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# Distribution and Molecular Diversity of *Paranoplocephala kalelai* (Tenora, Haukisalmi & Henttonen, 1985) Tenora, Murai & Vaucher, 1986 in Voles (Rodentia: *Myodes*) in Eurasia

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**Citation:** Krivopalov, A.; Vlasenko, P.; Abramov, S.; Akimova, L.; Barkhatova, A.; Dokuchaev, N.; Gromov, A.; Konyaev, S.; Lopatina, N.; Vlasov, E.; et al. Distribution and Molecular Diversity of *Paranoplocephala kalelai* (Tenora, Haukisalmi & Henttonen, 1985) Tenora, Murai & Vaucher, 1986 in Voles (Rodentia: *Myodes*) in Eurasia. *Diversity* **2022**, *14*, 472. https://doi.org/10.3390/d14060472

Academic Editors: Michael Wink and Sergey Shekhovtsov

Received: 25 May 2022
Accepted: 11 June 2022
Published: 12 June 2022

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**Abstract:** Cestodes *Paranoplocephala kalelai*, which parasitizes in the small intestine of *Myodes* voles and is distributed in northern Fennoscandia, was found in six habitats in the Asian part of Russia and eastern Kazakhstan, which indicates a wider distribution of *P. kalelai* on the continent. Analysis of mtDNA showed that *P. kalelai* is characterized by significant molecular variability in Eurasia. This study complements the data on the distribution of *P. kalelai* and provides the first molecular data from the territory of Russia and Kazakhstan. The sequence variability of two mitochondrial genes *cox1* and *nad1* of *P. kalelai* was studied in two species of voles: gray red-backed *Myodes rufocanus* and northern red-backed vole *Myodes rutilus*. Five haplotype groups in the *cox1* and *nad1* gene networks were identified, and the existence of two mtDNA lines in *P. kalelai* outside northern Fennoscandia was confirmed. The geographical distribution of the identified haplotypes suggests that the foothills of the Altai-Sayan mountains and southern West Siberia may serve as a refugium for *P. kalelai* during repeated glaciations.

**Keywords:** *Paranoplocephala kalelai*; Anoplocephalidae; Eurasia; voles; mtDNA; *Myodes rufocanus*; *Myodes rutilus*; phylogeography; diversity; genetic divergence; haplotype

## 1. Introduction

The *Paranoplocephala kalelai* (Tenora, Haukisalmi & Henttonen, 1985) parasitizes in the small intestine of red-backed voles (genus *Myodes*) and is therefore distributed in the forest belt. Infestation of voles by this cestode is significant. Thus, the prevalence in *M. rufocanus* averages 24% and reaches 90% in overwintered specimens [1]. The species has been described in Fennoscandia and there were no findings of *P. kalelai* to the east of this region. In the northeastern part of European Russia (Komi Republic) covered by taiga, there is no information on the parasitization of *P. kalelai* in rodents [2]. In the taiga zone beyond the Ural, there is no information on *P. kalelai* in the literature. However, *P. kalelai* was found in northern and gray red-backed voles near the city of Magadan and on Zavyalov Island (northern coast of the Sea of Okhotsk, far east of Russia) [3], indicating a wider distribution of *P. kalelai* on Eurasia. These identifications were made only based on morphological characters. When studying the helminth collection of the Laboratory of Parasitology of the IS&EA SB RAS, we found preparations of cestodes previously defined as *P. omphalodes* or *P. macrocephala*, but corresponding to the morphological criteria of *P. kalelai* from *Myodes* voles of Western Siberia.

Phylogenetic analysis showed that cestodes of *P. kalelai* from two different habitats of northern Fennoscandia form two divergent sublines according to mtDNA [4] which allowed us to assume taxonomic independence of these lines. However, *P. kalelai* has not been divided into two separate species because of the failure to identify morphological groups associated with the available molecular datasets [5]. Thus, it is obvious that *P. kalelai* in the northern territories of Finland and Norway is characterized by cryptic molecular variability which not registered in the study of morphological characters.

The aim of our study was to clarify the distribution of *P. kalelai* in the Asian part of Russia and to identify intraspecific molecular variability. Material from various remote regions of the Asian part of Northern Eurasia was collected and examined. Our study complements the data on *P. kalelai* distribution and its hosts and provides the first molecular data from the territory of Russia and Kazakhstan.

## 2. Materials and Methods

Data collection. During fieldwork in the Asian part of the continent, all rodents were dissected and examined for helminths immediately after trapping with live or snap traps. Cestodes *P. kalelai* were recorded in *M. rufocanus* and *M. rutilus* in seven habitats of four Asian regions: Khanty-Mansi Autonomous Okrug (KhMAO), Western Sayan, Magadan Oblast (Russia), and Western Altai (Kazakhstan) (Figure 1). The systematic position and Latin names of the definitive hosts are given according to [6].

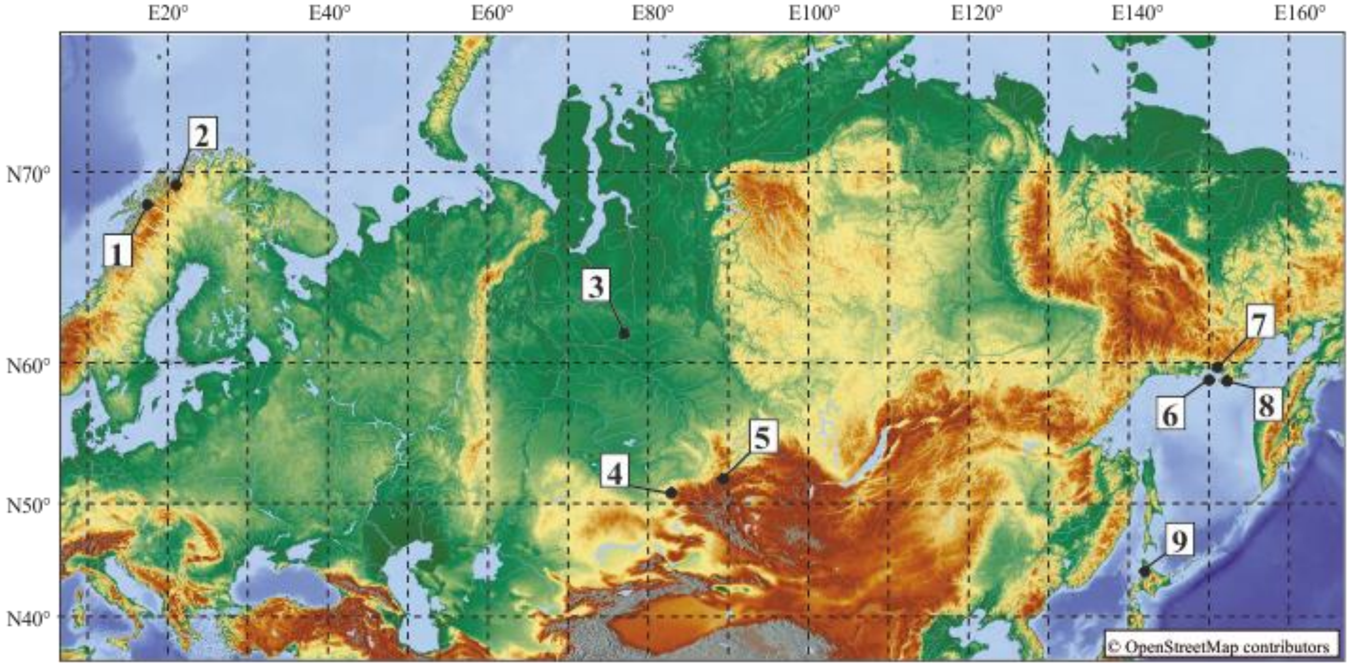

**Figure 1.** Geographical distribution of the sampling localities for *Paranoplocephala kalelai*. The samples from the localities 3–8 were collected by the authors. The samples from the localities 1, 2, 9 are given according to publications [4,5,7], respectively. The locality numbers refer to Table 1. The green, yellow, and orange colors indicate the elevation of the terrain in order from green to orange.

Molecular identification and phylogenetic analysis. Total DNA was extracted from tissue by using a "PREP-NA" kit (DNA-Technology Company, Moscow, Russia). Fragments of two mitochondrial genes *cox1* and *nad1* were amplified using primers and PCR conditions given in Haukisalmi et al. (2014). The amplicons were purified and sequenced at the Genomics Core Facility ICBFM SB RAS (Novosibirsk, Russia). Twenty new mtDNA sequences of *P. kalelai* from 12 samples from various hosts were submitted to GenBank (Table 1). In addition, 14 sequences of *P. kalelai* from the GenBank database were used. The sequences were aligned using ClustalW in MEGA 11 [8]. Haplotype networks were constructed for two mitochondrial genes, *cox1* and *nad1*. The length of the alignments was 544 (for the *cox1* gene) and 719 (*nad1*) nucleotides. The number of haplotypes was calculated using the program DNASP 6 [9]. Popart 1.7 software (https://popart.otago.ac.nz/downloads.shtml accessed on 12 June 2022) was used to calculate and visualize the median-joining network of phylogenetic relationships among haplotypes [10]. Analysis of genetic distances was conducted in MEGA 11.

**Table 1.** List of examined specimens of *Paranoplocephala kalelai* and GenBank accession numbers according to geographical origin. Specimens with GenBank access numbers beginning with the letters ON were collected and sequenced by the authors. The other samples are given according to [4,5,11].

| Locality (Number) | GenBank acc. no. (cox1/nad1) | Final Host |
|---|---|---|
| Ola, Magadan Oblast, Russia (7) | ON533413/ON548169 | *Myodes rufocanus* |
| Zavyalov Island, Magadan Oblast, Russia (6) | ON533414/ON548171 | *Myodes rufocanus* |
| Zavyalov Island, Magadan Oblast, Russia (6) | ON533415/ON548172 | *Myodes rufocanus* |
| Ola, Magadan Oblast, Russia (7) | ON533416/ON548168 | *Myodes rufocanus* |
| Koni Peninsula, Magadan Oblast, Russia (8) | ON533417/ON548173 | *Myodes rufocanus* |
| Pozarym, Western Sayan, Russia (5) | ON533418/ON548175 | *Myodes rutilus* |
| Pozarym, Western Sayan, Russia (5) | ON533419/ON548176 | *Myodes rufocanus* |
| Raduzhny, KhMAO, Russia (3) | ON533420/ON548177 | *Myodes rutilus* |
| Ridder, Western Altai, Kazakhstan (4) | ON533421/— | *Myodes rufocanus* |
| Ridder, Western Altai, Kazakhstan (4) | —/ON548178 | *Myodes rufocanus* |
| Ola, Magadan Oblast, Russia (7) | —/ON548174 | *Myodes rufocanus* |
| Ola, Magadan Oblast, Russia (7) | —/ON548170 | *Myodes rutilus* |
| Kilpisjärvi, Finland (2) | AY181511/— | *Myodes rufocanus* |
| Kilpisjärvi, Finland (2) | AY181512/— | *Myodes rufocanus* |
| Kilpisjärvi, Finland (2) | EF583963/KJ778953 | *Myodes rufocanus* |
| Kilpisjärvi, Finland (2) | EF583962/— | *Myodes rufocanus* |
| Kilpisjärvi, Finland (2) | EF583961/— | *Myodes rufocanus* |
| Narvik, Norway (1) | AY181513/— | *Myodes rufocanus* |
| Narvik, Norway (1) | AY189959/— | *Myodes rufocanus* |
| Asahikawa, Hokkaido, Japan (9) | LC535262/— | *Myodes rufocanus* |
| Asahikawa, Hokkaido, Japan (9) | LC535263/— | *Myodes rufocanus* |
| Asahikawa, Hokkaido, Japan (9) | LC535264/— | *Myodes rufocanus* |
| Asahikawa, Hokkaido, Japan (9) | LC535265/— | *Myodes rufocanus* |
| Asahikawa, Hokkaido, Japan (9) | LC535266/— | *Myodes rufocanus* |
| Asahikawa, Hokkaido, Japan (9) | LC535267/— | *Myodes rufocanus* |

## 3. Results and Discussion

We have obtained the sequences of two mitochondrial genes of the cestode *P. kalelai* (*cox1* and *nad1*) from two rodent species from Russia and Kazakhstan (Table 1). It was previously shown that *P. kalelai* mainly parasitizes *M. rufocanus* [5]. We also found cestode *P. kalelai* in gray red-backed vole in six localities in the Asian part of Russia and eastern Kazakhstan (Figure 1; Table 1). It was also noted that in two localities, *P. kalelai* was recorded in the northern red-backed vole *M. rutilus* (Table 1).

A total of 22 *cox1* gene sequences and 12 *nad1* sequences were involved in the study (Table 1). Thirteen *cox1* haplotypes and nine *nad1* haplotypes were identified.

The maximum pairwise Kimura 2-parameter distances in the *cox1* gene between the studied samples of *P. kalelai* was 0.042 ± 0.0089 (*cox1*: ON533416 and LC535248) with an average value of 0.023. Distances with other *Paranoplocephala* spp. [4,5] varied from 0.063 to 0.116, with an average value of 0.0849. The distance between haplogroups I and II was 0.0356 ± 0.0075, which coincides with the previously shown result for a smaller sample of *P. kalelai* of 0.037 [5].

The examination of the haplotype networks constructed by means of median-joining (MJ) and reduced median constructed by mtDNA showed that there were five major haplogroups unrelated to the host species (Figure 2). All five groups (I–V) were identified in the *cox1* gene network. Group I consists of the cestodes from the Western Sayan (H7c), Magadan Oblast (H5c and H4c), and Finland (H9c, H11c, H12c). Group II consists of the specimens from the Khanty-Mansi Autonomous Okrug (H6c), Norway (H6c, H10c), and Finland (H6c, H13c). Groups I and II correspond to the two clades previously established by the cox1 phylogenetic analysis, "Kilpisjärvi" and "Narvik," respectively [5]. Closely related to both of them is the H8c haplotype, which we identified in the Western Altai territory as a separate haplogroup III. Group IV consists of two haplotypes (H1c, H2c) found in six specimens of *P. kalelai* from voles from Hokkaido (Japan). Group V is directly related to group IV and consists of the H3c haplotype detected in three specimens of *P. kalelai* from the Magadan Oblast, which is directly related to it. The *nad1* haplotype network generally repeats the *cox1* groups, except for the missing data from Hokkaido and the "Kilpisjärvi" clade. Group I consists of the samples from the Western Sayan (H6n, H1n) and the Magadan Oblast (H3n-H5n). Group II consists of the samples from the Khanty-Mansi Autonomous Okrug (H7n) and Finland (H9n). Group III (Western Altai) is represented by the H8n haplotype, and group V is represented by the H2n haplotype from the Magadan Oblast.

The gray red-backed vole inhabits flat and mountain taiga and mountain-tundra areas [6]. *P. kalelai* is a host-specific parasite of gray red-backed and bank voles. Rare findings in bank voles are found only in areas where these host species cohabit. This indicates the possibility of *P. kalelai* parasitizing in other *Myodes* vole species, with the *M. rufocanus* remaining the main final host. Nevertheless, no molecularly confirmed findings of *P. kalelai* in other vole species of the genus *Myodes* are known, except for the gray red-backed and northern red-backed voles. Our findings of the *M. rutilus* beyond the Urals expand the potential range of its final hosts.

Evolutionary history and the complex intraspecific structure of *P. kalelai* are closely related to the dispersal history of the main definitive host, the gray red-backed vole. Most of the genetic diversity of the gray red-backed vole is concentrated in the southeastern part of the range, where representatives of all studied mitochondrial lines were found [12]. This pattern can be explained by the repeated fragmentation of the range associated with periodic glaciations in the Pleistocene and subsequent dispersal of voles. The mtDNA diversity of *P. kalelai* we analyzed suggests that the first gene flow known to us ("Kilpisjärvi" clade) came to northern Fennoscandia and northern Priokhotye (Magadan Oblast) from the Altai-Sayan mountains (Western Sayan), whose foothill parts, like the south of Western Siberia, were not exposed to glaciation [13,14].

The existence of very close haplotypes differing in less than ten substitutions (for both genes) suggests that there was a rapid expansion across a large area of the gray red-backed vole range. The next wave of dispersal passed to Fennoscandia through the taiga in the central part of Western Siberia, leaving its trace there in the form of close mtDNA haplotypes ("Narvik" clade). It is probably related to another refugium, also located in the south of Western Siberia. This is indicated by the existence of isolated haplotypes of *P. kalelai* in the foothills of western Altai (Kazakhstan). The further spread of *P. kalelai* probably went eastward. This gene flow gave rise to haplogroup IV which settled on the island of Hokkaido and haplogroup V re-populated the northern Priokhotye (Magadan Oblast).

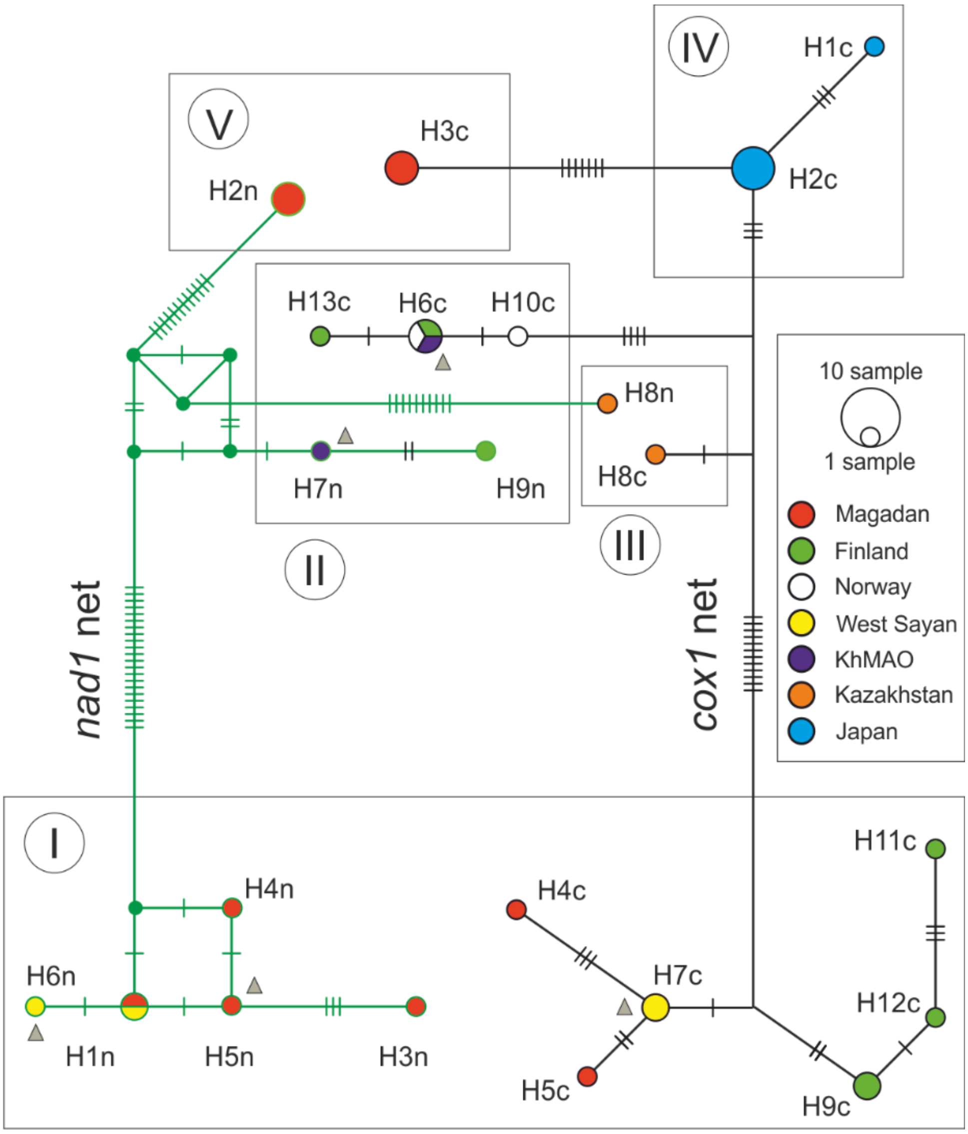

**Figure 2.** Median-joining network for *Paranoplocephala kalelai* constructed using the haplotypes of *nad1* (green line) and *cox1* (black line) fragment sequences. The size of the circles is proportional to the number of haplotypes. Dashes between haplotypes represent mutational steps between them. The color of the circle encodes the localities where the voles were captured. Haplogroup numbers are in Roman numerals. The triangle symbol next to the haplotype symbol indicates that the host is *Myodes rutilus*. In other cases, the host of the cestode is *M. rufocanus*.

Thus, the present study showed that two different mtDNA lines of *P. kalelai* also exist outside of Fennoscandia. This fact is not related to the parasitization of *P. kalelai* in different final hosts. The geographic distribution of the identified *P. kalelai* haplotypes suggests that the foothills of the Altai-Sayan Mountain and the South of Western Siberia, which were not covered by glaciers during the glaciation, served as refugia for *P. kalelai* and *M. rufocanus*. It is likely that at least two genetic flows emerged from these refugia, and these flows repopulated the territories freed from the ice as the glaciers retreated northward into Eurasia.

**Author Contributions:** Conceived and designed the experiments, A.K., S.A. and L.A.; collected the samples, N.D., P.V., N.L., S.K. and E.Z.; performed the experiments, A.B., E.V. and A.G.; analyzed the data, P.V., E.V. and A.G.; wrote and edited the paper, A.K., S.A., L.A., S.K., N.D., E.Z. and P.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** The reported study was funded by Russian Foundation for Basic Research, project number 20-54-00038 and Belarusian Republican Foundation for Fundamental Research: Б20Р-303 (4 May 2020).

**Institutional Review Board Statement:** Field procedures and protocols were approved by the Institutional Animal Care and Use Committees of the Institute of Systematics and Ecology of Animals (protocol #2020-02 dated 14 May 2020 and #2021-1 dated 28 April 2021). All wildlife field operations, including the responsible treatment of animals, met the guideline requirements of the order of the High and Middle Education Ministry (no. 742 issued on 13 November 1984) and by the Federal Law of the Russian Federation (no. 498-FZ issued on 19 December 2018). The study did not involve endangered or protected species.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Publicly available datasets were analyzed in this study. This data can be found in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/ (accessed on 24 May 2022); nucleotide sequence access numbers are given in Table 1).

**Acknowledgments:** We thank Marina Malkova and Zharkyn Kabdolov for their assistance in obtaining rodent cestode samples from Khanty-Mansi Autonomous Okrug and Kazakhstan.

**Conflicts of Interest:** The authors declare no conflict of interest.

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