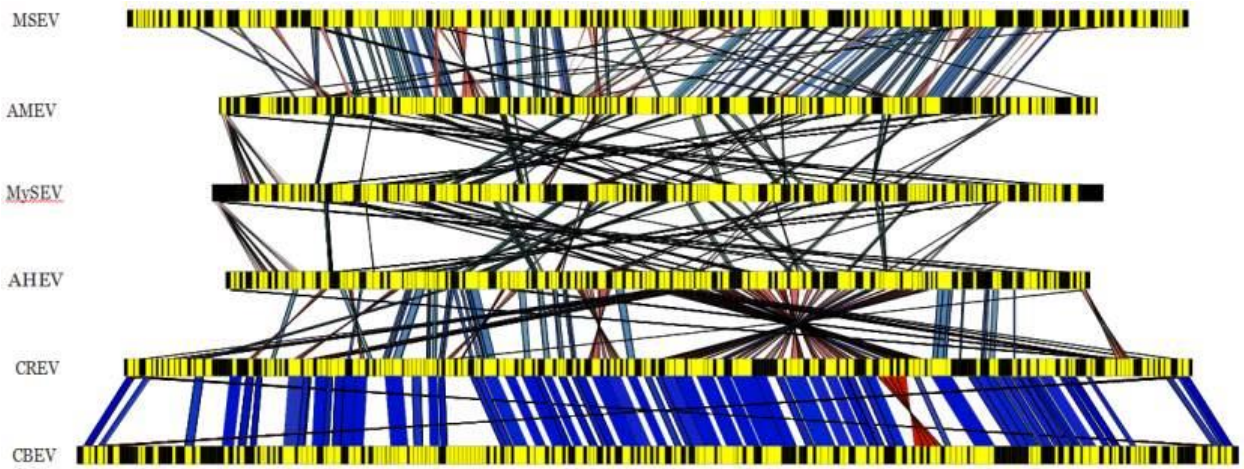


Fig. 6. Conservation in the central regions of poxvirus genomes

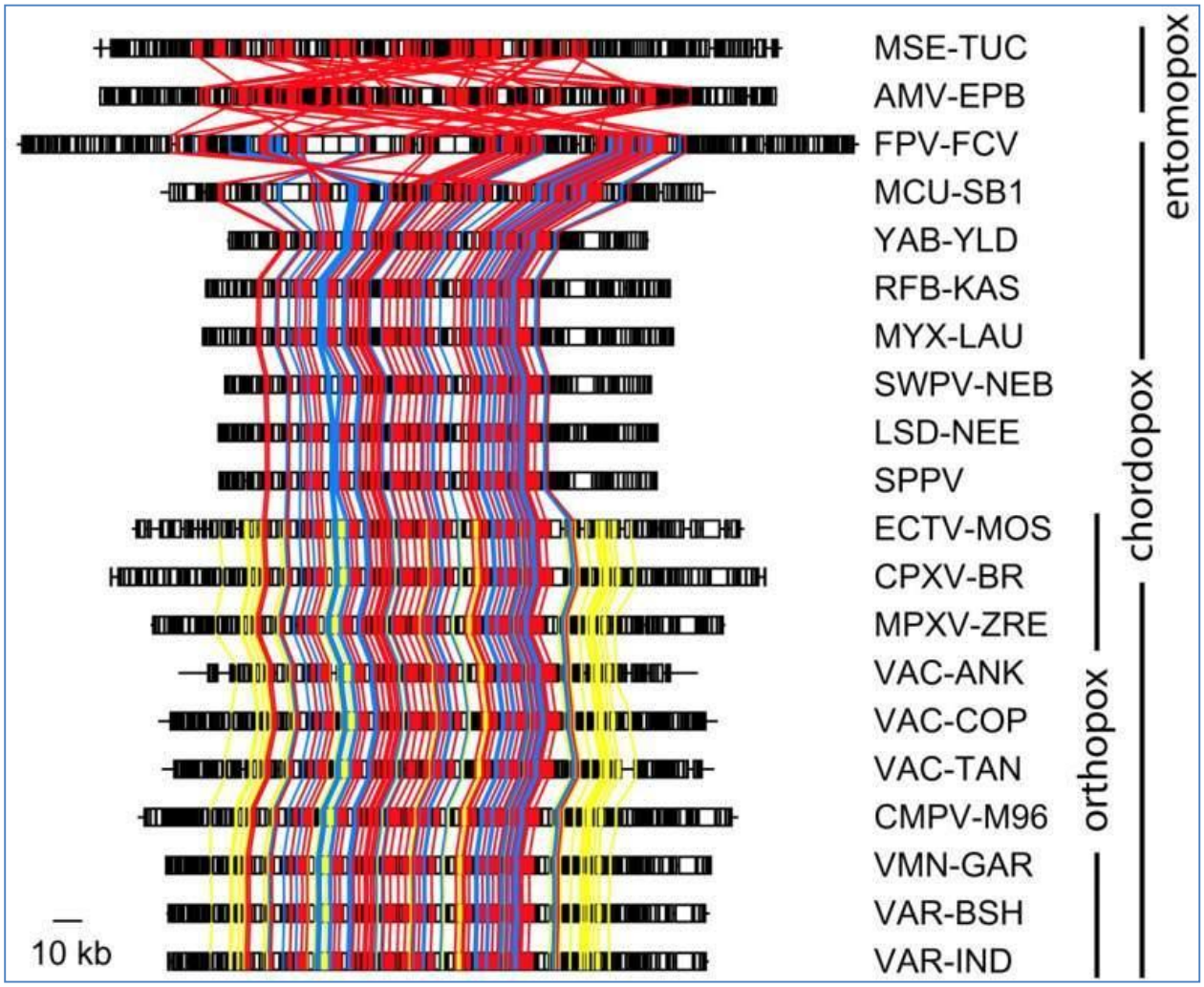


Fig. 7. Dot plot analysis between CREV and CBEV genomes (7a) and between Tanapoxvirus and Yaba monkey tumor virus genomes.