

Genotyping of *Echinococcus multilocularis* in Europe: final update



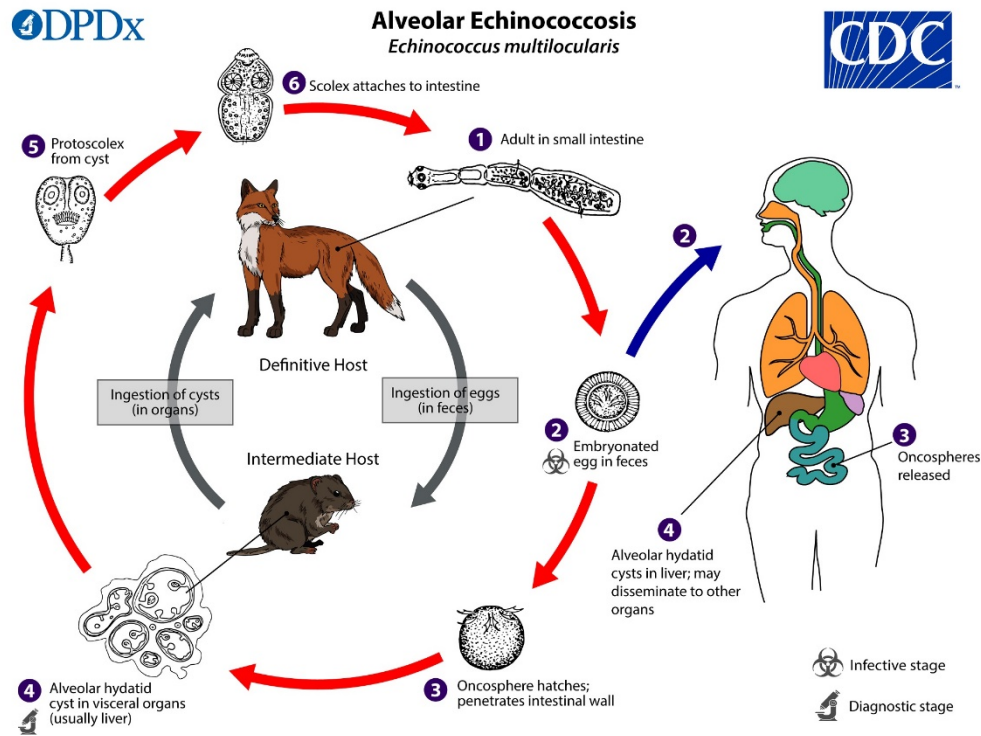
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ECHINOCOCCUS MULTILOCULARIS



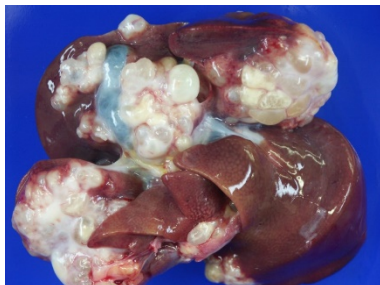
Echinococcus multilocularis,
Leuckart 1863

Phylum	Platyhelmyntes
Class	Cestoda
Order	Cyclophyllidea
Family	Taeniidae
Genus	<i>Echinococcus</i>

- Fox tapeworm, sustained by wild cycle
- Fox and other canids definitive hosts
- Rodents intermediate host
- Humans and other wild and domestic mammals dead-end hosts

2nd most important FB-parasitic disease at global level (FAO/WHO)

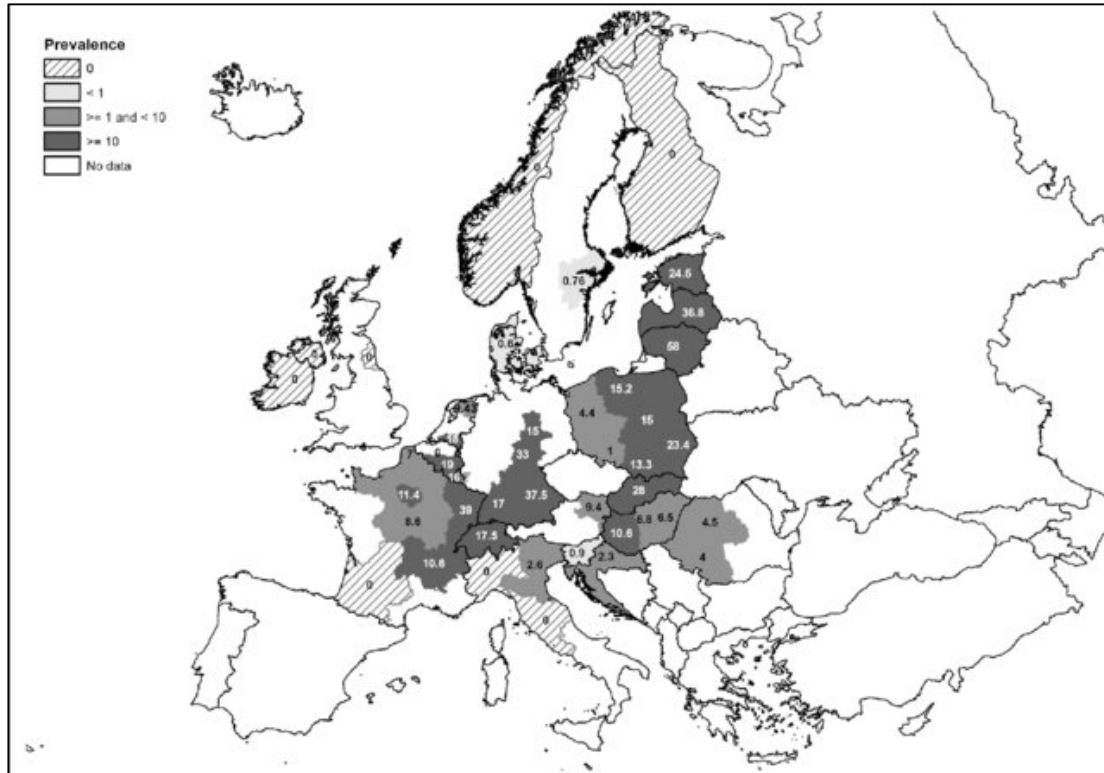
18.000 ANNUAL CASES WORLDWIDE
around 180 annual cases in Europe



Source: Deutsches Primatenzentrum

ALVEOLAR ECHINOCOCCOSIS

Echinococcus multilocularis prevalence in red foxes (EU)



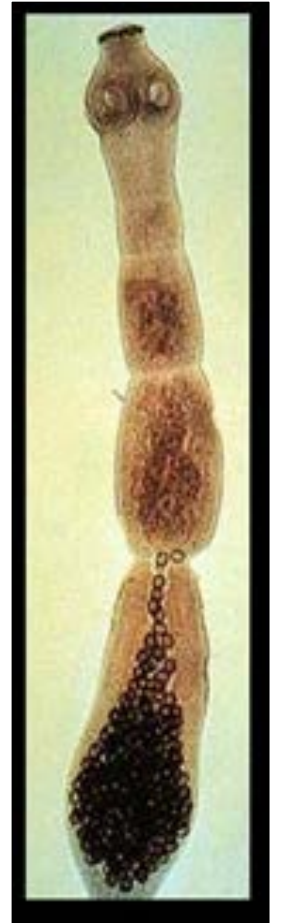
- ❖ A predator-prey cycle perpetuates Em in wildlife.
- ❖ The red fox (*Vulpes vulpes*) is the main reservoir in temperate parts of Europe.
- ❖ Most relevant target species for surveillance (EFSA).



Pooled prevalence of *Echinococcus multilocularis* in red foxes within the European Union and adjacent countries
Oksanen et al., 2016, Parasites & Vectors 9(1):519

AIM

To depict the current molecular epidemiology of *E. multilocularis* circulating in Europe, analysing samples from the most of the known European endemic countries and using mitochondrial markers comparable with reference data.



EURLP multi-year PROJECT

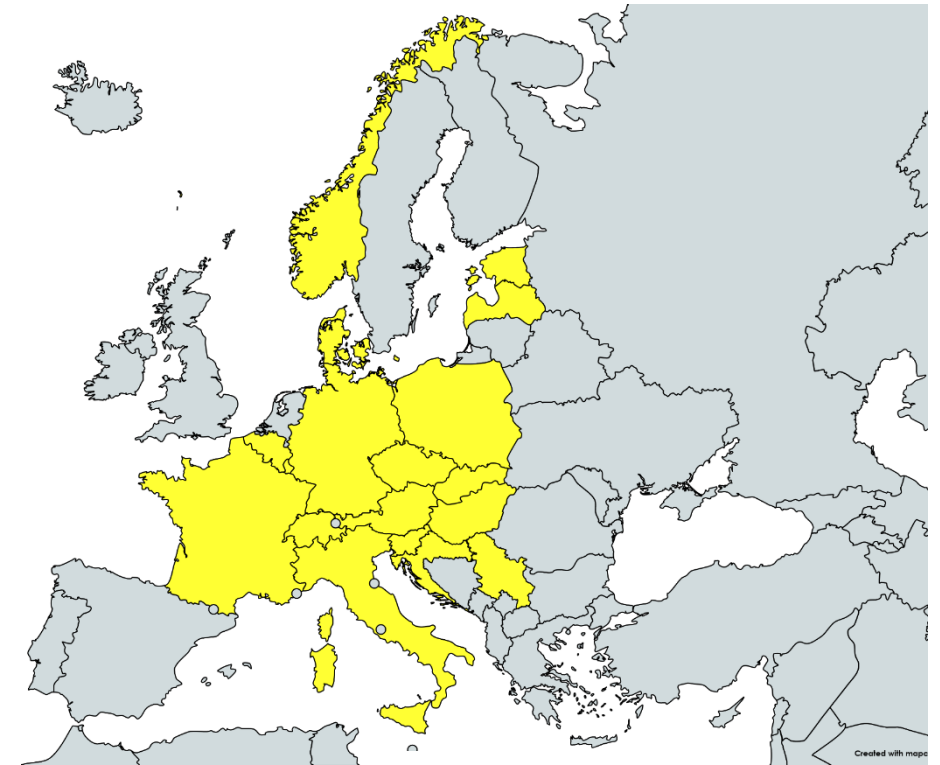
2019-2022

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18 COUNTRIES



20 INSTITUTIONS



GEOGRAPHIC VARIANTS BASED ON GENETIC DIVERSITY USING MITOCHONDRIAL MARKERS - A PIECE OF HISTORY:

- M1: China, Alaska, North America (later Japan and St. Lawrence Island)
- M2: Europe (Germany)

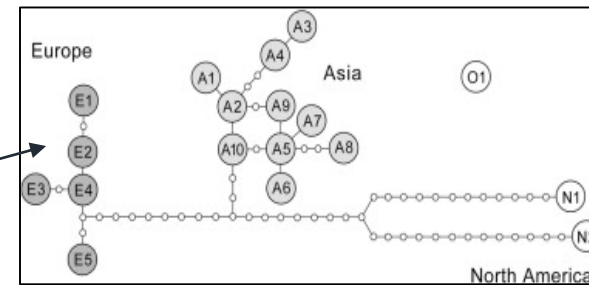
NAD1
COX1

Bowles et al., 1992
Bowles et al., 1993
Okamoto et al., 1995

- E1-E5: Europe
- A1-A10: Asia
- N1-N2: North America
- O1: Mongolia

NAD2
COB
COX1

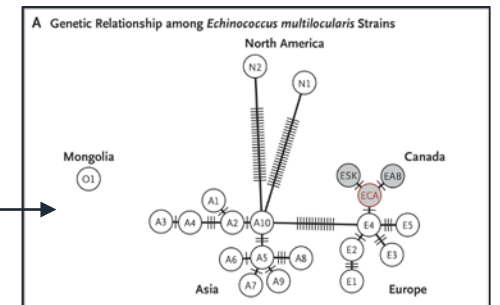
Nakao et al., 2009



European-like strains in Canada

NAD2, COB
COX1
Microsatellites

Jenkins et al., 2012
Gesy et al., 2013
Gesy et al., 2015
Massolo et al., 2019



Polish haplotype clusters with Asia

NAD2
COB
COX1

Karamon et al., 2017

Corroborated, and, admixture evidenced with EmsB

European-like strain in USA (Minnesota)

partial
COX1

Polish et al., 2020

Umhang et al., 2021

SAMPLES

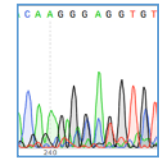
Country	Host species	n. isolates
Austria	7 humans, 4 red foxes, 1 macaque	12
Belgium	5 red foxes	5
Croatia	14 red foxes, 1 human	15
Czech Republic	15 red foxes	15
Denmark	2 red foxes, 1 raccoon dog	3
Estonia	2 red foxes, 1 beaver	3
France	15 red foxes, 1 mouse	16
Germany	8 red foxes, 2 humans, 2 common voles	12
Hungary	5 red foxes	5
Italy	5 red foxes	5
Latvia	27 red foxes, 2 raccoon dogs and 1 grey wolf	29
Luxembourg	3 muskrats	3
Norway (Svalbard)	3 arctic foxes	3
Poland	14 red foxes	14
Serbia	1 red fox	1
Slovakia	14 red foxes, 4 humans	18
Slovenia	1 red fox	1
Switzerland	13 dogs, 10 domestic pigs, 7 beavers, 4 humans, 3 lemurs, 2 gorillas, 2 mice, 2 macaques, 1 wild boar	44
total	-	204

METHODOLOGY

DNA extraction

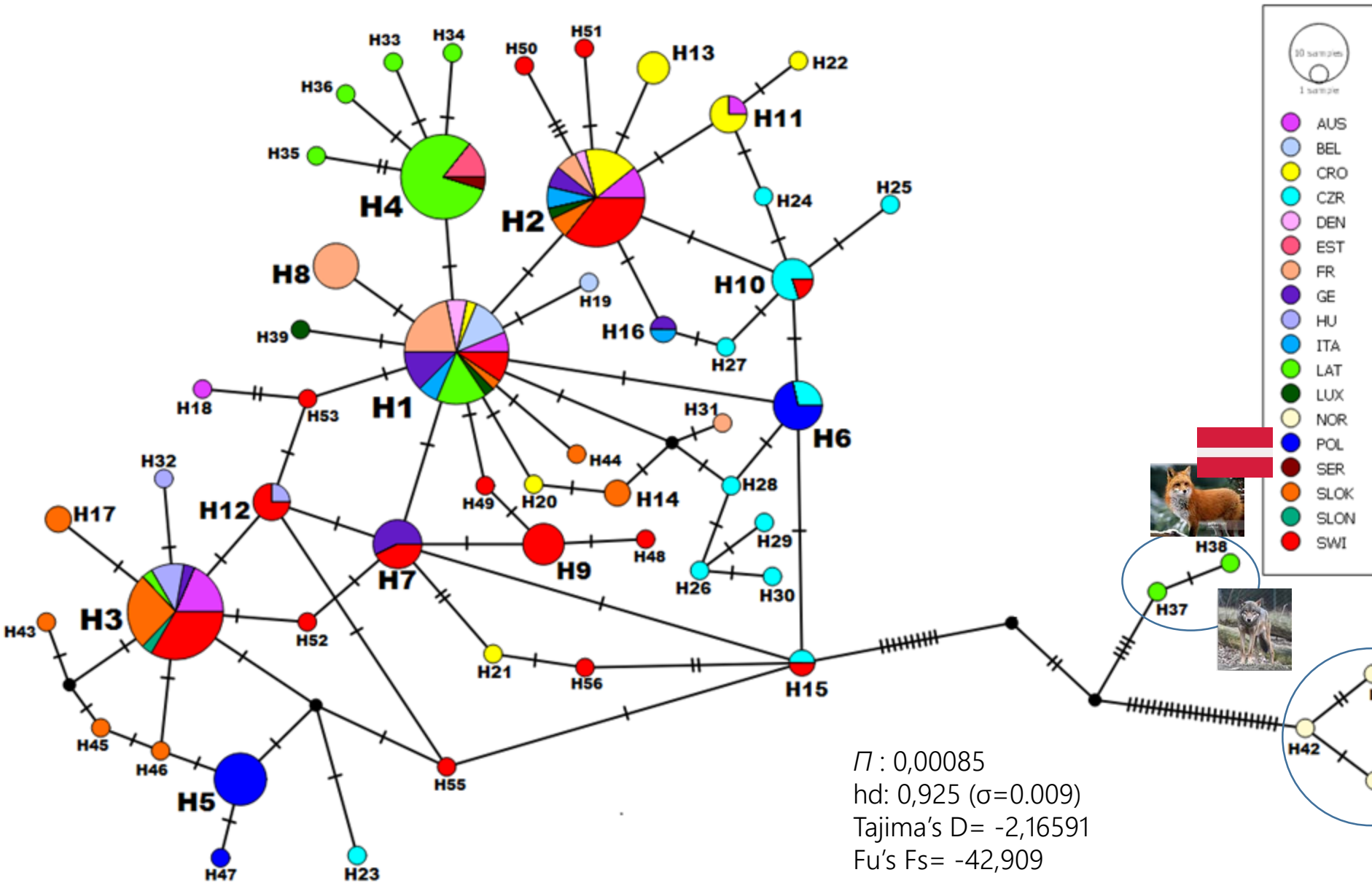
PCR

Sequencing



Final concatenated alignment: **4968** bp
COB (1068) + **ATP6** (516) + **NAD2** (882)
 + **NAD1** (894) + **COX1** (1608)

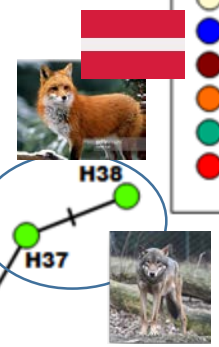
HAPLOTYPE ANALYSIS



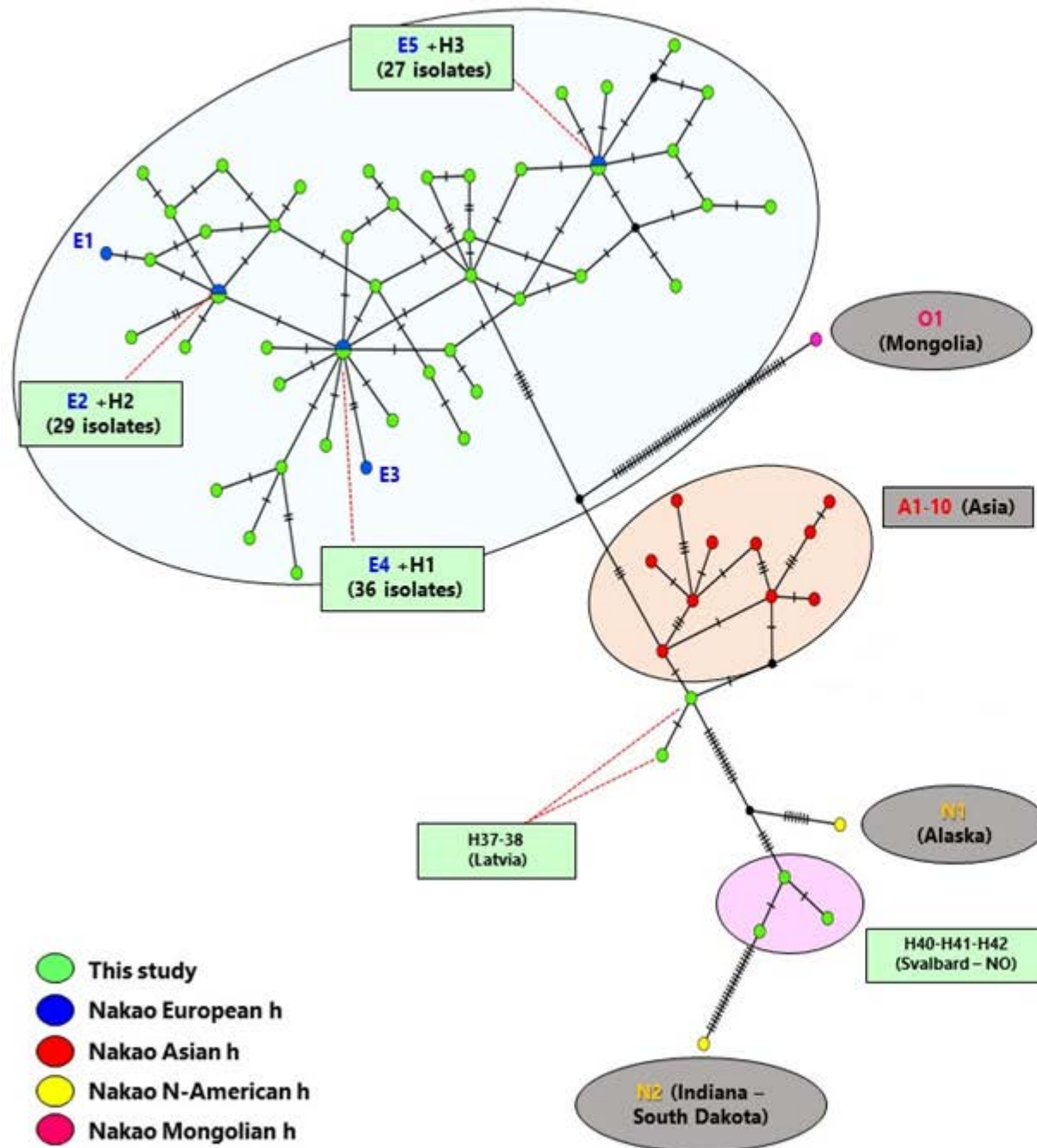
- 10 samples
- 1 sample
- AUS
- BEL
- CRO
- CZR
- DEN
- EST
- FR
- GE
- HU
- ITA
- LAT
- LUX
- NOR
- POL
- SER
- SLOK
- SLOV
- SWI

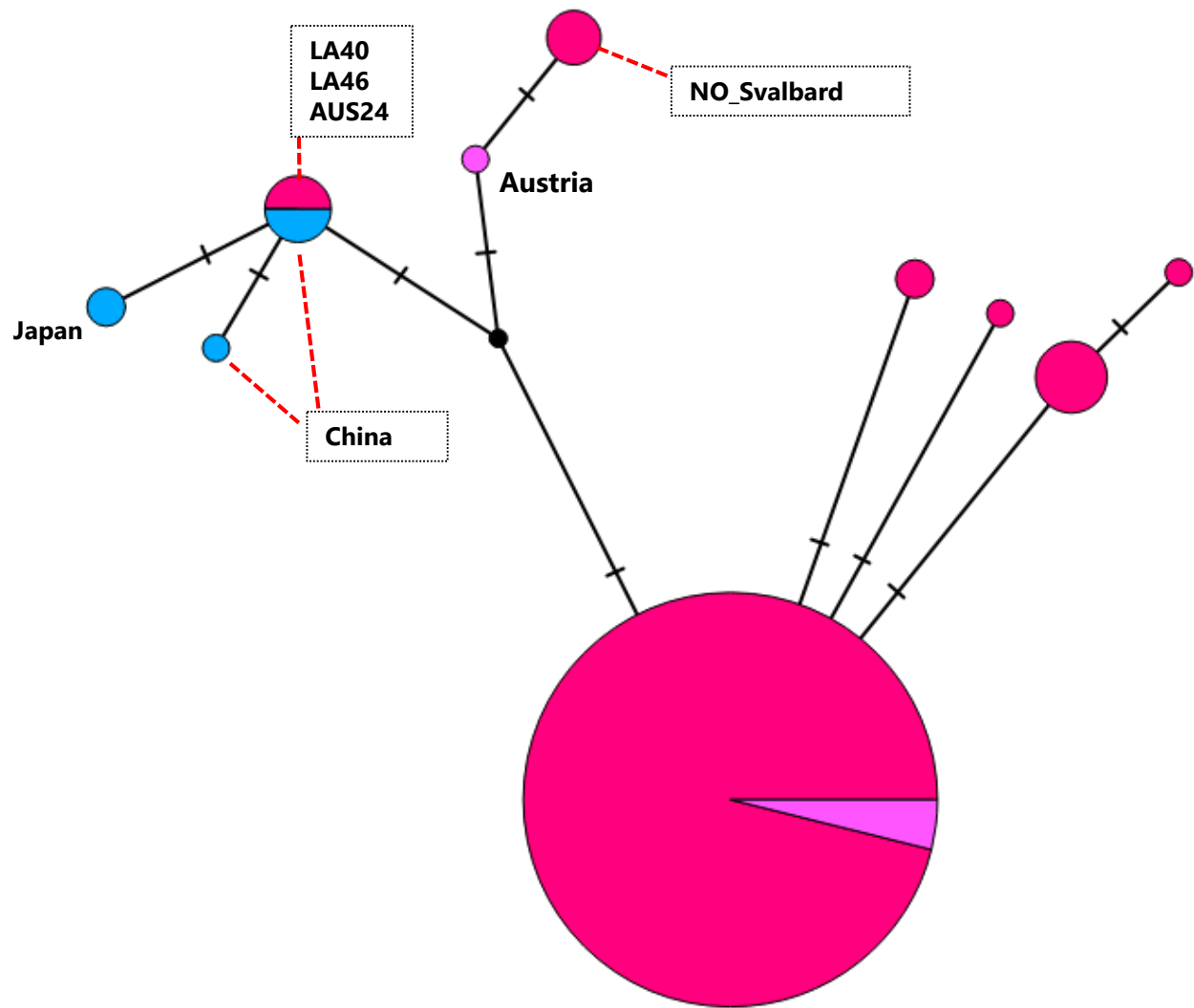
55 HAPLOTYPES

Over 204 isolates
 Sampled in 18 countries

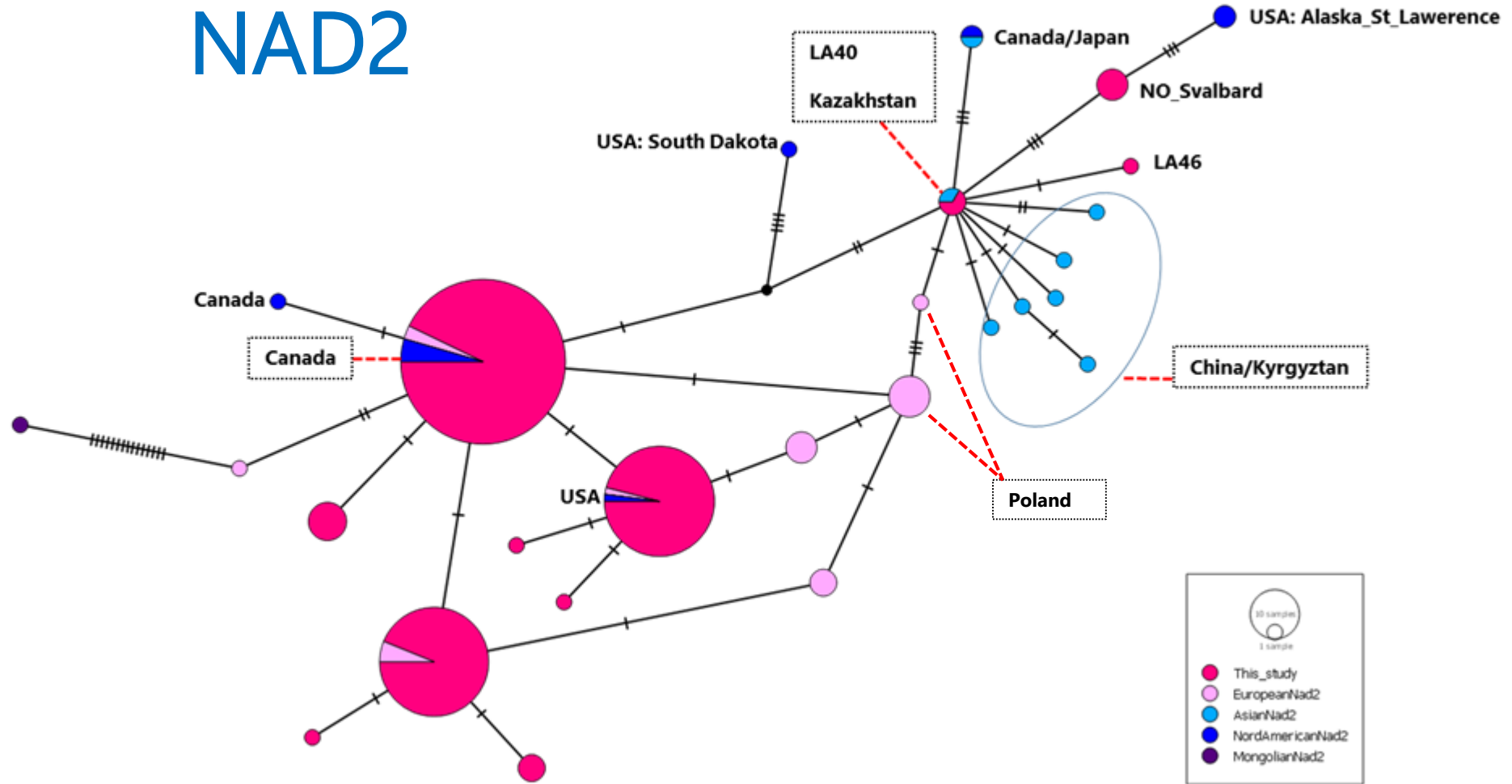


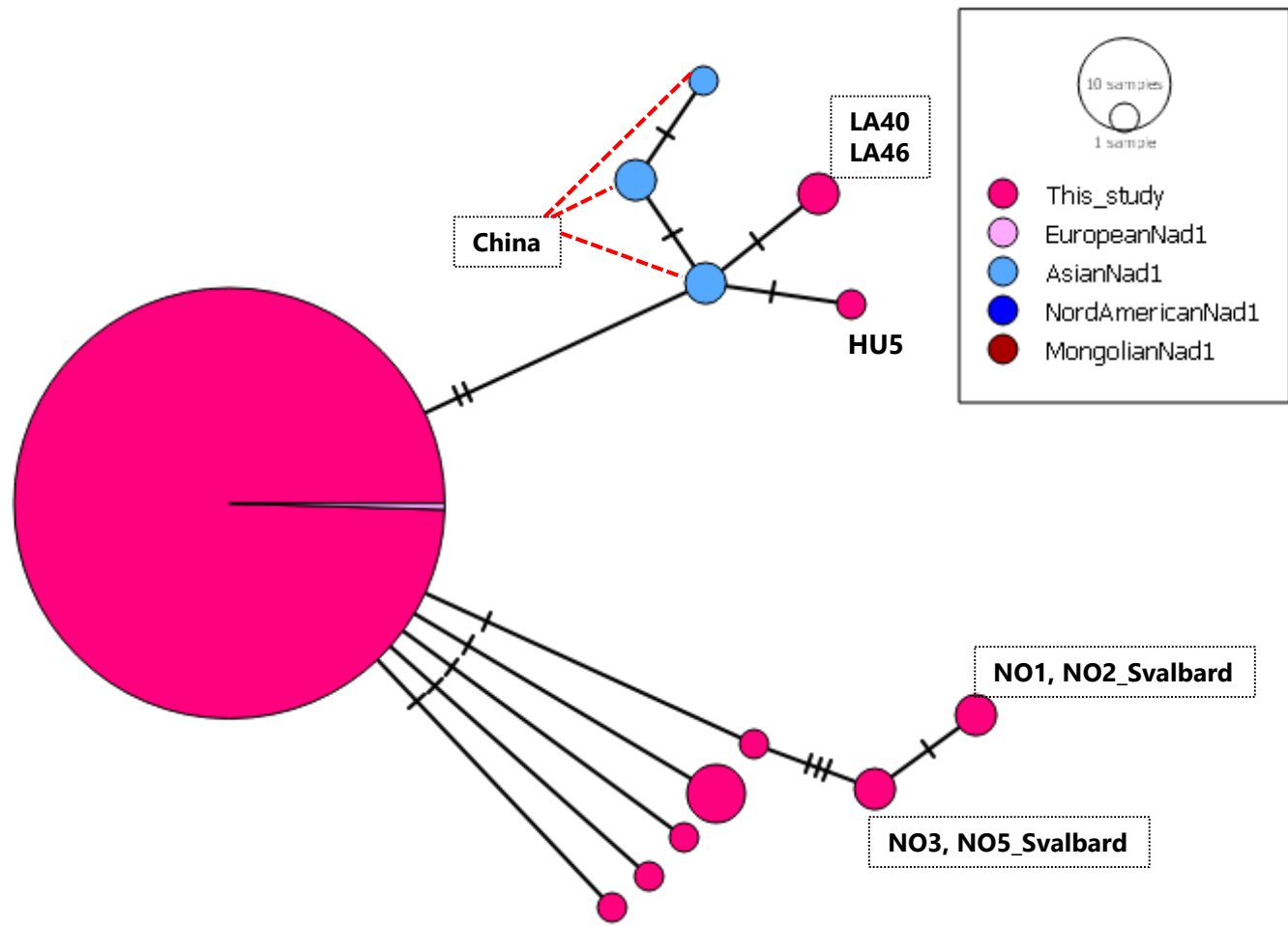
HAPLOTYPE NETWORK *sensu* NAKAO cytb+nad2+cox1





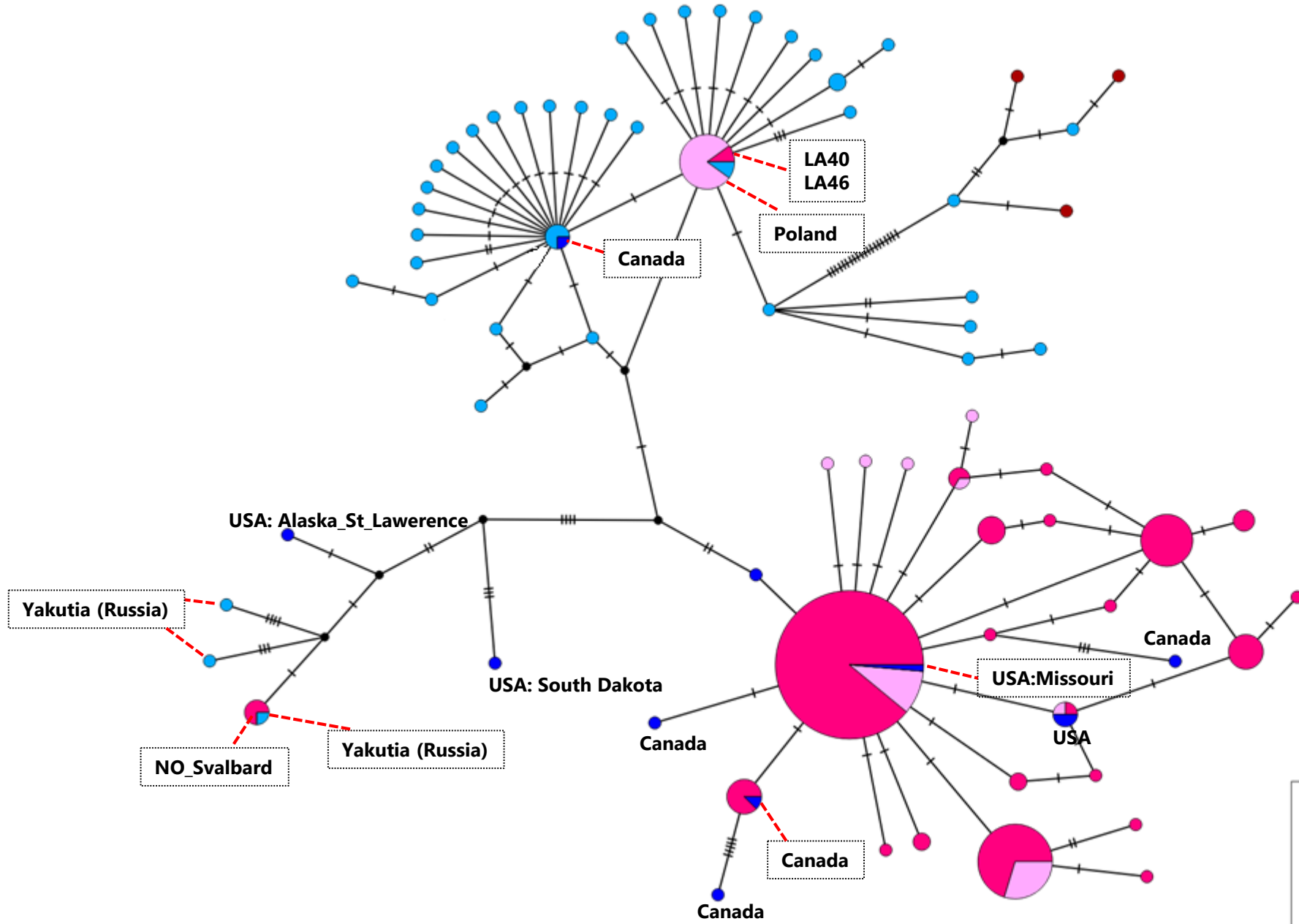
NAD2





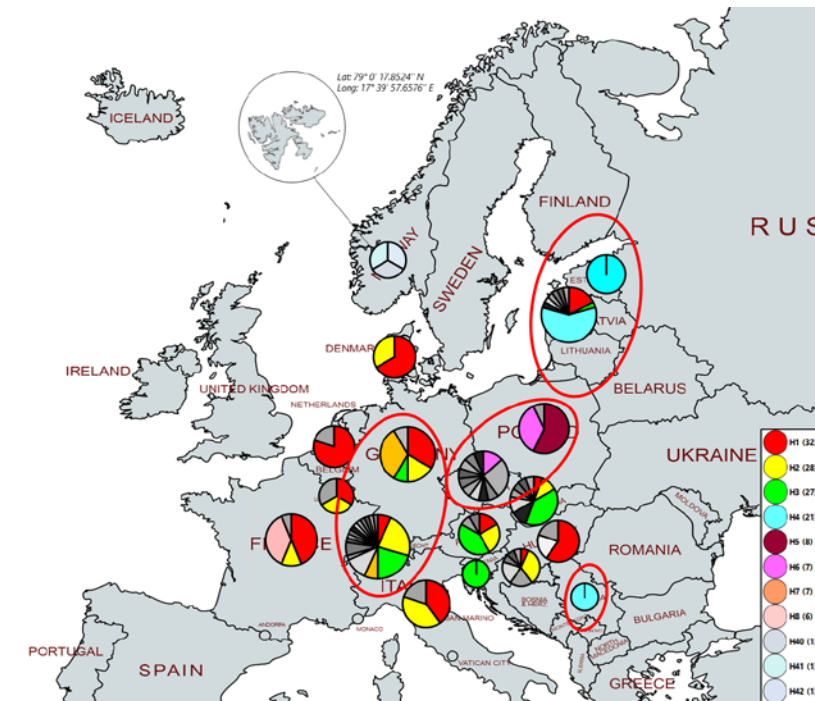
NAD1

COX1



CONCLUSIONS

1. Continental Europe is a homogenous assemble of Em genetic variants, characterized by highly-frequent, widespread haplotypes aside to medium-frequent, locally-spread haplotypes.
2. Switzerland, Czech Republic, Slovakia, Croatia and Latvia show high percentages of unique haplotypes.



CONCLUSIONS

2. Presence of Asian-like haplotypes. Not first time...

Institute of Parasitology, Biology Centre CAS
Folia Parasitologica 2017, 64: 007
doi: 10.14411/fp.2017.007


Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS


Genetic diversity of *Echinococcus multilocularis* in red foxes in Poland: the first report of a haplotype of probable Asian origin

Jacek Karamon, Krzysztof Stojęcki, Małgorzata Samorek-Pieróg, Ewa Biłska-Zajac, Mirosław Różycki, Ewa Chmurzyńska, Jacek Sroka, Jolanta Zdybel and Tomasz Cencek

Karamon et al., Folia Parasitologica 2017, 64: 007

 Asian-like haplotypes from this study

 Incomplete Asian-like haplotypes from this study

 Asian-like haplotypes from Karamon et al., 2017

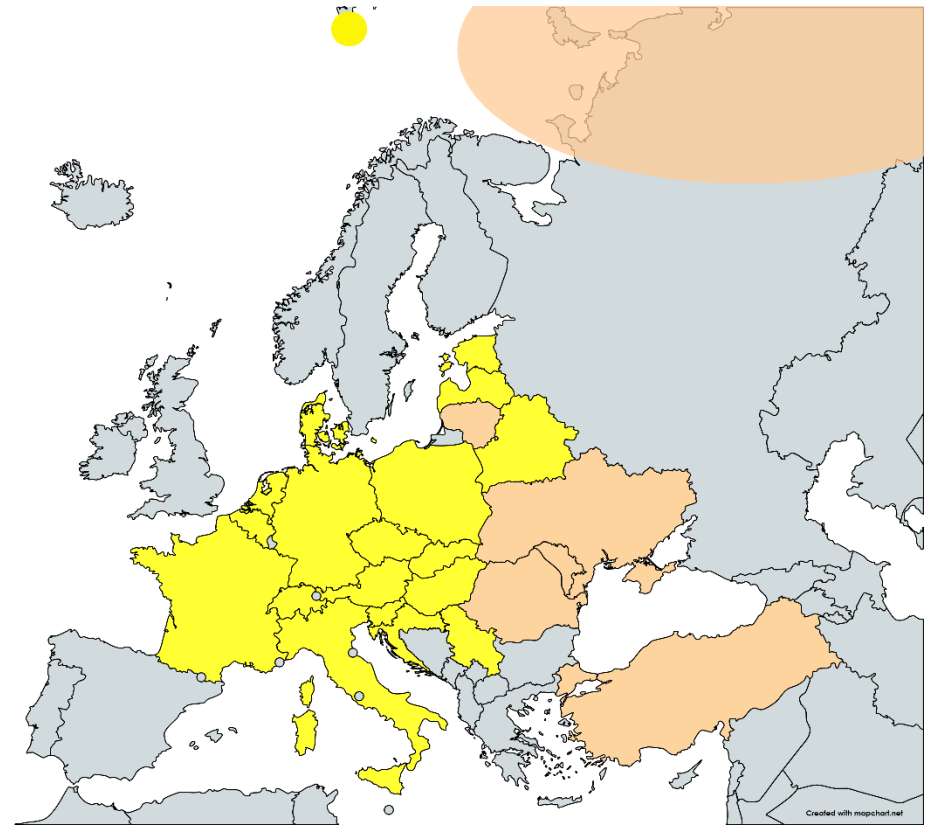


Which Asia?
Molecular data from:
CHINA*
KYRGYZSTHAN**
KAZAKHSTAN
JAPAN

*account for **91%** of the global HAE burden every year.

PERSPECTIVES

1. Fill the geographical gaps (Lithuania, Eastern Europe, Turkey, Arctic/Eastern Russia).
2. Whole mitogenome/genome approach on selected isolates.





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