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:	***2011.001a-gI***	(to be completed by ICTV officers)

Short title: Create a new family (*Mesoniviridae*), genus (*Alphamesonivirus*) and species (*Alphamesonivirus 1*) to accommodate insect nidoviruses
(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:

Alexander E. Gorbalenya (A.E.Gorbalenya@lumc.nl)
Chris Lauber (C.Lauber@lumc.nl)
Eric J. Snijder (E.J.Snijder@lumc.nl)
Phan Thi Nga (pnga_arboviruses@yahoo.com)
Kouichi Morita (moritak@nagasaki-u.ac.jp)
John Ziebuhr (John.Ziebuhr@viro.med.uni-giessen.de)
Christian Drosten (drosten@virology-bonn.de)
Sandra Junglen (junglen@virology-bonn.de)

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)	AEG, EJS and JZ are members of different SGs that are concerned with families of the order Nidovirales. The proposal was favorably discussed during meetings of the Coronaviridae SG at the XIth and XIIth Nidovirus meetings (May 2008, June 2011). Chairs of the Arteriviridae SG (Kay Faaberg), Coronaviridae SG (Raoul de Groot), and Roniviridae SG (Jeff Cowley) have approved the proposal.

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

Date first submitted to ICTV:
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. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	***2011.001aI***	(assigned by ICTV officers)

To create one new species within:

Genus:	***Alphamesonivirus* (new)**
Subfamily:	
Family:	***Mesoniviridae* (new)**
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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Alphamesonivirus 1	**HM746600** - Cavally virus isolate C79 (CavV); **DQ458789** - Nam Dinh virus isolate 02VN178 (NDiV)

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 new species is proposed to accommodate two newly identified and closely related viruses, Nam Dinh virus isolate 02VN178 (NDiV) and Cavally virus isolate C79 (CavV), isolated from mosquitoes. These are the first and only viruses that are placed in the newly created species, genus and family. The overall genomic and genetic similarity between these viruses is very high: genome size (20,192 and 20,187 nt), conservation of 7 open reading frames (ORFs) with identities ranging from 84.4 to 96.1% (at aa level) and from 87.5 to 93.7% (at nt level). They are separated from the next most closely related viruses, which belong to the *Roniviridae* and *Coronaviridae*, by evolutionary distances that are comparable with those separating viruses of the two latter families.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2011.001bI***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Mesoniviridae* (new)**
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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2011.001cI***	(assigned by ICTV officers)

To name the new genus: *Alphamesonivirus*

Assigning the type species and other species to a new genus

Code	***2011.001dI***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Alphamesonivirus 1

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

one

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

It is the first genus created in this newly proposed family

Origin of the new genus name:

Alphamesonivirus stands for **first (alpha- in the Greek alphabet) medium-size (meso- in Greek) nidovirus genus**

Reasons to justify the choice of type species:

Only one species has been recognized so far

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Although a species demarcation is not required when only one species is recognized, we have conducted an analysis of ***Alphamesonivirus 1*** for this proposal to ensure that the two viruses that form this species do not prototype separate species. A state-of-the-art framework, which was previously used to devise the taxonomy of the *Coronaviridae*, was applied to NDiV and CavV and a representative set of nidoviruses. The analysis was performed for two sets of proteins: the first included proteins conserved in all nidoviruses (3CLpro, RdRp, HEL1) and the second set

additionally included ExoN and OMT, which are conserved in large nidoviruses and NDiV/CavV. Pairwise evolutionary distances (PED) were compiled for all pairs of viruses. It was found that the PED separating NDiV and CavV is within the range of intra-species virus divergence in the *Coronaviridae* and *Roniviridae* (see Annex). Because of this observation, it is proposed to recognize NDiV and CavV as viruses of a single species that we called ***Alphamesonivirus 1*.**

MODULE 5: **NEW FAMILY**

creating and naming a new family

Code	***2011.001eI***	(assigned by ICTV officers)

To create a new family containing the subfamilies and/or genera listed below within the Order: *Nidovirales*

If there is no Order, write "**unassigned**" here.
If the Order has yet to be created (in Module 6) please write **"(new)"** after the proposed name.

Code	***2011.001fI***	(assigned by ICTV officers)

To name the new family: *Mesoniviridae*

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)

To assign the following subfamilies (if any) to the new family:
You may list several subfamilies here. For each subfamily, please state whether it is new or existing.

- If the subfamily is new, it must be created in Module 4
- If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family

Code	***2011.001gI***	(assigned by ICTV officers)

To assign the following genera to the new family:
You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

***Alphamesonivirus* (new)**

The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**

none

Reasons to justify the creation of the new family:
Additional material in support of this proposal may be presented in the Appendix, Module 9

Recently two closely related viruses, NDiV and CavV, were isolated by two groups of researchers from mosquitoes in Vietnam and Cote d'Ívoire, respectively. These ssRNA+ viruses were propagated in insect cells and characterized using different techniques. They have genome organizations, virion properties, mRNAs, and putative proteomes whose characteristics place them in the order *Nidovirales*. Phylogenetic and protein domain analyses indicated that NDiV and CavV consistently, albeit very distantly, cluster with viruses of the family *Roniviridae,* which also infect invertebrate hosts. Quantitative analysis of the relation of these newly identified viruses with the established nidoviruses in the Bayesian and Maximum Likelihood frameworks showed that the newly identified viruses form a deeply rooted lineage in the nidovirus tree comparable with the lineages occupied by *Coronaviridae* and *Roniviridae,* two

out of three previously established families in this order. The most distinct molecular characteristic of NDiV and CavV is the genome size of ~20 kb which is intermediate between the sizes of the *Arteriviridae* (small nidoviruses; 12.7-15.6 kb) on the one hand and *Coronaviridae* and *Roniviridae* (large nidoviruses; 25.6-31.7 kb) on the other. Together, these characteristics of NDiV and CavV provide a compelling basis for the creation of a new nidovirus family. This proposal was reported and discussed at the XIth and XIIth Nidovirus meetings in Oxford, UK (May 2008) and Acme, MI, USA (June 2011), respectively, which included discussions at meetings of the Coronavirus Study Group. The creation of a new family was also proposed in the two recent papers describing the identification and characterization of NDiV (Nga et al., 2011) and CavV (Zirkel et al., 2011).
Origin of the new family name:
Mesoniviridae stands for **Medium-size (Meso- in Greek) nidoviruses**

MODULE 9: **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.

Gorbalenya, A. E., Enjuanes, L., Ziebuhr, J. and E. J. Snijder (2006) Nidovirales: Evolving the largest RNA virus genome, Virus Research, 117: 17-37.

Nga, P. T., Parquet, M. D. C., Lauber, C., Parida, M., Nabeshima, T., Yu, F., Thuy, N. T., Inoue, S., Ito, T., Okamoto, K., Ichinose, A., Snijder, E.J., Morita, K., & Gorbalenya, A. E. (2011). Discovery of the first insect nidovirus, a missing evolutionary link in the emergence of the largest RNA virus genomes, PLoS Pathogens, 7(8): e1002215.

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.

We gladly acknowledge papers of (Nga et al., 2011) and (Zirkel et al., 2011) which were used to create this Annex.

The order *Nidovirales* includes positive-sense single-stranded RNA (ssRNA+) viruses of three families: *Arteriviridae* (12.7-15.7 kb genomes; "small nidoviruses") and *Coronaviridae* and *Roniviridae* (26.3–31.7 kb; the latter 2 families also referred to as "large nidoviruses") (Gorbalenya et al., 2006; de Groot et al., 2012) (Fig. 1). All other ssRNA+ viruses have genomes with sizes of less than 20 kb. Recently, two closely related viruses, NDiV and CavV, which are the subject of this proposal, were isolated by two groups of researchers from mosquitoes in Vietnam and Côte d'Ivoire, respectively (Nga et al., 2011; Zirkel et al., 2011).

These ssRNA+ viruses were propagated in insect cells and characterized using different techniques. They have a genome organization, virion properties, mRNAs, and putative proteome whose characteristics place them in the order *Nidovirales* (Fig. 1 and 2). Particularly they encode a complement to the replicative proteins characteristic of all nidoviruses: 3C-like main protease (3CLpro), RNA-dependent RNA polymerase (RdRp) and a superfamily 1 helicase (HEL1), and

also two additional replicase proteins, 3'-to-5'exoribonuclease (ExoN) and 2'-*O*-methyltransferase (OMT), that are characteristic for large nidoviruses. Phylogenetic and protein domain analyses indicated that NDiV and CavV consistently, albeit very distantly, cluster with viruses of the family *Roniviridae*, which also infect invertebrate hosts. However, this relation is limited to the domains common in large nidoviruses; particularly, no similarities were found between the structural proteins of NDiV and CavV virions and those of viruses of the *Roniviridae* or other nidoviruses. Quantitative analysis of the relation of these newly identified nidoviruses with the established nidoviruses in the Bayesian and Maximum Likelihood frameworks showed that they form a deeply rooted lineage in the nidovirus tree comparable with the lineages occupied by *Coronaviridae* and *Roniviridae* (Fig. 3; Table 1). The most distinct molecular characteristic of NDiV and CavV 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 NDiV and CavV provide a compelling basis for the creation of a new nidovirus family.

This proposal was reported and discussed at the XIth and XIIth Nidovirus meetings in Oxford, UK (May 2008) and Acme, MI, USA (June 2011), respectively, which included discussions at meetings of the Coronavirus Study Group. The creation of a new family was also proposed in the two recent papers describing the identification and characterization of NDiV (Nga et al., 2011) and CavV (Zirkel et al., 2011).

For this proposal we evaluated the overall genomic and genetic similarity between NDiV and CavV, also in the context of sequence divergence of previously established species in other nidovirus families. The overall similarity between NDiV and CavV was found to be very high: nearly identical genome sizes (20,192 and 20,187 nt, respectively), conservation of 7 ORFs with identities ranging from 84.4 to 96.1% at aa level and from 87.5 to 93.7% at nt level (Table 2). A state-of-the-art framework, which was previously used to devise the taxonomy of the *Coronaviridae*, was applied to NDiV and CavV and a representative set of nidoviruses. The analysis was performed for two sets of proteins: the first included proteins conserved in all nidoviruses (3CLpro, RdRp, HEL1) and the second set additionally included ExoN and OMT, which are conserved in large nidoviruses and NDiV/CavV. Pairwise evolutionary distances (PED) were compiled for all pairs of viruses. It was found that the PED separating NDiV and CavV is within the range of intra-species virus divergence in the *Coronaviridae* and *Roniviridae* (Fig. 4). Because of these observations, we propose to recognize NDiV and CavV as viruses of a single species *Alphamesonivirus 1*.

Table 1. Genome sequences of a representative set of the Nidovirus species.

species name[a]	virus abbreviation	(sub)family	acc. number
Alphamesonivirus 1	NDiV	Mesoni-	DQ458789
Alphamesonivirus 1	CavV	Mesoni	HM746600
Gill-associated virus	GAV	Roni-	AF227196
Yellow head virus	YHV	Roni-	EU487200
White bream virus	WBV-DF24	Toro-	NC_008516
Equine torovirus	EToV-Berne	Toro-	X52374
Bovine torovirus	BToV-Breda1	Toro-	NC_007447
Human coronavirus 229E	HCoV-229E	Corona-	NC_002645
Human coronavirus NL63	HCoV-NL63	Corona-	DQ445911
Miniopterus bat coronavirus 1	Mi-BatCoV-1A	Corona-	NC_010437
Rhinolophus bat coronavirus HKU2	Rh-BatCoV-HKU2	Corona-	NC_009988
Miniopterus bat coronavirus HKU8	Mi-BatCoV-HKU8	Corona-	NC_010438
Scotophilus bat coronavirus 512	Sc-BatCoV-512	Corona-	DQ648858
Porcine epidemic diarrhoea virus	PEDV-CV777	Corona-	NC_003436
Alphacoronavirus 1	FCoV	Corona-	NC_007025
SARS-related coronavirus	SARS-HCoV	Corona-	AY345988
Tylonycteris bat coronavirus HKU4	Ty-BatCoV-HKU4	Corona-	EF065505
Pipistrellus bat coronavirus HKU5	Pi-BatCoV-HKU5	Corona-	EF065509
Rousettus bat coronavirus HKU9	Ro-BatCoV-HKU9	Corona-	EF065513
Human coronavirus HKU1	HCoV-HKU1	Corona-	AY884001
Betacoronavirus 1	HCoV-OC43	Corona-	AY585228
Murine coronavirus	MHV-A59	Corona-	AY700211
Avian coronavirus	IBV-Beaud	Corona-	NC_001451
Beluga whale coronavirus SW1	BWCoV-SW1	Corona-	EU111742
Equine arteritis virus	EAV-CW	Arteri-	AY349167
Simian hemorrhagic fever virus	SHFV	Arteri-	NC_003092
Lactate dehydrogenase-elevating virus	LDV-P	Arteri-	U15146
Porcine respiratory and reproductive syndrome virus, type 2	PRRSV-NA	Arteri-	AF176348[b]
Porcine respiratory and reproductive syndrome virus, type 1	PRRSV-LV	Arteri-	M96262

[a] species names of coronaviruses taken from ICTV proposal 2008.085-122V.U that was approved by ICTV in 2009.
[b] sequence of the prototype virus is deposited in GenBank under U87392.
Red, taxons of this proposal.

Table 2. Comparison of genomes and ORFs of NDiV and CavV

	length NDiV [nt]	length CavV [nt]	frame[#] NDiV	frame[#] CavV	nucleotide identity [%]	amino acid identity [%]
ORF1a	7509	7497	0	0	88.3	90.0
ORF1b	7587	7587	-1	-1	92.6	96.1
ORF2a	2697	2700	-1	-1	90.7	87.5
ORF2b	636	642	+1	+1	88.8	90.2
ORF3a	474	474	-1	+1	91.1	93.0
ORF3b	348	348	0	-1	93.7	90.5
ORF4	96	87	-1	-1	87.5	84.4

[#] reading frame relative to that of ORF1a

ORF designation according to Table 2 in Zirkel et al., 2011.

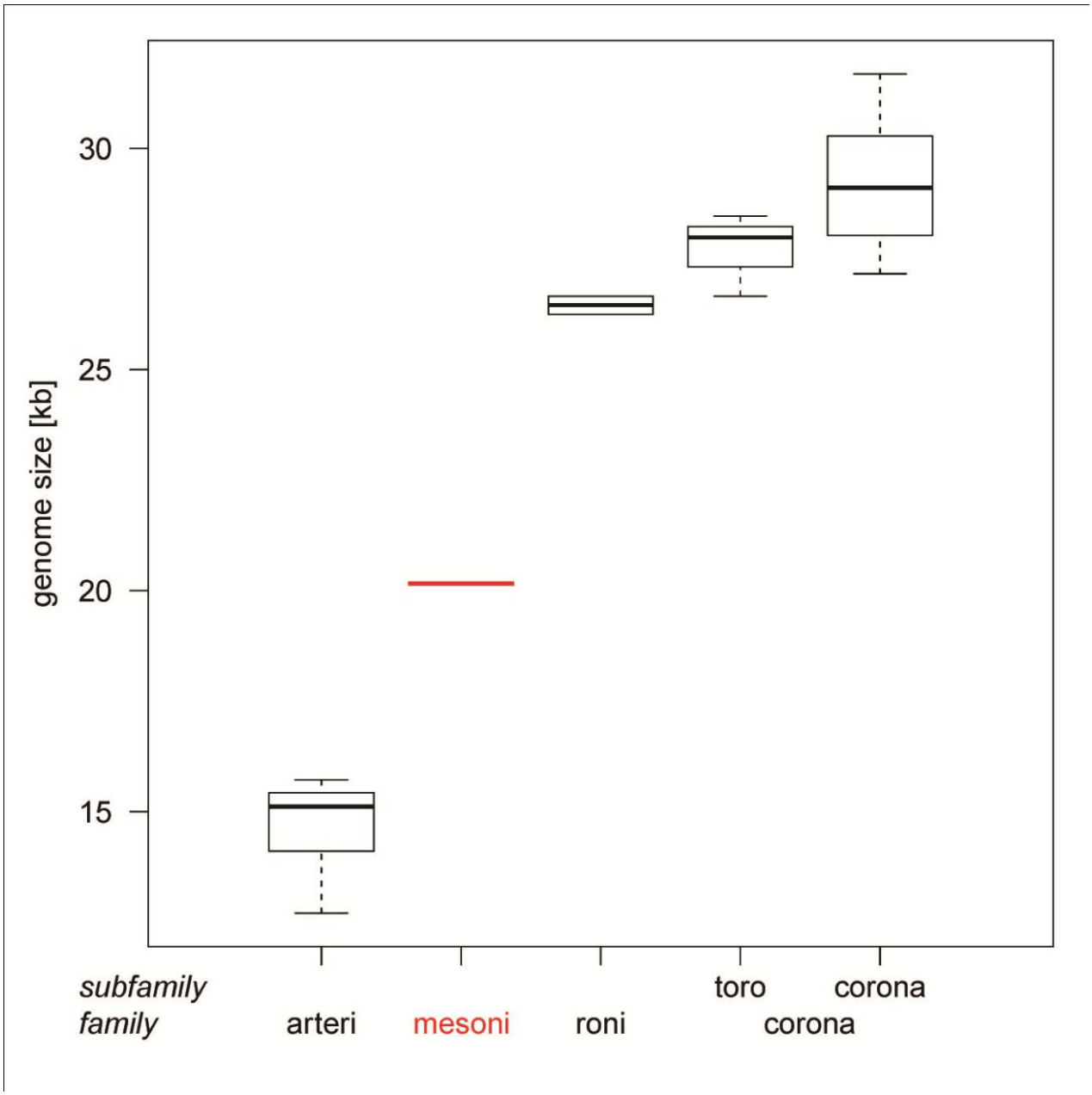

Fig. 1. Distribution of genome sizes of nidoviruses. Shown are distributions of genome sizes in 29 nidoviruses representing the two subfamilies (*Torovirinae* and *Coronavirinae*) of the family *Coronaviridae* as well as the families *Arteriviridae* and *Roniviridae*, and the newly proposed *Mesoniviridae* (CavV and NDiV). Taxon-specific box-and-whisker plots include: median (bold horizontal line), box (from the first to third quartile), whiskers (dashed lines, extending to the extremes).

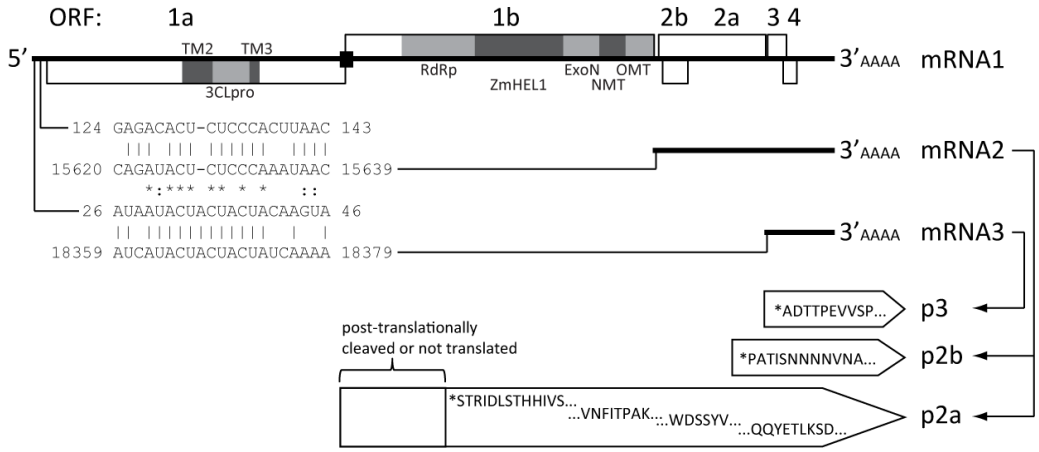

Fig. 2. NDiV genome organization and expression. Open reading frames (ORFs) are represented by open rectangles and ORF1a- and ORF1b-encoded protein domains identified by bioinformatics analyses are highlighted in grey. Peptide sequences of virion proteins were determined and mapped to the products of ORFs 2a, 2b, and 3 (bottom-right). ORFs 3 and 4 in this figure correspond to ORFs 3a and 3b, respectively, listed in Table 1. ORF4 listed in Table 1 is not shown in this figure. Experimentally determined N-terminal protein sequences are indicated by (*), other peptide sequences indicate experimentally determined inner sequences. Two pairs of conserved potential transcription regulator sequences (TRSs) – for sg mRNAs 2 and 3, respectively - were identified in the NDiV genome and aligned (bottom-left), with each pair consisting of a putative leader TRS in the 5'-UTR and a body TRS in the 3'-proximal region of the genome. Between these TRS pairs, eight and three positions include complete match (*) and nucleotide overlap (:), respectively. Adapted from Fig. 3 of Nga et al., 2011. Note that Zirkel et al., 2011 present experimentally verified TRS assignments for three sg mRNAs that partially deviate from those presented in this Figure.

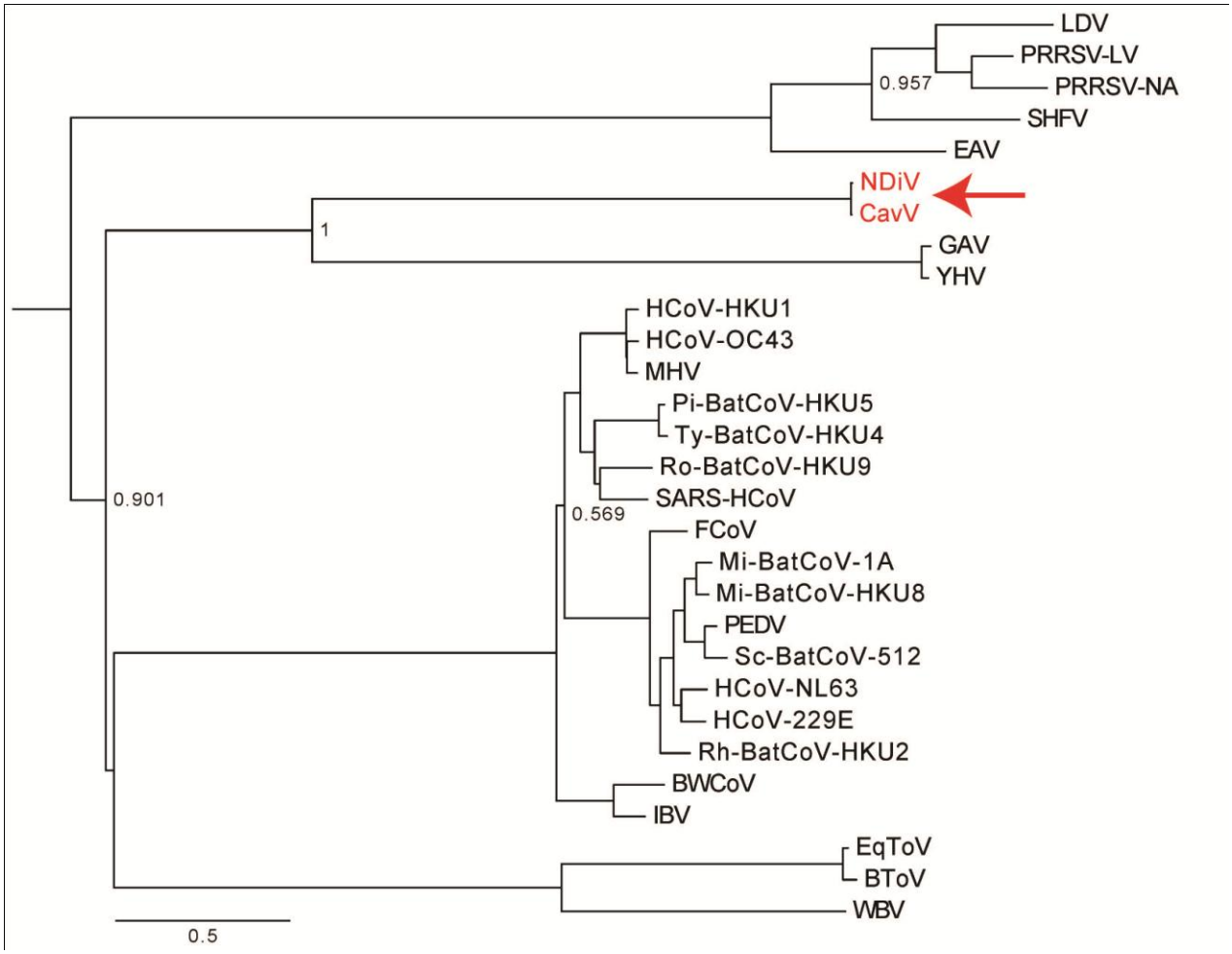

Fig. 3. Phylogeny of nidoviruses. To infer phylogenetic relationships between Nam Dinh virus isolate 02VN178 (NDiV**)** and Cavally virus isolate C79 (CavV**)** (red arrow) and other nidoviruses, a partially constrained tree was calculated using a concatenated alignment of the three nidovirus-wide conserved domains and a set of viruses representing currently recognized species. Numbers indicate posterior probability support values (at the scale from 0 to 1); all internal nodes for which no support value is provided have been fixed in the analysis based on prior analyses of nidovirus subsets (data not shown). The scale bars represent the number of substitutions per amino acid position on average. The tree was rooted on the arterivirus branch. For virus names abbreviations and further details see Table 1 of this proposal, and Fig. 6 and text of Nga et al., 2011.

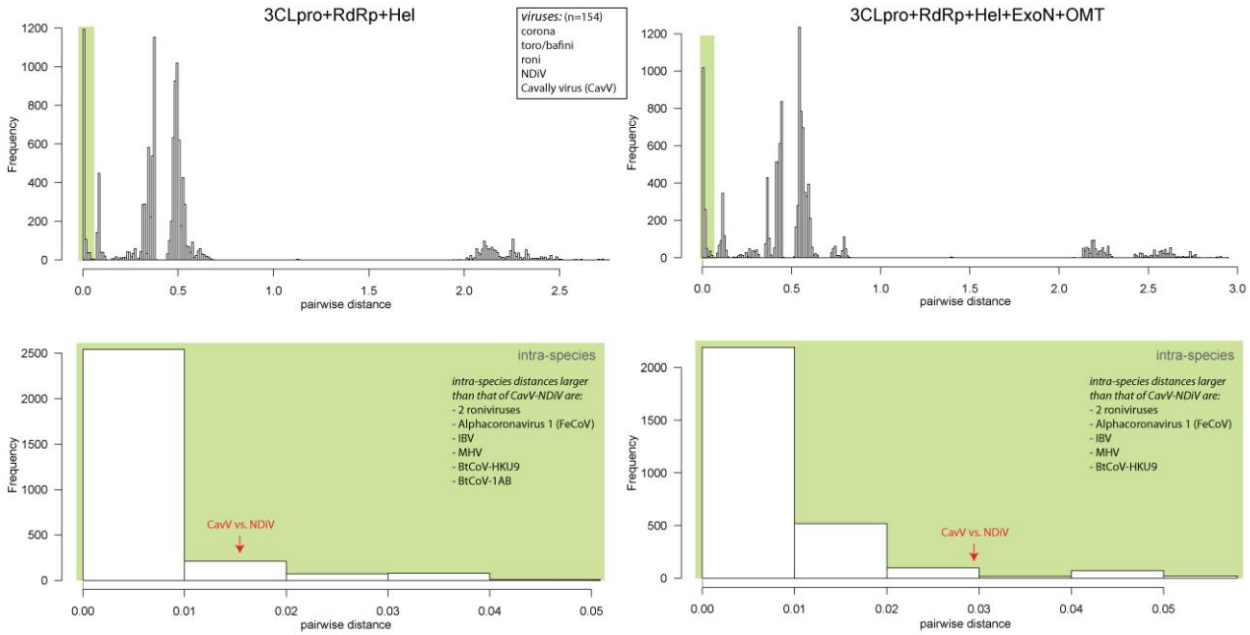

Fig. 4. Evolutionary distance between NDiV and CavV in relation to intra-species genetic divergence in large-sized nidoviruses. Multiple amino acid alignments for 154 nidoviruses with large genomes (all major nidovirus lineages except arteriviruses) comprising three nidovirus-wide conserved protein domains (left column) or five large-sized nidovirus-wide conserved domains (right column) were used to compile genetic distances between all virus pairs. These distances are shown in form of a frequency distribution (top row) and zoom-ins on small distances are provided (bottom row). The pair-wise distance between CavV and NDiV (indicated in red) is well within the intra-species distance range of other nidoviruses. A number of recognized nidovirus species show a maximum genetic divergence larger than that of CavV-NDiV; they are listed within the plot.