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:** | ***2014.004a,bS*** | (to be completed by ICTV officers) |

**Short title:** Create 4 new species in the genus *Alphamesonivirus* and 2 new unassigned species, all in the family *Mesoniviridae*
(e.g. 6 new species in the genus *Zetavirus*)

| | | | | | |
|---|---|---|---|---|---|
| **Modules attached** (modules 1 and 10 are required) | **1** ☒ | **2** ☒ | **3** ☐ | **4** ☐ | **5** ☐ |
| | **6** ☐ | **7** ☐ | **8** ☐ | **9** ☐ | **10** ☒ |

**Author(s):**


Alexander E. Gorbalenya, Chair
Anastasia Gulyaeva
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**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) | This proposal is filed by the Mesoniviridae Study Group (MSG) in consultation with the Nidovirales Study Group (NSG). |

**ICTV Study Group comments (if any) and response of the proposer:**

MSG and NSG agree with this proposal

| | |
|---|---|
| Date first submitted to ICTV: | 19/06/2015 |
| Date of this revision (if different to above): | 31/10/2015 |

**ICTV-EC comments and response of the proposer:**

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 | ***2014.004aS*** | (assigned by ICTV officers) |
|---|---|---|

**To create four new species within:**

| | |
|---|---|
| Genus: | ***Alphamesonivirus*** |
| Subfamily: | |
| Family: | ***Mesoniviridae*** |
| Order: | ***Nidovirales*** |

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.

| **Name of new species:** | **Representative isolate: (only 1 per species please)** | **GenBank sequence accession number(s)** |
|---|---|---|
| *Alphamesonivirus 2* | Karang Sari virus, KSaV | KC807171 |
| *Alphamesonivirus 3* | Dak Nong virus, DKNV | AB753015 |
| *Alphamesonivirus 4* | Casuarina virus, CASV | KJ125489 |
| *Alphamesonivirus 5* | Hana virus, HanaV | JQ957872 |

**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

The family ***Mesoniviridae*** with a single genus ***Alphamesonivirus,*** including a single species ***Alphamesonivirus 1,*** was founded 3 years ago (Lauber et al., 2012). It recognized the unique properties of two closely related mosquito viruses, NDiV (Nga et al., 2011) and CavV (Zirkel et al., 2011). That founding work established also a threshold on the pair-wise evolutionary distance (PED) for the intra-species divergence in the *Mesoniviridae* through comparison of these two viruses with viruses of two other nidovirus families, *Coronaviridae* and *Roniviridae.* The analysis was performed for the most conserved nonstructural proteins (nsps) or their equivalents shared by viruses of three families and using the state-of-the-art computational framework of DEmARC (Lauber & Gorbalenya, 2012ab), which was previously used to devise the taxonomy of the *Coronaviridae* (de Groot et al., 2012).

Since 2012, researchers have reported sequences of nearly full genomes for 18 viruses, which were recognized as mesoniviruses based on their overall similarity with NDiV and CavV (Zirkel et al., 2013; Kuwata et al., 2013; Vasilakis et al., 2014; Warrilow et al., 2014). A number of new mesonivirus species were proposed in these studies (Zirkel et al., 2013; Vasilakis et al., 2014; Warrilow et al., 2014). For this proposal, we conducted phylogenetic and DEmARC-based analyses involving all these viruses using either the entire replicase gene-encoded pp1ab polyprotein or a large subset of replicase gene-encoded pp1ab polyprotein containing all the highly conserved domains from 3CLpro to OMT, corresponding to the nsp5-to-nsp16 region of coronavirus replicase pp1ab polyprotein. To decide on a species structure to

be proposed, we analyzed all 11 candidate PED thresholds identified by DEmARC (clustering cost of zero and monophyly of clustering) and choose the one most close to the PED (0.092) that separates NDiV and CavV (see **Annex**). According to this criterion, the analyzed viruses form a total of 7 clusters, which could be split into two groups. The first group consists of five relatively closely connected clusters whose viruses are separated by PEDs less than 0.311. They are proposed to form five species of the genus ***Alphamesonivirus,*** including the established species ***Alphamesonivirus 1*** and four new species **Alphamesonivirus 2-5.** The second group includes two more distantly related viruses that are separated by PED exceeding the 0.311 threshold. They are proposed to prototype two unassigned species, **Mesonivirus 1** and **Mesonivirus 2.**

We believe that the further characterization of the natural diversity of mesoniviruses as well as additional studies of the functional and structural properties of these viruses will help to devise appropriate demarcation criteria for genera within the family *Mesoniviridae* that, at a later stage,will be used to establish genera for the species **Mesonivirus 1** and **Mesonivirus 2.** This future development of mesonivirus taxonomy will be conducted in close cooperation with other nidovirus SGs to benefit from a larger knowledge base and to ensure coherence of demarcation criteria and taxonomy structure across the order *Nidovirales*. In this process, the names of the new mesonivirus species may then be reviewed and, if necessary, revised**.**

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 | ***2014.004bS*** | (assigned by ICTV officers) |
|---|---|---|

**To create <u>two</u> new species within:**

| | |
|---|---|
| Genus: | ***unassigned*** |
| Subfamily: | |
| Family: | ***Mesoniviridae*** |
| Order: | ***Nidovirales*** |

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.

| **Name of new species:** | **Representative isolate: (only 1 per species please)** | **GenBank sequence accession number(s)** |
|---|---|---|
| *Mesonivirus 1* | Nsé virus, NséV | JQ957874 |
| *Mesonivirus 2* | Méno virus, MénoV | JQ957873 |

**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

Please see our comments to the species assigned to the genus ***Alphamesonivirus*** in the Module 2a.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

**References:**


de Groot, R.J., Cowley, J.A., Enjuanes, L., Faaberg, K.S., Perlman, S., Rottier, P.J.M., Snijder, E.J., Ziebuhr, J., and Gorbalenya, A.E. (2012) Order Nidovirales. In: Virus Taxonomy, the 9th Report of the International Committee on Taxonomy of Viruses, King, A., Adams, M., Carstens, E. & E.J Lefkowitz, Eds. Academic Press, pp. 753-763.

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Vasilakis N, Guzman H, Firth C, Forrester NL, Widen SG, Wood TG, Rossi SL, Ghedin E, Popov V, Blasdell KR, Walker PJ, Tesh RB. (2014). Mesoniviruses are mosquito-specific viruses with extensive geographic distribution and host range, Virol J.;11:97

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Zirkel, F., Kurth, A., Quan, P. L., Briese, T., Ellerbrok, H., Pauli, G., Leendertz, F. H., Lipkin, W. I., Ziebuhr, J., Drosten, C., & Junglen, S. (2011). An insect nidovirus emerging from a primary tropical rainforest. mBio, 2, e00077-11.

**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.

The newly recognized family *Mesoniviridae* (20-21 kb genomes) (Lauber et al., 2012) belongs to the order *Nidovirales* of positive-sense single-stranded RNA (ssRNA+) viruses that also includes three other families: *Arteriviridae* (12.7-15.7 kb genomes) and *Coronaviridae* and *Roniviridae* (26.3–31.7 kb) (Gorbalenya et al., 2006; de Groot et al., 2012) (Fig. 1). The family *Mesoniviridae* includes a single genus *Alphamesonivirus* with a single species *Alphamesonivirus 1* that currently includes two closely related viruses infecting mosquitoes, NDiV (Nga et al., 2011) and CavV (Zirkel et al., 2011), which were isolated in Vietnam and Côte d'Ivoire, respectively.

Most recently, several groups of researchers reported the identification, genome sequencing, and in most cases experimental characterization of new mesoniviruses isolated from different mosquito species on three continents (Zirkel et al., 2013; Kuwata et al., 2013; Vasilakis et al., 2014; Warrilow et al., 2014). They proposed that some of these viruses belong to the established species *Alphamesonivirus 1,* while others prototype new species. All new mesoniviruses have genome organizations, virion properties, mRNAs, and putative proteomes that are similar to those of *Alphamesonivirus 1* (Fig. 1). Quantitative analyses of the relation of these newly identified mesoniviruses with the established nidoviruses in the Bayesian and Maximum Likelihood (ML) frameworks showed that, in the nidovirus tree, the newly identified mesoniviruses clustered with NDiV and CavV in a deeply rooted lineageseparate from the lineages occupied by the *Coronaviridae* and *Roniviridae*. The most distinct molecular characteristic of all mesonoviruses is the genome size of ~20 kb which is intermediate between the size ranges of viruses of the *Arteriviridae* on the one hand and members of the *Coronaviridae* and *Roniviridae* on the other (**Fig. 1**). Together, these characteristics of the newly identified mesoniviruses provide a compelling basis for their formal recognition as members of the *Mesoniviridae* family.

For this proposal we evaluated the overall genomic and genetic similarity between all mesoniviruses, old and new. The overall similarity was found to be considerable, including genome sizes (from 19,980 nt for MénoV to 20,777 nt for KSaV, respectively), conservation of 7 ORFs with identities ranging from 56.6 to 100% at aa level and from 59.9 to 100% at nt level for the replicase ORF1ab region, which accounts for >80% of the genome size. For this proposal, we conducted quantitative analyses of evolutionary divergence of all these viruses using multiple sequence alignments of either the entire replicase gene-encoded pp1ab polyprotein or a large subset of it including the most conserved domains (from 3CLpro to OMT domains, corresponding to the nsp5-to-nsp16 polyprotein region in coronaviruses). In agreement with the published results, ML phylogenetic analysis by PhyML revealed an uneven distribution of viruses over several clusters (**Fig. 2**). To identify clusters corresponding to species, we employed the DEmARC framework (Lauber & Gorbalenya, 2012ab), which was previously used to devise the taxonomy of the *Mesoniviridae* and *Coronaviridae*. We have analyzed all 11 candidate PED thresholds identified by DEmARC (clustering cost of zero and monophyly of clustering) and selected one (0.092 PED) , which was most close to the PED that separates NDiV and CavV (**Fig. 3**). Based on this criterion, the analyzed viruses were found to form 7 clusters, which could be split into two groups. The first group consists of five relatively closely connected clusters whose viruses are separated by PEDs less than 0.311. They are proposed to form five species of the genus ***Alphamesonivirus,*** including the established species ***Alphamesonivirus 1*** and four new

species **Alphamesonivirus 2-5.** The second group includes two more distantly related viruses that are separated by PED exceeding the 0.311 threshold. They are proposed to prototype two unassigned species, **Mesonivirus 1** and **Mesonivirus 2.**

We believe that the further characterization of the natural diversity of mesoniviruses as well as additional studies into the functional and structural properties of these viruses will help to devise appropriate demarcation criteria for genera in the family *Mesoniviridae* which will then be used to establish genera for the species **Mesonivirus 1** and **Mesonivirus 2.** At this stage of poor virus sampling, we refrained from devising additional genera. This future development of mesonivirus taxonomy will be conducted in close cooperation with other nidovirus SGs to benefit from a larger knowledge base and to ensure coherence of the demarcation criteria and taxonomy structure across the order. In this process, the names of the new mesonivirus species may be reviewed and, if necessary, revised**.**

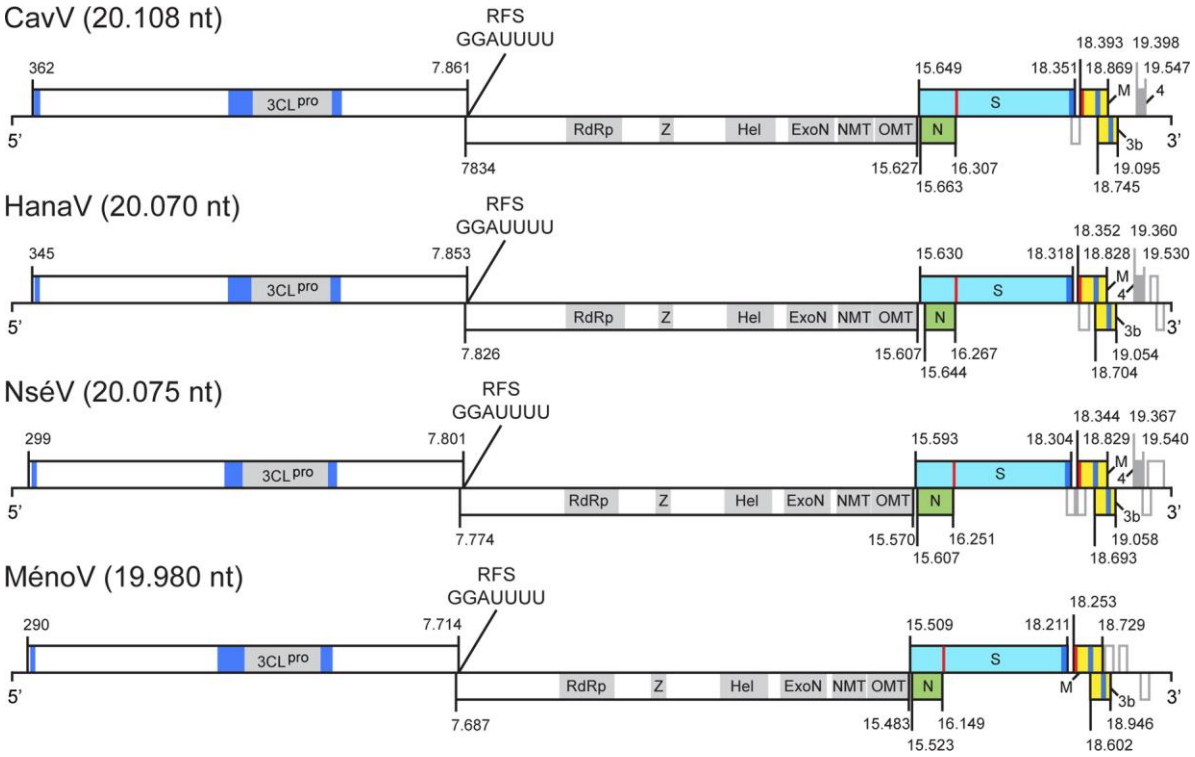
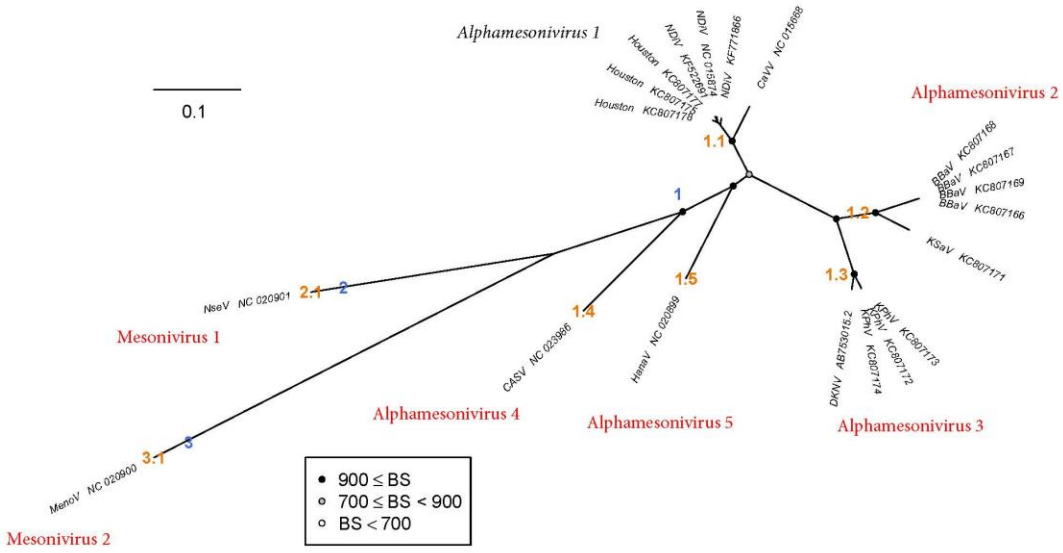



**Fig. 1** Mesonivirus genome organization. Shown is a schematic view of CavV (*Alphamesonivirus 1*), HanaV (new Alphamesonivirus 5), MénoV (new Mesonivirus 2), and NséV (new Mesonivirus 1) genomes. Open reading frames are shown by boxes, with nucleotide positions indicated. Conserved functional domains encoded by the replicase gene (ORF1a and -1b) as part of the pp1ab polyprotein are indicated by gray boxes. ORF2a is shown in light blue, ORF2b in green, and ORF3a and -3b in yellow. Hydrophobic (putative transmembrane) regions are marked in blue, and predicted signal peptides are shown in red. The light-gray boxes symbolize several small, nonconserved ORFs in the 3'-terminal regions of the four mesonivirus genomes. The solid gray box symbolizes ORF4, while other small ORFs are indicated by open gray boxes. (From Zirkel et al., 2013).




Mesoniviridae_20150612_nsp1to16_5362aa_0.092-0.311_PhyML_unrootedForProposal.pdf

**Fig. 2.** Phylogenetic analysis of mesoniviruses by PhyML. Virus acronyms, GenBank and RefSeq accession numbers, and species names are indicated in black, while names for the proposed species are in red. The indicated RefSeq IDs corresponds to the following GenBank IDs: NC_020900 to JQ957873; NC_020901 to JQ957874; NC_023986 to KJ125489; NC_020899 to JQ957872; NC_015668 to HM746600; NC_015874 to DQ458789. Clusters of different hierarchical levels according to DEmARC indicated with orange and blue numbers, which correspond to species and (tentative) genera, respectively (see Figure 3 for other details on clustering). Scale is in aa replacements per position, and support for internal nodes by bootstrap (BS) is indicated. (Gulyaeva & Gorbalenya, unpublished).

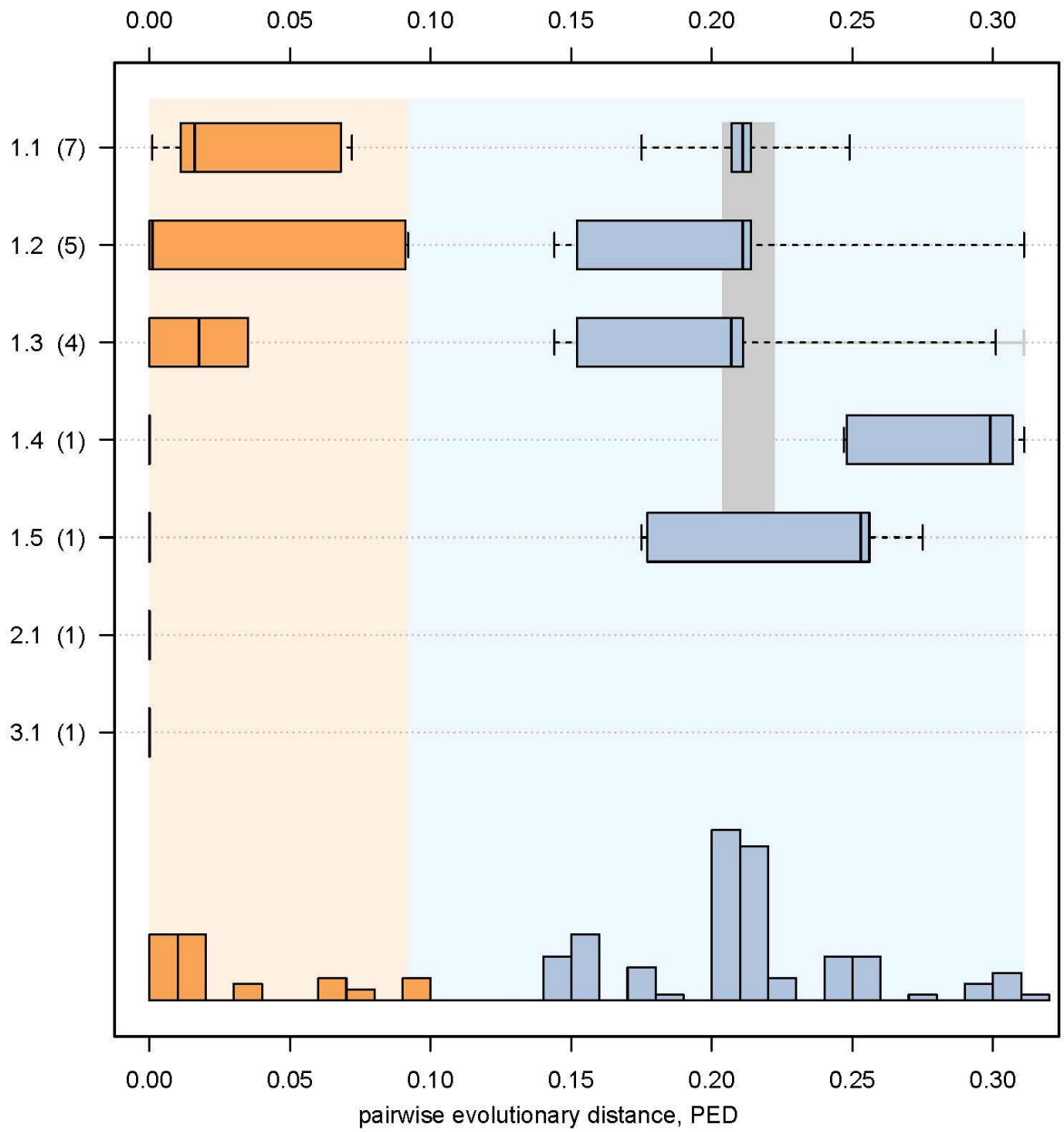



**Fig. 3.** Intra-group genetic divergence in the two-level hierarchical clustering of mesoniviruses by DEmARC. At the left axis, clusters numbering and number of viruses in respective clusters (bracketed) are indicated. They correspond to monophyletic clusters in the tree of Fig. 2. Box-and-whisker graphs were used to plot distributions of distances between viruses from the same species (orange), and between viruses from different species but the same genus (blue). The boxes span from the first to the third quartile and include the median (bold line), and the whiskers (dashed lines) extend to the extreme values. The corresponding first half of the PED distribution is depicted below. (Gulyaeva & Gorbalenya, unpublished).