



BARCODING INVASIVES: A NEW TOOL FOR INVASION MONITORING IN SOIL

David Porco, Louis Deharveng, Thibaud Decaens, Mark Stevens,
Sam James, Bettine Van Vuuren, Kevin Butt, Rodolphe Rougerie,
Benoit Richard and Paul Hebert





Biological invasions

- Species moving across long distance biogeographic barriers
 - Natural phenomenon (glaciations + tectonic mvt)
 - For the recorded data over only 500 years, current rate several order of magnitude higher
 - Rise of human transport and commerce
 - (Extensive air, sea and land transport network = massive introduction of taxa outside there natural range)



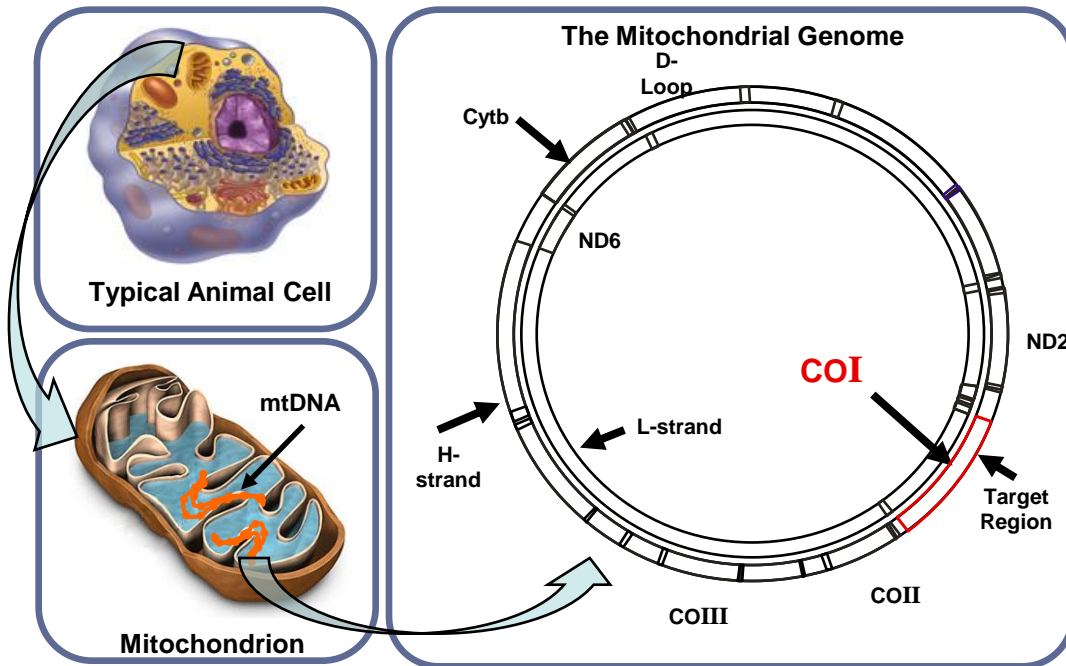


- Severe global consequences are foreseen:
 - loss of agricultural, forestry or fishery resources
 - disruption of ecosystems (unable to deliver services and goods)
 - Conservation issues
- All biota impacted
 - Soil biota is no exception but was almost ignored
 - Taxonomic impediment (difficult taxonomy, few specialists available for routine ID)
 - Limitation of morphological characters
 - » Adult specimens treated only
 -
 - Molecular taxonomy / DNA barcoding
 - Accurate ID, propagule pressure ?

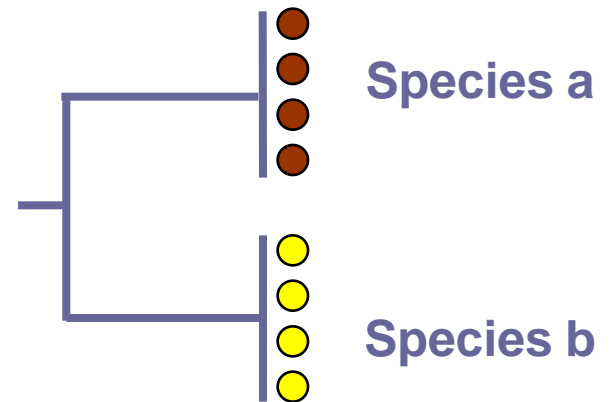
DNA Barcoding

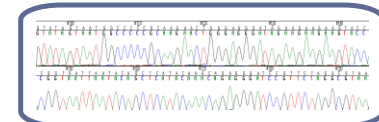
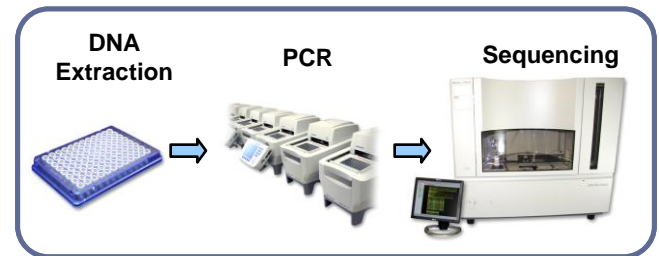
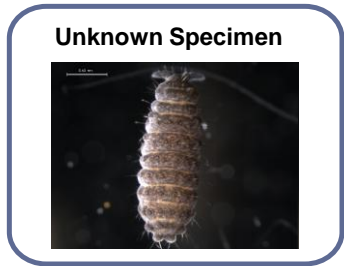
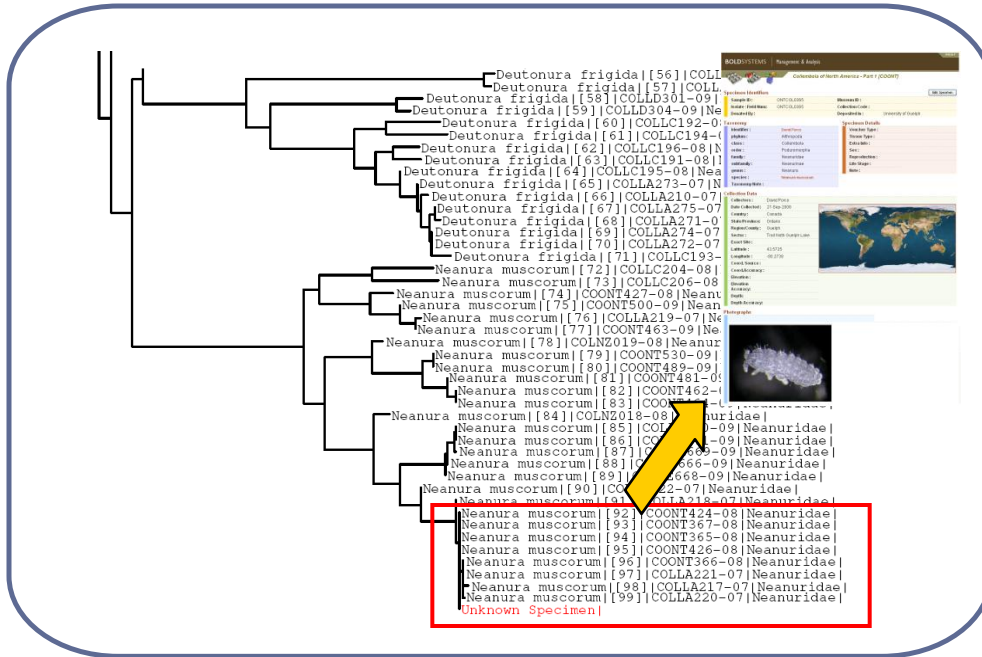
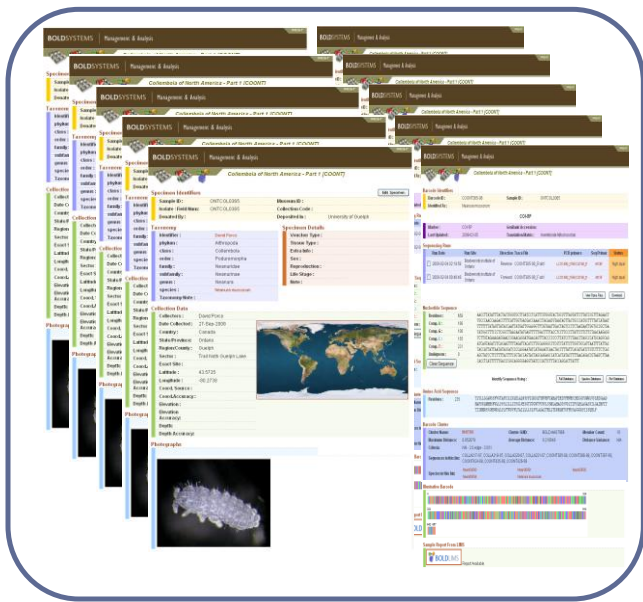
- Universal marker for animals - COI

An Internal ID System



- Intraspecific variation < Interspecific variation







- Lumbricidae: European invasion of North America
- Collembola: European invasion of North America (Africa and Oceania)



European Lumbricidae invasion in North America

- Last glaciations wiped out native species
- European species introduced both accidentally and intentionally (fish baits, vermicomposting, horticulture...)



- 823 specimens from 10 species
- +94 GenBank COI sequences

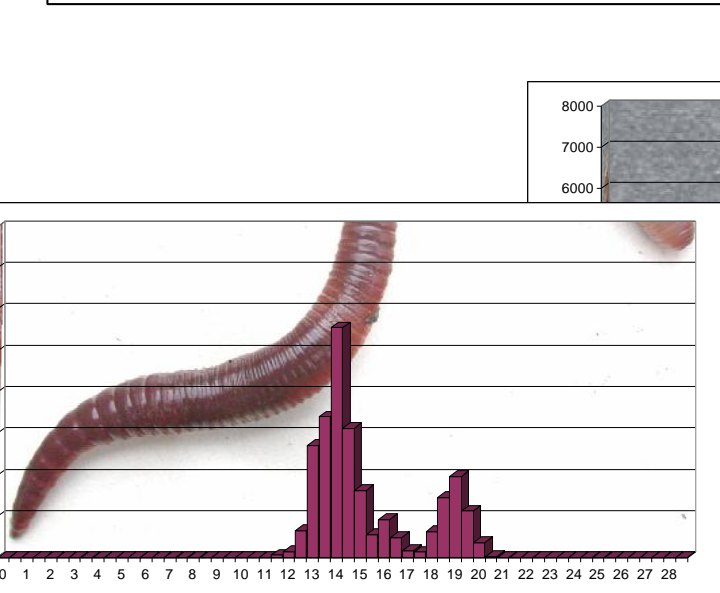
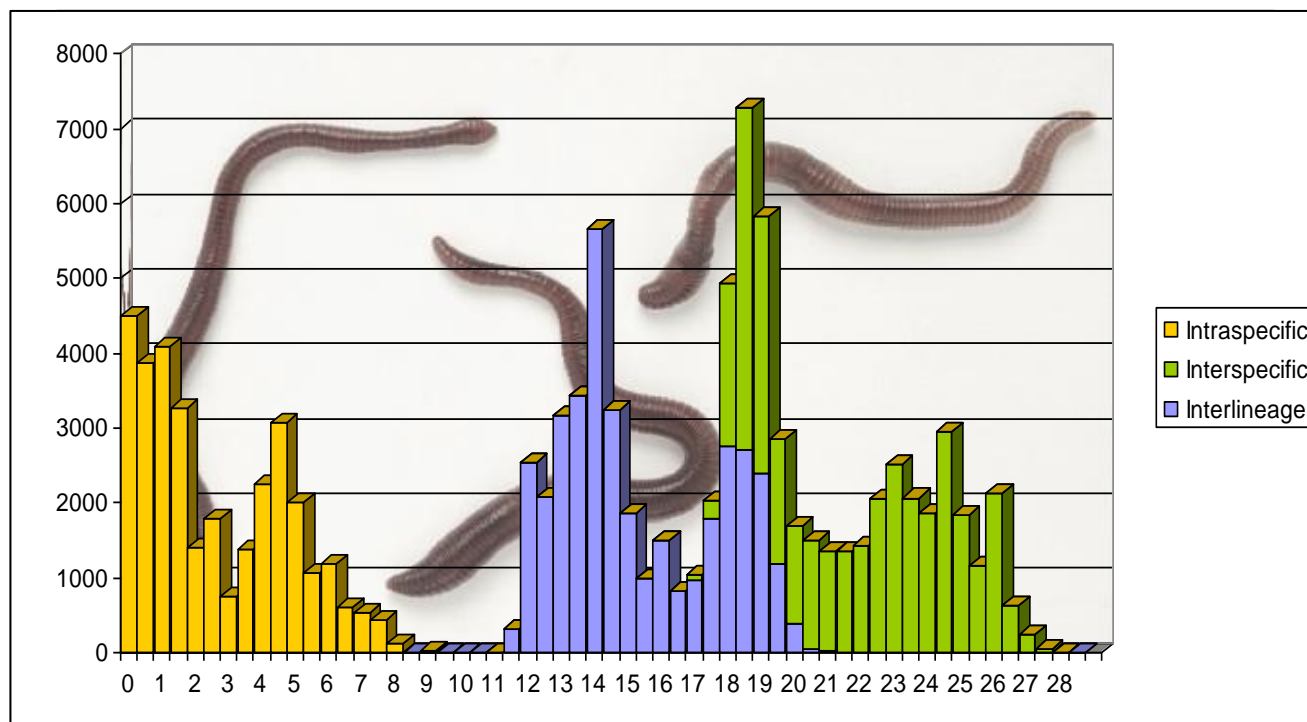


Species	Ni		Npop	Nlineages
<i>Allolobophora chlorotica</i>	264	France, UK, Norway, Romania, Denmark, Canada, US	13	5
<i>Aporrectodea caliginosa</i>	142	France, Canada, US, UK, Denmark	17	3
<i>Aporrectodea longa</i>	50	France, Canada, UK, Denmark	13	1
<i>Aporrectodea rosea</i>	68	France, Canada, US, Romania, Denmark	8	4
<i>Dendrobaena octaedra</i>	62	France, Canada, US, Denmark, Hungary	9	1
<i>Dendrodrilus rubidus</i>	35	France, Canada, US, Denmark	5	1
<i>Lumbricus castaneus</i>	33	France, Canada, US, Denmark, UK	9	1
<i>Lumbricus rubellus</i>	48	France, Canada, US, Denmark, Hungary, UK	13	2
<i>Lumbricus terrestris</i>	182	France, Canada, US, Denmark, UK	14	2
<i>Octolasion cyaneum</i>	30	France, Canada, US	4	1

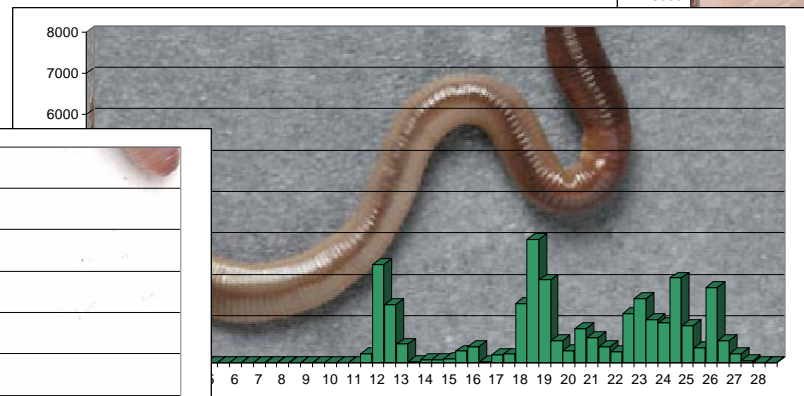
New 'lineages' in well known species

- Cryptic diversity
 - *Allolobophora chlorotica* (King et al. 2008)
 - *Aporrectodea caliginosa* (Perez-Losada et al. 2009)
 - *Lumbricus terrestris* ...
- Of interest for invasive species problematic

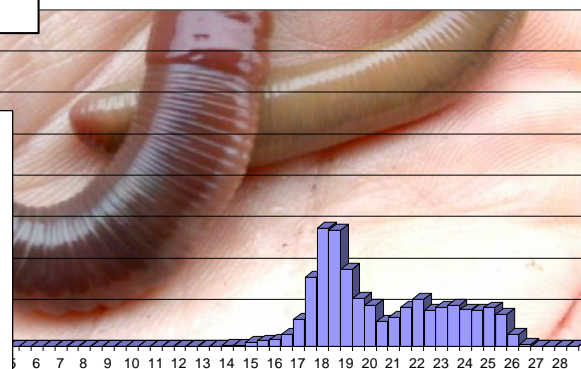




Allolobophora chlorotica
5 COI lineages

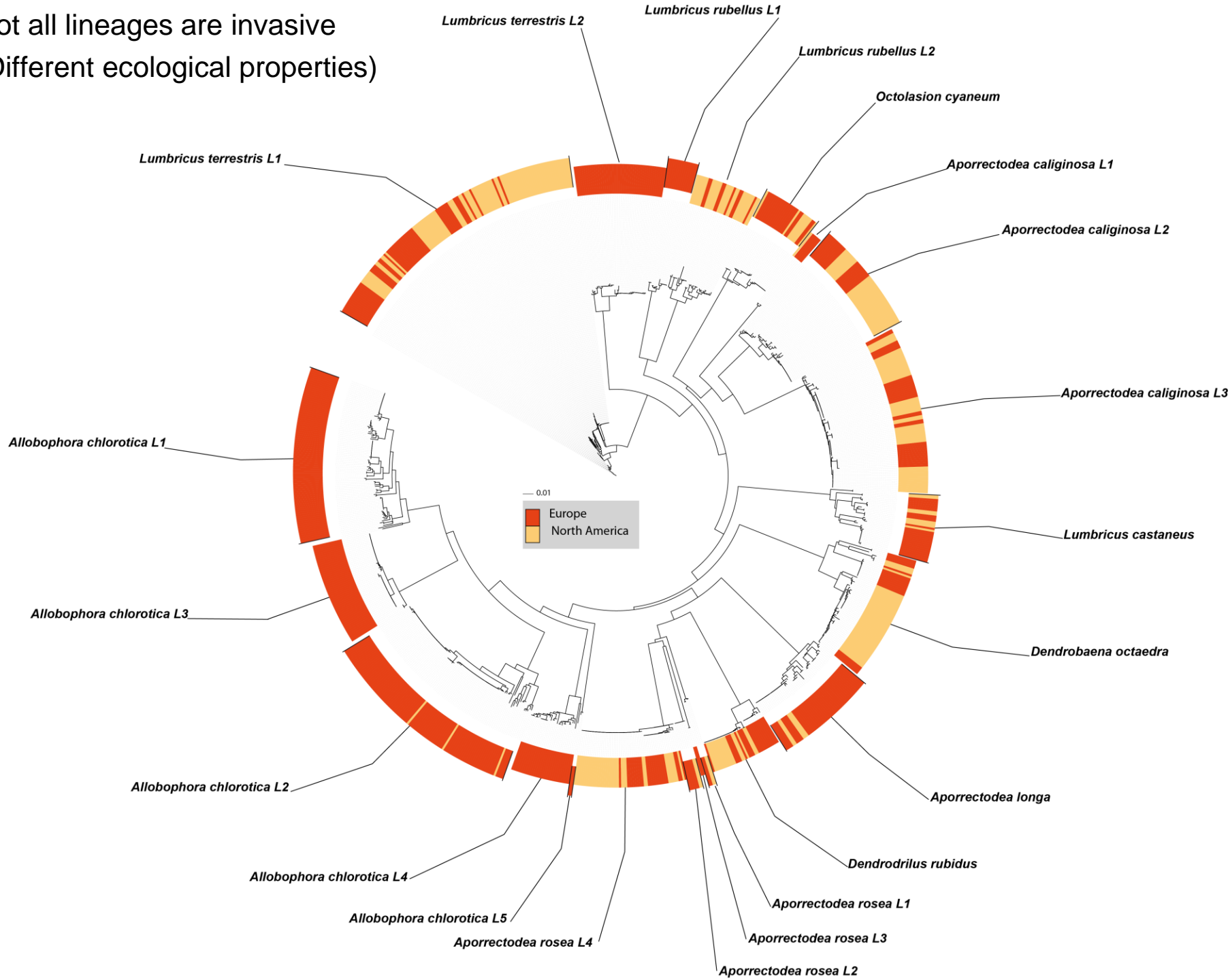


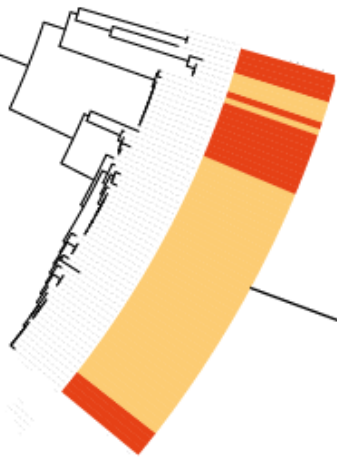
Aporrectodea
3 species + 7 COI lineages



Lumbricus
3 species + 4 COI lineages

Not all lineages are invasive
(Different ecological properties)





	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>Dendrobaena octaedra</i> Europe	0.600+-0.113	0.03795+-0.00633	4
<i>Dendrobaena octaedra</i> North America	0.919+-0.026	0.02030+-0.00449	5

Dendrobaena octaedra

- Epigeic (first, able to use undisturbed forest floor)
- Well established → $h >$ in North America
- Used as fishing bait → several introduction events
- Acid resistant species
- Very high resistance to freezing conditions (-20C)



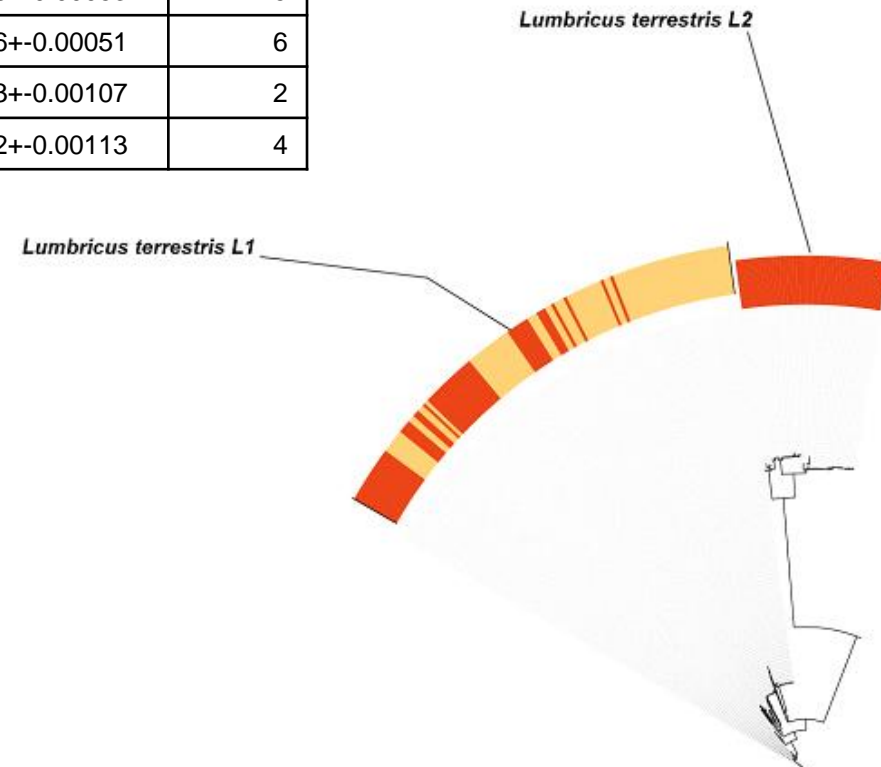
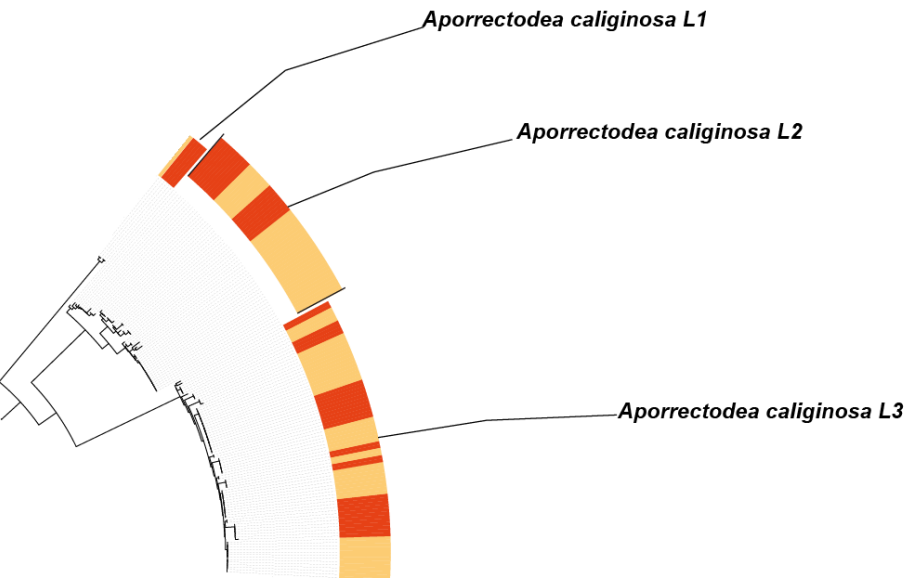
- Anecic
- only L1 in North America

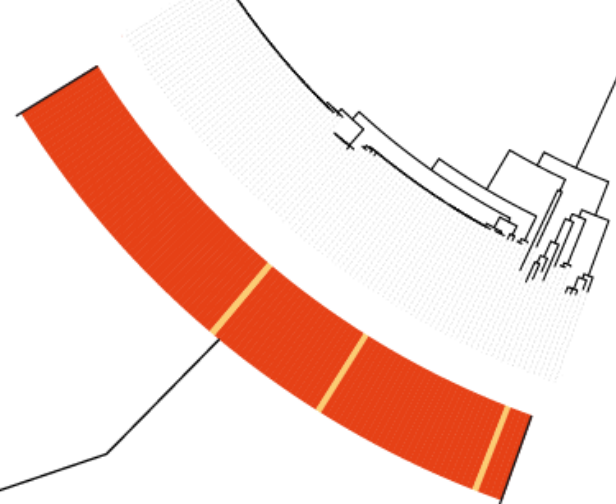
	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>L. terrestris</i> L1 Europe	0.845+-0.030	0.01802+-0.00261	6
<i>L. terrestris</i> L1 North America	0.836+-0.026	0.03494+-0.00142	6

- Well established \rightarrow h and π similar in Europe and North America
- Used as fishing bait \rightarrow several introduction events
- Acid tolerant



	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>Aporrectodea caliginosa</i> L3 Europe	0.832+-0.024	0.00558+-0.00053	5
<i>Aporrectodea caliginosa</i> L3 North America	0.877+-0.034	0.00676+-0.00051	6
<i>Aporrectodea caliginosa</i> L2 Europe	0.672+-0.075	0.00583+-0.00107	2
<i>Aporrectodea caliginosa</i> L2 North America	0.890+-0.049	0.01322+-0.00113	4





Allobophora chlorotica L2

- Only 3 specimens of L2 encountered in the whole North American sampling effort
- Already noted to be very rare (<1% in forest soil)
- Support badly acid soil
- Not resistant to freezing
- Not used as fishing bait or in vermicomposting

- No bottleneck (h and π even with Europe)

→ Characteristic of multiple introductions

- Already described for marine invasions
- Ecological resistance overwhelmed by propagule pressure



These worms are LUMBRICUS TERRESTRIS, also known as Canadian Nightcrawlers. We weigh the worms first then add a bunch of bedding into a breathable bag and ship them to you fast! These worms are **great for fishing**. They are the largest worm we sell, bigger than both the European and African nightcrawlers. These are the same worms you may buy at the bait shop. These are large worms, the perfect bait for that trophy fish.



Ma\$\$ive introduction

African Nightcrawlers

DESCRIPTION

These worms are Eudrilus Eugeniae, also known as African Nightcrawlers. They are great for composting and can eat half their weight a day in food. They will take your table scraps and turn them into great compost for your garden and plants. These are bedrun which means they come in all different sizes. There will be some adult breeders as well as juveniles and cocoons.

We weigh the worms first then add a bunch of bedding into a breathable bag and ship them to you fast! We are expert worm farmers and are willing to answer any questions our customers have about raising them. These worms are also **great for fishing**. They are the second largest worm we sell, only slightly smaller than the Canadian nightcrawlers. These are the same worms you may buy at the bait shop. These are large worms, the perfect bait for that trophy fish.

DO NOT deliver worms to HAWAII, due to legal restrictions from the Hawaii Department of Agriculture.

Item	Shipping	Total Price	Add to Cart
2.5 lb. (Approx 250 worms)	\$11.00 FREE!	\$67.99	Add to Cart
5 lbs.	\$15.00 FREE!	\$129.99	Add to Cart

BAIT Euro Nightcrawlers Fishing Worms 2 lb Pack



Our Price:\$48.95

Quantity:

[Add to Cart](#)

[Proceed to Checkout](#)

Other Images:



[click to enlarge](#)

Eisenia Fetida) Composting Worms

from Composting

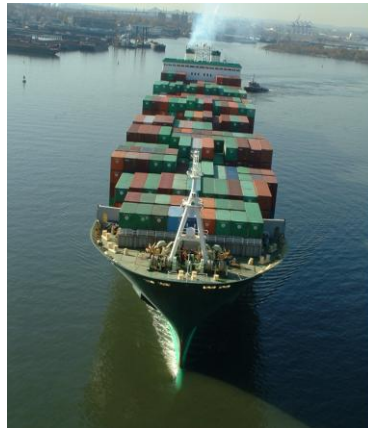
"ended" Composting Worms!

ow your source for
ns! Our customers love the
and now we are able to help



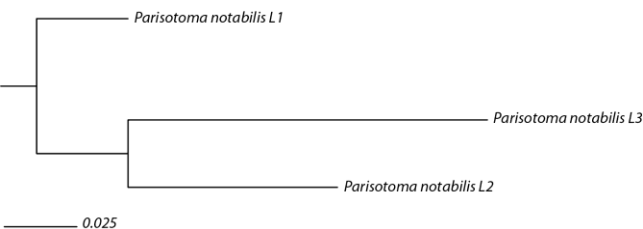
Collembola the unnoticed European invasion

- Unnoticed on continents
- Accidentally introduced with plants/soil/organic material import



- 7 species
- 293 barcodes + 38 Genbank sequences

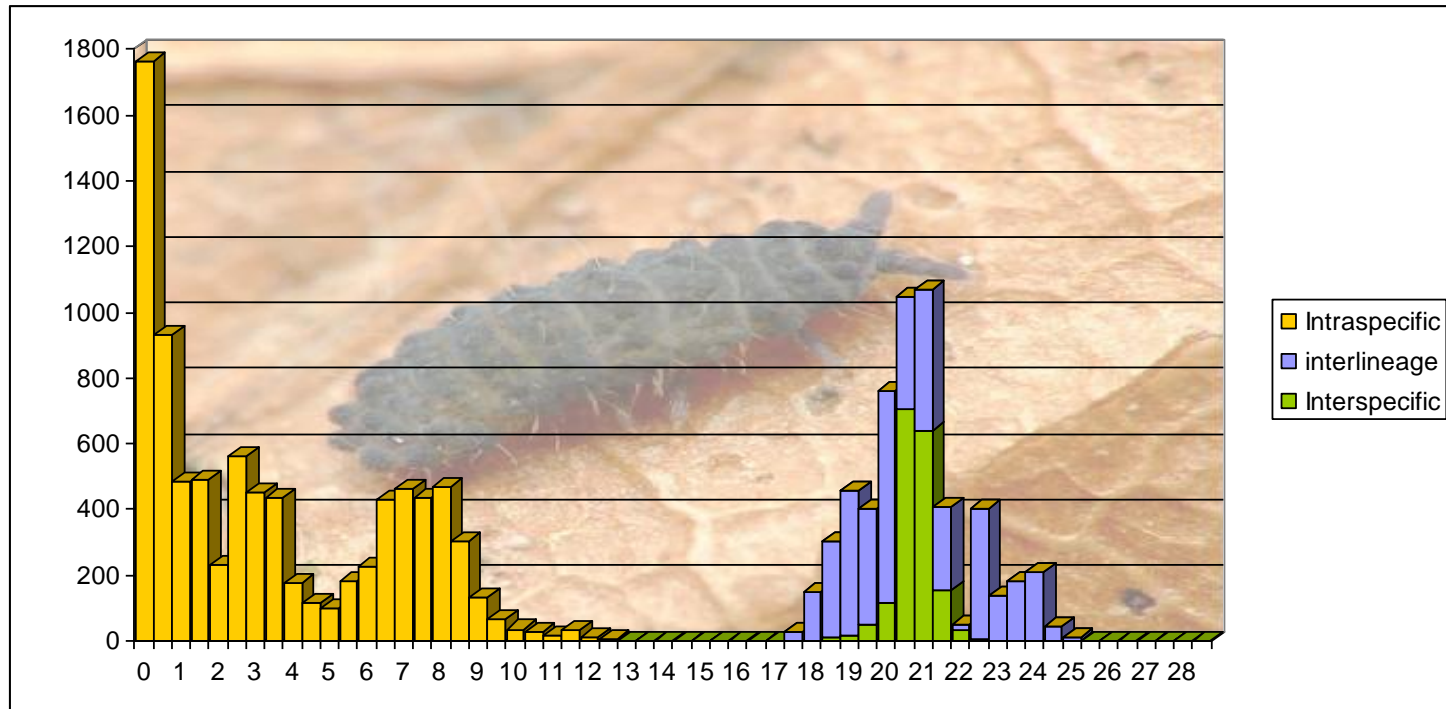
Species	Ni	Npop		Nlineages
<i>Isotomurus maculatus</i>	47	3	Belgium, South Africa, France	1
<i>Isotomurus unifasciatus</i>	7	2	Belgium, Australia	1
<i>Neanura muscorum</i>	43	7	France, New Zealand, Canada	1
<i>Orchesella cincta</i>	42	5	France, Denmark, Canada	1
<i>Orchesella villosa</i>	33	5	Belgium, France, Canada	1
<i>Parisotoma notabilis</i>	133	10	France, Canada	3
<i>Sminthurinus elegans</i>	26	6	France, Canada, US	1



Intraspecific distances 2.2%

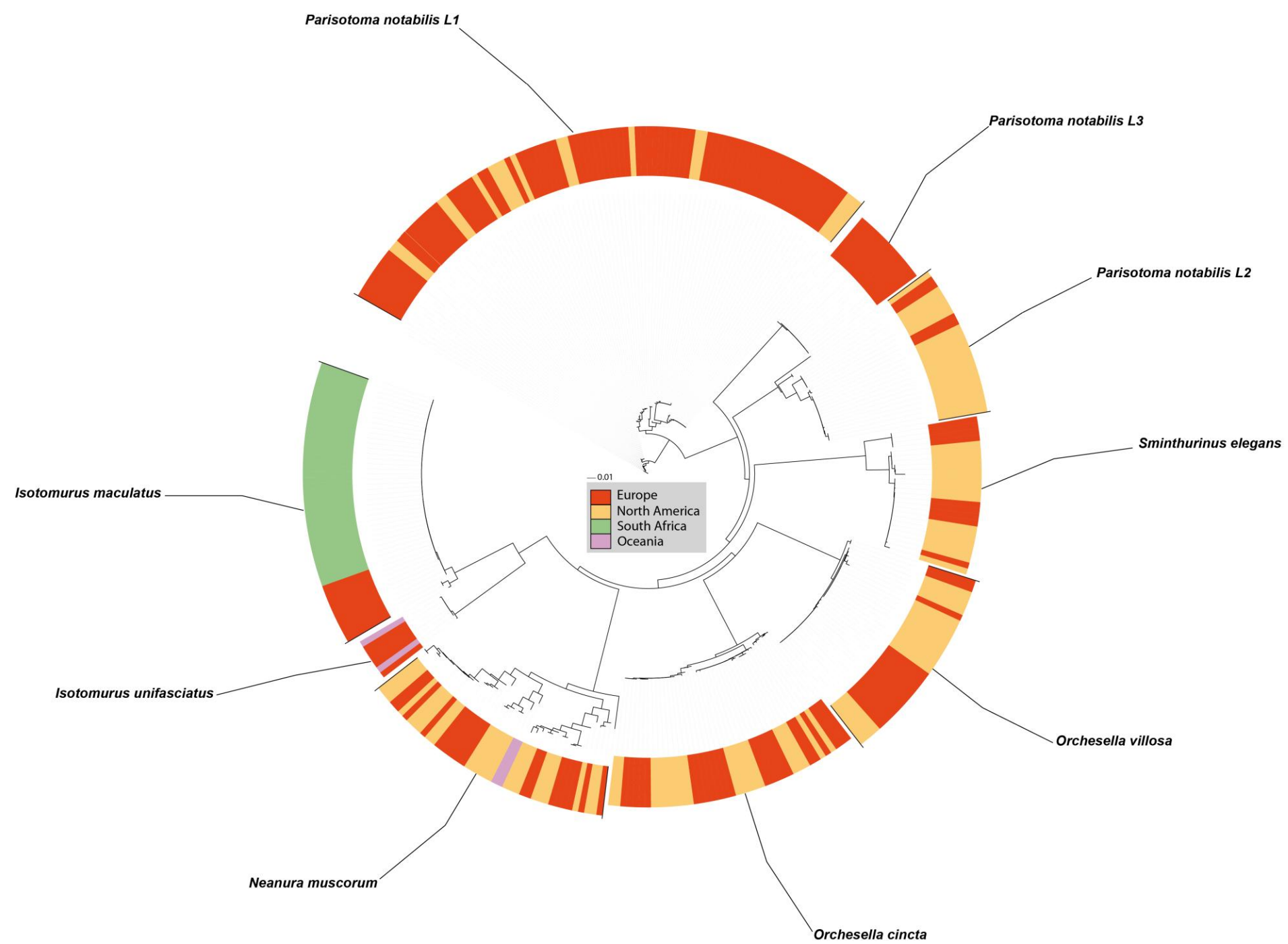
Interspecific distances 17.8%





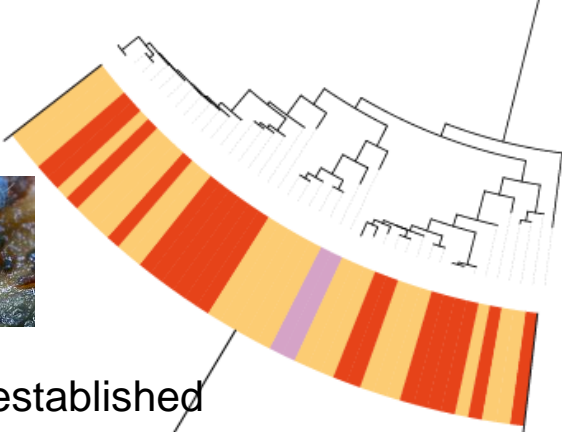
Mean Intraspecific = 1.8%

Mean Interspecific = 19.23%



	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>Neanura muscorum</i> Europe	0.869+-0.049	0.01398+-0.00203	4
<i>Neanura muscorum</i> North America	0.842+-0.049	0.02140+-0.00362	2

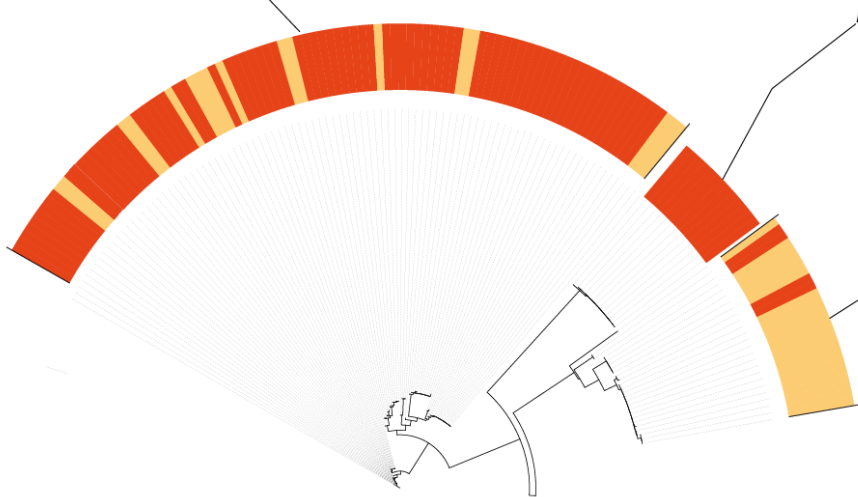
- L3 not present in North America
- h and π even with European populations → large populations well established



Neanura muscorum

	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>Parisotoma notabilis</i> L1 Europe	0.722+-0.070	0.02840+-0.00561	4
<i>Parisotoma notabilis</i> L1 North America	0.856+-0.017	0.03115+-0.00109	3

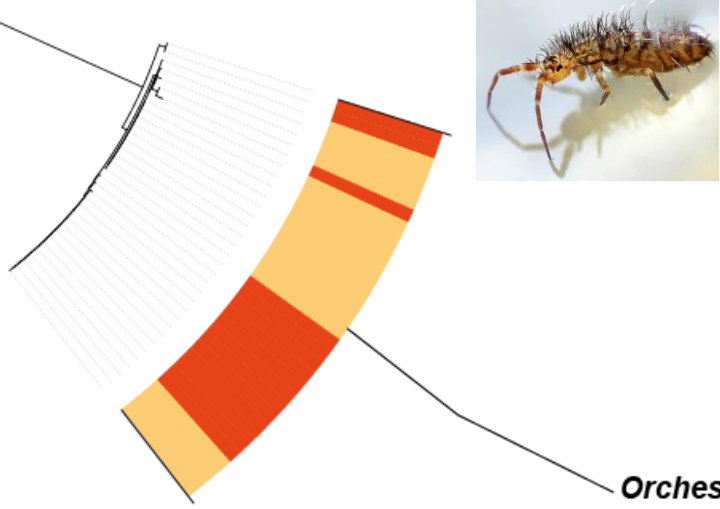
Parisotoma notabilis L1



Parisotoma notabilis L3

Parisotoma notabilis L2

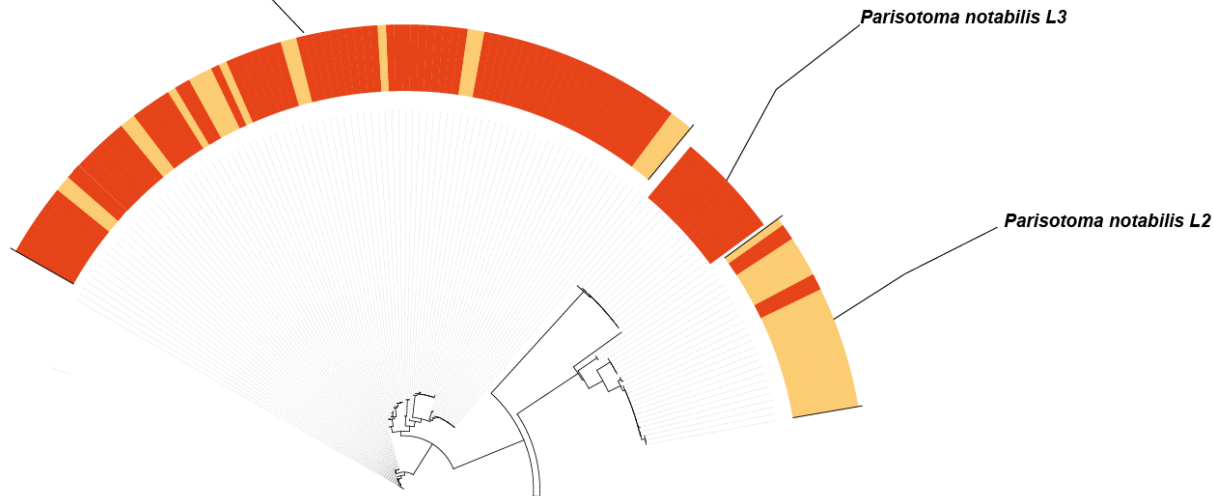




	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>Orchesella villosa</i> Europe	0.362+-0.145	0.00126+-0.00055	3
<i>Orchesella villosa</i> North America	0.366+-0.112	0.00073+-0.00022	2

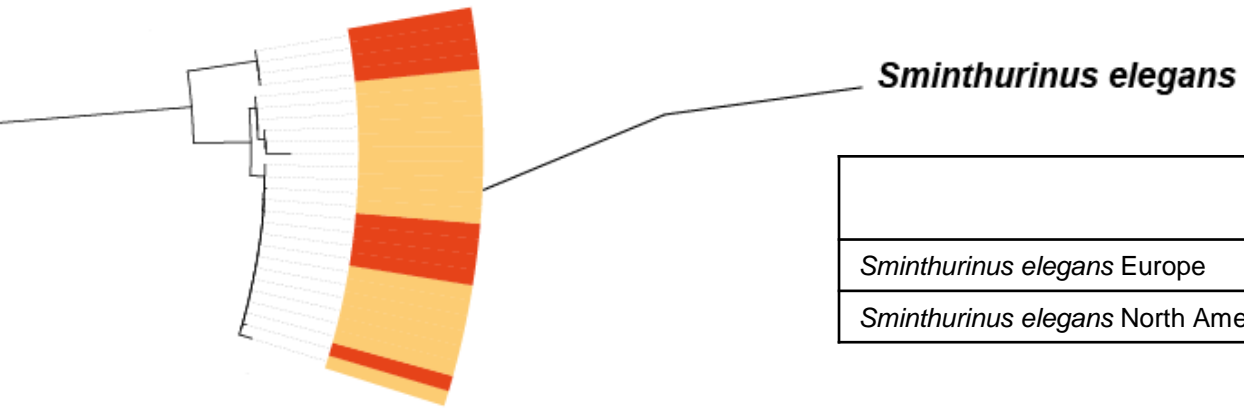
- h and π even with European populations → large populations well established

Parisotoma notabilis L1



	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>Parisotoma notabilis</i> L1 Europe	0.722+-0.070	0.02840+-0.00561	4
<i>Parisotoma notabilis</i> L1 North America	0.856+-0.017	0.03115+-0.00109	3

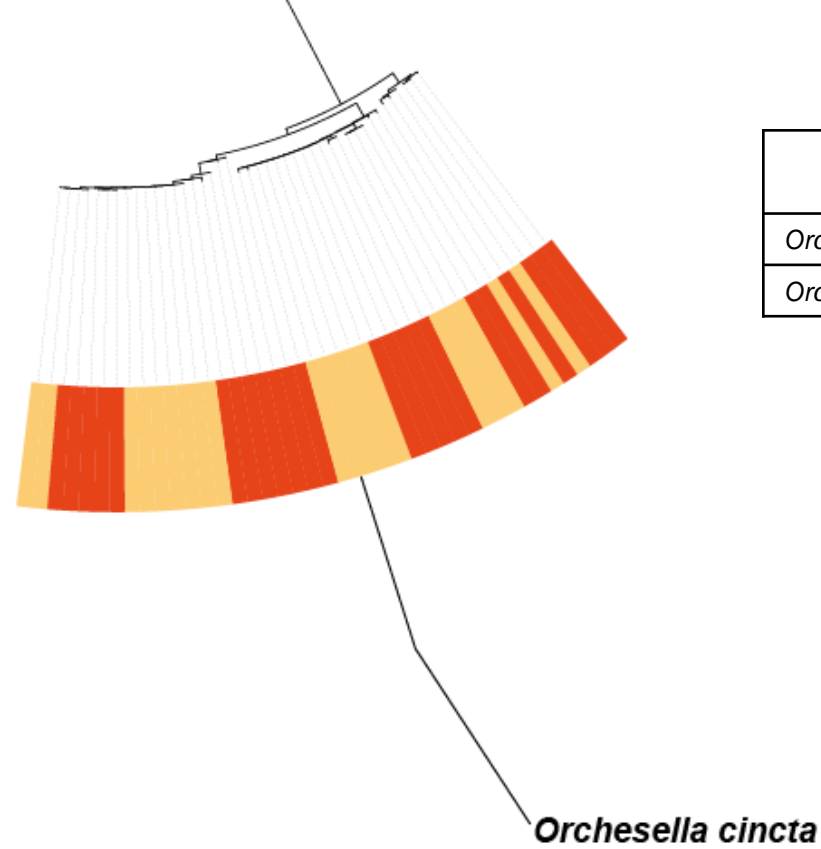




	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>Sminthurinus elegans</i> Europe	0.667+-0.105	0.03716+-0.00571	3
<i>Sminthurinus elegans</i> North America	0.522+-0.101	0.00434+-0.00125	3



- lower h and π for North American populations
→ recent colonization



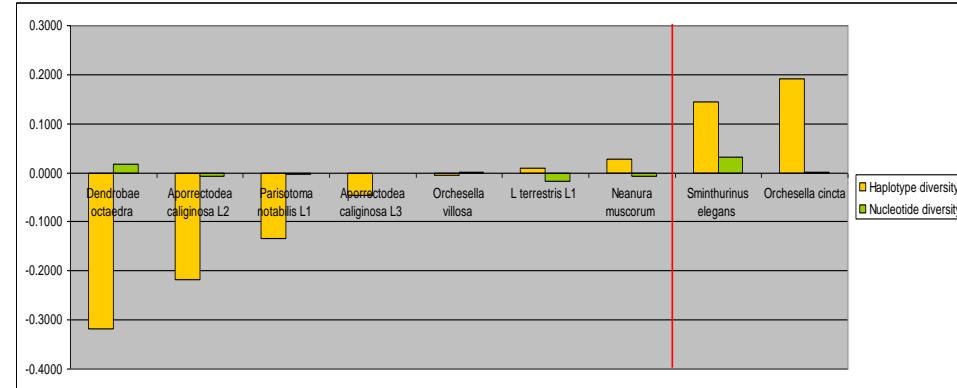
	Haplotype diversity (h)	Nucleotide diversity (π)	Npop
<i>Orchesella cincta</i> Europe	0.818+-0.057	0.00946+-0.00077	4
<i>Orchesella cincta</i> North America	0.626+-0.067	0.00869+-0.00114	3



- $h_{NA} < h_{Europe} \rightarrow$ settled from a limited amount of populations

Pattern Collembola / Lumbricidae

- Large populations well established



- Collembola\Unintentional = species spreading from a limited number of populations or low effective populations
- Link between the 2 Invasions?





Disrupt soil stratification
Change structure
Change organic matter availability
pH...

Indirect impact on the more
specialized native species

Pressure on mesofauna
Epigeic → less organic matters available
Endogeic → No layer devoid of organic matter

Invasive earthworms

Direct competition with native for resources

Disequilibrium with specific impact
- Increase density (temp)
- Decrease diversity (def)
Specialist/endemic species

Good conditions for invasive species
in mesofauna





Invasions of various groups linked together

→ A more global consideration of the biota?





A good tool for invasion monitoring in soil

- Remove the taxonomic impediment (well documented reference libraries) and morphologic characters limitations
 - All life stages
 - All conditions
- Allows to monitor more finely (cryptic diversity)
- Allows survey in different groups at the same time + Propagule pressure
- Low cost
- Fast
 - Ideal for massive routine ID
 - Critical to uncover early invasion events
- Massive parallel sequencing techniques...



Many thanks to the lab and collection department teams



Funded by



GenomeCanada



OntarioGenomicsInstitute



**NSERC
CRSNG**