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:	***2009.012a-fP***	(to be completed by ICTV officers)

Short title: Create new ssRNA virus species and genus for virus infecting marine fungoid protist thraustochytrids (Labyrinthulomycetes)
(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:

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Has this proposal has been seen and agreed by the relevant study group(s)? Please select answer in the box on the right	**Yes**

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**

Part (a) to create and name one or more new species.
If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.012aP***	(assigned by ICTV officers)

To create new species with the name(s):

Aurantiochytrium single-stranded RNA virus 01

Part (b) assigning new species to higher taxa
All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.012bP***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	***Labyrnavirus (new)***
Subfamily:	***unassigned***
Family:	***unassigned***
Order:	***Picornavirales***

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.

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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

We have reported on a new virus (SssRNAV) infectious to the marine fungoid protist *Schizochytrium* sp. NIBH N1-27 (Takao et al., 2005 & 2006). Genus *Schizochytrium* had been classified in the class *Labyrinthulomycetes*, family *Thraustochytriaceae* within the kingdom *Chromista* (Cavalier-Smith, 1997). In our papers, this virus was tentatively named as *Schizochytrium* single-stranded RNA virus (SssRNAV); however, the taxonomy of the host (*Schizochytrium*) has been recently redefined more precisely by integrating morphology, physiology and molecular phylogenetic data [Yokoyama and Honda (2007) and Yokoyama *et al.* (2007)]. As a result of this taxonomy rearrangement, the genus *Schizochytrium* was divided into three genera: genus *Aurantiochytrium,* genus *Oblongichytrium* and genus *Schizochytrium* (Yokoyama & Honda, 2007). All SssRNAV-sensitive host strains were classified in the genus *Aurantiochytrium.* **Therefore, here we submit the formal proposal to establish a new species for this virus and to name this species '*Aurantiochytrium single-stranded RNA virus 01* (AuRNAV01)'.**

AuRNAV01 was isolated from the coastal water of Kobe Harbor, Japan in July 2000 (Takao et al., 2005). The virus particle is icosahedral, lacking a tail, and is ca. 25 nm in diameter (Annexes Fig. 1). Virus particles form crystalline arrays and random assemblies within the cytoplasm of host cells (Annexes Fig. 2). The lytic cycle and the burst size were estimated at < 8 h and 5.8×10^3 – 6.4×10^4 infectious units per host cell, respectively. SDS-PAGE analysis revealed AuRNAV01 has three major structural proteins (37 kDa, 34 kDa and 32 kDa in molecular mass) and one minor protein (18 kDa) (Takao et al., 2005). The viral RNA is single-stranded with a positive sense; and is 9,018 nt in length (excluding the 3' poly A tail). Full nucleotide sequence of AuRNAV01 genome is registered with GenBank accession number AB193726. It contains two long open reading frames (ORFs), which are separated by an intergenic region of 92 nt. The 5' ORF (ORF 1) is preceded by an un-translated leader sequence of 554 nt. The downstream large ORF (ORF 2) and an additional ORF (ORF 3) overlaps by 431 nucleotides; ORF 3 is followed by an un-translated region of 70 nt (excluding the 3' poly A tail). The three ORFs encode putative replication proteins (ORF1), capsid proteins (ORF2) and a protein of unknown function (ORF3) (Annexes Fig. 3). The results of northern blot analysis suggest that AuRNAV01 synthesizes sub-genomic RNAs to translate ORF2 and ORF3 (Takao et al., 2006). The ORF3 sub-genomic RNA is expressed at high levels. Putative replication proteins' and capsid proteins' sequences showed remarkable similarities to those of the recently discovered three diatom-infecting viruses (*Rhizosolenia setigera RNA virus 01* [RsetRNAV01], *Chaetoceros tenuissimus RNA virus* [CtenRNAV01] and *Chaetoceros socialis f. radians RNA virus* [CsfrRNAV01]), as well as those of *Heterosigma akashiwo RNA virus SOG 263* (HaRNAV-SOG263: family *Marnaviridae*) and dicistroviruses (family *Dicistroviridae*) (Takao et al., 2006, Tomaru et al., 2009). However, some properties of this new virus are clearly distinct from those of the related viruses. (1) In dicistroviruses the downstream ORF is translated using an internal ribosome entry site (IRES) and subgenomic RNAs are not produced (Mayo, 2002). (2) HaRNAV-SOG263 genome harbors only one ORF (Lang et al., 2004). (3) Diatom-infecting viruses (RsRNAV01, CtenRNAV01, CsfrRNAV01) lack ORF3 (Shirai et al., 2006, 2008; Tomaru et al., 2009). (4) The AU ratio of AuRNAV01 is 50.2 %, while those of the three diatom-infecting ssRNA viruses (RsRNAV01, CtenRNAV01, CsfrRNAV01) are higher ranging between 60.4-63.7 % (Takao et al., 2006, Shirai et al., 2006, Shirai et al., 2008, Tomaru et al., 2009). (5) In a phylogenetic analysis based on both the nucleotide sequence and amino acid sequence of the putative RNA-dependent RNA polymerase (RdRp), AuRNAV01 forms a separate branch that is distinct from that of related viruses with the closest related viruses being the three diatom-infecting viruses (Takao et al., 2006, Tomaru et al., 2009 and see Annexes Fig. 4). These features clearly distinguish this virus from previously known virus genera. **Therefore, we also propose a new genus "*Labyrnavirus*" unassigned in the order *Picornavirales* for this single species '*Aurantiochytrium single-stranded RNA virus 01*' (see module 3).**

MODULE 3: **NEW GENUS**

creating and naming a new genus

Code	***2009.012cP***	(assigned by ICTV officers)

To create a new genus to contain the species listed below

Aurantiochytrium single-stranded RNA virus 01

Code	***2009.012dP***	(assigned by ICTV officers)

To name the new genus: *Labyrnavirus*

assigning a new genus to higher taxa

Code	***2009.012eP***	(assigned by ICTV officers)

To assign the new genus as follows: Ideally, a genus should be placed within a higher taxon, but if not, write "**unassigned**" in the box below.

Subfamily:	***unassigned***
Family:	***unassigned***
Order:	***Picornavirales***

If any of these taxa has yet to be created (in module 4, 5 or 6) please write "**(new)**" after its proposed name.

assigning type species and other species to a new genus

Code	***2009.012fP***	(assigned by ICTV officers)

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

Aurantiochytrium single-stranded RNA virus 01

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

Code		(assigned by ICTV officers)

To assign the following as additional species of the new genus:

Reasons to justify the creation of a new genus:

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

As mentioned in the preceding clause (module 2), *Aurantiochytrium single-stranded RNA virus* should be classified in a single new genus. **Therefore, we propose to create new genus *Labyrnavirus* (originated from ''ss*RNA virus* infecting *Labyr*inthulids'').**

The amino acid sequences of AuRNAV01 structural and non-structural proteins showed some similarities to those of HaRNAV-SOG263 and the diatom-infecting ssRNA viruses mentioned above; still, it may be too rough-and-ready to determine the family to which the new genus *Labyrnavirus* belongs (data not shown).

Origin of the new genus name:

ss*RNA virus* infecting *Labyr*inthulids→ *Labyrnavirus*

Reasons to justify the choice of type species:

AuRNAV01 is the only characterized member of this new genus *Labyrnavirus*.

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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequence
-

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Cavalier-Smith, T. (1997) Sagenista and Bigyra, two phyla of heterotrophic heterokont chromists. Archiv Protistenk 148: 253-267.

Honda, D., Yokochi, T., Nakahara, T., Raghukumar, S., Nakagiri, A., Schaumann, K., and Higashihara, T. (1999) Molecular phylogeny of labyrinthulids and thraustochytrids based on the sequence of 18S ribosomal RNA gene. J Eukaryot Microbiol 46: 637-647.

Lang, A. S., Culley, A. I., Suttle, C. A. (2004) Genome sequence and characterization of a virus (HaRNAV) related to picorna-like viruses that infects the marine toxic bloom-forming alga Heterosigma akashiwo. Virology 320: 206-217.

Mayo, M. A. (2002) Virus Taxonomy – Houston 2002. Arch Virol 147, 1071-1076.

Shirai, Y., Takao, Y., Mizumoto, H., Tomaru, Y., Honda, D., Nagasaki, K. (2006) Genomic and phylogenetic analysis of a single-stranded RNA virus infecting Rhizosolenia setigera (Stramenopiles: Bacillariophyceae). J. Mar. Biol. Ass. U.K., 86: 475-483.

Shirai, Y., Tomaru, Y., Takao, Y., Suzuki, H., Nagumo, T., Nagasaki, K. (2008) Isolation and characterization of a single-stranded RNA virus infecting the marine planktonic diatom Chaetoceros tenuissimus Meunier. Appl. Environ. Microbiol. 74(13): 4022-4027.

Takao, Y., Nagasaki, K., Mise, K., Okuno, T., Honda, D. (2005) Isolation and characterization of a novel single-stranded RNA virus (SssRNAV) infectious to a marine fungoid protist Schizochytrium sp. (Thraustochytriaceae, Labyrinthulea). Appl. Environ. Microbiol. 71: 4516-4522

Takao, Y., Mise, K., Nagasaki, K., Okuno, T., Honda, D. (2006) Complete nucleotide sequence and genome organization of a single-stranded RNA virus infecting the marine fungoid protist Schizochytrium sp. J. Gen. Virol. 87: 723-733

Tomaru Y, Takao Y, Suzuki H, Nagumo T, Nagasaki K (2009) Isolation and characterization of a single-stranded RNA virus infecting the bloom forming diatom Chaetoceros socialis. Appl. Environ. Microbiol. 75: 2375-2381.

Yokoyama, R., and Honda, D. (2007) Taxonomic rearrangement of the genus Schizochytrium sensu lato based on morphology, chemotaxonomical characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for Schizochytrium and erection of Aurantiochytrium and Oblongichytrium gen. nov. Mycoscience 48: 199-211.

Yokoyama, R., Salleh, B., and Honda, D. (2007) Taxonomic rearrangement of the genus Ulkenia sensu lato phylogeny based on morphology, chemotaxonomical characteristics and 18S rRNA gene (Thraustochytriaceae, Labyrinthulomycetes): emendation for Ulkenia and erection of Botryochytrium, Parietichytrium and Sicyoidochytrium gen. nov. Mycoscience 48: 329-341.

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.

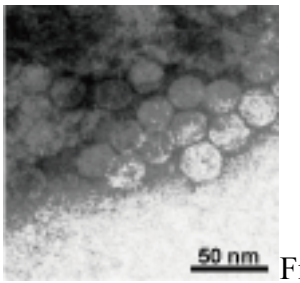

Fig. 1. Negatively-stained virions of AuRNAV01. (reprinted with copyright permission from American Society for Microbiology: Takao, Y. et al. *Appl. Environ. Microbiol.*, **71(8):** 4516-4522. [published in August 2005])

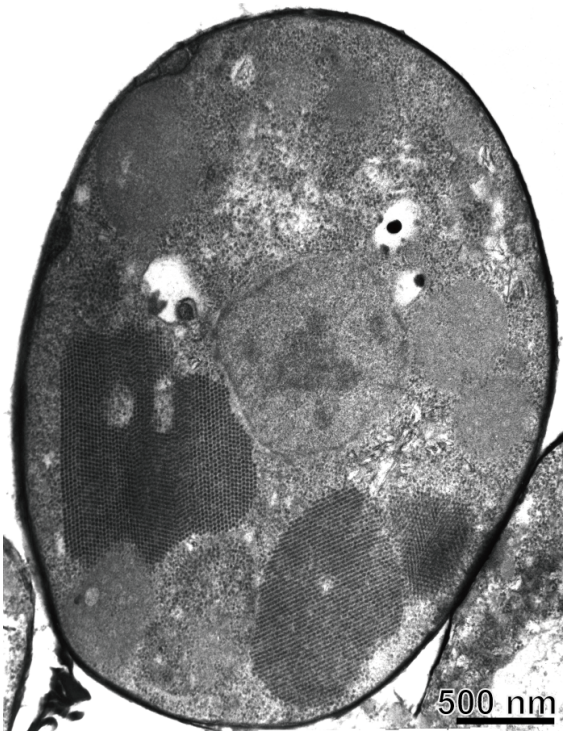

Fig. 2. Transmission electron microphotographs of *Aurantiochytrium* sp. NIBH N1-27 infected by AuRNAV01. (reprinted with copyright permission from American Society for Microbiology: Takao, Y. et al. (2005) *Appl. Environ. Microbiol.*, **71(8):** 4516-4522. [published in August 2005])

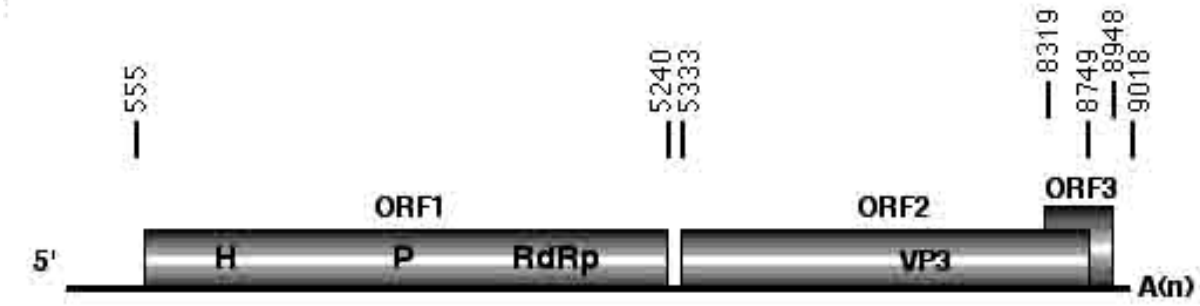

Fig. 3. Schematic genome structure of AuRNAV01. Numbers indicate base positions from the 5' terminus in the nucleotide sequence. H, RNA helicase domain; P, protease domain; RdRp, RNA-dependent RNA polymerase domain. (reprinted with copyright permission from the Society for General Microbiology: Takao, Y.et al. *J. Gen. Virol*., **87:** 723-733. [published in March 2006])

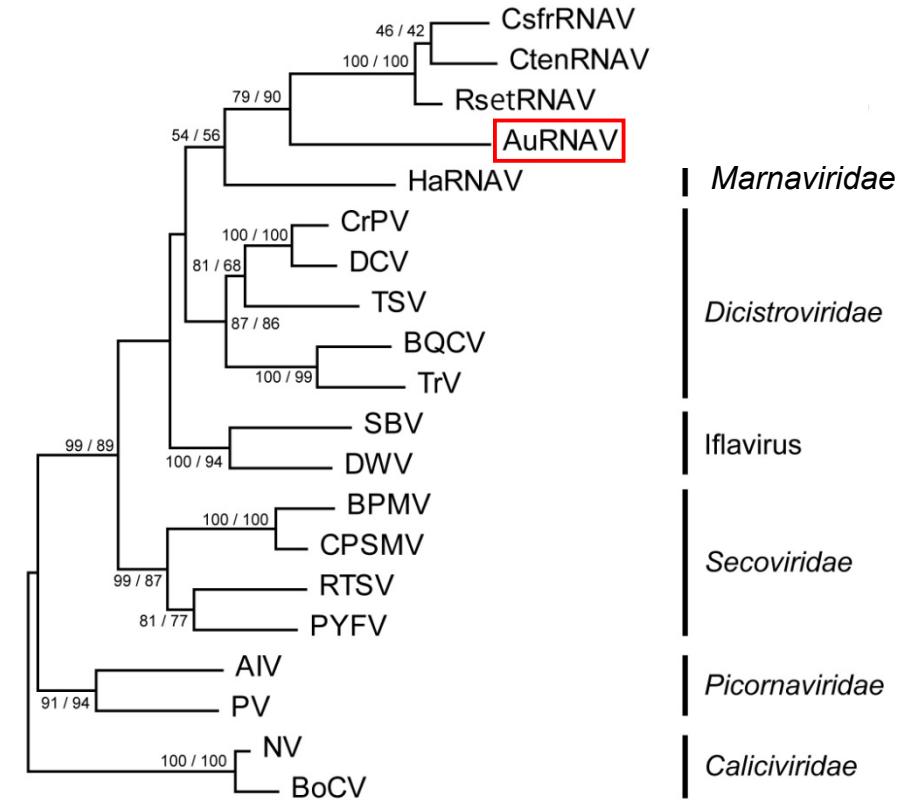

Fig. 4. ML tree based on deduced amino acid sequences of the RdRp whole domain. ML bootstrap values (%) from 100 samples are shown at the nodes, followed by bootstrap values based on the NJ analysis (%) from 100 samples. The ML distance scale bar is shown. The amino acid sequences used for comparison in the analyses are as follows with the NCBI accession numbers: Aichi virus (AIV), AB010145; Aurantiochytrium single-stranded RNA virus 01 (AuRNAV: red highlighted), BAE47143; Bovine enteric calicivirus (BoCV), AJ011099; Bean pod mottle virus (BPMV), AF394608; Black queen cell virus (BQCV), AF183905; Chaetoceros socialis f. radians RNA virus 01 (CsfrRNAV), AB469874; Chaetoceros tenuissimus RNA virus 01 (CtenRNAV), AB375474; Cowpea severe mosaic virus (CPSMV), M83830; Cricket paralysis virus (CrPV), M21938; Drosophila C virus (DCV), AF014388; Deformed wing virus (DWV), AY292384; Heterosigma akashiwo RNA virus SOG 263 (HaRNAV), AY337486; Norwalk virus (NV), M87661; Human poliovirus 1 Mahoney (PV), V01149; Parsnip yellow fleck virus (PYFV), D14066; Rhizosolenia setigera RNA virus 01 (RsetRNAV), BAE79742; Rice turgo spherical virus (RTSV), AAA66056; Sacbrood virus (SBV), AF092924; Triatoma virus (TrV), AF178440; and Taura syndrome virus (TSV), AF277675. (reprinted with copyright permission from the American Society for Microbiology: Tomaru, Y. et al. (2009) *Appl. Environ. Microbiol.*, **75:** 2375–2381. [published in April 2009])