Genotyping of *Echinococcus multilocularis* in Europe: achievements and future perspectives

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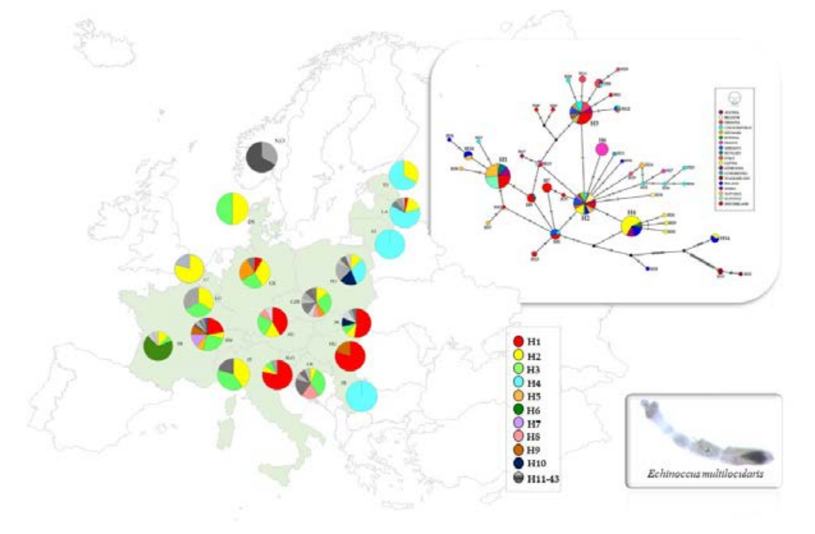

XVIII Workshop of National Reference Laboratories for Parasites
Rome, 16th-17th November 2023

ISTITUTO SUPERIORE DI SANITA', ROME, ITALY

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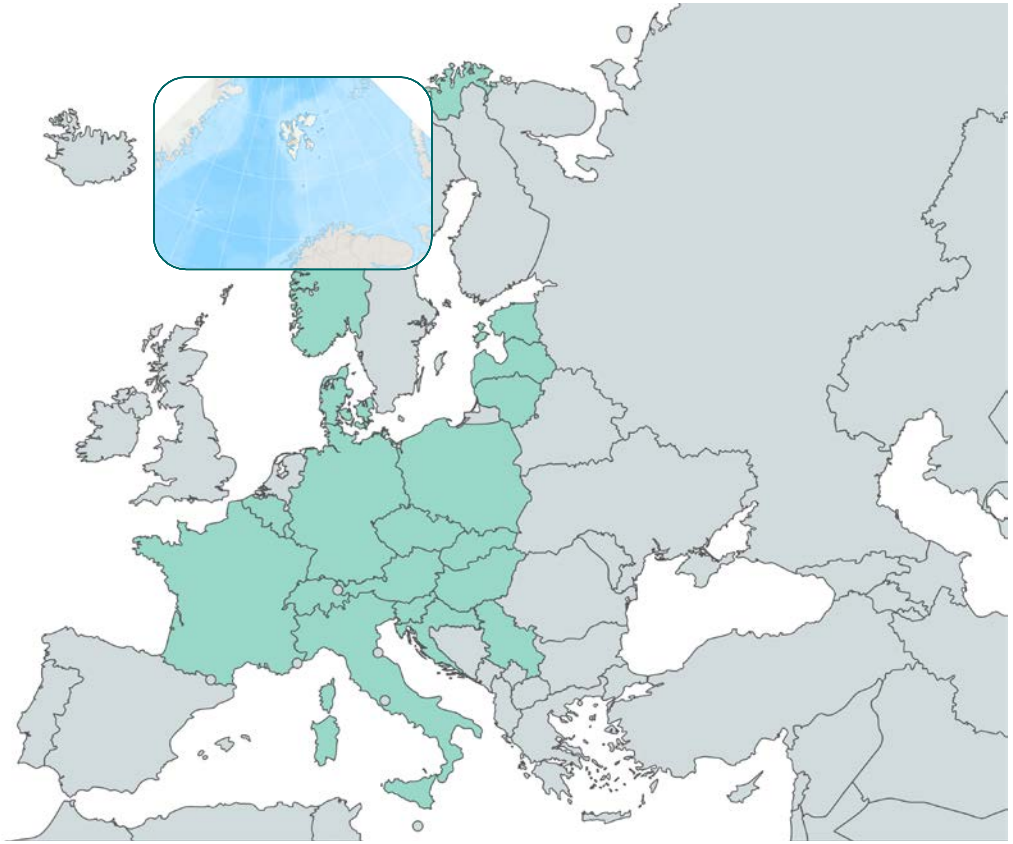
University of Ljubljana, Gerbičeva 60, 1000, Ljubljana, Slovenia.
Vetsuisse faculty, University of Bern, Bern, Switzerland.

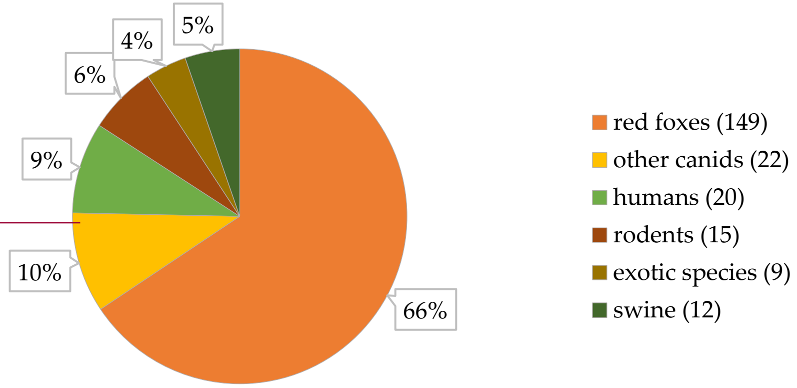
THE FINAL COLLECTION

Country	Host species	n. isolates
Austria	8 humans, 3 red foxes, 1 macaque	12
Belgium	5 red foxes	5
Croatia	14 red foxes, 1 human	15
Czech Republic	15 red foxes	15
Denmark	3 red foxes, 1 raccoon dog	4
Estonia	2 red foxes, 1 beaver	3
France	15 red foxes, 1 mouse	16
Germany	8 red foxes, 2 humans, 1 common vole, 1 mouse	12
Hungary	5 red foxes	5
Italy	5 red foxes	5
Latvia	25 red foxes, 3 raccoon dogs, 1 grey wolf	29
Lithuania	4 red foxes	4
Luxembourg	3 muskrats	3
Norway (Svalbard)	3 arctic foxes	3
Poland	16 red foxes	16
Serbia	1 red fox	1
Slovakia	15 red foxes, 6 humans	21
Slovenia	13 red foxes, 1 jackal	14
Switzerland	13 dogs, 11 domestic pigs, 7 beavers, 3 humans, 3 lemurs, 3 macaques, 2 gorillas, 1 mouse, 1 wild boar	44
total 19	-	227



EURLP biobank

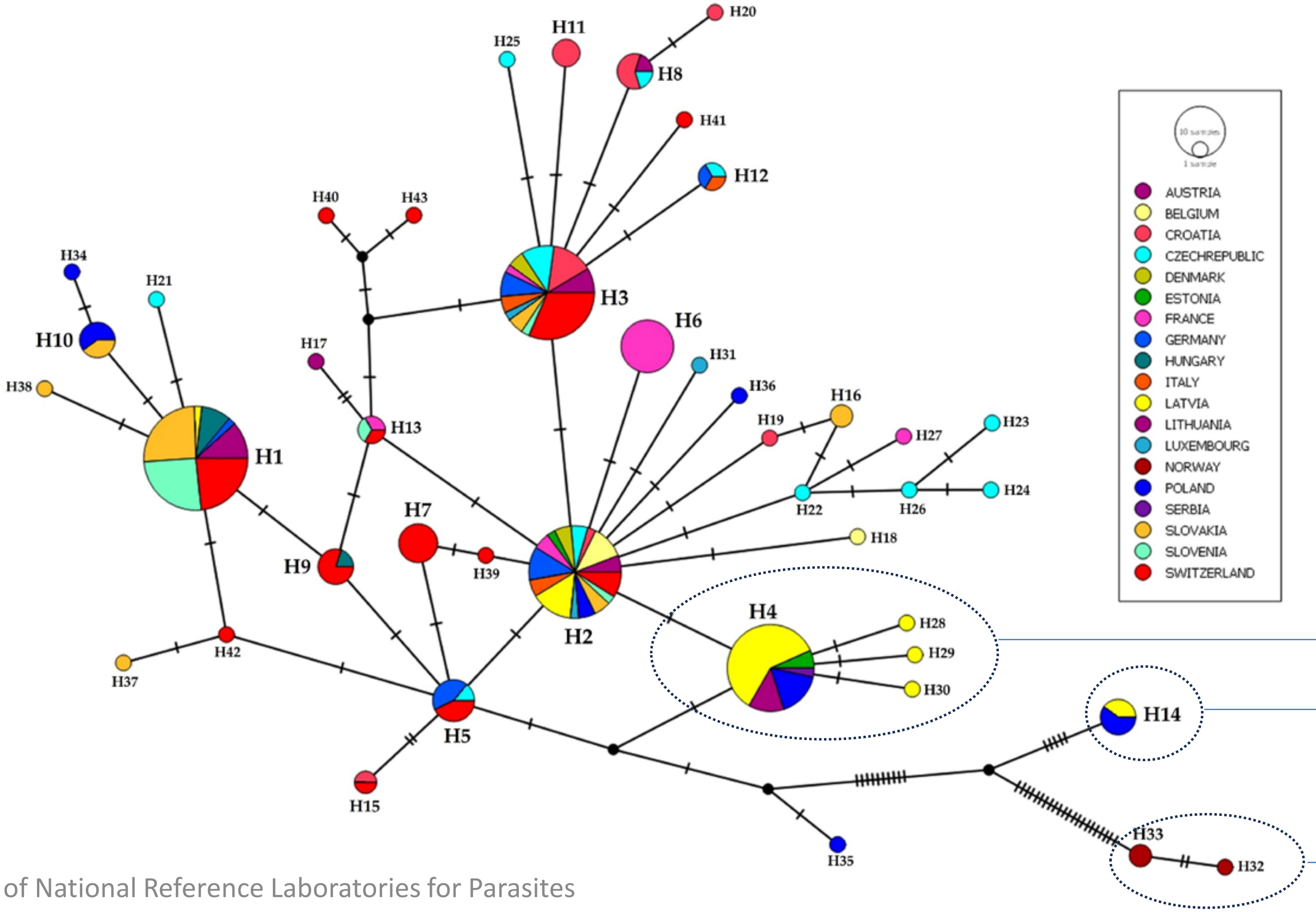
1 isolate = 1 specimen from 1 host



Results in summary

Analysis based on 5 Mt genes

1. The genetic diversity of *Echinococcus multilocularis* from 19 European countries was explored. Concatenated mtDNA sequences (4,968bp) of 227 isolates were compared. **43 haplotypes were found, four of them widely spread.**

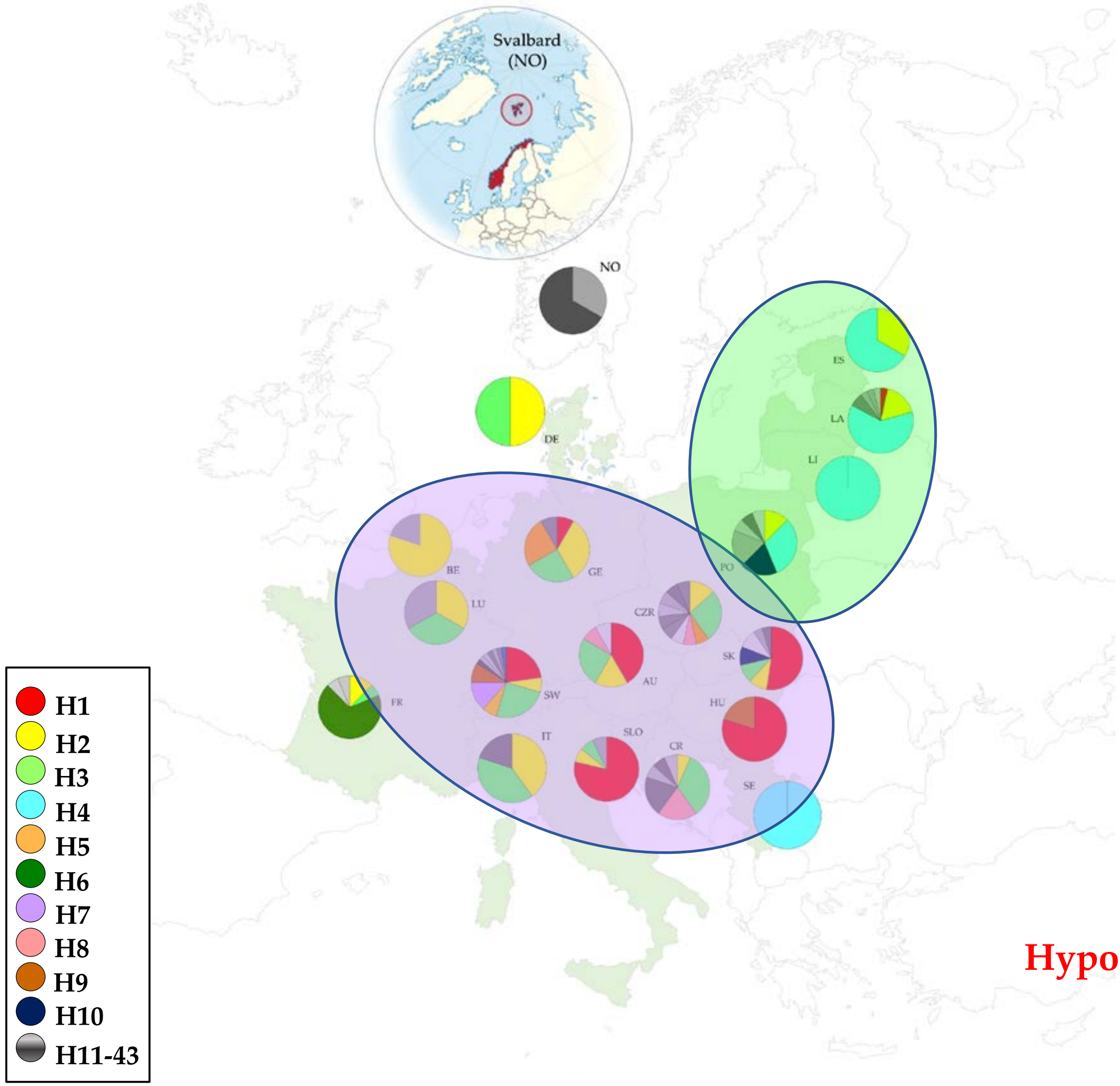


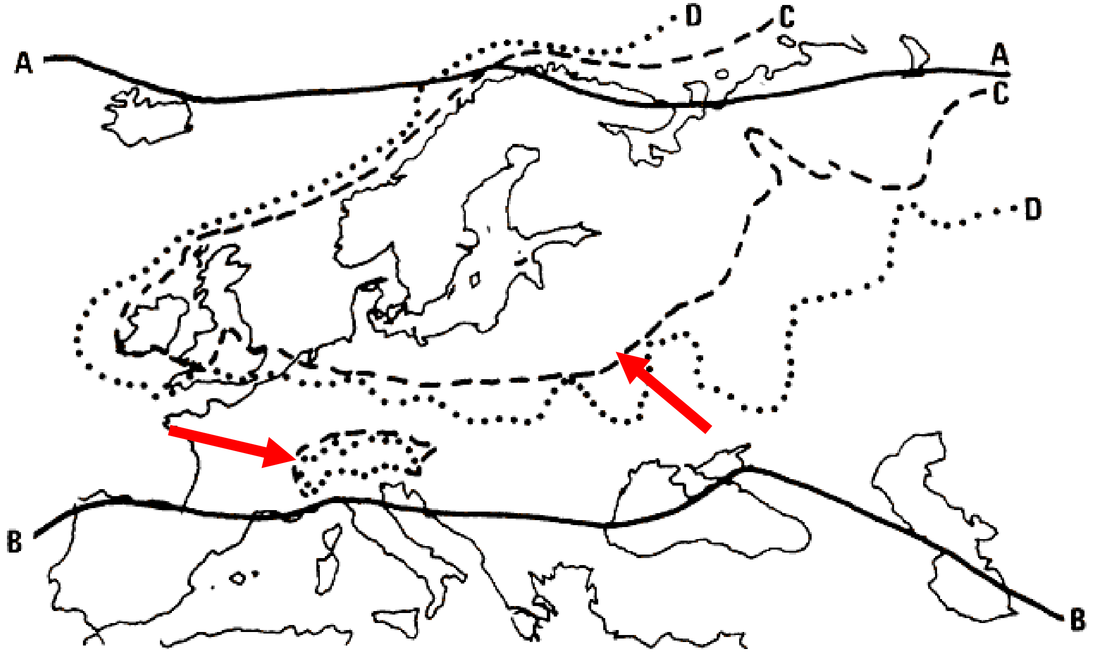
Estonia
Latvia
Lithuania
Poland
Serbia

Latvia
Poland (NE)

Svalbard (NO)

2. Mainland Europe appeared to be dominated by **two main clusters**.



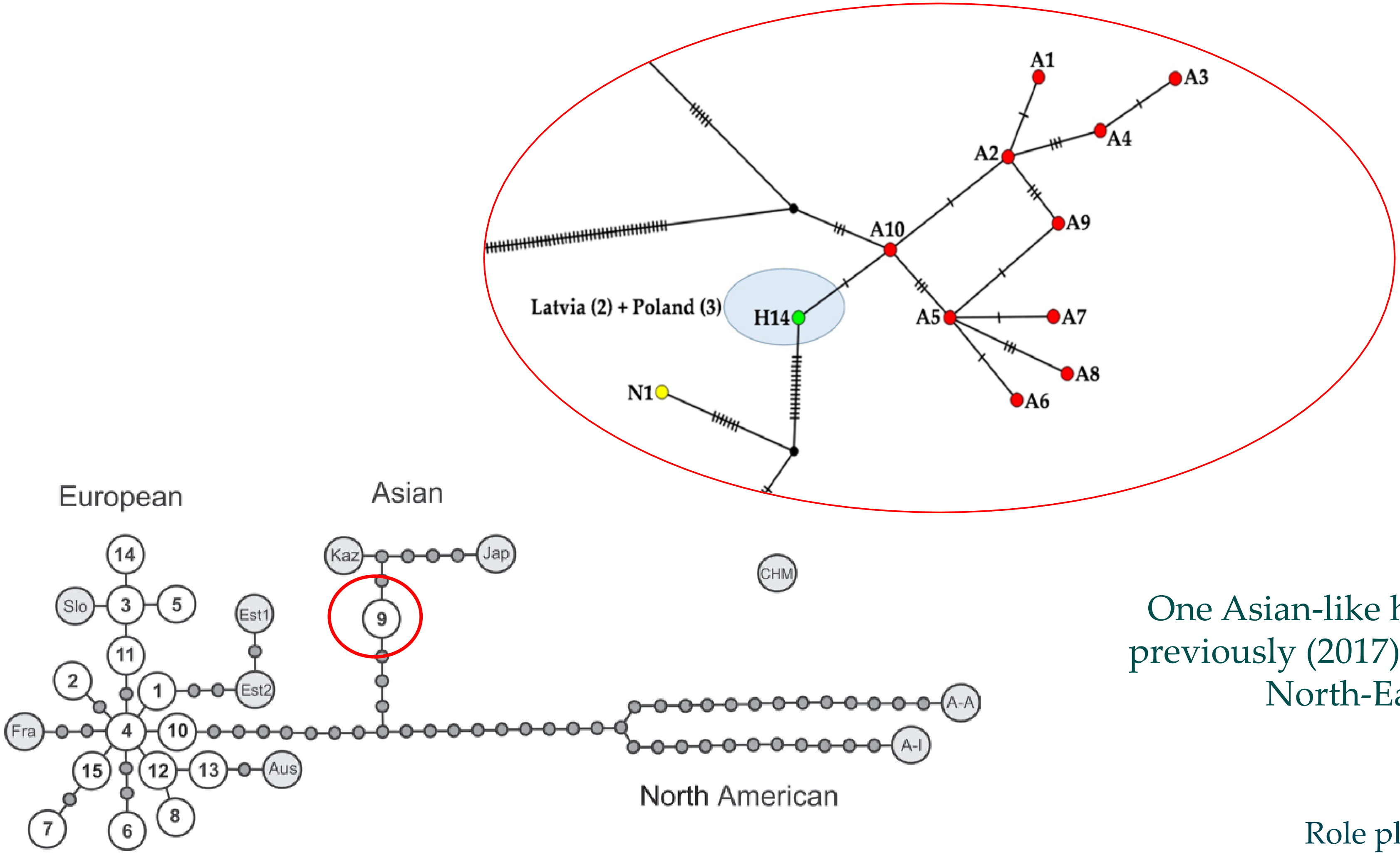


→ C: The limits of glacial debris deposited during the Würm Ice Age (LGM, 22,000-18,000 BC) (Evan Hadingham, 1980)

Hypotheses:

- a: Em maintained and isolated in different LGM refugia by the red fox?
- b: Em maintained and isolated in different LGM ice-covered areas by the Arctic fox?

3. An **Asian-like** haplotype was identified in Latvia and North-Eastern Poland.



Source: **Karamon et al., Folia Parasitol (Praha).9;64:2017.**

H14 finding locations

One Asian-like haplotype (named EmPl9) previously (2017) found in 7 red foxes in the North-East Poland as well.

Role played by the invasive raccoon dog?

?

4. The haplotypes from Svalbard were unrelated to the European haplotypes.

Rather, they showed some similarities with North-American and Asian haplotypes, **but a strong similarity with Em cox1 sequences from Yakutia (Eastern Siberia).**

Em was recently introduced in the Svalbard archipelago. Previous studies suggested that it was imported by Arctic foxes migrating from Siberia through the Arctic Sea ice (Knapp et al., 2012).

Here it established its life cycle thanks to the presence of the sibling vole, introduced from Europe (Henttonen et al., 2001).

A similar dynamic was described for the introduction of rabies.

FUTURE DIRECTIONS

Enrich the analysis:

SAMPLING
-missing territories
-low represented territories
Belarus, Hungary, Ukraine, Romania, European Russia, Serbia, and Sweden

DATASET (mitogenome and other markers)
-depict more precisely something about the spreading
-explore the relations among these two geographic clusters
-understand what is worthy to look at to find diversity and for which purpose

to provide a better insight into the population structure of *E. multilocularis* at European scale

New data:

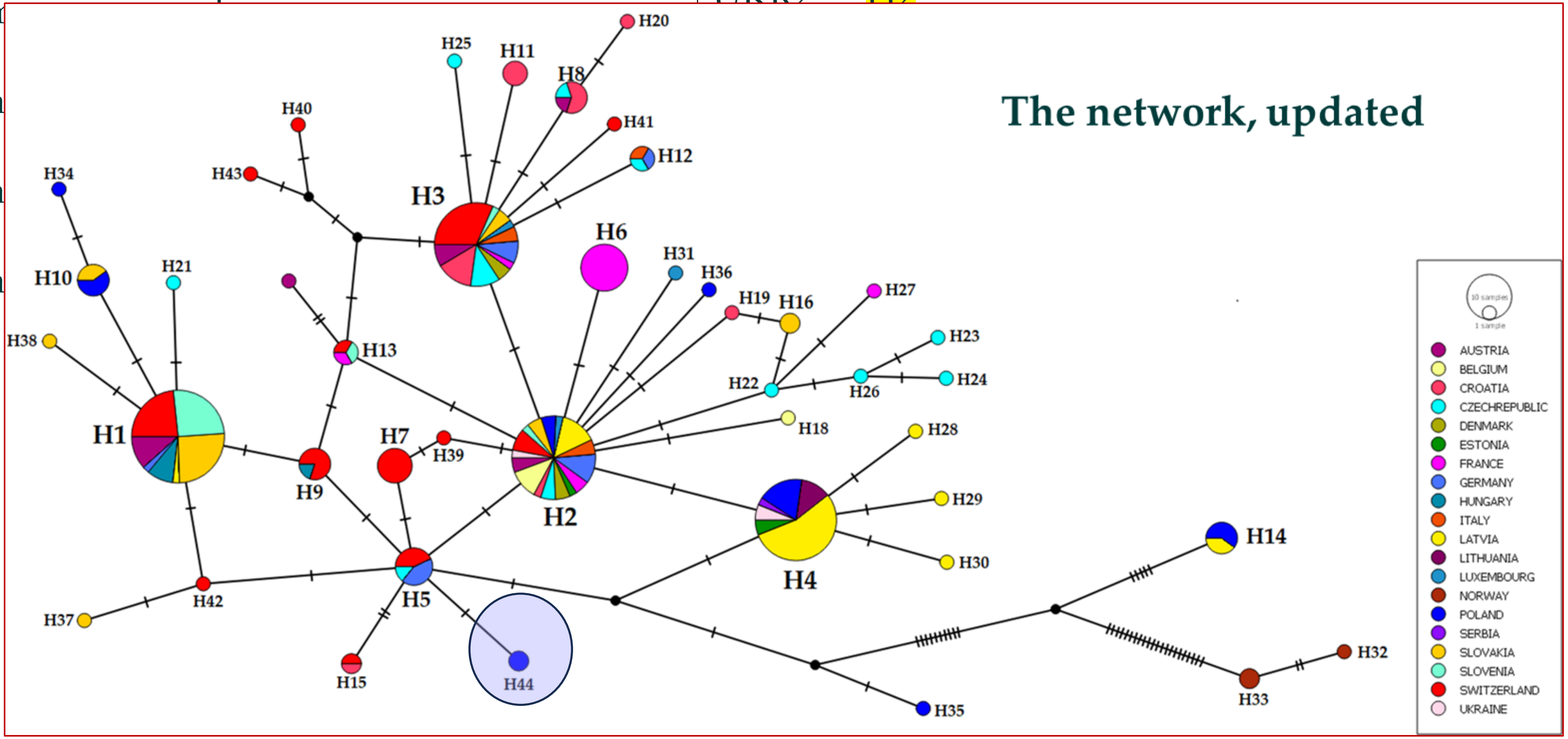
UKR1: H4
UKR2: H2

Som

3 sa

3 sa

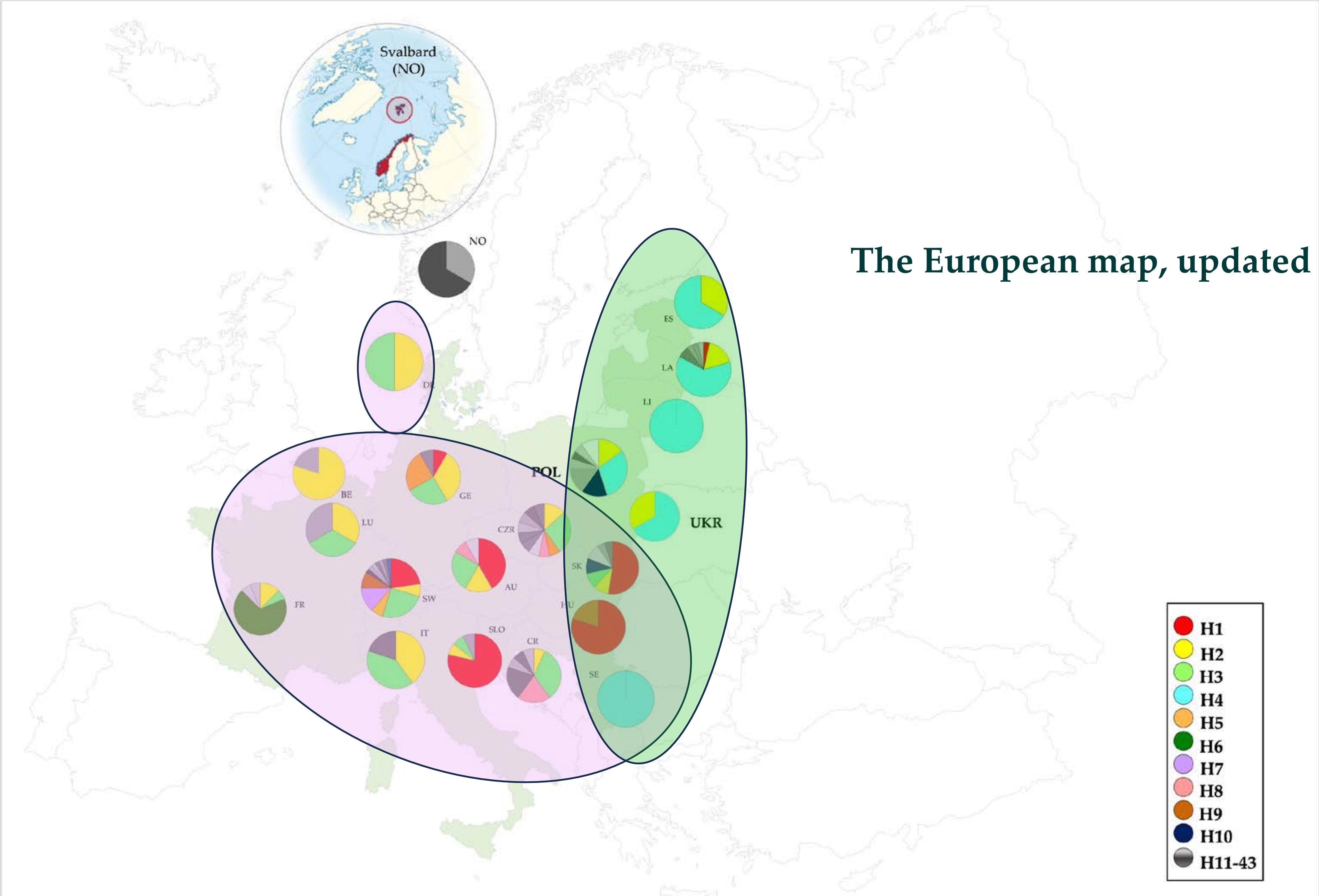
1 sa



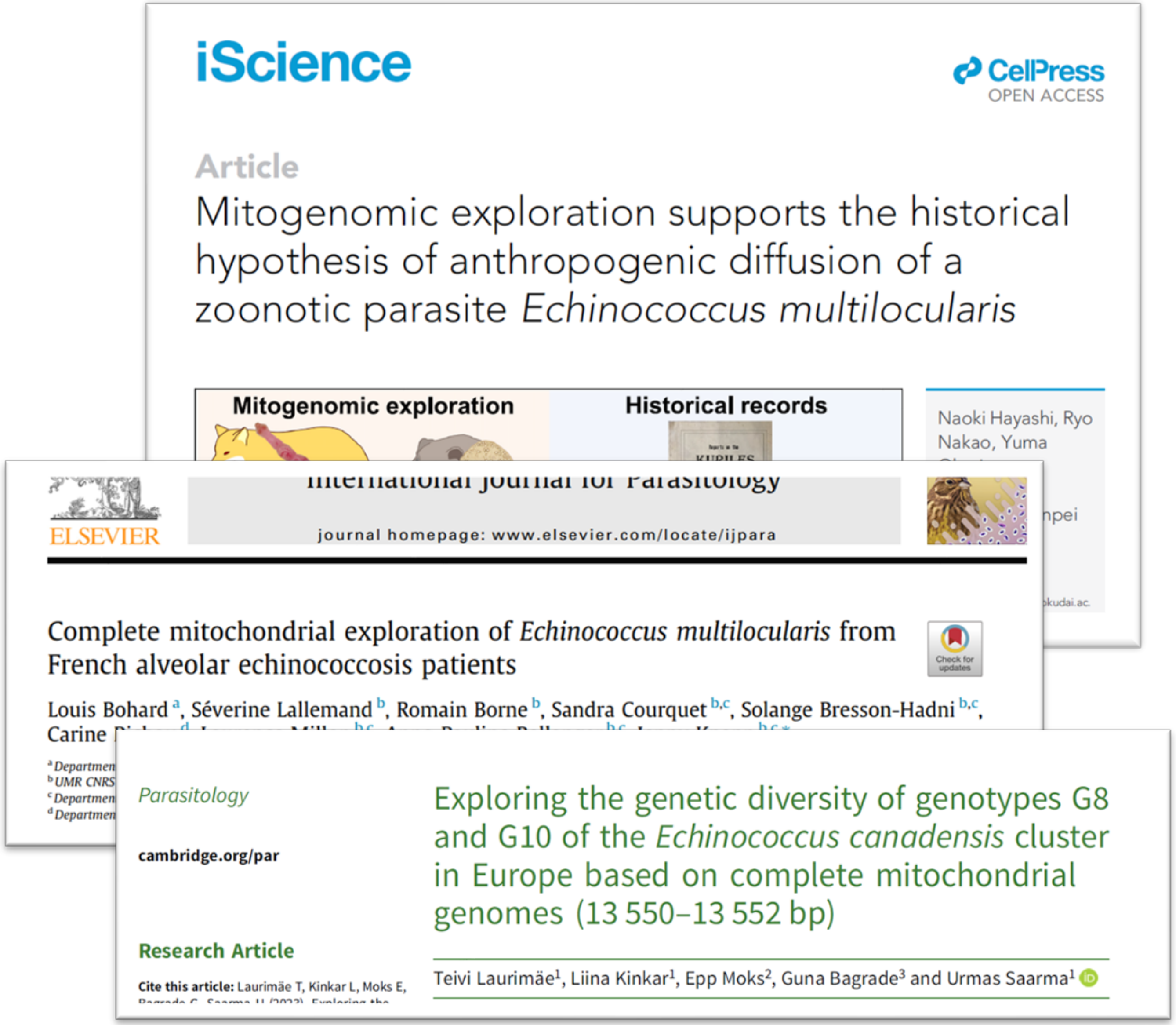
The network, updated

Poland, the buffer zone

The European map, updated



THE EXPLORATION OF COMPLETE MITOGENOMES



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Article

Mitogenomic exploration supports the historical hypothesis of anthropogenic diffusion of a zoonotic parasite *Echinococcus multilocularis*

Naoki Hayashi, Ryo Nakao, Yuma

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International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Complete mitochondrial exploration of *Echinococcus multilocularis* from French alveolar echinococcosis patients

Louis Bohard[a], Séverine Lallemand[b], Romain Borne[b], Sandra Courquet[b,c], Solange Bresson-Hadni[b,c],
Carine

Parasitology

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Research Article

Cite this article: Laurimäe T, Kinkar L, Moks E,

Exploring the genetic diversity of genotypes G8 and G10 of the *Echinococcus canadensis* cluster in Europe based on complete mitochondrial genomes (13 550–13 552 bp)

Teivi Laurimäe¹, Liina Kinkar¹, Epp Moks², Guna Bagrade³ and Urmas Saarma¹

From the current classification (5 genes haplotypes)

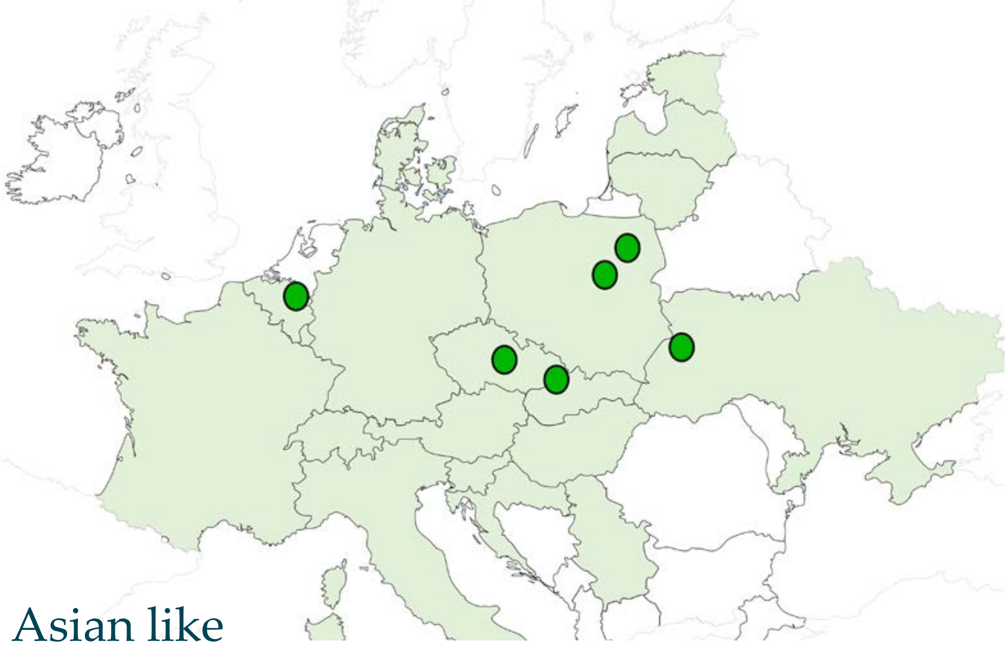
Questions

- Do **relations** among common haplotypes change?
- 2 isolates of the **same haplotype**, from 2 different countries, are **further differentiated** when looking at the whole mitogenome?
- What are the **most informative regions**?
- Are there geographic specific segregating polymorphisms?
- Is there a transition zone between Asia and Europe? What is the origin of the Asian-like haplotypes circulating in North-East Europe?

THE EXPLORATION OF COMPLETE MITOGENOMES

4,968 bp (cob, atp6, nad2, nad1, cox1) haplotypes →

1. SLOK3: H1
2. BEL7: H2
3. CZR10: H3
4. POL20: H4
5. UKR3: H4
6. POL23: H14 Asian like



By PCR and sequencing

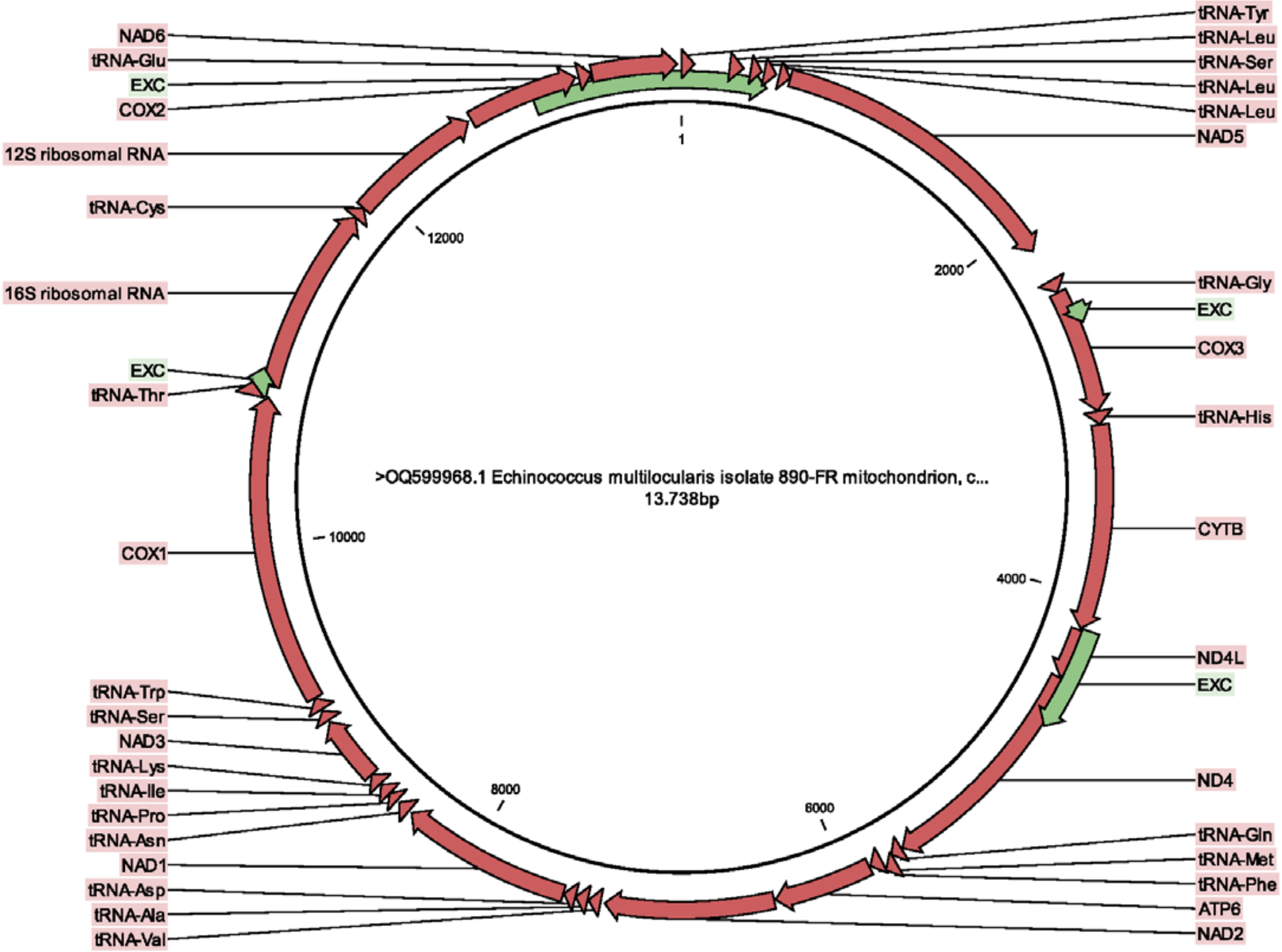
Assay developed by
Prof. **Urmas Saarma**
Chair of Mammalogy
Department of Zoology
Institute of Ecology and Earth
Sciences University of Tartu

Limit: analysing few isolates, the differences could be related to individuals rather to haplotype populations (the whole H1/H2 etc groups)

THE EXPLORATION OF *ALMOST* FULL MITOGENOMES
Preliminary results

11,705 over 13,738 bp (exclusion by sequence quality in green)

Exclusions:
0…460 (tRNA)
2481…2582 (cox3)
4180…4709 (nad4)
10774…10914 (tRNA)
12941…13738 (cox2, nad6)



THE EXPLORATION OF *ALMOST* FULL MITOGENOMES
Preliminary results

11,705 over 13,738 bp (exclusion by quality)

HAPLOTYPE DATA

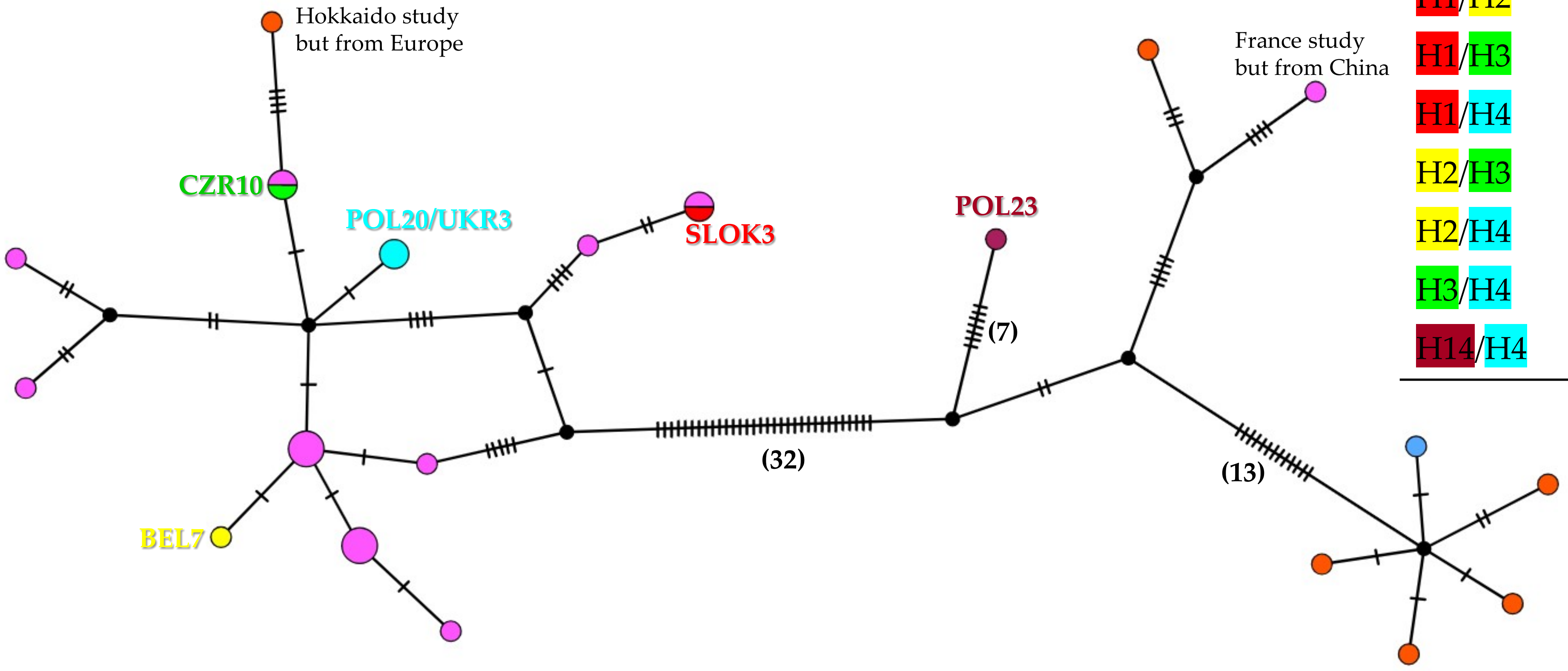
- **54** variables sites
- 5 haplotypes

Excluding POL23 –Asian like isolate:

- **14** variables sites
- 4 haplotypes

THE EXPLORATION OF *ALMOST* FULL MITOGENOMES
Preliminary results

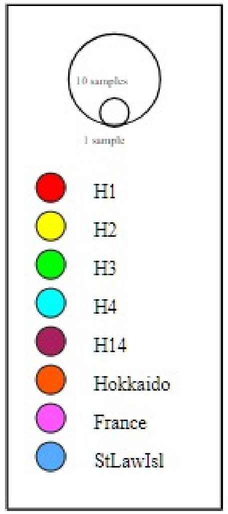
TCS NETWORK



How do the relations change?

	4968bp	11705bp
H1/H2	3	12
H1/H3	4	11
H1/H4	4	11
H2/H3	1	3
H2/H4	1	3
H3/H4	2	2
H14/H4	17	44

mutations



THE EXPLORATION OF *ALMOST* FULL MITOGENOMES
Preliminary results – informative regions?

bp	marker	N° of mutations	
1575	**NAD5**	**1 H1-H4** **10 Asian H***	6.3%
545	**COX3**	**1 H1-H4** **1 Asian H**	3.6%
1068	**COB**	**2 H1-H4** **4 Asian H***	4.6%
1214	**NAD4**	**3 H1-H4** **3 Asian H**	4.9%
516	**ATP6**	**2 Asian H**	3.9%
882	**NAD2**	**2 H1-H4**	

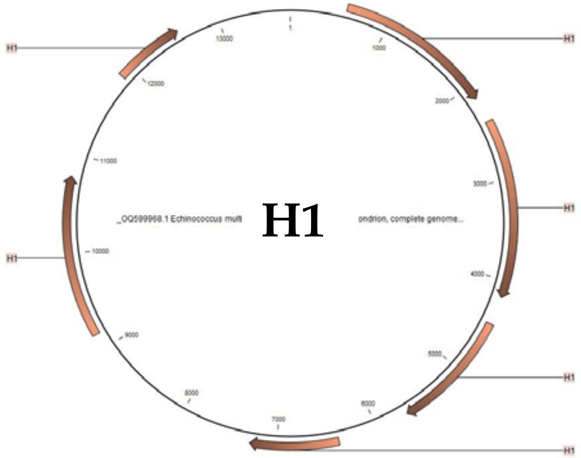
*one is shared

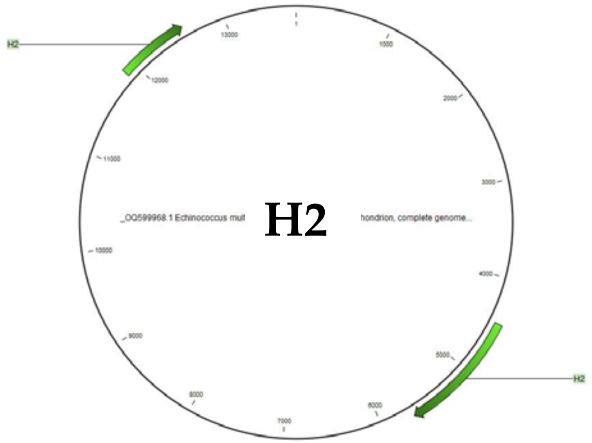
- To differentiate Asian from European haplotypes:
-potentially each region
-NAD5 most «powerful»

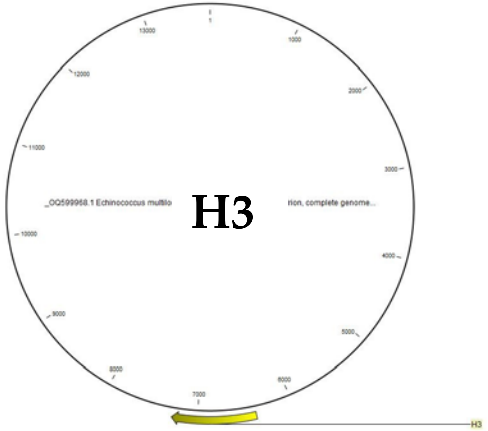
- To differentiate among very common European haplotypes:
-12S, NAD4 (+++), COB, NAD2 (++)

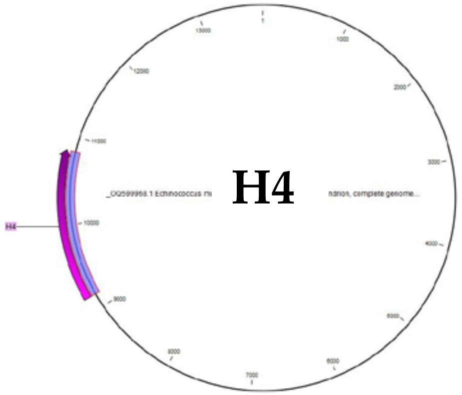
CONCLUSIONS (preliminary):

- Apparently, relations among the previously identified commonest European haplotypes (H2-H3-H4) do not change significantly increasing the number of analysed loci, but it could be the case of haplotype **H1**.
- The mutations distinguishing the <u>Asian</u> and the <u>European</u> clusters are distributed along all the Em mitogenome (at least within protein coding genes and ribosomal 12S and 16S), but they seem particularly concentrated in the NAD5 gene.
- The mutations distinguishing the commonest European haplotypes are distributed almost all along the Em mitogenome for H1, but they are fewer for H2, H3 and H4.









Thank you

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Photo by Birger Strahl