



HAL
open science

Challenging the phylogenetic relationships among *Echinococcus multilocularis* isolates from main endemic areas

Séverine Lallemand, Jorge Oyhenart, Benoit Valot, Romain Borne, Louis Bohard, Gérald Umhang, Jacek Karamon, Sergey Konyaev, Caroline Rönnberg, Bruno Gottstein, et al.

► To cite this version:

Séverine Lallemand, Jorge Oyhenart, Benoit Valot, Romain Borne, Louis Bohard, et al.. Challenging the phylogenetic relationships among *Echinococcus multilocularis* isolates from main endemic areas. *International Journal for Parasitology*, 2024, 54 (11), pp.569-582. 10.1016/j.ijpara.2024.05.004 . anses-04862405

HAL Id: anses-04862405

<https://anses.hal.science/anses-04862405v1>

Submitted on 3 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Challenging the phylogenetic relationships among *Echinococcus multilocularis* isolates from main endemic areas [☆]



S  verine Lallemand ^a, Jorge Oyhenart ^b, Benoit Valot ^a, Romain Borne ^a, Louis Bohard ^c, G  rald Umhang ^d, Jacek Karamon ^e, Sergey Konyaev ^f, Caroline R  nnberg ^g, Bruno Gottstein ^h, Delphine Weil-Verhoeven ⁱ, Carine Richou ⁱ, Solange Bresson-Hadni ^{a,j}, Laurence Millon ^{a,j}, Anne-Pauline Bellanger ^{a,j}, Jenny Knapp ^{a,j,*}

^a UMR CNRS 6249 Chrono-Environnement, University of Franche-Comt  , 16 Route de Gray, 25030 Besan  on cedex, France

^b Instituto de Ciencias de la Tierra y Ambientales de La Pampa, Consejo Nacional de Investigaciones Cient  ficas y T  cnicas – Facultad de Ciencias Naturales Universidad Nacional de La Pampa, La Pampa, Argentina

^c Department of Infectiology, University Hospital of Besan  on, 25030 Besan  on, France

^d INTERFAS Unit, National Reference Laboratory for *Echinococcus* spp., Rabies and Wildlife Laboratory, ANSES, Malz  ville, France

^e Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Pu  awy, Poland

^f Institute of Systematics and Ecology of Animals, SB RAS, Novosibirsk, Russia

^g Public Health Agency of Sweden, Department of Microbiology, Unit for Parasitology, Solna, Sweden

^h Institute of Infectious Diseases, Faculty of Medicine, University of Bern, CH-3012 Bern, Switzerland

ⁱ Department of Hepatology and Intensive Cares, University Hospital of Besan  on, 25030 Besan  on, France

^j Department of Parasitology-Mycolology, Reference National Centre for Echinococcoses, University Hospital of Besan  on, 25030 Besan  on, France

ARTICLE INFO

Article history:

Received 16 January 2024

Received in revised form 5 April 2024

Accepted 23 May 2024

Available online 28 May 2024

Keywords:

Echinococcus multilocularis

Whole mitogenome sequencing

Haplogroup

Phylogenetic inference

ABSTRACT

Alveolar echinococcosis (AE) is a rare but severe disease that affects more than 18,000 people worldwide per year. The complete sequencing of the mitochondrial genome of *Echinococcus multilocularis* has made it possible to study the genetic diversity of the parasite and its spatial and temporal evolution. We amplified the whole mitochondrial genome by PCR, using one uniplex and two multiplex reactions to cover the 13,738 bp of the mitogenome, and then sequenced the amplicons with Illumina technology. In total, 113 samples from Europe, Asia, the Arctic and North America were analyzed. Three major haplogroups were found: HG1, which clustered samples from Alaska (including Saint-Lawrence Island), Yakutia (Russia) and Svalbard; HG2, with samples from Asia, North America and Europe; and HG3, subdivided into three micro-haplogroups. HG3a included samples from North America and Europe, whereas HG3b and HG3c only include samples from Europe. In France, HG3a included samples from patients more recently diagnosed in a region outside the historical endemic area. A fourth putative haplogroup, HG4, was represented by only one isolate from Olkhon Island (Russia). The increased discriminatory power of the complete sequencing of the *E. multilocularis* mitogenome has made it possible to highlight four distinct geographical clusters, one being divided into three micro-haplogroups in France.

   2024 The Author(s). Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Echinococcus multilocularis is a flatworm of the Class Cestoda, Family Taeniidae. Its life cycle involves members of the Order Carnivora (mainly red foxes in Europe) as the definitive hosts (DH) and small rodents as the intermediate hosts (IH). Humans act as aberrant intermediate and dead-end hosts. The parasite is the causative agent of alveolar echinococcosis (AE), a rare but serious zoonosis,

primarily affecting the liver, more rarely other organs and potentially secondarily metastasizing. In IH, the metacestode stage of the parasite leads to the formation of an infiltrating and destructive parasitic tumour of varying size, sometimes with central necrosis. The resulting AE is a disease that develops slowly, clinically manifesting between 5–15 years after infection, mainly affecting the liver (96%) but also other organs in approximately 4% of cases, e.g., the lungs, brain, or bones (Thompson and McManus, 2002; Eckert and Deplazes, 2004; Piarroux et al., 2011). When the first clinical signs appear (pain, general alteration of the physical state, jaundice, hepatomegaly, cholangitis, pruritus), the stage of parasite development is often far advanced and thus also the metacestode mass. The main first-line diagnostic tool

^{  } Note: Nucleotide sequence data reported in this paper are available in the GenBank™ database under the accession numbers [OR911371-OR911453](https://doi.org/10.1016/j.ijpara.2024.05.004).

* Corresponding author at: Department of Parasitology-Mycolology, University Hospital of Besan  on, 3 boulevard Alexander Fleming, F-25003 Besan  on, France.

E-mail address: jknapp@univ-fcomte.fr (J. Knapp).

is ultrasound which, coupled with specific serology, confirms 95% of cases (Bresson-Hadni et al., 2021).

More than 18,000 new human cases occur annually worldwide, mostly in China (Torgerson et al., 2010). In Europe, the historical endemic zone covers the countries near the Alps (southern Germany, Switzerland, Austria, and eastern France) and was described as early as 1900 by Posselt (Eckert and Thompson, 2017). In France, the incidence of AE has risen in recent years, with an increasing number of diagnoses outside the historical endemic area (data from the National Reference Centre of Echinococcoses (NRC-E) University Hospital, Besan  on, France). This area was described by Piarroux et al. (2013) and consists of two regions in the east and centre of France, including a total of 22 d  partements (second largest administrative areas in France, corresponding to the European NUTS3 level), known as d  partements at risk (DAR). The annual number of declared French AE cases has risen from seven to approximately 50 (data from the NRC-E), in the last 40 years. Simultaneously, the proportion of immunocompromised AE patients increased from 1% to 18% between 1982 and 2012. Chauchet and co-workers (2014) suggest a link between the increase in the number of immunocompromised AE patients due to immunosuppressive treatment and a change in therapeutic choices for cancer and chronic inflammatory diseases (Chauchet et al., 2014).

In North America, few human cases had been described outside St. Lawrence Island. However, human cases have been increasingly described in recent years. Houston and co-workers (2021) reported a cluster of 17 human cases in Alberta, Canada, between 2013 and 2020, partly associated with the emergence of a European-type strain in local wildlife. However, the strains from these patients have not been sequenced (Houston et al., 2021). The infectivity of strains according to origin has been little studied. Bartel and co-workers (1992) compared *E. multilocularis* specimens from Montana and Alaska in a rodent model. The specimen from Alaska (St. Lawrence Island) was found to be more pathogenic, with faster development, earlier formation of lung metastases, and a high number of deaths versus none for those infected with the Montana isolate (Bartel et al., 1992).

Studying the genetic diversity of *E. multilocularis* mitochondrial DNA (mtDNA) allows determination of haplotypes, as defined by unique sets of variants, followed by haplogroups, defined as collections of similar haplotypes, identified in phylogenetic trees as major branch points (Mitchell et al., 2014). Studies on genetic diversity have advanced considerably over the last 10 years. The genetic diversity of the parasite includes the study of single nucleotide polymorphisms (SNPs), insertion-deletions (indels), and heteroplasmy. Although the non-Mendelian inheritance pattern of mtDNA reduces its informative value in population genetics, it is widely used due to its haploid nature, high copy number, and faster mutation rate than nuclear markers (Ballard and Whitlock, 2004). In addition to the genes usually processed, determination of the complete mitochondrial genome provides a larger genetic diversity by including areas more susceptible to variations than genes, as the non-coding regions I and II and ribosomal DNA. (Nakao et al., 2002).

Mitochondrial sequence analyses have enabled the identification of distinct groups of *E. multilocularis* (Bowles et al., 1992; Bowles and McManus, 1993). Three mitochondrial genes have been particularly used: cytochrome *b* (*cob*), NADH dehydrogenase subunit 2 (*nad2*), and cytochrome *c* oxidase subunit 1 (*cox1*). Based on the study of these three genes, four genetically well-differentiated clades have been described at the continental level (Asian, European, North American, and Inner-Mongolian clades) (Nakao et al., 2009). Other mitochondrial genes have also been studied, such as NADH dehydrogenase subunit 1 (*nad1*) and the 12S gene (Geszy et al., 2014; Wang et al., 2018; Heidari et al., 2019). Santoro and co-authors (2024) described 43 haplotypes,

including four main ones, from a collection of European *E. multilocularis* isolates, one haplotype being found in all the countries investigated but Svalbard. Additionally, two main haplotypes seemed to dominate, the first one mostly in western, central and eastern European countries, and the other in Baltic areas and north-eastern Poland. An Asian-type haplotype was also found in Latvia and north-eastern Poland (Santoro et al., 2024). In North America, the recent description of human cases (17 in the province of Alberta and one in Saskatchewan (Canada), and three in Vermont and Minnesota states, USA) (Yamasaki et al., 2008; Polish et al., 2022, 2021), has led Santa and co-authors (2023) to study the genetic diversity of *E. multilocularis* in wild coyotes and red foxes from western Canada. The genetic variants were closely related to the European clade and supported the hypothesis of a relatively recent invasion with various founder events (Santa et al., 2023).

To date, nuclear markers (Haag et al., 1997; Knapp et al., 2012) and microsatellite markers (Bretagne et al., 1996; Nakao et al., 2003; Bart et al., 2006; Knapp et al., 2020; Umhang et al., 2021) have also been used. Nuclear markers are generally highly conserved and therefore less suitable for establishing phylogeny. Microsatellite markers such as the tandem repeated multi-locus microsatellite EmsB, are highly discriminating from the local to the continental scale, but can be complex to interpret and do not permit phylogenetic considerations (Valot et al., 2015; Knapp et al., 2020).

The whole mitochondrial genome of *E. multilocularis* was sequenced in 2002. It is a closed circular molecule of 13,737 to 13,738 bp. The mitochondrial genome of *E. multilocularis* contains 12 protein-coding genes, two rRNA genes (l-rRNA, s-rRNA), and 22 tRNA genes. The ATP8 gene is missing from the mtDNA of cestodes (Nakao et al., 2002).

In a previous study (Bohard et al., 2023), a new functional protocol for the complete sequencing of the *E. multilocularis* mitochondrial genome was developed, allowing an improvement in our knowledge of genetic diversity among AE lesions from French patients.

The main objective of the present study was to refine the phylogenetic relationships, using complete mtDNA sequencing, among *E. multilocularis* isolates from different endemic areas and different hosts and, in particular, in French patients with AE. The secondary objective was to (i) investigate the existence of sub-haplogroups among those French strains and (ii) to examine whether clinical features varied according to those sub-haplogroups.

2. Materials and methods

2.1. Sample collection

In total, 113 samples of various origin were selected, of which 57 were from a collection of biological French human samples submitted to the NRC-E for molecular diagnosis and 56 selected from a worldwide collection or from previous studies (Table 1). Of the 113 samples, 74 were of human origin and 39 of animal origin (13 foxes (individual worms), 21 rodents, three monkeys, and two dogs (metacestode lesions). Thirty patients' mitogenomes had already been sequenced as part of a previous study (Bohard et al., 2023) (GenBank accession numbers **OQ599939-OQ599968**). The French patients were diagnosed with AE between 1987 and 2022. For each, retrospective information was collected, including epidemiological data (gender, age at diagnosis, residence at diagnosis, year of diagnosis, residence in a DAR, zone of residence), immunocompetence status, clinical data (organ involved, with or without extra-hepatic localization, presence of metastases, invasion of neighbouring organs). For geographical considerations, Europe has been considered in the present study as bounded to the east by the Ural Mountains and to the southeast by the Carpathian Mountains.

Table 1
Characteristics of *Echinococcus multilocularis* samples and results of haplotypic analysis.

ID	Sample name	Geographical origin	Original host	DH/IH	Sampling year	Reference	Haplotype	Haplogroup	VCF Acc. No.	GenBank Acc. No.
FE-007	NRC-E-007	France	<i>Homo sapiens</i>	IH	2000	this study	MtG_h1	HG3c	9710–007	OR911371
FE-191	NRC-E-191	France	<i>Homo sapiens</i>	IH	2011	this study	MtG_h2	HG3b	11652–191	OR911372
FE-238	NRC-E-238	France	<i>Homo sapiens</i>	IH	1999	this study	MtG_h1	HG3c	9908–238	OR911373
FE-270	NRC-E-270	France	<i>Homo sapiens</i>	IH	1985	this study	MtG_h2	HG3b	0011–270	OR911374
FE-276	NRC-E-276	France	<i>Homo sapiens</i>	IH	2001	this study	MtG_h3	HG3c	0105–276	OR911375
FE-292	NRC-E-292	France	<i>Homo sapiens</i>	IH	2004	this study	MtG_h4	HG3b	0403–292	OR911376
FE-333	NRC-E-333	France	<i>Homo sapiens</i>	IH	2005	this study	MtG_h5	HG3c	0412–333	OR911377
FE-351	NRC-E-351	France	<i>Homo sapiens</i>	IH	2005	this study	MtG_h4	HG3b	0502–351	OR911378
FE-355	NRC-E-355	France	<i>Homo sapiens</i>	IH	2005	this study	MtG_h1	HG3c	0503–355	OR911379
FE-363	NRC-E-363	France	<i>Homo sapiens</i>	IH	2006	this study	MtG_h3	HG3c	0506–363	OR911380
FE-366	NRC-E-366	France	<i>Homo sapiens</i>	IH	2005	this study	MtG_h6	HG3c	0512–366	OR911381
FE-371	NRC-E-371	France	<i>Homo sapiens</i>	IH	2007	this study	MtG_h7	HG3b	0512–371	OR911382
FE-404	NRC-E-404	France	<i>Homo sapiens</i>	IH	2007	this study	MtG_h3	HG3c	0707–404	OR911383
FE-423	NRC-E-423	France	<i>Homo sapiens</i>	IH	2008	this study	MtG_h3	HG3c	0707–423	OR911384
FE-427	NRC-E-427	France	<i>Homo sapiens</i>	IH	2010	this study	MtG_h4	HG3b	0804–427	OR911385
FE-478	NRC-E-478	France	<i>Homo sapiens</i>	IH	2010	this study	MtG_h8	HG3c	10529–478	OR911386
FE-491	NRC-E-491	France	<i>Homo sapiens</i>	IH	2019	Bohard et al., 2023	MtG_h3	HG3c	19746–491	OQ599939
FE-652	NRC-E-652	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h3	HG3c	18685–652	OQ599947
FE-653	NRC-E-653	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h9	HG3a	18818–653	OQ599962
FE-655	NRC-E-655	France	<i>Homo sapiens</i>	IH	2017	this study	MtG_h10	HG3b	17166–655	OR911387
FE-735	NRC-E-735	France	<i>Homo sapiens</i>	IH	2017	this study	MtG_h11	HG3b	17792–735	OR911388
FE-749	NRC-E-749	France	<i>Homo sapiens</i>	IH	2017	Bohard et al., 2023	MtG_h12	HG3c	17872–749	OQ599942
FE-755	NRC-E-755	France	<i>Homo sapiens</i>	IH	2020	Bohard et al., 2023	MtG_h13	HG3c	20084–755	OQ599950
FE-763	NRC-E-763	France	<i>Homo sapiens</i>	IH	2017	this study	MtG_h14	HG3b	17124–763	OR911389
FE-766	NRC-E-766	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h1	HG3c	18083–766	OQ599940
FE-779	NRC-E-779	France	<i>Homo sapiens</i>	IH	2017	this study	MtG_h15	HG3b	17857–779	OR911390
FE-780	NRC-E-780	France	<i>Homo sapiens</i>	IH	2017	Bohard et al., 2023	MtG_h15	HG3b	17068–780	OQ599953
FE-781	NRC-E-781	France	<i>Homo sapiens</i>	IH	2017	this study	MtG_h16	HG3c	17062–781	OR911391
FE-782	NRC-E-782	France	<i>Homo sapiens</i>	IH	2017	this study	MtG_h17	HG3a	17853–782	OR911392
FE-790	NRC-E-790	France	<i>Homo sapiens</i>	IH	2017	this study	MtG_h18	HG3a	17077–790	OR911393
FE-791	NRC-E-791	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h3	HG3c	18317–791	OQ599948
FE-792	NRC-E-792	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h12	HG3c	18488–792	OQ599943
FE-796	NRC-E-796	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h17	HG3a	18328–796	OQ599965
FE-800	NRC-E-800	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h15	HG3b	18250–800	OQ599954
FE-805	NRC-E-805	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h3	HG3c	18718–805	OQ599949
FE-826	NRC-E-826	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h1	HG3c	18544–826	OQ599944
FE-829	NRC-E-829	France	<i>Homo sapiens</i>	IH	2018	Bohard et al., 2023	MtG_h19	HG3b	18022–829	OQ599951
FE-836	NRC-E-836	France	<i>Homo sapiens</i>	IH	2019	Bohard et al., 2023	MtG_h20	HG3a	19104–836	OQ599963
FE-840	NRC-E-840	France	<i>Homo sapiens</i>	IH	2022	this study	MtG_h21	HG3b	22694–840	OR911394
FE-841	NRC-E-841	France	<i>Homo sapiens</i>	IH	2019	Bohard et al., 2023	MtG_h4	HG3b	19674–841	OQ599955
FE-842	NRC-E-842	France	<i>Homo sapiens</i>	IH	2021	Bohard et al., 2023	MtG_h22	HG3c	21798–842	OQ599941
FE-860	NRC-E-860	France	<i>Homo sapiens</i>	IH	2019	Bohard et al., 2023	MtG_h17	HG3a	19518–860	OQ599964
FE-878	NRC-E-878	France	<i>Homo sapiens</i>	IH	2021	Bohard et al., 2023	MtG_h9	HG3a	21229–878	OQ599961
FE-879	NRC-E-879	France	<i>Homo sapiens</i>	IH	2021	Bohard et al., 2023	MtG_h23	HG3b	21939–879	OQ599956
FE-884	NRC-E-884	France	<i>Homo sapiens</i>	IH	2020	Bohard et al., 2023	MtG_h24	HG3b	20298–884	OQ599960
FE-888	NRC-E-888	France	<i>Homo sapiens</i>	IH	2020	Bohard et al., 2023	MtG_h25	HG3b	20659–888	OQ599959
FE-889	NRC-E-889	France	<i>Homo sapiens</i>	IH	2022	this study	MtG_h15	HG3b	22986–889	OR911395
FE-890	NRC-E-890	France	<i>Homo sapiens</i>	IH	2020	Bohard et al., 2023	MtG_h39	HG2	20928–890	OQ599968
FE-892	NRC-E-892	France	<i>Homo sapiens</i>	IH	2020	Bohard et al., 2023	MtG_h4	HG3b	20381–892	OQ599957
FE-900	NRC-E-900	France	<i>Homo sapiens</i>	IH	2020	Bohard et al., 2023	MtG_h1	HG3c	20549–900	OQ599945
FE-907	NRC-E-907	France	<i>Homo sapiens</i>	IH	2021	Bohard et al., 2023	MtG_h17	HG3a	21012–907	OQ599966
FE-908	NRC-E-908	France	<i>Homo sapiens</i>	IH	2020	Bohard et al., 2023	MtG_h1	HG3c	20742–908	OQ599946
FE-921	NRC-E-921	France	<i>Homo sapiens</i>	IH	2022	this study	MtG_h26	HG3c	22963–921	OR911397
FE-922	NRC-E-922	France	<i>Homo sapiens</i>	IH	2022	this study	MtG_h17	HG3a	22958–922	OR911398

(continued on next page)

Table 1 (continued)

ID	Sample name	Geographical origin	Original host	DH/IH	Sampling year	Reference	Haplotype	Haplogroup	VCF Acc. No.	GenBank Acc. No.
FE-935	NRC-E-935	France	<i>Homo sapiens</i>	IH	2021	Bohard et al., 2023	MtG_h27	HG3b	21006–935	OQ599952
FE-938	NRC-E-938	France	<i>Homo sapiens</i>	IH	2021	Bohard et al., 2023	MtG_h17	HG3a	21516–938	OQ599967
FE-988	NRC-E-988	France	<i>Homo sapiens</i>	IH	2022	this study	MtG_h1	HG3c	22993–988	OR911396
S1	St Lawrence Isl_3	Alaska (St Lawrence island)	<i>Microtus oeconomus</i>	IH	1995	Knapp et al., 2007	MtG_h40	HG1	SLI-S1	OR911399
S2	St Lawrence Isl_5	Alaska (St Lawrence island)	<i>Microtus oeconomus</i>	IH	1995	Knapp et al., 2007	MtG_h40	HG1	SLI-S2	OR911400
S3	St Lawrence Isl_6	Alaska (St Lawrence island)	<i>Microtus oeconomus</i>	IH	1995	Knapp et al., 2007	MtG_h40	HG1	SLI-S3	OR911401
S4	StLawrence Isl_7	Alaska (St Lawrence island)	<i>Microtus oeconomus</i>	IH	1995	Knapp et al., 2007	MtG_h40	HG1	SLI-S4	OR911402
S5	StLawrence Isl_9	Alaska (St Lawrence island)	<i>Microtus oeconomus</i>	IH	1995	Knapp et al., 2007	MtG_h40	HG1	SLI-S5	OR911403
S33	SLI60	Alaska (St Lawrence island)	<i>Homo sapiens</i> ^a	IH	1990	Gottstein et al., 1991	MtG_h40	HG1	SLI-S33	OR911417
S31	27_Alaska.r	Alaska	<i>Microtus oeconomus</i> ^a	IH	1989	Bretagne et al., 1996	MtG_h40	HG1	ALK-S31	OR911415
S34	SLI144	Alaska (St Lawrence island)	<i>Homo sapiens</i> ^a	IH	1990	Gottstein et al., 1991	MtG_h43	HG2	SLI-S34	OR911418
S64	13,264	Armenia	<i>Homo sapiens</i>	IH	ND	Umhang et al., 2021	MtG_h51	HG2	ARM-S64	OR911444
S20	1-Human-HP-102039	Belgium	<i>Homo sapiens</i>	IH	2010	Knapp et al., 2020	MtG_h29	HG3c	BLG-S20	OR911408
S16	CND85_b43	Canada	rodent	IH	1985	Knapp et al., 2007	MtG_h41	HG2	CND-S16	OR911406
S35	CND-84_b36	Canada	rodent ¹	IH	<1980	Knapp et al., 2007	MtG_h41	HG2	CND-S35	OR911419
S36	CND-85_b36	Canada	rodent ¹	IH	<1980	Knapp et al., 2007	MtG_h41	HG2	CND-S36	OR911420
S17	52-Dog-09D2679	Canada (British Columbia)	<i>Canis lupus familiaris</i> ^b	IH	2009	Peregrine et al., 2012	MtG_h28	HG3a	CND-S17	OR911407
S44	Em Chine 1.2	China	<i>Canis lupus familiaris</i>	IH	ND	this study	MtG_h45	HG2	CHN-S44	OR911426
S28	3_Germany.r	Germany (Stuttgart 50 km west)	<i>Ondatra zibethicus</i>	IH	1984	Bretagne et al., 1996	MtG_h32	HG3b	GER-S28	OR911414
S26	Germany monkey 1 – 2022	Germany	monkey	IH	2022	this study	MtG_h4	HG3b	GER-S26	OR911412
S25	Em genomic DNA 01	Japan (Hokkaido)	rodent	IH	<2009	Knapp et al., 2011	MtG_h42	HG2	JPN-S25	OR911411
S27	15_Japan.r	Japan (Hokkaido)	<i>Clethrionomys rufocanus</i>	IH	1979	Bretagne et al., 1996	MtG_h42	HG2	JPN-S27	OR911413
S47	A50	Japan (Hokkaido)	<i>Vulpes vulpes</i>	DH	ND	this study	MtG_h42	HG2	JPN-S47	OR911427
S48	E50	Japan (Hokkaido)	<i>Vulpes vulpes</i>	DH	ND	this study	MtG_h46	HG2	JPN –S48	OR911428
S49	I50	Japan (Hokkaido)	<i>Vulpes vulpes</i>	DH	ND	this study	MtG_h42	HG2	JPN –S49	OR911429
S50	M50	Japan (Hokkaido)	<i>Vulpes vulpes</i>	DH	ND	this study	MtG_h42	HG2	JPN –S50	OR911430
S51	N50	Japan (Hokkaido)	<i>Vulpes vulpes</i>	DH	ND	this study	MtG_h42	HG2	JPN –S51	OR911431
S40	LUX	Luxembourg	<i>Homo sapiens</i>	IH	2021	Bohard et al., 2023	MtG_h4	HG3b	21169-LX1	OQ599958
S37	Em1 Norv Spitzberg	Norway (Svalbard)	<i>Microtus levis</i>	IH	2004–2006	Knapp et al., 2012	MtG_h44	HG1	SVB-S37	OR911421
S42	Em7 Norv Spitzberg	Norway (Svalbard)	<i>Microtus levis</i>	IH	2004–2006	Knapp et al., 2012	MtG_h44	HG1	SVB-S42	OR911424
S43	Em22 Norv Spitzberg	Norway (Svalbard)	<i>Microtus levis</i>	IH	2004–2006	Knapp et al., 2012	MtG_h44	HG1	SVB-S43	OR911425
S52	Fox3	Poland (Warmi�nsko-Mazurskie)	<i>Vulpes vulpes</i>	DH	2009–2013	Karamon et al., 2017	MtG_h47	HG2	POL-S52	OR911432
S53	Fox7	Poland (Zachodniopomorskie)	<i>Vulpes vulpes</i>	DH	2009–2013	Karamon et al., 2017	MtG_h34	HG3a	POL-S53	OR911433
S54	Fox12	Poland (Lubuskie)	<i>Vulpes vulpes</i>	DH	2009–2013	Karamon et al., 2017	MtG_h35	HG3a	POL-S54	OR911434
S55	Fox28	Poland (�wietokrzyskie)	<i>Vulpes vulpes</i>	DH	2009–2013	Karamon et al., 2017	MtG_h36	HG3c	POL-S55	OR911435
S56	Fox33	Poland (�laskie)	<i>Vulpes vulpes</i>	DH	2009–2013	Karamon et al., 2017	MtG_h17	HG3a	POL-S56	OR911436
S57	Fox47	Poland (Warmi�nsko-Mazurskie)	<i>Vulpes vulpes</i>	DH	2009–2013	Karamon et al., 2017	MtG_h37	HG3c	POL-S57	OR911437
S58	Fox64	Poland (Podlaskie)	<i>Vulpes vulpes</i>	DH	2009–2013	Karamon et al., 2017	MtG_h17	HG3a	POL-S58	OR911438
S59	Fox78	Poland (Mazowieckie)	<i>Vulpes vulpes</i>	DH	2009–2013	Karamon et al., 2017	MtG_h47	HG2	POL-S59	OR911439
S60	13,254	Russia (Altai)	<i>Homo sapiens</i>	IH	ND	Umhang et al., 2021	MtG_h48	HG2	RUS-S60	OR911440
S61	13,256	Russia (Altai)	<i>Homo sapiens</i>	IH	ND	Umhang et al., 2021	MtG_h49	HG2	RUS-S61	OR911441
S62	13,259	Russia (Altai)	<i>Homo sapiens</i>	IH	ND	Umhang et al., 2021	MtG_h50	HG2	RUS-S62	OR911442
S63	13,260	Russia (Altai)	<i>Homo sapiens</i>	IH	ND	Umhang et al., 2021	MtG_h50	HG2	RUS-S63	OR911443
S65	13,269	Russia (Altai)	<i>Homo sapiens</i>	IH	ND	Umhang et al., 2021	MtG_h52	HG2	RUS-S65	OR911445
S66	13,270	Russia (Altai)	<i>Homo sapiens</i>	IH	ND	Umhang et al., 2021	MtG_h53	HG2	RUS-S66	OR911446
S67	13,271	Russia (Altai)	<i>Homo sapiens</i>	IH	ND	Umhang et al., 2021	MtG_h54	HG2	RUS-S67	OR911447
S70	13,276	Russia (Ryazan Oblast)	<i>Meriones unguiculatus</i>	IH	ND	Umhang et al., 2021	MtG_h55	HG2	RUS-S70	OR911448
S71	13,277	Russia (Ryazan Oblast)	<i>Meriones unguiculatus</i>	IH	ND	Umhang et al., 2021	MtG_h38	HG3c	RUS-S71	OR911449
S72	13,278	Russia (Ryazan Oblast)	<i>Meriones unguiculatus</i>	IH	ND	Umhang et al., 2021	MtG_h56	HG2	RUS-S72	OR911450
S73	13,279	Russia (Anabar-Yakutia)	<i>Microtus gregalis</i>	IH	ND	Umhang et al., 2021	MtG_h57	HG1	RUS-S73	OR911451
S74	13,280	Russia (Anabar-Yakutia)	<i>Microtus gregalis</i>	IH	ND	Umhang et al., 2021	MtG_h57	HG1	RUS-S74	OR911452
S76	13,286	Russia (Olkhon Island-Irkutsk Oblast)	<i>Alticola olchonensis</i>	IH	2010–2012	Konyaev et al., 2013	MtG_h58	c	RUS-S76	OR911453
S41	SW1	Sweden	<i>Homo sapiens</i>	IH	2017	this study	MtG_h17	HG3a	SWD-S40	OR911423
S13	ADN_42	Switzerland (Z�rich zoo)	<i>Chlorocebus pygerythrus</i>	IH	1989	Knapp et al., 2007	MtG_h17	HG3a	SWT-S13	OR911404
S21	WR	Switzerland	<i>Homo sapiens</i>	IH	2014	this study	MtG_h30	HG3b	SWT-S21	OR911409

Table 1 (continued)

ID	Sample name	Geographical origin	Original host	DH/IH	Sampling year	Reference	Haplotype	Haplogroup	VCF Acc. No.	GenBank Acc. No.
S23	SJ	Switzerland	<i>Homo sapiens</i>	IH	2013	this study	MTC_h31	HG3b	SWT-S21	OR911410
S39	9_Z��rich.h	Switzerland	<i>Homo sapiens</i>	IH	1981	Bretagne et al., 1996	MTC_h9	HG3a	SWT-S39	OR911422
S32	CH5	Switzerland	<i>Homo sapiens</i> ^a	IH	ND	Gottstein et al., 1991	MTC_h33	HG3a	SWT-S32	OR911416
S14	ADN_43	Switzerland (Z��rich zoo)	<i>Chlorocebus pygerythrus</i>	IH	1990	Knapp et al., 2007	MTC_h17	HG3a	SWT-S14	OR911405

DH, definitive host; IH, intermediate host; VCF, variant call format; Acc. No, accession number.

^a Maintained in rodents.

^b Dog with hepatic AE, from British Columbia (BC) province (Quesnel area), Canada, without stay abroad declared.

^c Unique representative of a new group: putative haplotype HG4.

2.2. PCR and sequencing

A previously described protocol was applied to amplify the entire mitochondrial genome of *E. multilocularis* (13,738 bp) (Bohard et al., 2023). Briefly, PCR was carried out in a final volume of 20 µl containing 30 to 150 ng of purified DNA, 0.5 µM of each primer, and 0.5 U AmpliTaq Gold 360 DNA Polymerase master mix (Thermo Fisher Scientifics, Vilnius, Lithuania), and nuclease-free water. Seven pairs of previously described primers (Bohard et al., 2023), were used to amplify the whole mitochondrial genome of *E. multilocularis* (Table 2). The PCR conditions were an initial denaturation at 95 °C for 5 min, followed by 40 cycles consisting of a denaturation step at 98 °C for 20 s, an annealing step at 55 °C for 15 s, and an elongation step at 65 °C for 3 min, and a final elongation at 65 °C for 5 min. All PCRs were performed with a Biometra T3 thermocycler (Whatman Biometra, G  ttingen, Germany). PCR products were revealed by electrophoresis on a 1% agarose gel.

PCR products were purified separately and then mixed together in equimolar amounts after fluorometric measurement, (Qubit, Invitrogen, Thermo Fisher Scientific, Wilmington, DE, USA), to obtain a minimum concentration of mixed PCR products of 20 ng/µL. Sequencing was performed using Illumina technology (NextSeq 500 System, Illumina, San Diego, USA) after preparation of the library with a Nextera XT DNA Library Prep Kit (Illumina) by the microbiology core laboratory of the Pasteur Institute (P2M, Paris, France).

2.3. Data analyses

2.3.1. Determination of the consensus sequences

Raw paired-end reads of 150 bp were obtained by Illumina sequencing and trimmed using Sickle v1.33.2 (Joshi and Fass, 2011) at a quality threshold of 20 and a length threshold of 75 bp. Quality control of the reads was performed using FastQC v0.11.9 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Trimmed reads were mapped against GenBank accession number **AB018440.2** using the Burrows-Wheeler Aligner (BWA)-MEM algorithm (v0.7.17.2) (Li and Durbin, 2010). Variant calling was performed using FreeBayes (v1.3.6) (Garrison and Marth, 2012). A minimum mapping quality score of 20 and a minimal alternate fraction of observations supporting an alternate allele of 0.2 were retained. This means that mutations occurring in at least 20% of reads were analysed. This threshold allowed

Table 2

Oligonucleotide sequences used to amplify the complete mitochondrial genome of *Echinococcus multilocularis*. Primer positions are given according to the *Echinococcus multilocularis* reference mitochondrial genome, GenBank accession number **AB018440.2**.

Primer name	Primer sequence (5' – 3')	Primer position	PCR product length (bp)
E1f	TTCAAAGAGTGTGGATTTC	1081...1100	2153
E1r	CAATACTCCAGTAAAAATTGAAGAATC	3206...3233	
E2f	GAAGTAGTGGGTTTGTATTGTC	3175...3196	1887
E2r	CACCCAACAAACCTAACTTC	5042...5061	
E3f	CATGCTGAGGCTACTAGAG	4992...5010	2072
E3r	GTATCCCTTTAAATCTGTTGAATCTG	7038...7063	
E4f	GGGGTTATGTAGAATTATTTATTTTG	7002...7028	2098
E4r	TACACCAACCAAAGAGAAAG	9080...9099	
E5f	ATAGGTGTGGTTAGAGAAAATTG	9018...9040	1827
E5r	CAGTATACAACCAGGTTAATG	10823...10844	
E6f	ACCTGGTTGTATAGCTGTGAAG	10828...10849	2099
E6r	CACCAACAGGAAACGAATC	12908...12926	
E7f	GACTATAAAGATTGTTGGTCATCAG	12847...12871	1997
E7r	GAAAGGAAATCCAGCACTCTTTG	1083...1105	

the examination of heteroplasmy (Pakendorf and Stoneking, 2005). Mutations occurring in 20 to 80% of reads were considered to be ambiguous sites and those occurring in more than 80% of reads were considered to be real mutations. Ambiguous sites appearing in nucleotide sequences were coded using the standard International Union of Pure and Applied Chemistry (IUPAC) for combinations of two or more bases. To obtain consensus sequences, a variant call format (VCF) filter was then applied to conserve only the mutations with a quality score $Q > 500$. The VCF files resulting from this mapping have been published in the European Variation Archive (EVA) (<https://www.ebi.ac.uk/eva/?eva-study=PRJEB52628>). Mutations related to primer sequences were found using IGV viewer (Thorvaldsdóttir et al., 2013) and were not considered for the remaining analysis. A consensus sequence in FASTA formatted files was obtained for each sample using BCFtools consensus (v1.9) (Li et al., 2009).

2.3.2. Determination of haplotypes and population diversity

All *E. multilocularis* mitogenome sequences and the *E. multilocularis* mitogenome reference sequence (GenBank accession number **AB018440**), were aligned using MAFFT v.7.508 (Katoh and Standley, 2013). All loci given in this article are numbered relative to this reference. Haplotypes were determined using DNA sequence polymorphism v.6.12.03 (Rozas et al., 2017), indels not being considered. Arlequin software v3.5.2.2 (Excoffier and Lischer, 2010) was used to calculate population diversity indices, such as the number of haplotypes (Hn), the haplotype diversity (Hd), corresponding to the probability that two randomly sampled genome specimens are different, nucleotide diversity (defined as the average number of nucleotide differences per site in pairwise comparisons among DNA sequences) and neutrality indices based on Tajima's D (Tajima, 1989) and Fu's F_s (Fu and Li, 1993) tests to assess the random evolution of a DNA sequence.

2.3.3. Phylogeny and determination of haplogroups

A phylogenetic tree was constructed using all the complete mtDNA sequences to determine the genetic relationships between samples and compare them with data available in the literature. The tree was generated with MrBayes (Ronquist et al., 2012) using the previously complete sequence alignment. A generalized time-reversible (GTR) model with variable sites and a gamma distribution of evolution rates were used. The *Echinococcus shiquicus* sequence (**NC 009460.1**) (Nakao et al., 2007) was used as an outgroup. One million iterations were computed with a sampling every 500, on two independent Markov chains. Convergence was verified using an effective sample size (ESS) above 100 and a potential scale reduction factor (PSRF) close to 1 (Gelman and Rubin, 1992). A consensus tree was determined after removing the first 25% of samples as burn-in. Visualization and annotation of the tree were performed using ItoI v.6 (Letunic and Bork, 2021). A median-joining network was then created to visualize the relationship between the complete mtDNA sequences of samples from the French AE patients in greater detail. Previously defined French haplotypes were used to construct a haplotype network with PopART software (<https://popart.otago.ac.nz>) using the median-joining algorithm as the inference method (Bandelt et al., 1999).

2.3.4. Statistical analyses

Statistical analyses were performed using Jamovi (v.2.2.3) (<https://www.jamovi.org>). A potential association between the clinical forms of AE and the haplogroup of the *E. multilocularis* strains in French AE patients was investigated. The variables analysed were for (i) a quantitative variable: age at diagnosis, for (ii) qualitative variables: diagnosis after 2013, gender, immunosup-

pression status prior to diagnosis, and for (iii) clinical forms: hepatic forms alone, extra-hepatic forms alone, intra and extra-hepatic forms, invasion of neighbouring tissues and/or organs and presence of metastases. For quantitative variables, normality (Shapiro-Wilk) and homogeneity of variance (Levene) tests were assessed. If normality and/or homogeneity were not verified, a non-parametric test was applied (Kruskal-Wallis). Otherwise, Fisher's test was used. For qualitative variables, verification of the expected number of observations led to the use of the chi-square test or Fisher's exact test when the number of observations was less than five. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Description of polymorphism

In total, 113 DNA extracts were sequenced and analysed by alignment and variant calling. The sizes of the mitogenomes ranged from 13,735 to 13,740 bp. Among the 13,747 sites (corresponding to the size of the multiple alignment including indels), 13,311 were invariable (sequence conservation $C = 0.969$). Of the 436 polymorphic sites, 17 were indels and 419 were substitutions. Among the substitutions, 207 were parsimony informative sites (205 with two variants and two with three variants at loci 3798 and 6811 according to the reference sequence **AB018440**) and 212 were singleton variable sites (210 with two variants and two with three variants at loci 11,948 and 13,526). The nucleotide content was: T (48.42%), A (20.59%), G (23.36%) C (7.63%). The molecular diversity index values are summarized in Table 3. Selective neutrality tests were not significant (Tajima's $D = -1.27271$, $P = 0.065$; Fu's $F_s = 0.71923$, $P = 0.664$).

SNPs were evenly distributed throughout the mitogenome. Parsimony informative sites concerned 0 (*nd4L*) to 2.9% (*cox3*) of the nucleotide sites in the gene sequences and up to 3.8% in intergenic region 1 (Fig. 1). The nucleotides at positions 112 and 3490 differed from those in the reference sequence, which suggested that it was rather the reference sequence that contained the SNPs. One major insertion of four bases was detected at locus 8376, at the end of the *nad1* gene (position 886 in the gene), for all Yakutia (Russia) and Svalbard samples and for 6/7 samples from Alaska.

3.2. Determination of haplotypes and phylogenetic inference

From the 113 aligned mitochondrial sequences with the mitogenome reference sequence **AB018440**, 58 haplotypes were deter-

Table 3
Molecular and haplotypic diversity indices for 113 *Echinococcus multilocularis* mitogenome samples.

Molecular parameters and index	Value
Sample size	113
Number of observed nucleotide sites	13,747
Number of polymorphic sites (%)	436 (3.1%)
Number of parsimony informative sites	207
Number of singleton variable sites	212
Number of indels sites	17
Number of substitutions	419
Number of observed transitions	348
Number of observed transversions	71
Number of INDELS	10
GC content	31%
π (mean number of pairwise differences)	52.020494 ± 22.662985
average over loci ± S.D.	0.003785 ± 0.001826
Hn (number of haplotypes)	58
Hd (haplotype diversity) ± S.D.	0.971 ± 0.006

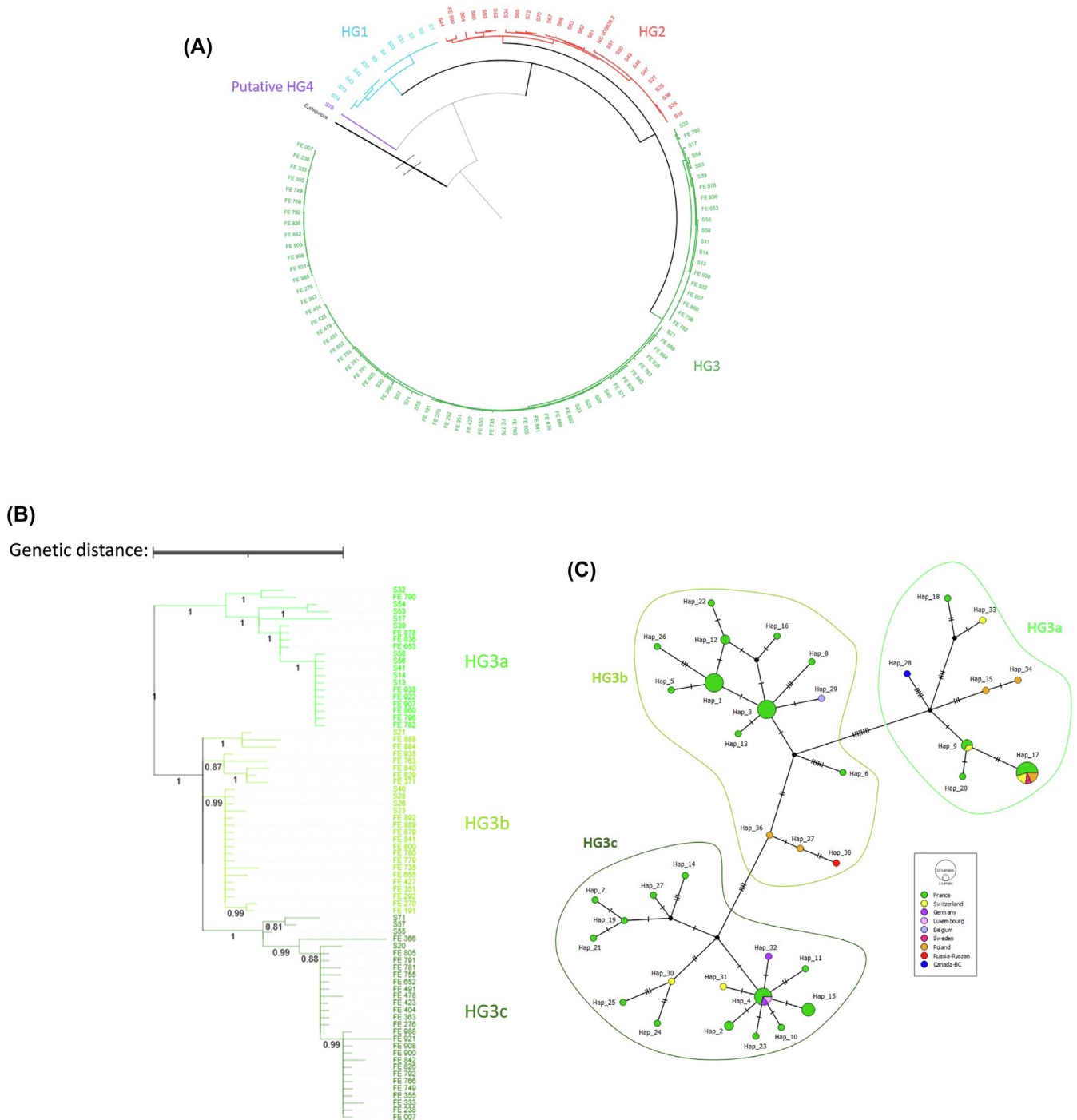


Fig. 2. Phylogenetic tree constructed using the Bayesian method on whole mitogenome sequences from *Echinococcus multilocularis* isolates and the reference sequences of *Echinococcus shiquicus* [NC_009460.1](#) and *E. multilocularis* [AB018440](#). (A) The branch representing *E. shiquicus* has been deliberately shortened by a factor of 10 to facilitate readability. (B) Subtree representing the haplogroup HG3. The bold lines represent posterior probability values $\geq 90\%$. (C) Entire mitochondrial haplotype network of *Echinococcus multilocularis* based on the median-joining method. Dashes on the lines represent mutations. Each circle represents one of the 38 haplotypes. The sizes of the circles depend on the numbers of samples representing the haplotypes and the colours the geographical origins. BC, British Columbia.

3.3. Comparison of epidemiological and clinical data according to haplogroups among French AE patients

Among the 57 French AE patients, 56 (98%) presented an AE lesion belonging to the HG3 haplogroup, 10 (18%) in the micro-haplogroup HG3a, 21 (37%) in HG3b, and 25 (45%) in HG3c. One patient presented an AE lesion clustered in the HG2 haplogroup after reporting several visits to China years before. Comparisons of the results of epidemiological and clinical data for French

patients for the HG3 micro-haplogroups are summarized in [Table 5](#). Briefly, the proportion of diagnoses after 2013 differed between the HG3a and HG3c micro-haplogroups (from 2014 to 2022 for HG3a versus 1997 to 2022 for HG3c, Fisher’s exact test, $P = 0.007$).

4. Discussion

Sequencing of the complete *E. multilocularis* mtDNA based on a worldwide collection of AE lesions and *E. multilocularis* adult

Table 4

Single nucleotide polymorphisms (SNPs) described for the *cox1* gene in samples belonging to the *Echinococcus multilocularis* Mongolian clade. Shaded columns report SNP loci present in all samples and which appear to be clade-specific. Positions are given according to (i) the *E. multilocularis* reference mitochondrial genome, GenBank accession number **AB018440.2**, and (ii) according to the *cox1* gene in **AB018440.2**.

AB018440	A	A	T	A	G	T	A	A	T	G	C	A	T	C	T	G	T	C	A	G	A	T	G	G	A	A	T	T	
Position in AB018440	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	1	1	1	1	1	1	1	1	1	1	1	1	1	
	2	2	3	3	4	4	4	5	6	6	8	8	8	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	4	2	6	4	5	9	8	5	7	5	5	9	0	5	1	1	2	3	3	4	4	4	5	5	6	6	7	
	1	3	9	3	0	3	7	8	0	1	2	4	9	6	9	0	7	7	1	7	1	7	9	6	9	5	7	1	
																9	9	1	6	9	3	8	3	2	8	2	3	2	
Position in <i>cox1</i> gene	5	7	1	1	2	2	3	4	4	5	6	6	7	7	7	9	1	1	1	1	1	1	1	1	1	1	1	1	
	7	9	6	9	7	8	3	2	8	0	8	9	3	4	9	4	0	1	1	2	2	3	3	3	4	4	5	5	
				5	9	6	9	3	4	6	7	8	0	5	2	5	5	1	0	5	1	4	1	2	9	3	8	0	4
																		5	7	2	5	9	4	9	8	4	8	9	8
AB510023 (9199-10746)	.	G	C	.	A	C	T	.	G	.	T	G	G	T	G	A	G	G	A	A	T	G	C	C	
AB510025 (9165-10772)	G	G	.	G	A	C	T	G	G	A	T	G	G	T	G	.	C	T	G	A	G	G	A	A	T	G	C	C	
AB777920 (9165-10772)	G	G	.	G	A	C	T	.	G	A	T	G	G	T	G	.	C	T	G	A	G	G	A	A	T	G	C	C	
AB777921 (9165-10772)	.	G	.	G	A	C	T	.	G	.	T	G	G	T	G	.	C	T	G	A	G	G	A	A	T	G	C	.	
AB461420 (9165-10772)	.	G	C	G	A	C	T	.	G	A	T	G	G	T	G	.	C	T	G	A	G	G	A	A	T	G	C	C	
AB813186 (9165-10772)	.	G	.	G	A	C	T	.	G	.	T	G	G	T	G	.	C	T	G	A	G	G	A	A	T	G	C	.	
AB813187 (9165-10772)	.	G	C	G	A	C	T	.	G	A	T	G	G	T	G	.	C	T	G	A	G	G	A	A	T	G	C	C	
AB813188 (9165-10772)	.	G	.	G	A	C	T	.	G	.	T	G	G	T	G	T	C	T	G	A	G	G	A	A	T	G	C	.	
OR911453 (9165-10772) S76	.	G	.	G	A	C	T	.	G	.	T	G	G	T	G	.	C	T	G	A	G	G	A	A	T	G	C	.	

worms has enabled us to establish a phylogeny of *E. multilocularis*. Until now, the common use of concatenated genes has not allowed a distinction between haplogroups in France. Bohard et al. (2023) showed that concatenation of the *cob*, *cox1*, and *nad2* genes made it possible to distinguish just six haplotypes, versus 13 based on the complete mitochondrial genome obtained from samples collected from 30 French AE patients. Here, the study of the complete mitochondrial genome of a panel including 113 samples revealed 419 mutations and enabled us to differentiate 58 haplotypes and three major haplogroups. The classic use of concatenated genes made it possible to solely determine continental clusters as European, Asian, central or Arctic North American, and Alaskan clades (Nakao et al., 2009; Wu et al., 2017; Santoro et al., 2024). Because the complete mitochondrial DNA of *E. multilocularis* was sequenced in the present study, the term ‘‘haplogroup’’ instead of the ‘‘clade’’ was preferred, which is generally applied in the literature.

Haplogroup HG1 included haplotypes from locations close to the Arctic Circle: Svalbard Island (Norway), St. Lawrence Island (Alaska), and Yakutia (Russia). Given the geographical origin of these samples and their genetic distance one to another, we hypothesized that the subspecies described by Rausch and Schiller could belong to haplogroup 1 (Rausch and Schiller, 1954). It was named *Echinococcus sibiricensis* and characterized by shorter rostellar hooks in arctic foxes, *Vulpes lagopus*, and sled dogs on St. Lawrence Island in the Bering Sea. Moreover, in 1957, Vogel (Vogel, 1957) considered *E. sibiricensis* to be a geographic subspecies of *E. multilocularis*. In 1985 and 1998–1999, parasites from

the Hulunbeier Pasture in Inner Mongolia, northeastern China, were investigated and differences in the evolution between *E. multilocularis* and *E. sibiricensis* were reported (Tang et al., 2001). However, molecular studies confirmed that these two parasites belong to the same species (Nakao et al., 2009). Moreover, the place of the recently described species *Echinococcus russicensis* in phylogeny also has to be studied with regard to the present findings (Romig et al., 2015).

Haplogroup HG2 included samples from Asia (Russia, Armenia, Japan, and China), as well as North America (Canada and Alaska) and Europe (Poland, France). From the present findings, it appears to be the most frequently distributed haplogroup worldwide.

Haplogroup HG3 combined mainly European samples (France, Switzerland, Germany, Luxembourg, Belgium, Sweden, and Poland), as well as Russian (Ryazan oblast) and Canadian samples. This haplogroup accounted for the majority of the present studied samples (65%) and analysis of the phylogenetic tree enabled us to determine the three micro-haplogroups HG3a, HG3b, and HG3c. In France, HG3b and HG3c constituted the two major micro-haplogroups found in the historical endemic zone of AE (Piarroux et al., 2013). Of note, mitochondrial intergenic zone 1 (positions 67 to 249 according to AB018440) features a variable zone specific to European micro-haplogroups HG3a and HG3c. Santoro and co-workers described a predominant haplotype in French red fox samples, which is characterized by the presence of an ‘‘A’’ in position 222 in the *nad2* gene (position 6610 according to AB018440) (Santoro et al., 2024). This SNP was not found in any of the present

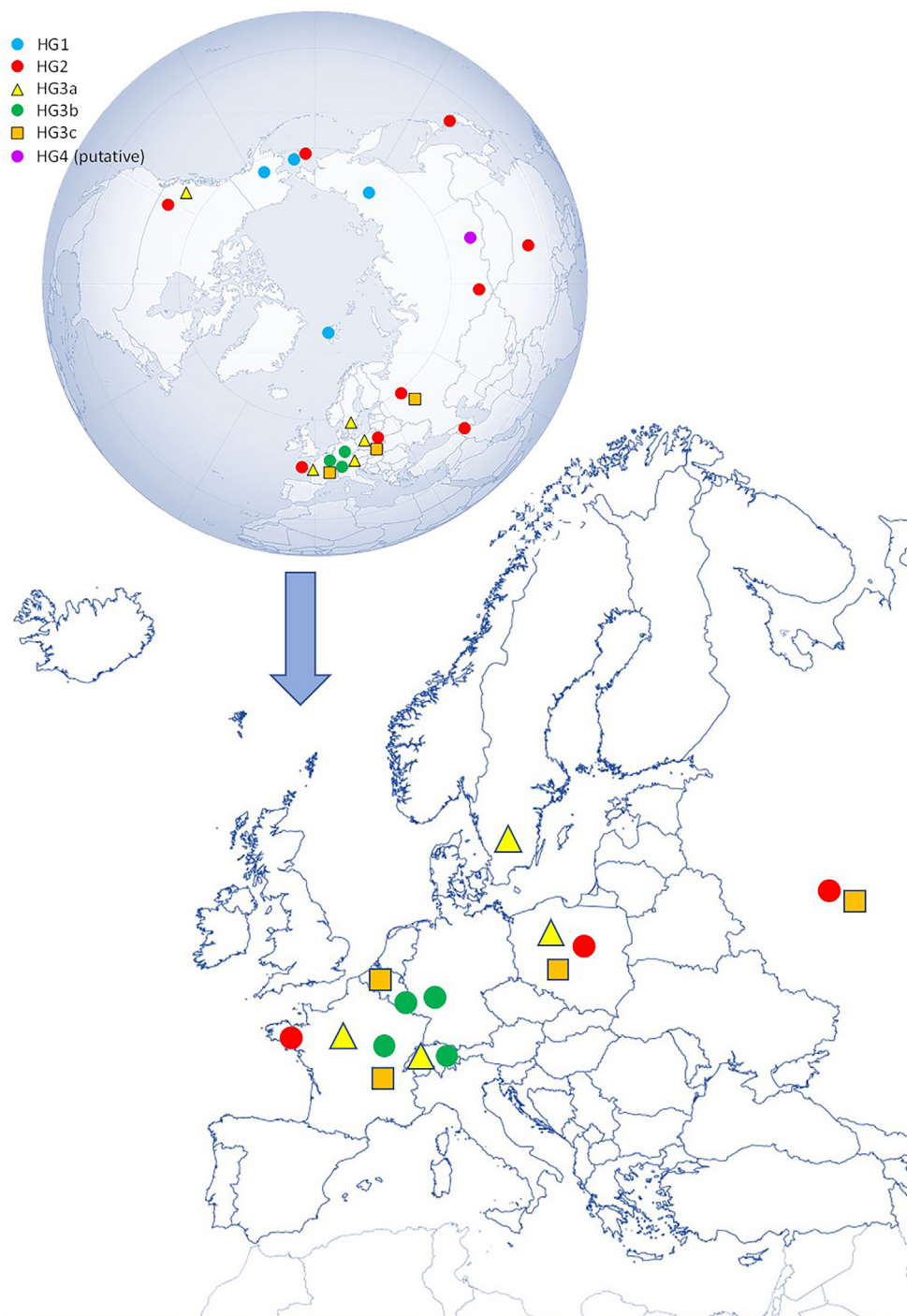


Fig. 3. Schematic representation of *Echinococcus multilocularis* haplogroup distribution. Each dot indicates the presence of a haplogroup in a given country.

French samples considered, although heteroplasmy occurrence was studied. This difference could be due to the fox origin in the former paper and the human origin of the present collection of samples. Haplogroup HG3a included all samples from more recently diagnosed patients included in the present study and all were from north-western France, an area with a recent emergence of AE cases. This region of France is not part of the historical endemic zone. It is, thus, difficult to know whether these cases i) are only better recorded today with the active registration of AE cases, as this is the case e.g. in France with the NRC-E, mandated by government authorities, ii) were detected due to improved diagnostic techniques for the parasite in humans and animals, or iii) indicate

the presence of an emerging area contaminated by *E. multilocularis* with the spreading of foxes, as previously observed (Combes et al., 2012; Umhang et al., 2014). Of note, all patients from this western area presented specimens genetically clustering in the same haplotype, resulting in low genetic diversity, which could occur, for example, after a bottleneck event. Haplogroup HG3a was also described in two monkeys from a zoo in Z rich (Switzerland), in a Swedish patient, and in two Polish foxes from the region of Sileskie and Podlaskie. For the isolates of the six patients with HG3a for whom the information was available, all had an identical microsatellite EmsB profile (P04 in Knapp and co-workers (2020)). This EmsB profile was also found to be predominant in

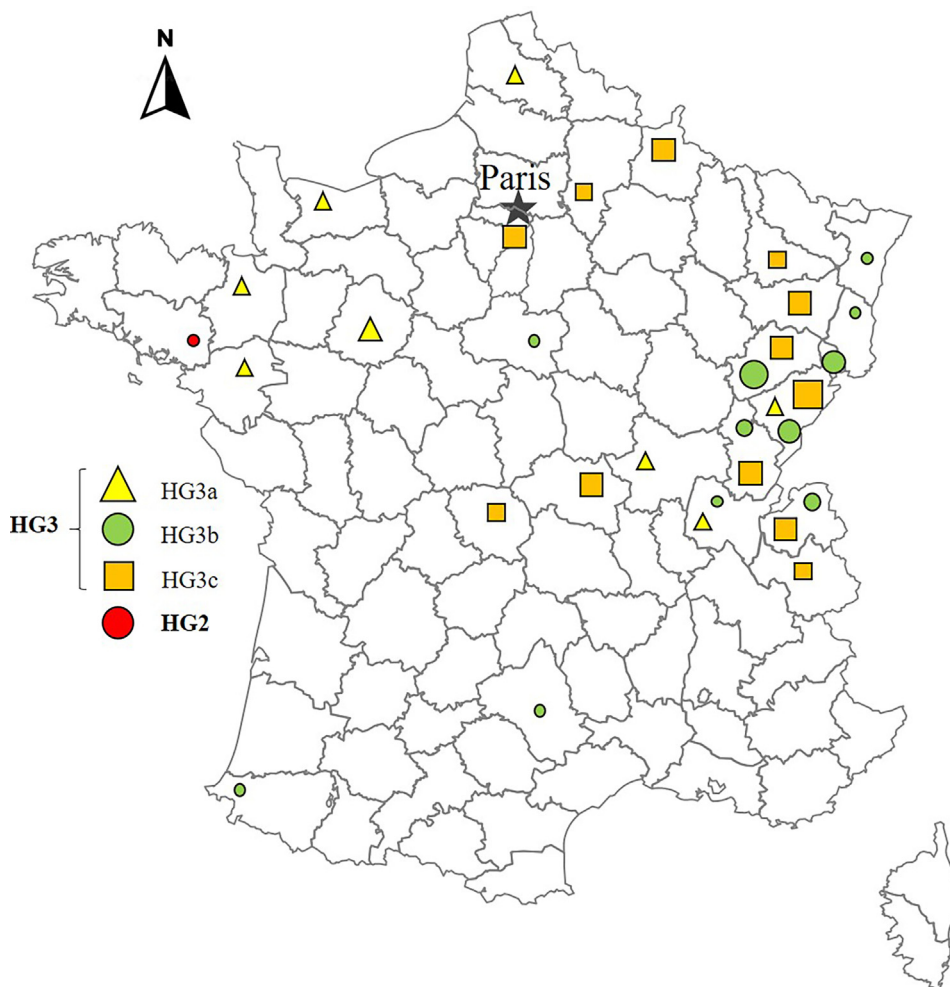


Fig. 4. French *Echinococcus multilocularis* haplogroup distribution map based on the patients' "d partement" of residence. The areas delimit the repartition zones of haplogroups in France in this study. The sizes of the circles are proportional to the numbers of patients in a haplogroup in a given French "d partement" (administrative division). The red (black) dot indicates a patient known to have probably been contaminated in the Tibetan region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Comparison of epidemiological and clinical data between haplogroups in French patients.

	No of patients	Sub-haplogroup			Statistical test
		HG3a (n = 10)	HG3b (n = 21)	HG3c (n = 25)	
Epidemiology					
Proportion of males	56	0.60 (6/10)	0.52 (11/21)	0.48 (12/25)	SI ^a
Median age at diagnosis [min–max]	56	66 [15–76]	58 [11–81]	57 [10–76]	K = 2.96, SI ^b
AE diagnoses after 2013	56	1 (10/10)	0.71 (15/21)	0.52 (13/25)	P = 0.016 ^a
Clinical status					
Immunosuppression	43	0.40 (4/10)	0.36 (5/14)	0.37 (7/19)	SI ^a
Clinical form					
Hepatic form only	52	0.80 (8/10)	0.47 (9/19)	0.52 (12/23)	SI ^a
Extra-hepatic form only	55	0.00 (0/10)	0.10 (2/21)	0.17 (4/24)	SI ^a
Intra- and extra- hepatic forms	52	0.20 (2/10)	0.42 (8/19)	0.35 (8/23)	SI ^a
Invasion neighbour organs/tissues					
Metastasis	44	0.00 (0/9)	0.27 (4/15)	0.25 (5/20)	SI ^a

AE, alveolar echinococcosis; SI, statistically insignificant.

^a Fisher's exact test, ^b Kruskal-Wallis test.

isolates from red foxes in France in a previous study (corresponds to P17 in Umhang (2014)).

A fourth putative haplogroup was described in a single Asian sample obtained from a rodent native of the Olkhon Island in Rus-

sia. *Alticola olchonensis* is an endemic species of the Lake Baikal area with an extremely restricted geographical distribution. The red fox is known as the DH of *E. multilocularis* in the area and the winter ice bridge allows free island-continent migration (Konyaev

et al., 2013a). However, no other representatives of this haplogroup have been found in any of the seven Altai patients in the present collection of samples. In previous studies, this specimen was identified as part of the Mongolian clade, based on partial mitochondrial and microsatellite EmsB studies (Konyaev et al., 2013; Umhang et al., 2021). Moreover, genetic relationships with Mongolian samples previously isolated from humans has to be investigated (Ito et al., 2010).

Four distinct haplogroups have been identified, each with an apparently distinct geographic origin, Arctic, Asian, European and ‘‘Mongolian’’. However, their distribution in the endemic area of the Northern hemisphere shows a more or less marked expansion of natural or anthropic origin. The natural migration of red foxes over long periods would explain the east–west gradient of strains of Asian origin, as far as the Ural Mountains (Ryazan oblast near Moscow), Poland, Latvia (Karamon et al., 2017; Santoro et al., 2024). In Turkey, strains of Asian origins were also described (Avcioglu et al., 2021; Santoro et al., 2024). In the opposite west-east direction, the expansion of Asian strains could be partly explained by the fact that Arvicoline rodents most likely crossed the Beringia land bridge during the Pleistocene, carrying with them Asian strains of *E. multilocularis* to North America (Rausch, 1994). Hayashi and co-workers (2023) described transfers of DH and DI between St. Lawrence, Bering, Kurile, Rebun and Hokkaido Islands, leading to the colonisation by arctic-type strains (Hayashi et al., 2023). In Alaska, in addition to Arctic and Asian strains, two European-type strains were present in this study. In recent decades, several authors have demonstrated the presence of European-type strains in Canada (British Columbia (Jenkins et al., 2012), Alberta and Saskatchewan (Geszy and Jenkins, 2015) and in the United States, as in New York State (Conlon et al., 2023), Vermont (Polish et al., 2021), Virginia (Zajac et al., 2020), Missouri, (Kuroki et al., 2022)). Various anthropogenic factors may explain this colonization. The importation of red foxes of European origin for sport hunting (in the mid-1700 s in the eastern USA and over the following 150 years), followed by other intentional imports over the last century (fur trade, for example) importing dogs, may have led to the establishment of these strains (Kamler and Ballard, 2002; Davidson et al., 2012).

The Arctic fox harboured a proper strain and the Svalbard example is an obvious simple system with one DH (*Vulpes lagopus*), one IH (*Microtus levis*), and a well-defined and restricted area. Henttonen et al. (2001) concluded that the migrating arctic fox was the source of spread of *E. multilocularis* to Svalbard and that the introduction of an IH through human activities was the reason for the establishment of the parasite. A microsatellite EmsB study found a profile similar to some described in St. Lawrence Island isolates. A circumpolar origin of the parasite, harboured by arctic foxes and transmitted to imported rodents, was hypothesized (Knapp et al., 2012). Our study confirms this result with the close relative relationship between samples from Svalbard and those from St. Lawrence Island clustering in HG1.

Distinguishing haplogroups could help in the investigation of genetic diversity related to infectivity and nuclear gene expression. Among the 56 French patients belonging to the HG3 haplogroup, there were no differences in the type of organ affected (liver versus other organs) according to the HG3a, 3b, or 3c micro-haplogroup ($P = 0.459$). In addition, the immunocompetent status of the patients did not differ between micro-haplogroups (all with approximately 40% immunosuppressed patients). These micro-haplogroups may be too close genetically to show a true difference in infectivity. It could be informative to compare the infectivity of *E. multilocularis* between the main haplogroups (HG1, HG2, and HG3). To date and to our knowledges, no study has demonstrated a correlation between *E. multilocularis* genetic characteristics and clinical data in patients with alveolar echinococcosis. It could be

informative to compare the pathogenicity of the haplogroups determined by mtDNA studies through the analysis of functional markers of parasite infectivity, as expression, or regulation studies of nuclear genes involved in the variation of the pathogenicity of the parasite.

In conclusion, from a worldwide collection of *E. multilocularis* samples, we describe three haplogroups that are distributed differently around the world and a putative fourth haplogroup represented by only one sample originating from the Inner Mongolian region. Haplogroup HG1 appears to be restricted to the polar region, whereas haplogroup HG2 is distributed worldwide. The third haplogroup, HG3, is composed of three micro-haplogroups. The micro-haplogroups HG3b and HG3c were only found in Europe in the present collection of samples, whereas micro-haplogroup HG3a was also present in North America. HG3a also appears to predominate in north-western France, outside the historical endemic area.

CRedit authorship contribution statement

S  verine Lallemand: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft. **Jorge Oyhenart:** Formal analysis, Methodology, Software. **Benoit Valot:** Methodology, Software, Validation, Writing – review & editing. **Romain Borne:** Formal analysis, Methodology, Software, Writing – review & editing. **Louis Bohard:** Methodology, Writing – review & editing, Conceptualization. **G  rard Umhang:** Data curation, Writing – review & editing. **Jacek Karamon:** Data curation, Writing – review & editing. **Sergey Konyaev:** Data curation, Writing – review & editing. **Caroline R  nnberg:** Data curation, Resources. **Bruno Gottstein:** Data curation, Resources, Visualization, Writing – review & editing. **Delphine Weil-Verhoeven:** Data curation, Visualization. **Carine Richou:** Visualization, Data curation. **Solange Bresson-Hadni:** Visualization, Data curation. **Laurence Millon:** Funding acquisition, Writing – review & editing. **Anne-Pauline Bellanger:** Supervision, Writing – review & editing. **Jenny Knapp:** Data curation, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the National Reference Centre for Echinococcoses, University Hospital, Besan  on, France.

References

- Avcioglu, H., Guven, E., Balkaya, I., Kirman, R., Akyuz, M., Mebarek Bia, M., Gulbeyen, H., Yaya, S., 2021. The situation of echinococcosis in stray dogs in Turkey: the first finding of *Echinococcus multilocularis* and *Echinococcus ortleppi*. *Parasitology* 148, 1092–1098.
- Ballard, J.W.O., Whitlock, M.C., 2004. The incomplete natural history of mitochondria. *Mol. Ecol.* 13, 729–744.
- Bandelt, H.J., Forster, P., R  hl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37–48.
- Bart, J.M., Knapp, J., Gottstein, B., El-Garch, F., Giraudoux, P., Glowatzki, M.L., Berthoud, H., Maillard, S., Piarroux, R., 2006. EmsB, a tandem repeated multi-loci microsatellite, new tool to investigate the genetic diversity of *Echinococcus multilocularis*. *Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis.* 6, 390–400.
- Bartel, M.H., Seese, F.M., Worley, D.E., 1992. Comparison of Montana and Alaska isolates of *Echinococcus multilocularis* in gerbils with observations on the cyst growth, hook characteristics, and host response. *J. Parasitol.* 78, 529–532.

- Bohard, L., Lallemand, S., Borne, R., Courquet, S., Bresson-Hadni, S., Richou, C., Millon, L., Bellanger, A.-P., Knapp, J., 2023. Complete mitochondrial exploration of *Echinococcus multilocularis* from French alveolar echinococcosis patients. *Int. J. Parasitol.* S0020-7519(23)00076-0.
- Bowles, J., Blair, D., McManus, D.P., 1992. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Mol. Biochem. Parasitol.* 54, 165–173.
- Bowles, J., McManus, D.P., 1993. NADH dehydrogenase 1 gene sequences compared for species and strains of the genus *Echinococcus*. *Int. J. Parasitol.* 23, 969–972.
- Bresson-Hadni, S., Spahr, L., Chappuis, F., 2021. Hepatic Alveolar Echinococcosis. *Semin. Liver Dis.* 41, 393–408.
- Bretagne, S., Assouline, B., Vidaud, D., Houin, R., Vidaud, M., 1996. *Echinococcus multilocularis*: microsatellite polymorphism in U1 snRNA genes. *Exp. Parasitol.* 82, 324–328.
- Chauchet, A., Grenouillet, F., Knapp, J., Richou, C., Delabrousse, E., Dentan, C., Millon, L., Di Martino, V., Contreras, R., Deconinck, E., Blagosklonov, O., Vuitton, D.A., Bresson-Hadni, S., Virginie, V., Karine, B., Brigitte, B., Isabelle, B.-C., Oleg, B., Solange, B.-H., Pascale, B.M., Sylvie, C., Remy, C., Eric, D., Vincent, D.M., Philippe, E., Sophie, F., Patrick, G., Frédéric, G., Bruno, H., Séverine, V.-D., Jenny, K., Stéphane, K., Georges, M., Laurence, M., Francis, R., Carine, R., Claire, V., Angèle, V.D., Lucine, V., Patricia, P., Claire, G., Armand, A., Jean, B., Monique, C., François, B.J., Bernadette, C., Patrick, H., Anne, M., Odile, F., Christian, L., Jérôme, D., Olivier, G., Christian, P., Meja, R., Martine, W., Martine, P., Eric, C., Marc, J., Jean-Jacques, R., Daniel, S., Alain, G., Lorraine, L., Marie, M., Jérôme, W., Olivier, F., Didier, S., Cathy, C., Francois, D.J., Bruno, G., Monique, D., Ahmed, A.-B., Maxime, A., Yves, H., Nicolas, L., Véronique, L., Blandine, A.-L., for the FrancEchino Network, 2014. Increased incidence and characteristics of alveolar echinococcosis in patients with immunosuppression-associated conditions. *Clin. Infect. Dis.*, 59.
- Combes, B., Comte, S., Raton, V., Raoul, F., Boué, F., Umhang, G., Favier, S., Dunoyer, C., Woronoff, N., Giraudoux, P., 2012. Westward spread of *Echinococcus multilocularis* in foxes, France, 2005–2010. *Emerg. Infect. Dis.* 18, 2059–2062.
- Conlon, C.L., Schuler, K.L., Lejeune, M., Whipps, C.M., 2023. Novel report of the European variant of *Echinococcus multilocularis* coyotes (*Canis latrans*) in the New York State. *J. Parasitol.* 109, 357–361.
- Davidson, R.K., Romig, T., Jenkins, E., Tryland, M., Robertson, L.J., 2012. The impact of globalisation on the distribution of *Echinococcus multilocularis*. *Trends Parasitol.* 28, 239–247.
- Eckert, J., Deplazes, P., 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin. Microbiol. Rev.* 17, 107–135.
- Eckert, J., Thompson, R.C.A., 2017. Historical aspects of echinococcosis. *Adv. Parasitol.* 95, 1–64.
- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10, 564–567.
- Fu, Y.X., Li, W.H., 1993. Statistical tests of neutrality of mutations. *Genetics* 133, 693–709.
- Gelman, A., Rubin, D.B., 1992. Inference from iterative simulation using multiple sequences. *Stat. Sci.* 7, 457–472.
- Gesy, K.M., Jenkins, E.J., 2015. Introduced and native haplotypes of *Echinococcus multilocularis* in wildlife in Saskatchewan, Canada. *J. Wildl. Dis.* 51, 743–748.
- Gesy, K.M., Schurer, J.M., Massolo, A., Liccioli, S., Elkin, B.T., Alisaukas, R., Jenkins, E. J., 2014. Unexpected diversity of the cestode *Echinococcus multilocularis* in wildlife in Canada. *Int. J. Parasitol. Parasites Wildl.* 3, 81–87.
- Haag, K., Zaha, A., Araújo, A., Gottstein, B., 1997. Reduced genetic variability within coding and non-coding regions of the *Echinococcus multilocularis* genome. *Parasitology* 115 (Pt 5), 521–529.
- Hayashi, N., Nakao, R., Ohari, Y., Irie, T., Kouguchi, H., Chatanga, E., Mohamed, W.M. A., Moustafa, M.A.M., Kinoshita, G., Okamoto, M., Yagi, K., Nonaka, N., 2023. Mitogenomic exploration supports the historical hypothesis of anthropogenic diffusion of a zoonotic parasite *Echinococcus multilocularis*. *iScience* 26, 107741.
- Heidari, Z., Sharbatkhori, M., Mobeidi, I., Mirhendi, S.H., Nikmanesh, B., Sharifdini, M., Mohebbi, M., Zarei, Z., Arzamani, K., Kia, E.B., 2019. *Echinococcus multilocularis* and *Echinococcus granulosus* in canines in North-Khorasan Province, northeastern Iran, identified using morphology and genetic characterization of mitochondrial DNA. *Parasit. Vectors* 12, 606.
- Henttonen, H., Fuglei, E., Gower, C.N., Haukisalmi, V., Ims, R.A., Niemimaa, J., Yoccoz, N.G., 2001. *Echinococcus multilocularis* on Svalbard: introduction of an intermediate host has enabled the local life-cycle. *Parasitology* 123, 547–552.
- Houston, S., Belga, S., Buttenschoen, K., Cooper, R., Girgis, S., Gottstein, B., Low, G., Massolo, A., MacDonald, C., Müller, N., Preiksaitis, J., Sarlie, P., Vaughan, S., Kowalewska-Grochowska, K., 2021. Epidemiological and clinical characteristics of alveolar Echinococcosis: An Emerging Infectious Disease in Alberta, Canada. *Am. J. Trop. Med. Hyg.*
- Ito, A., Agvaandaram, G., Bat-Ochir, O.-E., Chuluunbaatar, B., Gonchigsenghe, N., Yanagida, T., Sako, Y., Myadagsuren, N., Dorjsuren, T., Nakaya, K., Nakao, M., Ishikawa, Y., Davaaajav, A., Dulmaa, N., 2010. Histopathological, serological, and molecular confirmation of indigenous alveolar echinococcosis cases in Mongolia. *Am. J. Trop. Med. Hyg.* 82, 266–269.
- Jenkins, E.J., Peregrine, A.S., Hill, J.E., Somers, C., Gesy, K., Barnes, B., Gottstein, B., Polley, L., 2012. Detection of European Strain of *Echinococcus multilocularis* in North America. *Emerg. Infect. Dis.* 18, 1010–1012.
- Kamler, J.F., Ballard, W.B., 2002. A Review of Native and Nonnative Red Foxes in North America. *Wildl. Soc. Bull.* 1973–2006 (30), 370–379.
- Karamon, J., Stojcecki, K., Samorek-Pieróg, M., Bilska-Zajac, E., Rózycki, M., Chmurzyńska, E., Sroka, J., Zdybel, J., Cencek, T., 2017. Genetic diversity of *Echinococcus multilocularis* in red foxes in Poland: the first report of a haplotype of probable Asian origin. *Folia Parasitol.* 64, 007.
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780.
- Knapp, J., Staebler, S., Bart, J.M., Stien, A., Yoccoz, N.G., Drögemüller, C., Gottstein, B., Deplazes, P., 2012. *Echinococcus multilocularis* in Svalbard, Norway: microsatellite genotyping to investigate the origin of a highly focal contamination. *Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis.* 12, 1270–1274.
- Knapp, J., Gottstein, B., Bretagne, S., Bart, J.-M., Umhang, G., Richou, C., Bresson-Hadni, S., Millon, L., 2020. Genotyping *Echinococcus multilocularis* in human alveolar echinococcosis patients: an EmsB microsatellite analysis. *Pathog. Basel Switz.* 9, 282.
- Konyaev, S.V., Yanagida, T., Nakao, M., Ingovatova, G.M., Shoykhet, Y.N., Bondarev, A. Y., Odnokurtsev, V.A., Loskutova, K.S., Lukmanova, G.I., Dokuchaev, N.E., Spiridonov, S., Alshinecky, M.V., Sivkova, T.N., Andreyanov, O.N., Abramov, S. A., Krivopalov, A.V., Karpenko, S.V., Lopatina, N.V., Dupal, T.A., Sako, Y., Ito, A., 2013. Genetic diversity of *Echinococcus* spp. in Russia. *Parasitology* 140, 1637–1647.
- Kuroki, K., Morishima, Y., Dorr, L., Cook, C.R., 2022. Alveolar echinococcosis in a dog in Missouri, USA. *J. Vet. Diagn. Invest.* 34, 746–751.
- Letunic, I., Bork, P., 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 49, W293–W296.
- Li, H., Durbin, R., 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinforma. Oxf. Engl.* 26, 589–595.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25, 2078–2079.
- Mitchell, S.L., Goodloe, R., Brown-Gentry, K., Pendergrass, S.A., Murdock, D.G., Crawford, D.C., 2014. Characterization of mitochondrial haplogroups in a large population-based sample from the United States. *Hum. Genet.* 133, 861–868.
- Nakao, M., Yokoyama, N., Sako, Y., Fukunaga, M., Ito, A., 2002. The complete mitochondrial DNA sequence of the cestode *Echinococcus multilocularis* (Cyclophyllidae: Taeniidae). *Mitochondrion* 1, 497–509.
- Nakao, M., Sako, Y., Ito, A., 2003. Isolation of polymorphic microsatellite loci from the tapeworm *Echinococcus multilocularis*. *Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis.* 3, 159–163.
- Nakao, M., McManus, D.P., Schantz, P.M., Craig, P.S., Ito, A., 2007. A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology* 134, 713–722.
- Nakao, M., Xiao, N., Okamoto, M., Yanagida, T., Sako, Y., Ito, A., 2009. Geographic pattern of genetic variation in the fox tapeworm *Echinococcus multilocularis*. *Parasitol. Int.* 58, 384–389.
- Pakendorf, B., Stoneking, M., 2005. Mitochondrial DNA and human evolution. *Annu. Rev. Genomics Hum. Genet.* 6, 165–183.
- Peregrine, A.S., Jenkins, E.J., Barnes, B., Johnson, S., Polley, L., Barker, I.K., De Wolf, B., Gottstein, B., 2012. Alveolar hydatid disease (*Echinococcus multilocularis*) in the liver of a Canadian dog in British Columbia, a newly endemic region. *Can. Vet. J.* 53, 870–874.
- Piarroux, M., Piarroux, R., Giorgi, R., Knapp, J., Bardonnnet, K., Sudre, B., Watelet, J., Dumortier, J., Gérard, A., Beytout, J., Abergel, A., Manton, G., Vuitton, D.A., Bresson-Hadni, S., 2011. Clinical features and evolution of alveolar echinococcosis in France from 1982 to 2007: results of a survey in 387 patients. *J. Hepatol.* 55, 1025–1033.
- Piarroux, M., Piarroux, R., Knapp, J., Bardonnnet, K., Dumortier, J., Watelet, J., Gerard, A., Beytout, J., Abergel, A., Bresson-Hadni, S., Gaudart, J., Network, F.S., 2013. Populations at risk for alveolar echinococcosis. *France. Emerg. Infect. Dis.* 19, 721–728.
- Polish, L.B., Pritt, B., Barth, T.F.E., Gottstein, B., O'Connell, E.M., Gibson, P.C., 2021. First European haplotype of *Echinococcus multilocularis* Identified in the United States: an emerging disease? *Clin. Infect. Dis. off. Publ. Infect. Dis. Soc. Am.* 72, 1117–1123.
- Polish, L.B., O'Connell, E.M., Barth, T.F.E., Gottstein, B., Zajac, A., Gibson, P.C., Bah, A., Kirchgessner, M., Estrada, M., Seguin, M.A., Ramirez-Barrios, R., 2022. European Haplotype of *Echinococcus multilocularis* in the United States. *N. Engl. J. Med.* 387, 1902–1904.
- Rausch, R.L., 1994. Transberingian dispersal of cestodes in mammals. *Int. J. Parasitol.* 24, 1203–1212.
- Rausch, R., Schiller, E.L., 1954. Studies on the helminth fauna of Alaska. XXIV. *Echinococcus sibiricensis* n. sp., from St. Lawrence Island. *J. Parasitol.* 40, 659–662.
- Romig, T., Ebi, D., Wassermann, M., 2015. Taxonomy and molecular epidemiology of *Echinococcus granulosus* sensu lato. *Vet. Parasitol.* 213, 76–84.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542.
- Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* 34, 3299–3302.
- Santa, M.A., Umhang, G., Klein, C., Grant, D.M., Ruckstuhl, K.E., Musiani, M., Gilleard, J.S., Massolo, A., 2023. It's a small world for parasites: evidence supporting the

- North American invasion of European *Echinococcus multilocularis*. Proc. Biol. Sci. 290, 20230128.
- Santoro, A., Santolamazza, F., Cacciò, S.M., La Rosa, G., Antolová, D., Auer, H., Bagrade, G., Bandelj, P., Basso, W., Beck, R., Citterio, C.V., Davidson, R.K., Deksne, G., Frey, C.F., Fuglei, E., Glawischnig, W., Gottstein, B., Harna, J., Huus Petersen, H., Karamon, J., Jansen, F., Jarošová, J., Jokelainen, P., Lundström-Stadelmann, B., Maksimov, P., Miljević, M., Miterpáková, M., Moks, E., Origg, F., Ozolina, Z., Ryser, M.-P., Romig, T., Šarkūnas, M., Scorrano, N., Saarma, U., Šnábel, V., Sréter, T., Umhang, G., Vengušt, G., Žele Vengušt, D., Casulli, A., 2024. Mitochondrial genetic diversity and phylogenetic relationships of *Echinococcus multilocularis* in Europe. Int. J. Parasitol. 54, 233–245.
- Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.
- Tang, C.T., Chen, J.A., Tang, L., Cui, G.W., Qian, Y.C., Kang, Y.M., Lu, H.C., 2001. Comparison observation on the mature alveolar of *Echinococcus sibiricensis* and *Echinococcus multilocularis* in the experimentally infected white mice. Shi Yan Sheng Wu Xue Bao 34, 261–268.
- Thompson, R.C.A., McManus, D.P., 2002. Towards a taxonomic revision of the genus *Echinococcus*. Trends Parasitol. 18, 452–457.
- Thorvaldsdóttir, H., Robinson, J.T., Mesirov, J.P., 2013. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief. Bioinform. 14, 178–192.
- Torgerson, P.R., Keller, K., Magnotta, M., Ragland, N., 2010. The global burden of alveolar Echinococcosis. PLoS Negl. Trop. Dis. 4, e722.
- Umhang, G., Knapp, J., Hormaz, V., Raoul, F., Boué, F., 2014. Using the genetics of *Echinococcus multilocularis* to trace the history of expansion from an endemic area. Infect. Genet. Evol. 22, 142–149.
- Umhang, G., Bastid, V., Avcioglu, H., Bagrade, G., Bujanić, M., Bjelić Čabrilo, O., Casulli, A., Dorny, P., van der Giessen, J., Guven, E., Harna, J., Karamon, J., Kharchenko, V., Knapp, J., Kolarova, L., Konyaev, S., Laurimaa, L., Losch, S., Miljević, M., Miterpakova, M., Moks, E., Romig, T., Saarma, U., Snabel, V., Sréter, T., Valdmann, H., Boué, F., 2021. Unravelling the genetic diversity and relatedness of *Echinococcus multilocularis* isolates in Eurasia using the EmsB microsatellite nuclear marker. Infect. Genet. Evol. 92, 104863.
- Valot, B., Knapp, J., Umhang, G., Grenouillet, F., Millon, L., 2015. Genomic characterization of EmsB microsatellite loci in *Echinococcus multilocularis*. Infect. Genet. Evol. J. Mol. Epidemiol. Evol. Genet. Infect. Dis. 32, 338–341.
- Vogel, H., 1957. *Echinococcus multilocularis* in South Germany. I. The tapeworm stage of strains from humans and animals. Z. Tropenmed. Parasitol. 8, 404–454.
- Wang, X., Liu, J., Zuo, Q., Mu, Z., Weng, X., Sun, X., Wang, J., Boufana, B., Craig, P.S., Giraudoux, P., Raoul, F., Wang, Z., 2018. *Echinococcus multilocularis* and *Echinococcus shiquicus* in a small mammal community on the eastern Tibetan Plateau: host species composition, molecular prevalence, and epidemiological implications. Parasit. Vectors 11, 302.
- Wu, C., Zhang, W., Ran, B., Fan, H., Wang, H., Guo, B., Zhou, C., Shao, Y., Zhang, W., Giraudoux, P., Knapp, J., Wen, H., Kuang, L., Li, J., 2017. Genetic variation of mitochondrial genes among *Echinococcus multilocularis* isolates collected in western China. Parasit. Vectors 10, 265.
- Yamasaki, H., Nakao, M., Nakaya, K., Schantz, P.M., Ito, A., 2008. Genetic analysis of *Echinococcus multilocularis* originating from a patient with alveolar echinococcosis occurring in Minnesota in 1977. Am. J. Trop. Med. Hyg. 79, 245–247.
- Zajac, A., Fairman, D., McGee, E., Wells, B., Peregrine, A., Jenkins, E., LeRoith, T., St John, B., 2020. Alveolar echinococcosis in a dog in the eastern United States. J. Vet. Diagn. Investig. 32, 742–746.