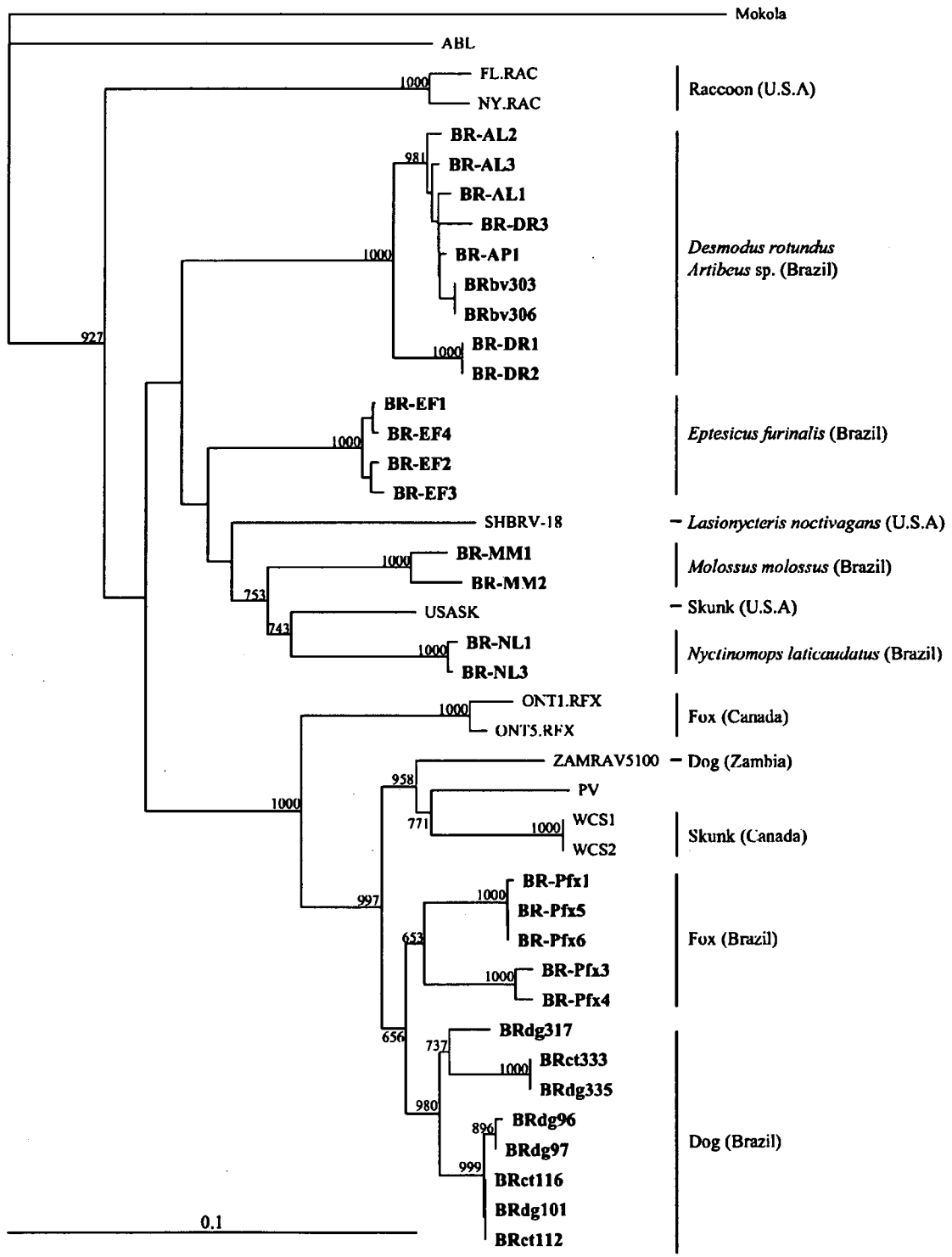


Fig. 4. Phylogenetic tree based on the M gene (2496–3104 compared to PV strain). Bold font indicates the samples for which nucleotide sequences were determined in this study. Bootstrap values are shown at major branches.

protein length, and did not group with carnivora-related RV isolates from other regions that encoded P proteins of different length. In addition, specific amino acid substitutions with respect to host species and geographic origin were found. Taken together, these findings suggest that phylogenetic analyses using these two genes are capable of identifying epidemiological characteristics of Brazilian RVs.

Conservation of the P protein in Brazilian RV isolates was low, with two conserved (CD1 and 2) and two variable domains (VD1 and 2), which corroborates the findings of Nadin-Davis *et al.* [30, 32]. Variable amino acid substitutions characteristic of Brazilian RV variants occurred at high frequencies in the variable domains, and this was reflected in the hydrophilic regions of the observed hydropathic profiles. Additionally, the LC8-binding motif, involved in viral nucleocapsid axoplasmic transport [21, 39], formed consensus sequence K(S/A)TQT in the VD2 region. With the exception of several RV isolates, the Ser and Ala differences were observed in the consensus sequences of isolates from either the carnivora- or chiroptera- related RV isolates, as reported by Nadin-Davis *et al.* [32]. The variable domains thought to be located on the surface structure of the P protein have been suggested to be involved in host/viral interactions and adaptation to the host environment [31, 32]. Our results support this hypothesis and studies of the variable domains are expected to be useful in elucidating the adaptive evolution of the virus.

At least 2 independent site of the P protein that confer N protein-binding activity have been identified; one is located within the N-terminal half of the protein, and another within 50 residues of the C-terminus [3, 11]. The first 19 N-terminal residues of the P protein confer L protein-binding ability, as does the N-terminal region containing the L protein-binding site [5]. Conserved domains contain N and L protein-binding sites and the conserved lysine-rich motif, FSKKYKF, which is an important component in N protein-binding [22, 32]. These two conserved domains containing the lysine-rich motif were observed in all Brazilian RV isolates, supporting the hypothesis that they may be essential to activities such as granule formation and general functioning of the virus.

Phosphorylation of the P protein is essential for transcription to occur in the vesicular stomatitis virus (VSV) [18], which belongs to the *Rhabdoviridae* family. In five serine residues which constitute the phosphoacceptors in the P protein of the CVS strain [13], $Ser_{210}$ and $Ser_{271}$ within the PKC phosphoacceptor target were notably conserved in all lyssavirus genotypes (GT 1 to 7) [32], including the Brazilian RV variants examined in this study. Studies on VSV and paramyxovirus have shown that P gene-encoded multiple proteins are important in the viral replication cycle and pathogenicity of the virus [12, 24, 37]. The four methionine residues located in-frame of single P protein sequences are translated into four proteins of various lengths with no known function in the CVS strain [4]. Nonetheless $Met_{20}$ has been retained in all lyssavirus genotypes [32] and conservation of $Met_{20}$ was observed in all Brazilian RV variants. Notably these conserved amino acid residues in the wild-type RVs are likely to be associated with important viral functions. Further laboratory research of these conserved amino acid residues would facilitate the identification of the biological characteristics of potential functions in wild-type RVs.

The M protein binds to the RNP core, and collaborates with the viral G protein in the infected cells to produce progeny virions by budding at the cell membrane [34, 35]. The M protein is also required for the development of a typical bullet-shaped rhabdovirus [29]. Consequently, the M protein is involved in binding to several viral components. In this study, although the M protein exhibited RV variant-associated amino acid substitutions, the hydropathic profiles conformed closely with each other. In addition, the PPxY motif, which interacts with the WW domains of cellular components [15], was conserved in all Brazilian RV variants. Laboratory-adapted and carnivora RV strains from North America have been characterized as having identical hydropathic M-protein profiles [17, 30], suggesting that the primary structure of the M protein may be important for the retention of viral structure and function.

The present study reveals that Brazilian RV isolates possess sufficient variability with respect to host species and geographic origin, while retaining conserved sequences (motifs) thought to be associated with a viral function in the P and M genes of RV. Studies on wild-type RVs are likely to increase our understanding of the relationship between the virus and the host, as well as the biological characteristics of the virus in the wild. Such genetic data would be helpful in investigating pathogenicity associated with RV.

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