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NEW INSIGHTS INTO THE GENETIC VARIABILITY OF EUROPEAN TRICHINELLA SPIRALIS

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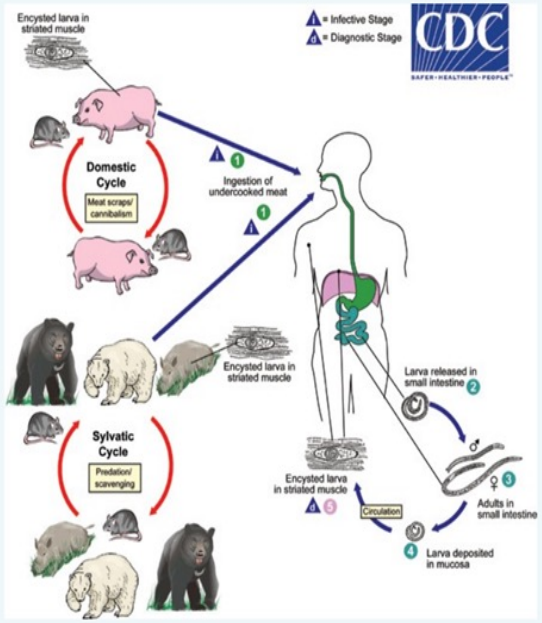
Why Trichinella spiralis still matters

A foodborne parasite maintained across domestic and wildlife cycles

FOOD SAFETY CONTEXT

- **Zoonotic nematode** transmitted when infected meat is eaten
- **Highly adapted to swine** and historically important for pork safety
- Circulates in both **domestic and sylvatic** settings
- **Backyard pig production, wild boar, rodents** and **improper disposal** or **feeding of hunting waste** can bridge domestic and sylvatic cycles

The key question is not only detection, but source attribution and transmission pathways.



Domestic ↔ sylvatic interface

What is the source of infection on pig farms?

Several routes can produce the same diagnostic result



Slaughter leftovers



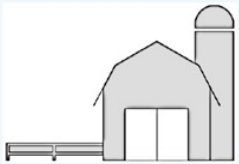
Wild-boar waste



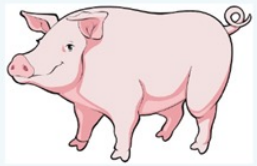
Fox carcasses



Rodents / vectors



?



Are farms linked by one source, or are these independent events?

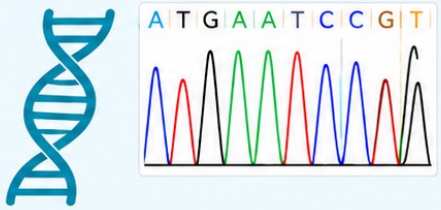
Molecular epidemiology can use DNA sequence to separate distinct infection sources.

Challenge: genetically homogeneous parasite

Earlier molecular studies showed very low genetic variability in *Trichinella spiralis*.

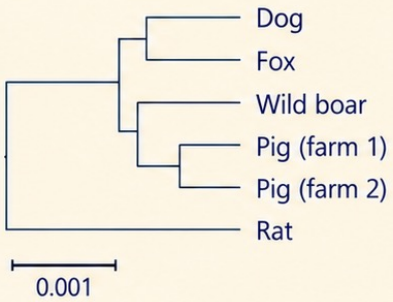
Earlier molecular studies

- Different multilocus marker systems repeatedly showed low genetic variability and a highly homogeneous population.



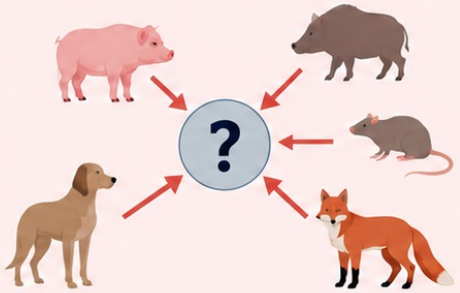
Closely related isolates

- Isolates of *T. spiralis* from different hosts and regions are genetically very similar.



Consequence for outbreaks

- It is difficult to distinguish sources of infection and link cases in outbreaks, because isolates are genetically very similar.



LOW GENETIC VARIABILITY → **LOW EPIDEMIOLOGICAL DISCRIMINATION**

Why standard markers are not enough

T. spiralis was long regarded as genetically uniform at common molecular resolution

Single markers

Often too little information to distinguish closely related or inbred isolates.

Low cost
Low resolution

Whole genome

Would provide all information, but may be expensive and unnecessary for outbreak tracing.

Maximum data
High cost

ddRADseq

Samples limited DNA from many places in the genome, balancing resolution and cost.

Genome-wide loci
Practical scale

Trich-tracker fills the gap between too little information and whole-genome overkill.

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Trich-tracker – a practical tool to trace *Trichinella spiralis* transmission based on rapid, cost-effective sampling of genome-wide genetic variation ☆

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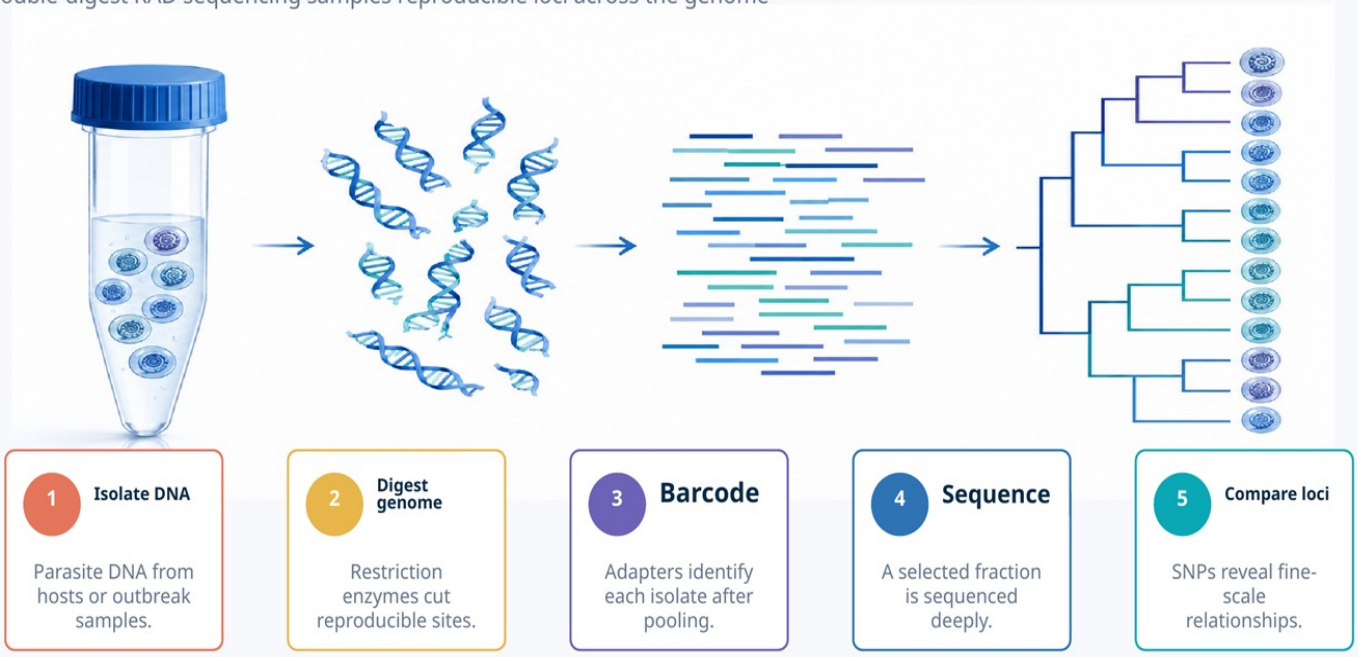
ABSTRACT

Molecular epidemiology using traditional sequencin
due to a lack of genetic variation available for discrim
ing techniques offer a solution to this problem by in
Here, we introduce Trich-tracker, a tool that make
throughout the genome of *Trichinella spiralis* to m
and distinctions among focal outbreaks of *T. spiralis*.
ation among a sample of parasites from Polish farms
ble digest restriction site-associated DNA sequenc
sequence were generated from each sample and sho



Trich-tracker: a practical high-resolution workflow

Double-digest RAD sequencing samples reproducible loci across the genome



Concept: many independent loci make subtle genetic differences measurable.

THE NEXT QUESTION

From outbreak tracing to European population structure

Can the same high-resolution approach detect geographic structure and long-range connections among T. spiralis isolates?

Poland | Germany | Croatia | Romania

A continental comparison expands Trich-tracker from local outbreak reconstruction to population-level inference.



Sample selection for population genetic analysis

From the initial European sample set to the final dataset used for UPGMA and STRUCTURE

Hosts / sample origin: Domestic pig • Wild boar • Bear • Rat

INITIAL DATASET
42 samples

- Poland 16
- Germany 9
- Romania 10
- Croatia 7

Library preparation

Sequencing

Bioinformatic processing

Mapping to the T. spiralis reference genome

QUALITY FILTER
≥30% of the genome mapped to the T. spiralis reference
Samples below 30% were excluded

FINAL DATASET
31 samples

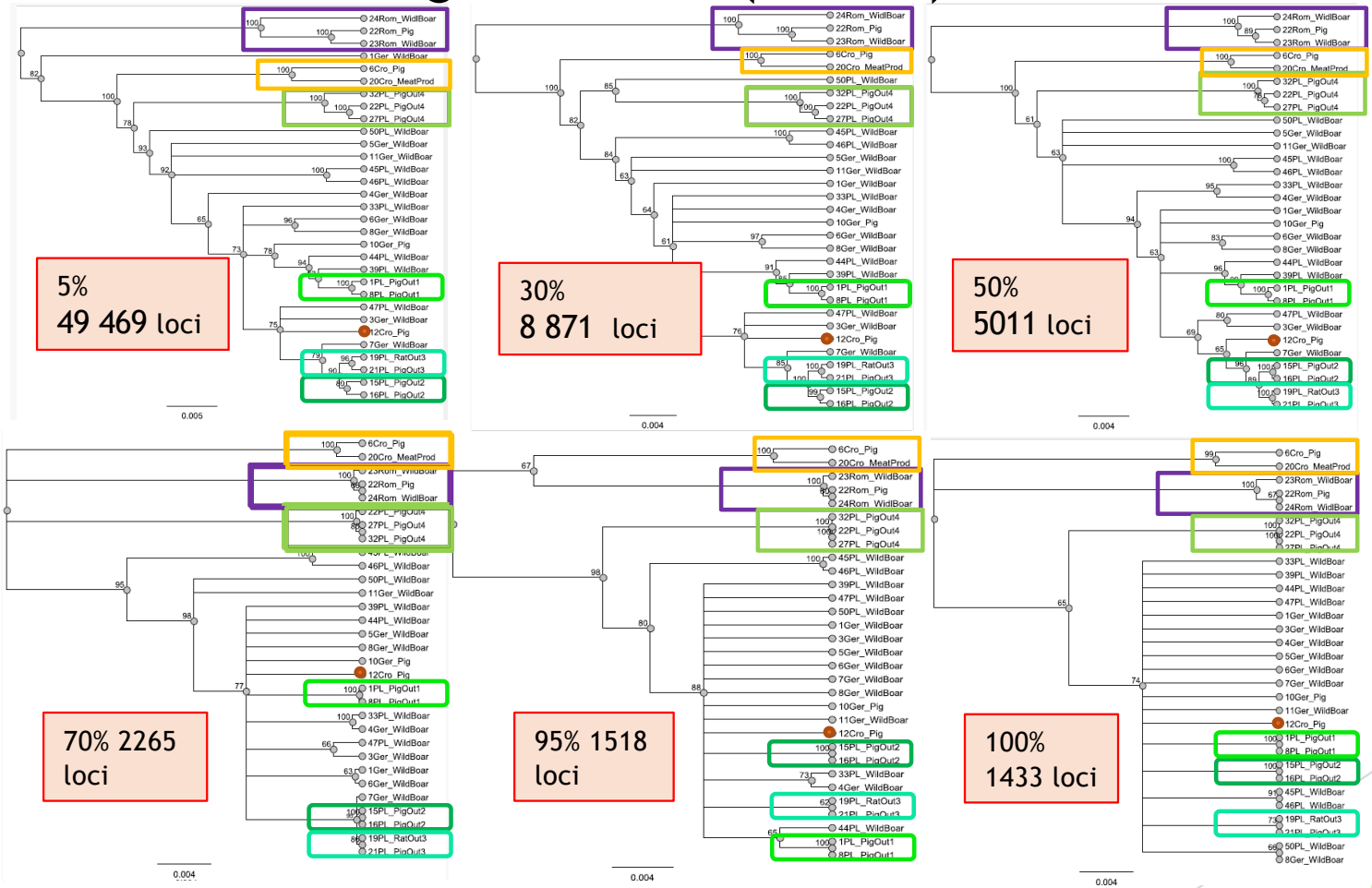
- Poland 16
- Germany 9
- Romania 3
- Croatia 3

UPGMA + STRUCTURE

11 samples were excluded after mapping-based quality filtering (42 → 31).



Comparison of UPGMA Trees Based on Different Locus Presence Filtering Thresholds (5-100%)



STABLE GROUPS IDENTIFIED



Romania

All Romanian isolates cluster together at every threshold (5–100%).



Croatia

6Cro_Pig and 20Cro_MeatProd consistently cluster together.



Polish outbreaks

- Outbreak 1 (PL) – stable
- Outbreak 2 (PL) – stable
- Outbreak 3 (PL) – stable
- Outbreak 4 (PL) – stable

All Polish outbreak groups remain intact across all thresholds.



Germany

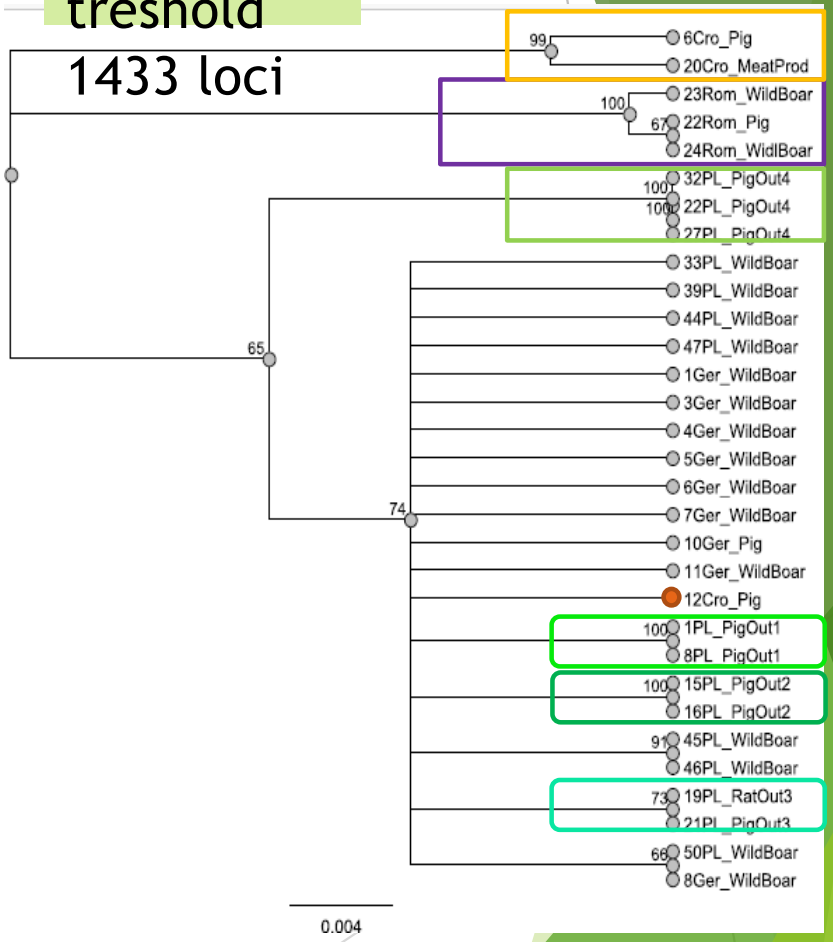
German isolates form a cluster, but internal relationships are less stable.

OVERALL TAKE-HOME MESSAGE



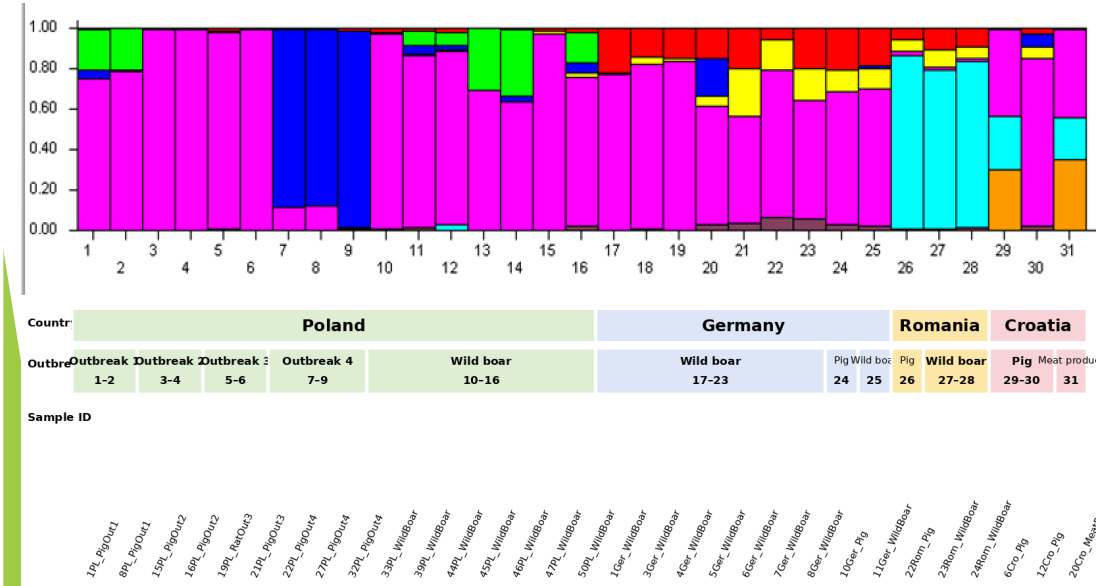
- ✓ Main clusters are robust to locus filtering (5–100%).
- ✓ Polish outbreak groups show strong internal similarity.
- ✓ Polish and German isolates cluster together, indicating similarity, but can be better distinguished with a higher number of analyzed loci.
- ✓ Stable topology supports shared ancestry or epidemiological connection within clusters.

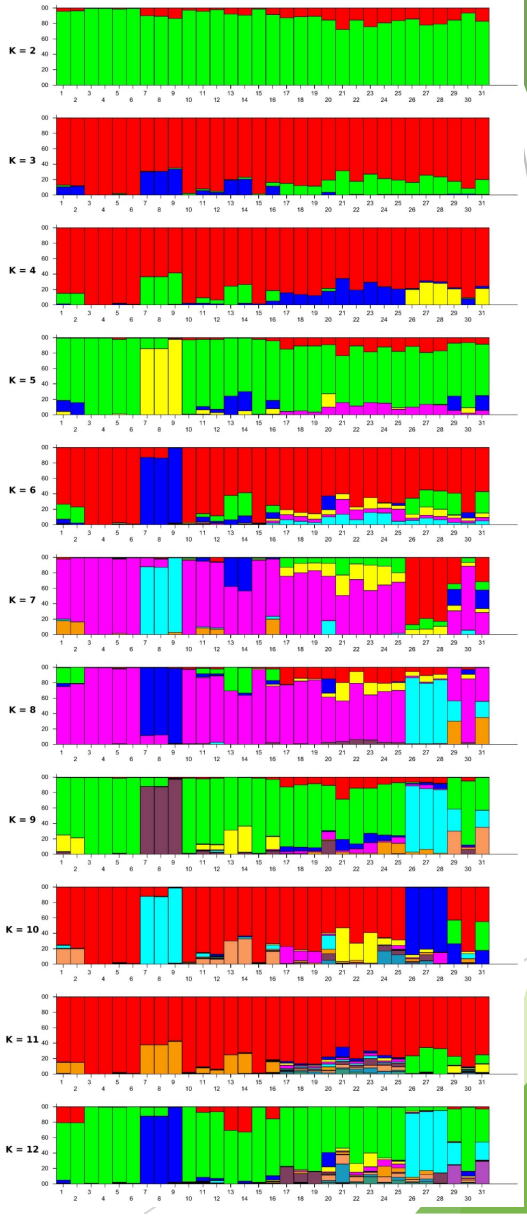
100% treshold
1433 loci



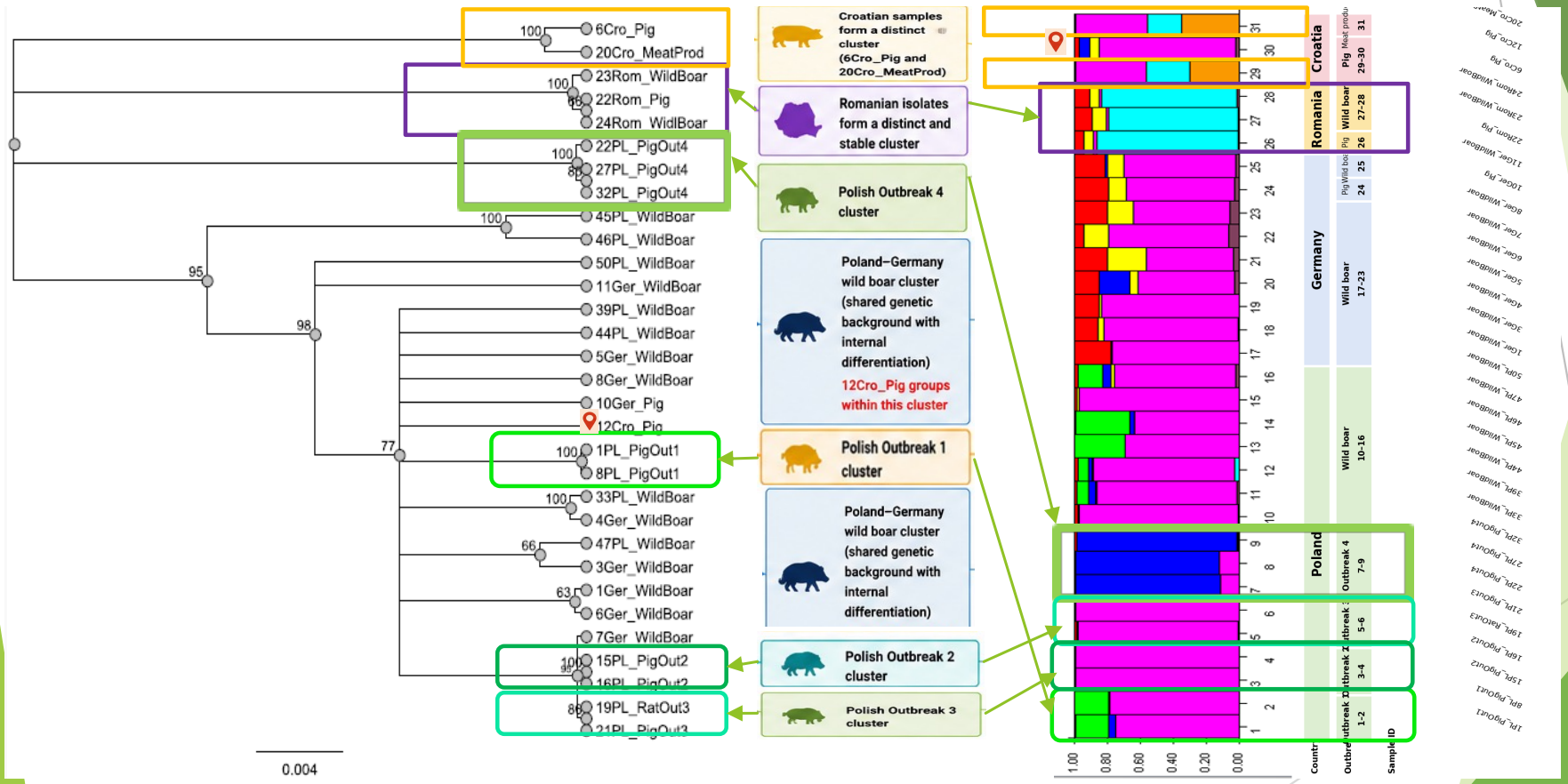
STRUCTURE

K = 5 was the best-supported solution (ΔK Evanno = 36,33), whereas higher K values revealed increasingly biologically meaningful hierarchical structure, with K = 8 providing the clearest geographic differentiation while retaining the same major population divisions at higher resolution.



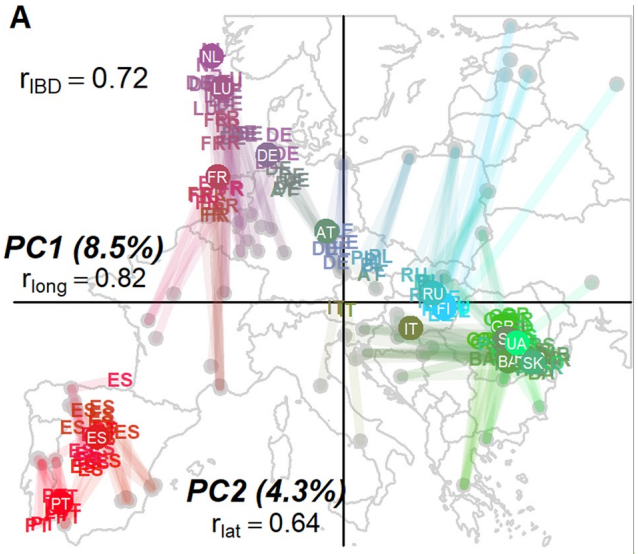


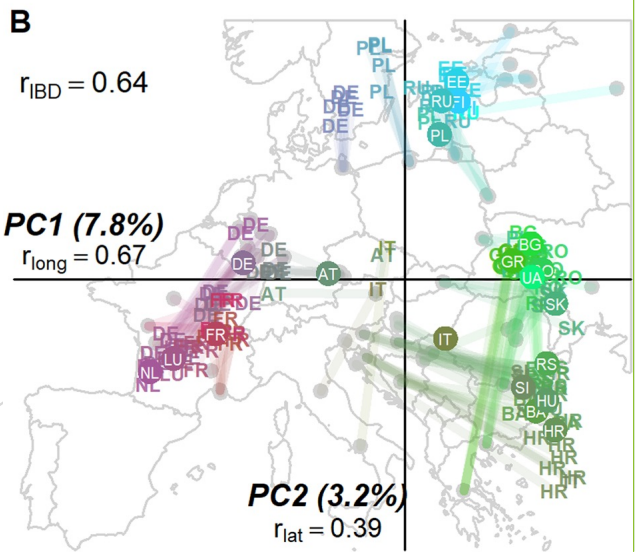
Concordance between UPGMA and STRUCTURE (K=8)



Why Romanian *T. spiralis* are so distinct?

1. The distinct clustering of Romanian samples is consistent with previously reported regional genetic structuring of European wild boar, in which Romanian populations were assigned to an Eastern European-Balkan genetic cluster distinct from the Central European cluster including Poland and Germany.
2. ???
3. ???





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ARTICLE OPEN

Check for updates

Spatial genetic structure of European wild boar, with inferences on late-Pleistocene and Holocene demographic history

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European wildlife has been subjected to intensifying levels of anthropogenic impact throughout the Holocene, yet the main genetic partitioning of many species is thought to still reflect the late-Pleistocene glacial refugia. We analyzed 26,342 nuclear SNPs of 464 wild boar (*Sus scrofa*) across the European continent to infer demographic history and reassess the genetic consequences of natural and anthropogenic forces. We found that population fragmentation, inbreeding and recent hybridization with domestic pigs have caused the spatial genetic structure to be heterogeneous at the local scale. Underlying local anthropogenic signatures, we found a deep genetic structure in the form of an arch-shaped cline extending from the Dinaric Alps, via Southeastern Europe and the Baltic states, to Western Europe and, finally, to the genetically diverged Iberian peninsula. These findings indicate that, despite considerable anthropogenic influence, the deeper, natural continental structure is still intact. Regarding the glacial refugia,

Why Croatian *T. spiralis* pig sample are not clustering with Croatian group??

1. 12Cro_Pig represents a genetically distinct Croatian pig individual that shares greater genomic affinity with part of the Central European cluster than with the other Croatian samples. This may reflect shared ancestral variation or historical gene flow.
2. ??
3. ???

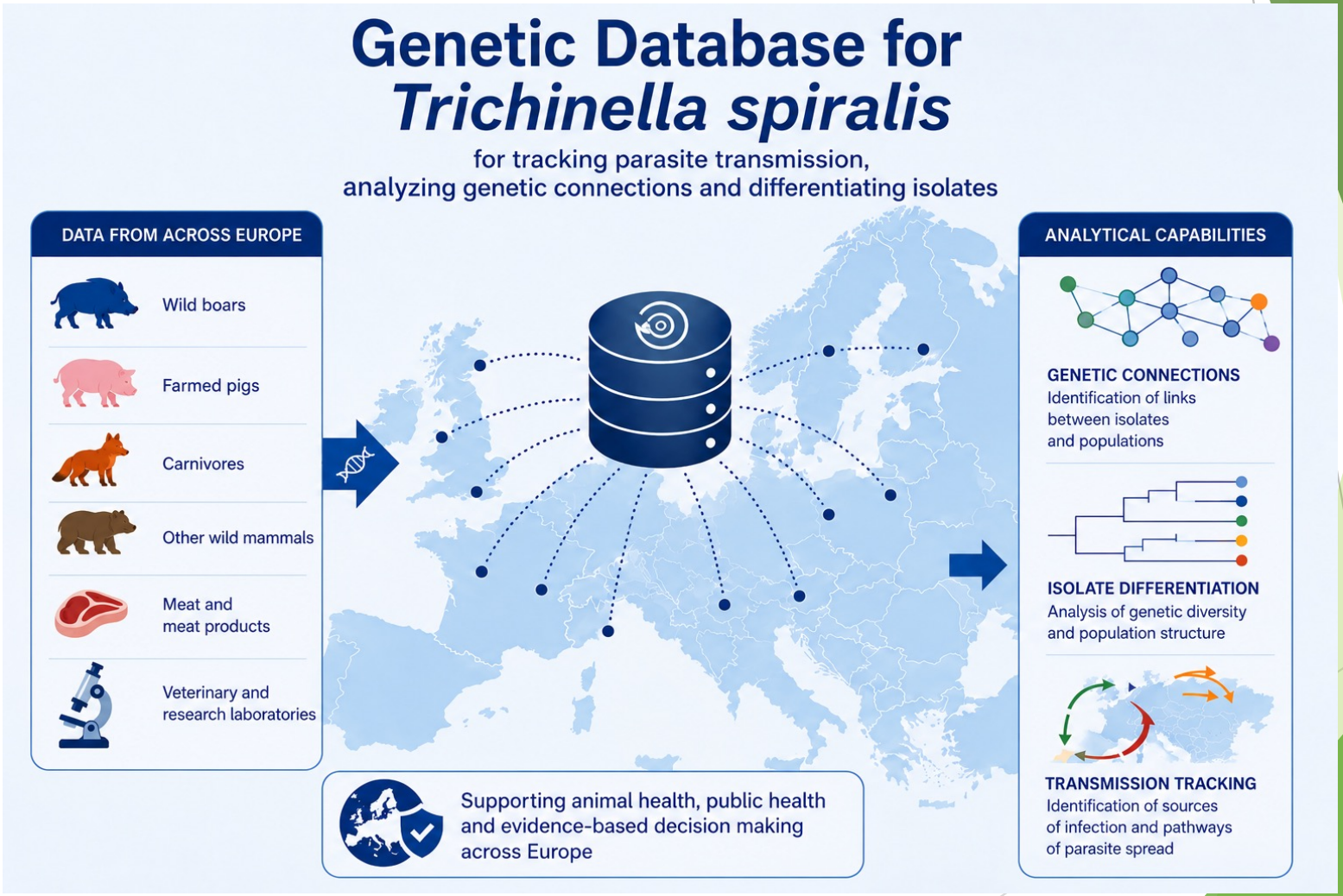
Conclusions

High-resolution genotyping reveals epidemiologically informative population structure in European T. spiralis

1. **High-resolution ddRADseq – Trich-tracker reveals genetic variation within European T. spiralis previously considered highly homogeneous.**
2. **Stable genetic clusters correspond to geographic origin and epidemiologically defined outbreaks.**
3. **Phylogenetic and STRUCTURE provide concordant evidence for the major population divisions.**
4. **Polish and German isolates share a common genetic background but we can find further internal differentiation.**
5. **Geographic origin alone does not fully predict genetic relatedness — 12Cro_Pig is a clear example.**

From a genetically homogeneous parasite to epidemiologically informative population structure.

Perspectives?







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