

COMMON MIDWIFE TOAD VIRUS: OUTBREAKS IN EUROPE, PATHOLOGY AND GENOMIC ANALYSES

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OUTBREAKS IN EUROPE

In Europe, the first known outbreak of common midwife toad virus (CMTV) occurred in northern Spain in 2007 (Balseiro et al., 2009). Since then, three more outbreaks have occurred in Spain and in The Netherlands (Balseiro et al., 2010; Kik et al., 2011, 2012), affecting both wild and captive amphibians, and indicating that the host range and geographic distribution of CMTV are much wider than previously suspected. CMTV is known to infect wild tadpoles of the common midwife toad (*Alytes obstetricans*) and juvenile alpine newts (*Triturus alpestris*) in Spain; and adult wild water frogs (*Pelophylax* spp.) and common newts (*Lissotriton vulgaris*) in The Netherlands. Recently it has been reported in captive frogs in the former country.



Outbreaks of CMTV in Europe: Spain and The Netherlands

PATHOLOGY

Common gross lesions include systemic hemorrhagic disease (Fig. 1). Electron microscopy of virus particles demonstrated enveloped iridovirus-like virus particles with hexagonal nucleocapsid morphology and approximately 160-180 nm in diameter (Fig. 2). Histological findings revealed the presence of intracytoplasmic inclusion bodies and the necrosis of epithelial and endothelial cells, which result in destruction of several organs, including skin, liver, pancreas, spleen, kidney and intestine (Fig. 3).

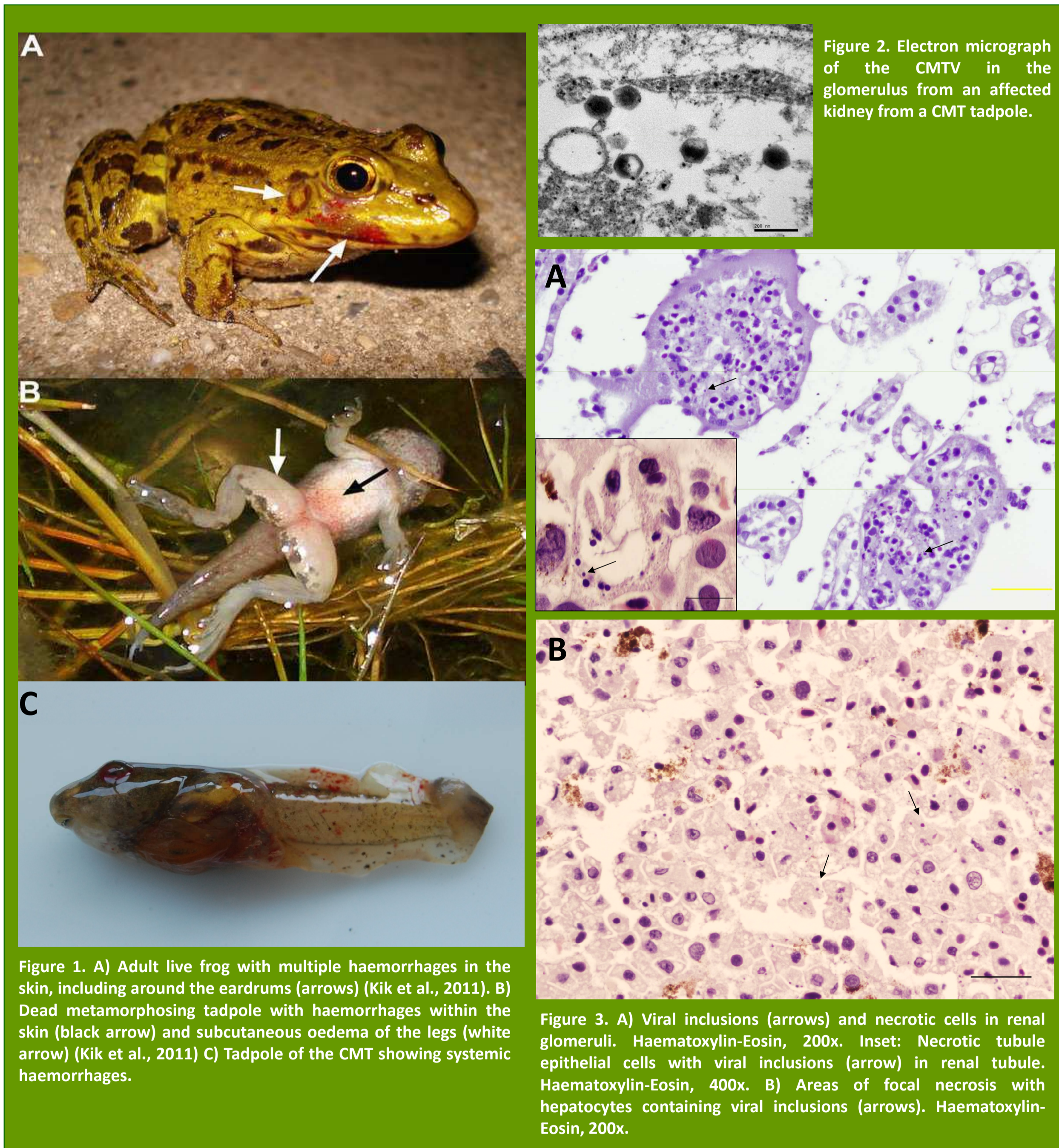


Figure 1. A) Adult live frog with multiple haemorrhages in the skin, including around the eardrums (arrows) (Kik et al., 2011). B) Dead metamorphosing tadpole with haemorrhages within the skin (black arrow) and subcutaneous oedema of the legs (white arrow) (Kik et al., 2011). C) Tadpole of the CMT showing systemic haemorrhages.

Figure 3. A) Viral inclusions (arrows) and necrotic cells in renal glomeruli. Haematoxylin-Eosin, 200x. Inset: Necrotic tubule epithelial cells with viral inclusions (arrow) in renal tubule. Haematoxylin-Eosin, 400x. B) Areas of focal necrosis with hepatocytes containing viral inclusions (arrows). Haematoxylin-Eosin, 200x.

DISCUSSION

As we have shown here, CMTV is an emerging pathogen infecting numerous species within continental Europe. Additionally, we have shown it to be a novel ALRVs that could represent an evolutionary intermediate between the two previously recognized groups within ALRVs, namely FV3-like and EHNV-like viruses. This information will be of importance both to further understand the evolutionary relationship among ranaviruses as well as to understand their mechanisms of spread and host shifts. Also, it should prove helpful to define the species and strain structure of ranaviruses, which will be crucial to determine the mechanisms of emergence of CMTV-caused disease and whether it may be linked to environmental factors, virus spread, or both. Better knowledge of the ranavirus species and strain structure will be crucial to set up detection methods and surveillance strategies in order to minimize their impact on amphibian wildlife and cultivated species. It is possible that the emergence of ranavirus disease on Europe is human or bird-mediated, and that the virus was naturally introduced from another region. It is important to remain vigilant in order to detect new outbreaks and to monitor the possible spread of this pathogen and its possible impact on wildlife biodiversity.

GENOMIC ANALYSES

Phylogenetic analysis of CMTV using the sequence of the major capsid protein, placed this virus as a close relative of FV3 and other amphibian-like ranaviruses (ALRVs) (Mavian et al., 2012), clustering with isolates from different hosts (fish and amphibian) and geographic origin (Fig. 4A and 4B). Analysis of the gene content of CMTV (Fig. 4C), showed that it contained all the ALRV specific genes, confirming its adscription to this group. Further, phylogenetic analysis of the concatenated sequence of the iridoviral core protein sequences of all completely sequenced ranaviruses, using the LCDV China sequence as a distantly related outgroup, positioned CMTV as a closer relative of the FV3-like virus group within the ranaviruses with high confidence (Fig. 4D, upper panel). Dot plot analysis comparing CMTV to FV3 or EHNV viruses (Fig. 4D, lower panel), however, showed that its genome was not fully collinear with either of them. Particularly, the position of the observed genomic rearrangements suggest that CMTV might constitute the type isolate of an evolutionary intermediate between both ALRV groups. Inversion of one segment from an EHNV-like precursor might have given rise to the CMTV-like structure, while a further large genomic inversion might have generated the precursor of current FV3-like viruses. The use of primers specific for the flanking sequences produced a larger PCR amplification product on CMTV DNA than on FV3 DNA, and that it can therefore be used for differentiation of these viruses (Fig. 4E).

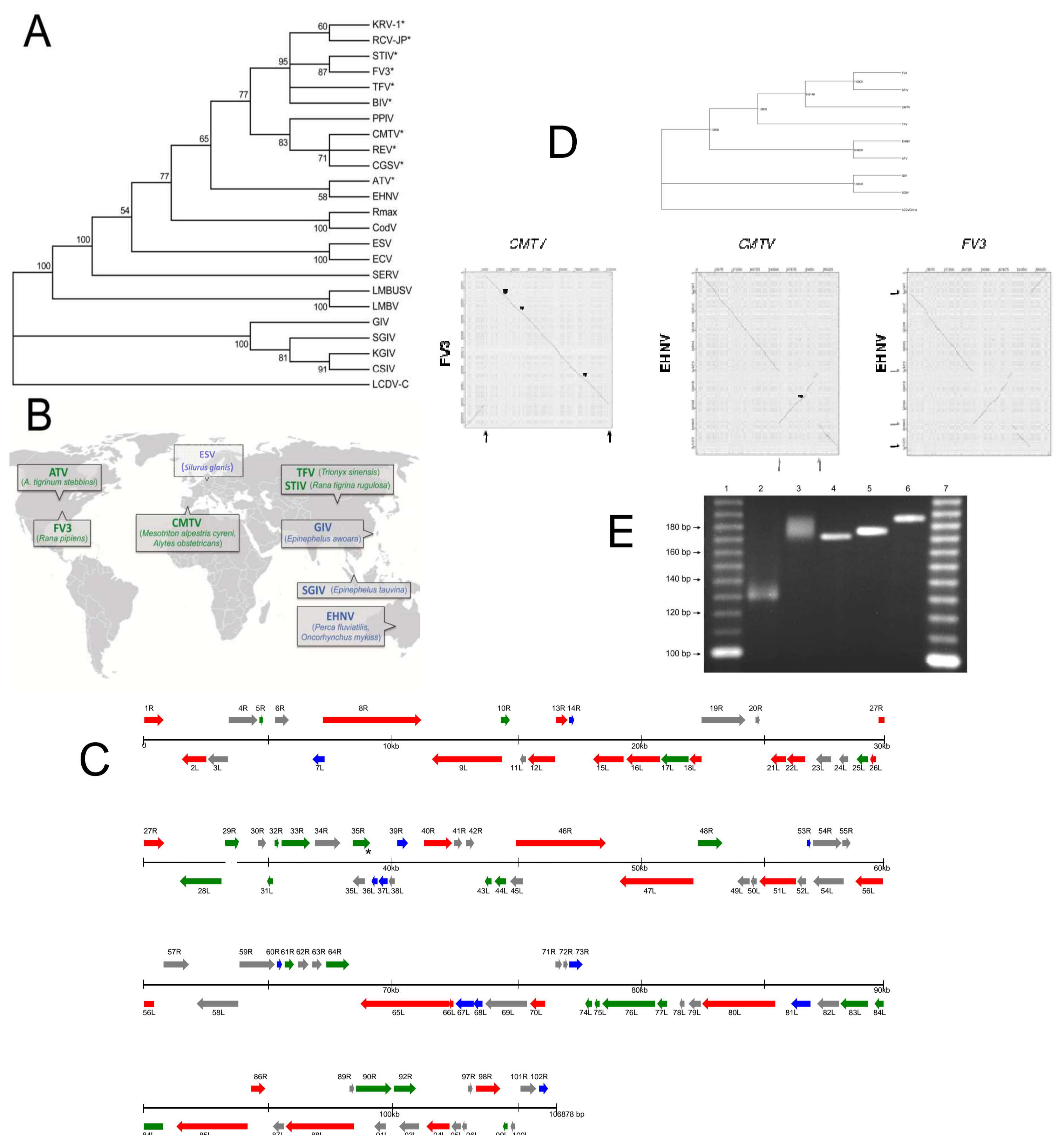


Figure 4: A) The CMTV is a novel amphibian-like ranavirus isolated in Europe. B) Name, location and host of isolation of currently fully sequenced ranavirus genomes. C) Linear systematic organization of the CMTV genome. Known conserved genes are labelled in red (iridovirus core genes), green (ranavirus-specific genes) and blue (ALRV-specific genes). D) The CMTV is an evolutionary intermediate in ALRV. E) PCR amplification of CA microsatellites in FV3 (lane 2) and CMTV (lane 3). The rest of the lanes correspond to known size markers which were used for determination of the actual size of the CA-microsatellite in CMTV.

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