



# APPLICATION OF MOLECULAR METHODS FOR THE DIAGNOSIS AND THE GENETIC CHARACTERIZATION OF INFECTIOUS PATHOGENS IN DOMESTIC AND WILD CARNIVORES

## OBJECTIVE

To detect infectious agents in domestic and wild carnivores using molecular techniques and to genetically analyze the pathogens identified, in order to investigate the circulation of these pathogens in carnivores and to evaluate their genetic characteristics.

## MATERIALS AND METHODS

Different biological samples of domestic [1,2,3] and wild carnivores [4,5,6] from Italy and Europe were tested between 2019 and 2022. After nucleic acid extraction, molecular assays (real-time PCR, end-point PCR, retrotranscription, rolling circle amplification) were carried out for the detection and the genetic characterization of DNA and RNA viruses and bacteria. Sequence analysis was performed using specific softwares.

## RESULTS

During the study period, it was possible to detect and genetically characterize DNA viruses, such as canine circovirus (**CanineCV**) in Italian dogs and wolves and Norwegian foxes (Fig. 1) [1,4,5]; canine adenovirus type 1 and 2 (**CAAdV-1** and **CAAdV-2**) in Italian wolves and dogs, respectively (Fig. 2) [1,4], canine parvovirus type 2 (**CPV-2**) in wolves from Italy and dogs from Romania [2,4]; RNA viruses, such as canine distemper virus (**CDV**) in stone martens from Italy (Fig. 3) [6]; and bacteria, such as *Anaplasma phagocytophilum* in Italian cats (Fig. 4) [3].

Fig. 2. Two enlargements of phylogenetic trees of CAAdV-1 (top) and of CAAdV-2 (bottom) constructed with the multiple gene approach: concatenated nucleotide sequences of the hexon and fiber genes. Phylogenetic trees were constructed using the Maximum Likelihood method and the Hasegawa-Kishino-Yano model with gamma distribution and invariable sites. Bootstrap values (1000 reps) are indicated. Top. **Highlighted**: sequence of CAAdV-1 generated in an Italian wolf [4]. The amino acid residues for the deduced hexon and fiber proteins are reported. Bottom. **Highlighted**: sequences of CAAdV-2 generated in dogs from Italy [1].

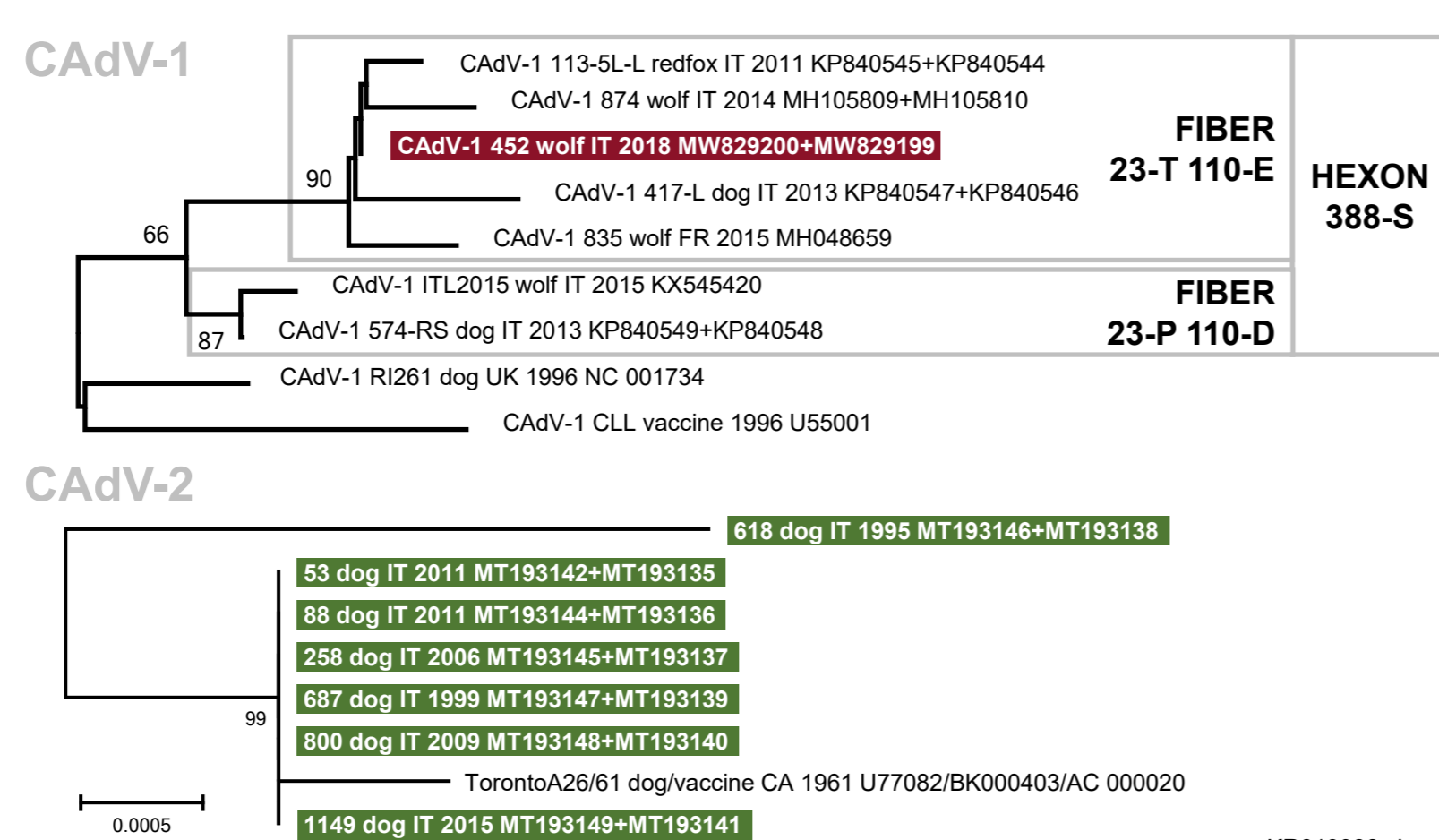


Fig. 1. Phylogenetic tree of CanineCV constructed using the Maximum Likelihood method and the General Time Reversible model with gamma distribution and invariable sites.

**Highlighted**: sequences of CanineCV generated in wolves from Italy [4]. **Highlighted**: sequences of CanineCV generated in foxes from Norway [5]. Numbers in black (right): clusters proposed by Urbani et al. [5]. Bootstrap values (1000 reps) are indicated.

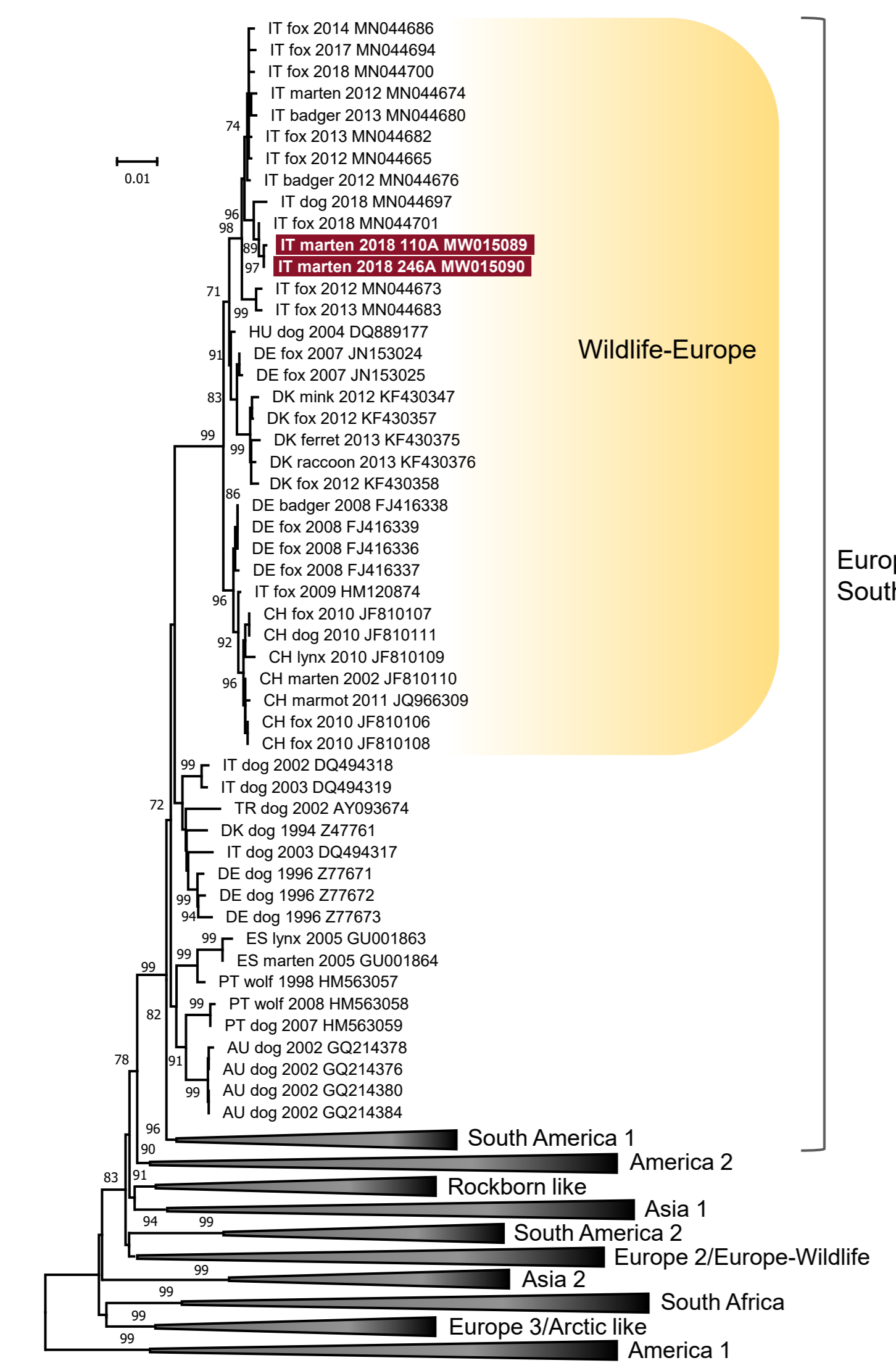
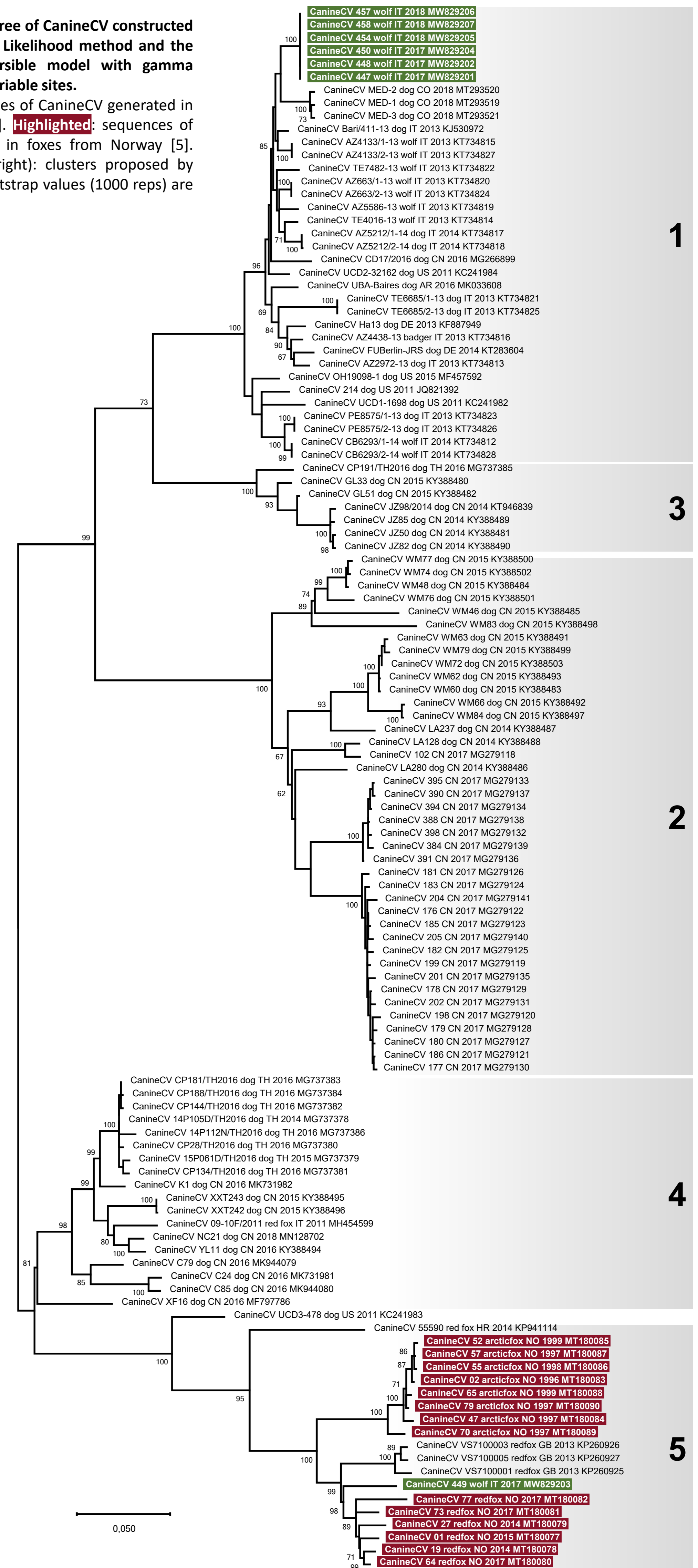


Fig. 3. Phylogenetic tree of CDV constructed using the Neighbor-Joining method and the Tamura 3-parameter model with gamma distribution. **Highlighted**: sequences of CDV generated in stone martens from Italy [6]. Orange: Wildlife Europe genetic subgroup. On the right canine morbillivirus genetic lineages are indicated. Bootstrap values (1000 reps) are indicated.

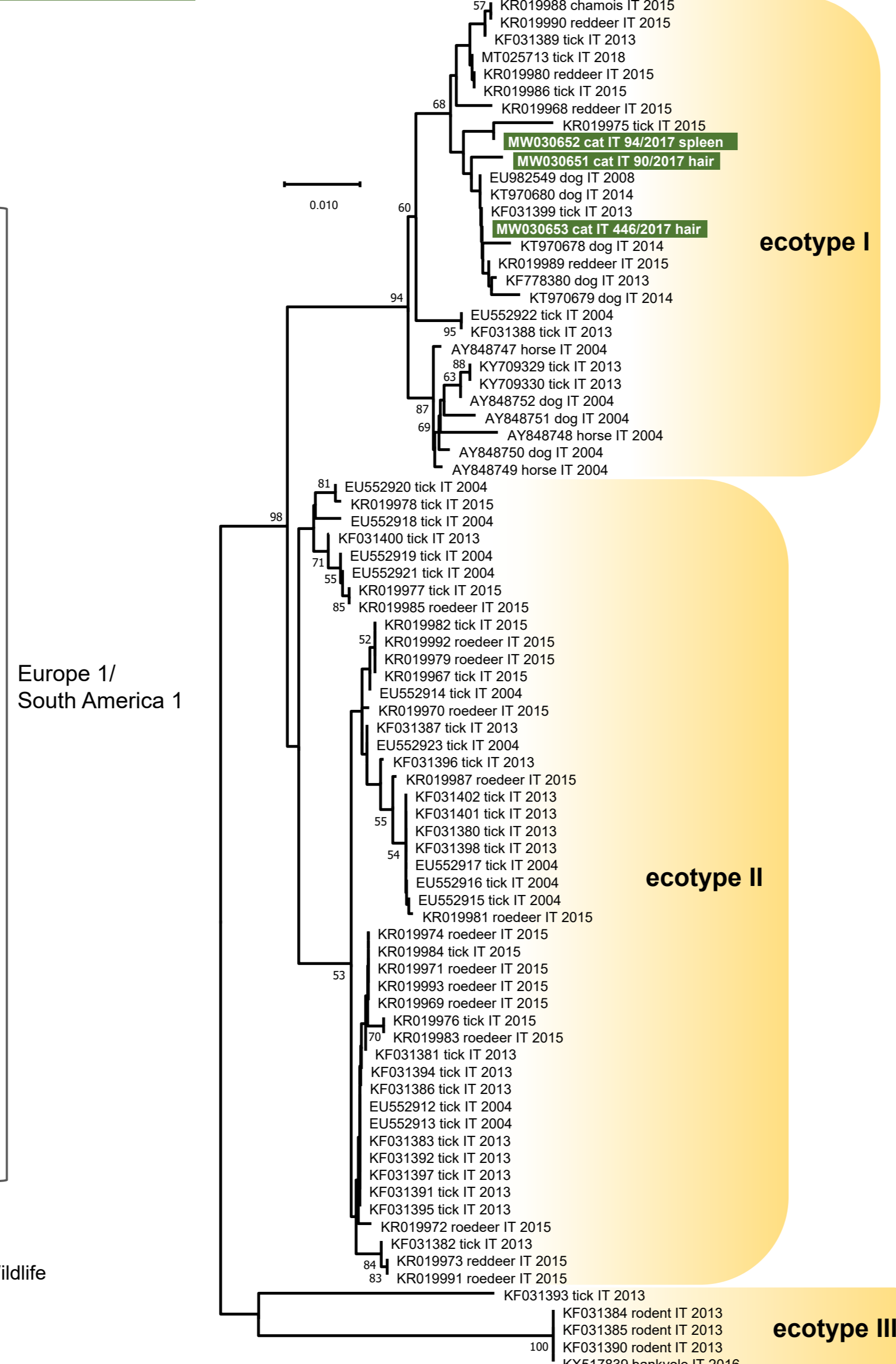


Fig. 4. Phylogenetic tree of *Anaplasma phagocytophilum* constructed using the Minimum Evolution method and the Tamura 3-parameter model with gamma distribution. **Highlighted**: sequences of *A. phagocytophilum* generated in cats from Italy [3]. On the right main clusters are labelled. Bootstrap values (1000 reps) are indicated.

## CONCLUSIONS

Taken together, the results obtained allowed stating that infectious agents circulate among domestic and wild carnivores, alone or in synergism with each other, causing co-infections and a worsening of the health status of the animals. Sequence analysis allowed clarifying aspects concerning transmission between domestic and wild hosts and the epidemiological role of wild carnivores. Prophylactic measures, such as core vaccinations and ectoparasite prevention treatments, should be adopted in domestic animals to reduce the potential transmission of pathogens.

## REFERENCES

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