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## MOLECULAR-GENETIC IDENTIFICATION OF TWO PARAMUSHIR VIRUS (*BUNYAVIRALES*, *NAIROVIRIDAE*, *ORTHONAIROVIRUS*) STRAINS ISOLATED ON THE TYULENIY ISLAND IN THE OKHOTSK SEA

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During arbovirus surveillance *Ixodes uriae* ticks were collected in the nest colonies of seabirds on the Tyuleny Island (48° 29' N, 144° 38' E) in the Okhotsk Sea near Sakhalin Terpeniya Cape. The *I. uriae* identity and sex of the ticks were determined based on their morphology. All of the ticks were stored alive in a wet chamber until separation. Ticks were separated by species, sex, developmental stages, and later processed in pools of 10 imago or 25 preimago.

Viruses were isolated using intracerebrally inoculated newborn mice. Two strains of Paramushir virus (PMRV) (*Bunyavirales*, *Nairoviridae*, *Orthonairovirus*) were obtained: PMRV/*Ixodes uriae*/Russia/Tyuleny Island/133/2015 and PMRV/*Ixodes uriae*/Russia/Tyuleny Island/137/2015. Biological samples were homogenized using Tissue Lyser LT (Qiagen, Germany) for 5 min in 100 µL of phosphate buffered saline (pH 7.0) before extraction, centrifuged at 2000 rpm for 10 min, and the supernatant was used for nucleic acid extraction. Total nucleic acids were extracted and purified using the RIBO-prep DNA/RNA extraction kit (k2-9-Et-100CE, AmpliSens, Russia), according to the recommendations of the manufacturer. DNA/RNA was eluted with 50 µL of the elution buffer (AmpliSens, Russia) and stored at -70 °C until evaluation. Broad-range RT-PCR was performed using a set of broadly reactive degenerate oligonucleotides designed to target each viral species that could be transmitted by ticks within the genera as follows: *Flavivirus*, *Orthonairovirus*, *Phlebovirus*, *Orthobunyavirus*, *Orthoreovirus*, *Orbivirus*. All PCR products of the expected size were cut out from the gel under UV control, extracted using a MinElute gel extraction kit (Qiagen, Germany) and ligated into the pGEM-T plasmid vector (Promega, USA). These plasmids were transformed into *Escherichia coli* (XL1 blue strain) and 10 white colonies were sequenced using standard M13R primers by means of the ABI Prism 3500 XL (Applied Biosystems, USA). Obtained se-

quences were examined using the Basic Local Alignment Search Tool (BLAST). As a result, 489 436 and 358 075 reads were obtained for the samples PMRV-133 and PMRV-137, respectively. However, obtained reads were distributed unequally. In particular, L-segments were covered at 95.7 % and 98.4 % (for PMRV-133 and PMRV-137, respectively), M-segments were covered at 23.0 % and 72.8 %, whereas S-segments were covered at 100 % for the both strains. Existing gaps were sequenced using Sanger sequencing technique by means of the ABI Prism 3500 XL (Applied Biosystems, USA). PMRV-133 and PMRV-137 strain nucleic acid sequences were submitted to GenBank under accession numbers MH124637, MH124638 and MH124634-MH124636, respectively. Phylogenetic analysis showed that both sequenced strains were closely related to PARV LEIV-1149K strain within the Sakhalin virus subgroup.

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