

Updated Phylogenetic Analysis of Arenaviruses Detected in Boid Snakes

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With great interest we read the manuscript entitled “Isolation, Identification, and Characterization of Novel Arenaviruses, the Etiological Agent of Boid Inclusion Body Disease” by Hetzel and colleagues (1). This very well performed and comprehensive study about the role of divergent arenaviruses in inclusion body disease (IBD) of boid snakes clearly shows a causal relationship between arenaviruses (Avs) and IBD *in vitro*.

Experiments were performed with a virus isolated from a *Boa constrictor* with inclusion body disease and tentatively called University of Helsinki virus (UHV). UHV was sequenced completely and compared with two other arenaviruses detected in boid snakes, Golden Gate virus (GGV) and California Academy of Sciences virus (CASV) (2). Since there were considerable genetic differences between these viruses, the authors suggested that UHV might belong to a new species.

Of interest, we recently published in the *Journal of General Virology* (online publication, 6 March 2013) a paper about the detection of novel divergent arenaviruses in boid snakes with IBD in the Netherlands, which included the nearly complete genome of a novel arenavirus (boa Av NL B3) detected in a *Boa constrictor* and several fragments of the nucleoprotein gene of other variants of arenaviruses present in boid snakes (3). Comparison of sequences of the major open reading frames of one of the arenaviruses described in our study (boa Av NL B3) with UHV-1, described by Hetzel and colleagues, revealed that the pairwise identities of the nucleoprotein (NP), glycoprotein precursor (GPC), RNA-dependent RNA polymerase (RdRp), and RING domain-containing zinc binding protein (Z) genes were, respectively, 77%, 67%, 89%, and 79% at the nucleotide level and 84%, 62%, 92%, and 89% at the deduced amino acid level.

Since (i) both viruses were detected in the same species (*Boa constrictor*), (ii) animals in which viruses were detected lived in neighboring countries (Germany and the Netherlands), and (iii) divergences of the NP and GPC gene products were 16% and 38%, respectively, at the deduced amino acid level, the two viruses share a number of the characteristics used by the International Committee on Taxonomy of Viruses to include different isolates of arenaviruses in the same species (http://ictvdb.bio-mirror.cn/Ictv/fs_arena.htm [accessed 15 August 2013]). Therefore, we have prepared updated phylogenetic trees, including all currently available (nearly) complete gene sequences of divergent arenaviruses detected in snakes with inclusion body disease, which confirms that UHV and Boa Av NL B3 are closely related (Fig. 1). Of interest, the discrepancy between the GPC- and NP-based phylogenetic trees might be caused by intrasegmental recombination, which is supported by analysis using various recombination detection methods of Recombination Detection Program 4.24 (4) and has been suggested previously for the S segment of arenaviruses (for a review, see reference 5).

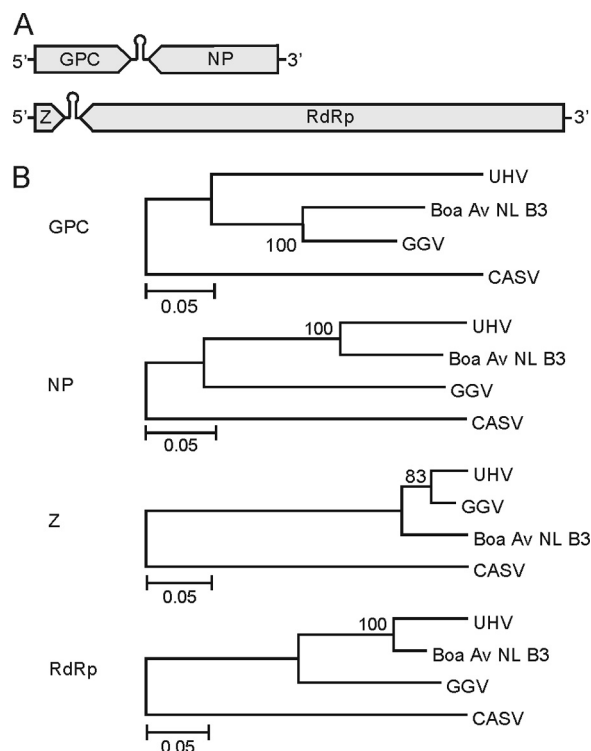


FIG 1 Genomic analysis of currently known divergent arenaviruses detected in boid snakes. (A) Schematic overview of the genome organization of arenaviruses of boid snakes, including the two gene segments, predicted coding regions, and intergenic hairpins. Not drawn to scale. (B) Phylogenetic trees of the deduced amino acid sequences of the GPC, NP, Z, and RdRp gene products of currently known full-length divergent arenavirus sequences identified from boid snakes with IBD generated with the neighbor-joining method using *p* distance (the proportion [*p*] of nucleotide sites at which two sequences being compared are different) and 1,000 bootstrap replicates (B). Bootstrap values are indicated. GenBank accession numbers (one for each viral segment) are as follows: for University of Helsinki virus (UHV-1), [KF297880](#) and [KF297881](#); for Boa Av NL B3, [KC508669](#) and [KC508670](#); for Golden Gate virus (GGV), [NC_018483](#) and [NC_018482](#); and for California Academy of Sciences virus (CASV), [NC_018484](#) and [NC_018481](#).

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