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LANDIRAS VIRUS, A NOVEL HANTAVIRUS HOSTED BY *TALPA AQUITANIA* N.SP, A RECENTLY DISCOVERED SOUTH EUROPEAN MOLE SPECIES

LE VIRUS LANDIRAS, UN NOUVEL HANTAVIRUS HÉBERGÉ PAR TALPA AQUITANIA *N. SP. RÉCEMMENT DÉCOUVERTE AU SUD-OUEST DE L'EUROPE*

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INTRODUCTION

During the last 20 years, numerous new hantaviruses belonging to different species have been discovered in non-rodent micromammals. In the following, using the results of a Bayesian analysis, we describe *Landiras* virus (LDRV), a new *Orthohantavirus* discovered in a recently described new mole species: *Talpa aquitania* (Nicolas *et al*. 2015, 2017). The new virus species is compared with other hantaviruses, collected from moles, rodents or shrews, which are classified in the subfamily Mammantavirinae.

MATERIALS AND METHODS

Sample collection

From 2011 to 2020, several hundred moles were captured in France and Northern Spain. The aim of this work was the search for hantaviruses likely to be hosted by moles. Moles are classi-fied pest species. This status authorizes different control methods, including trap killing, and no permits are required for fieldwork. Immediately after trapping, moles were stored at -20° C until further processing. Lung, kidney, heart, liver, and spleen tissues were aseptically removed and stored in RNA*later* Stabilization Solution (Ambion). The genetic study of the samples allowed the discovery of a new species of mole, present in France and in the north of Spain: *Talpa aquitania* (Hugot *et al*. 2014; Nicolas *et al*. 2017).

Hantavirus screening

One hundred and seventy (170) lung samples collected in mole specimens identified as *T. aquitania* were sent to the Virology laboratory of Leuven University (Belgium). Total RNA was extracted from mole tissue with the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. A nested degenerate RT-PCR was performed using the OneStep RT-PCR kit (Qiagen) with primers targeting a conserved region in the polymerase gene, as described previously (Klempa *et al*. 2006). PCR amplicons were purified using ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix) and sent to Macrogen for Sanger sequencing. Sequences were manually inspected using Chromas 2.6 (Technelysium) and a consensus sequence was derived with Seqman 7.0 (DNAstar). Two samples were found positive for Hantaviruses. Both were collected on July 17th, 2016 in Landiras (Girond department) in the south-west of France (N-44,5667; W-0,4167), identified as *T. aquitania* in the French National Museum of Natural History and referenced in the collections as MNHN-ZM-2017-2257 and MNHN-ZM-2017-2258.

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S segment sequencing

To obtain the complete sequence of the S (nucleocapsid) gene, an additional nested RT-PCR was performed on the same total RNA extract, using the OneStep RT-PCR kit. The first round of this PCR used a custom primer that contains an 8-nt motif targeting the conserved panhandle sequence present on both ends of the S segment and a long 5' overhang (5'-GTTTCCCACTGGAGGATATAGTAGTA-3'). The inner PCR reaction was performed using a primer targeting the overhang of the first-round primer (5'-GTTTCCCACTGGAG-GATA-3'). The resulting amplicon was used to prepare a sequencing library with the SQK-LSK109 and EXP-NBD104 kits (Oxford Nanopore Technologies) according to the manufacturer's instructions. The resulting library was run on a MinION R9.4.1 flow cell and the data were base called using the HAC model of Guppy v3.0.3. Fastq-files were assembled using Canu v1.8 (Koren *et al.* 2017) and the resulting contig was polished using Medaka v0.8. The resulting contig was checked for errors by mapping back the sequencing reads using CLC genomics work-bench 20.0.2 (Qiagen). Only MNHN-ZM-2017-2257 allowed to obtain a complete sequence (1966 nt) were submitted to GeneBank as ON944104 access number and used for the phylogenetic analysis.

Phylogenetic analysis

Multiple sequence alignments of hantavirus sequences were generated in MAFFT and manually corrected using SeaView 4.6.3 (Katoh *et al.* 2002; Gouy *et al.* 2010). An alignment of the complete coding region of the S segment was used in subsequent Bayesian inference using MrBayes (Huelsenbeck and Ronquist 2001) using a General Time Reversible (GTR) substitution model and a discretized distribution with default priors. Two independent Markov Chain Monte Carlo analyses, each using 3 heated and 1 cold chain, were run until adequate effective sample sizes (ESS > 200) were obtained. A consensus tree was built employing a burn-in of 25% and rooted using the "midpoint root" option.

RESULTS AND DISCUSSION

Analysis of the S (nucleocapsid) gene of *Landiras* virus shows that it is close to the *Orthohantavirus Bruges* (NC034394) previously described in several specimens of *T. europaea* captured in Belgium, Germany and the United Kingdom (Laenen *et al.* 2017) (Figure 1). Both are very different from Nova virus (FJ539168) also discovered in *T. europaea* (Kang *et al.* 2009; Hugot *et al.* 2014; Hun Gu *et al.* 2014). As *T. europaea* and *T. aquitania* have contiguous ranges, this raises the question of a possible virus host switching between the two mole species. Further investigations are needed to gain insights into hantaviruses ecology, transmission dynamics and virus–host evolution.

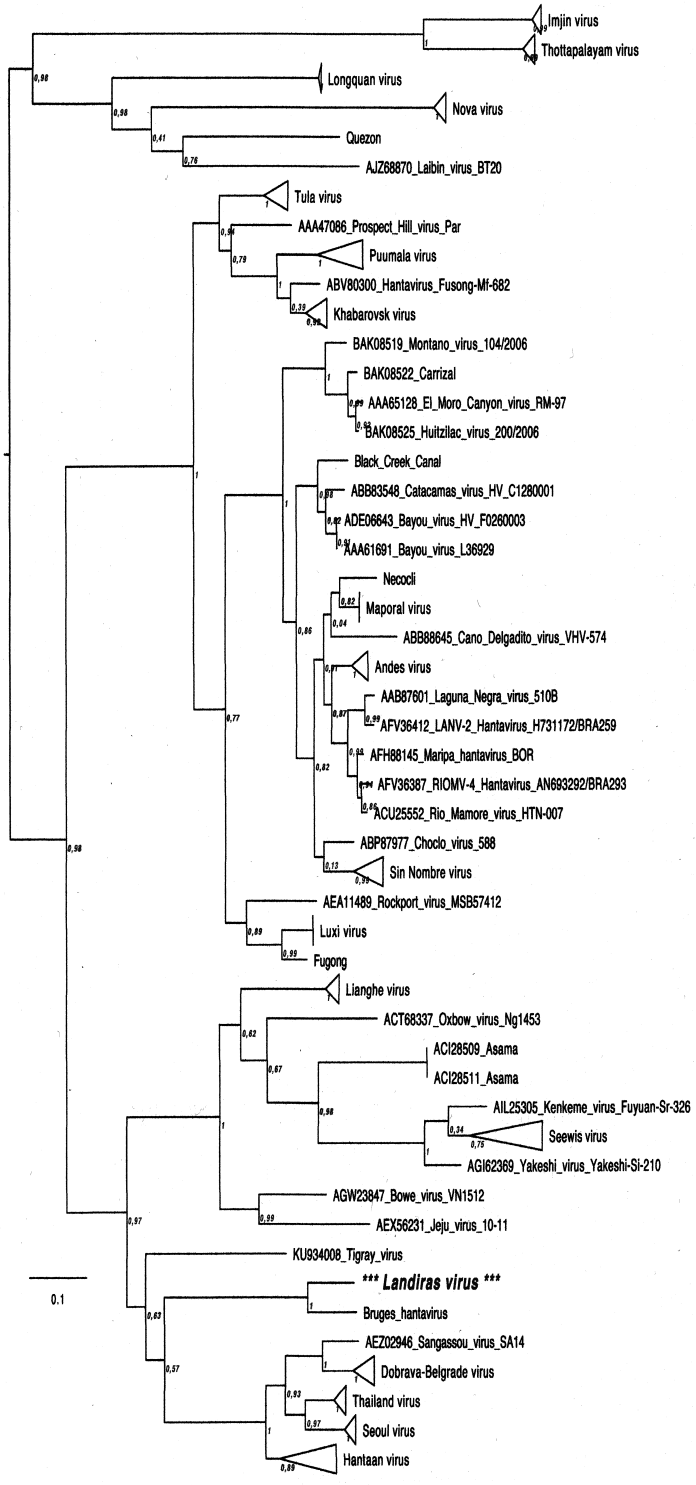

Figure 1 : *Bayesian phylogenetic analysis of the nucleocapsid (S) gene. Numbers at the nodes indicate posterior clade probability values.*

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