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Genetic population structure of Protostrongylidae nematodes from an isolated population of European brown hare (*Lepus europaeus*, Pallas 1778)

Objective – The aim of the project is to verify whether the genetic structure of the parasites population has a genetic variability compatible to the hypothesis of a geographic isolation of their host from Pianosa Island since the late Pleistocene (Mengoni et al., 2018).

Materials and Methods – 739 adult parasites were collected from 20 hares from Pianosa Island and 80 adults parasites were collected from 2 hosts from mainland (Grosseto, Tuscany) according to Lesage et al., 2014. The caudal part of all males (Fig.1) was used for the morphological identification of the species, later molecularly confirmed by the Internal Transcribe Spacer (ITS) on a subsample of nematodes. The assessment of intraspecific genetic structure of nematodes was then analyzed by the 2 mitochondrial markers Cytochrome Oxidase subunit 1 (COI) and NADH-dehydrogenase subunit 4 (NADH4) comparing sequences of 12 nematodes from Pianosa Island and 12 nematodes from mainland population of european hare (Grosseto).

Fig.1 – Detail of the caudal part of males

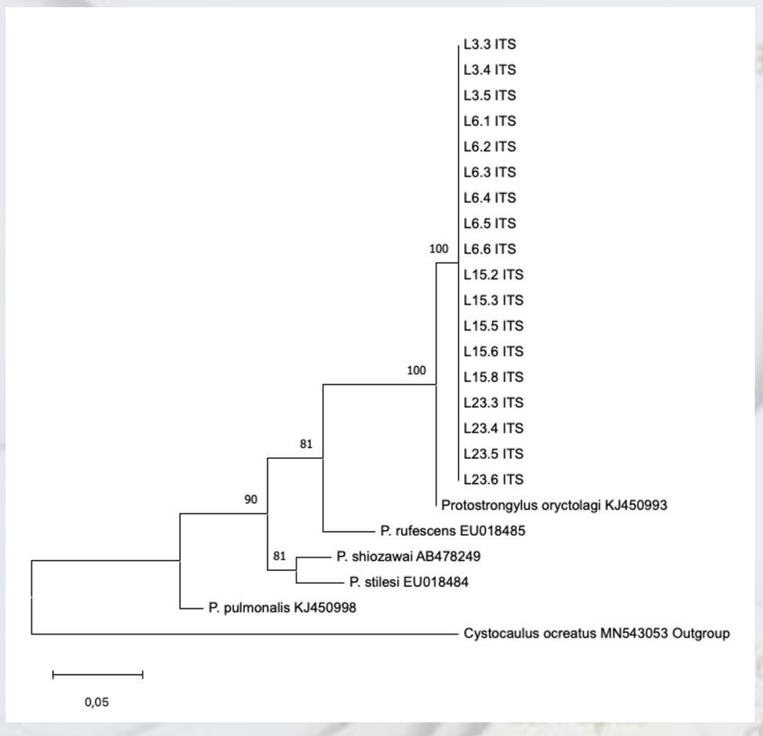

Fig.2 – Phylogenetic tree based on whole ITS gene of specimens from Pianosa Island.

C

Gene	COX													NADH4		
Position of Mutation		4	1	4	4	5	5	5	5	5	5	5	6	8	1 7	Haplotypes
	6	7	6	8	0	9	0	3	1	8	4	2	6	5	5	
L17.1_G	**G**	**G**	**A**	**T**	**G**	**A**	**A**	**A**	**A**	**C**	**T**	**T**	**G**	**T**	**C**	A
L17.2_G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
L17.6_G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
L17.7_G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
L6.3_P	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
L6.1_P	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	B
L6.2_P	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	B
L6.4_P	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	B
L23.5_P	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	B
L23.40_P	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	B
L50.1_G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	B
L50.3_G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	B
L50.6_G	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C
L50.7_G	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C
L17.5_G	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	D
L23.3_P	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	E
L23.4_P	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	F
L50.2_G	A	.	.	.	.	G	C	.	.	.	.	.	.	.	.	G
L17.8_G														.	.	A*
L6.5_P														C	A	B*
L6.6_P														.	.	A*
L50.4_G														.	.	A*
L23.6_P														.	.	A*
L23.112_P														.	.	A*

D

Haplotypes / Sites	A	B	C	D	E	F	G	Tot
Pianosa	4	6	0	0	1	1	0	12
Grosseto	6	2	2	1	0	0	1	12

Fig.4 – C= Highlights of intraspecific variable sites between specimens from the analysis of mithocondrial genes; Haplotypes shown from the most frequent; area in grey show sequences obtained only for NADH4 gene; * = samples with uncertain haplotype due to absence of COI gene sequences; D= Haplotypes frequences between the two sampling sites.

Results – The obtained results confirm that the ITS gene is optimal for species identification. 100% of analyzed subsample, belonged to a single species, *Protostrongylus oryctolagi* with 100% bootstrap support and a 100% BLAST sequence identity, (Fig.2), supporting thus the morphological identification. As regard the population structure, the preliminary analyzes on sequences variation of COI and NADH4 markers showed a slight but detectable variability.

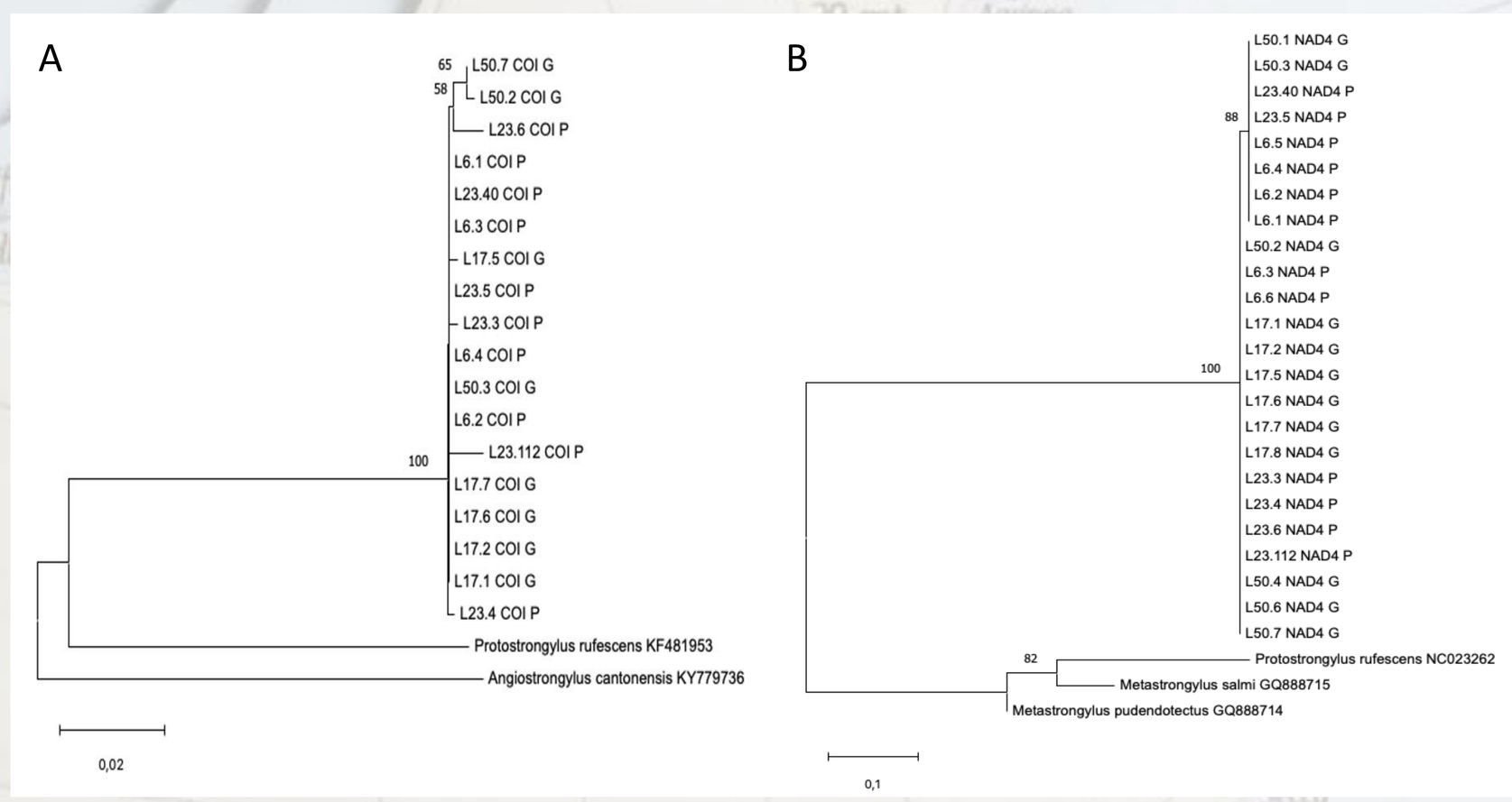

Fig.3 – Comparing analysis between specimens from Pianosa Island (P) and the mainland area near Grosseto (G); A= Phylogenetic tree based on COI gene (706bp); B= Phylogenetic tree based on NADH4 gene (209bp).

Conclusions – The mithocondrial markers used for population structure analyzes, confirm they represent useful targets to deepen intraspecific variability. Due to the low level of variability between isolated and mainland populations (Fig. 3 and 4), we can suggest that the isolation status of the hosts could not be attested to the late Pleistocene as described in literature but should be the result of a recent hosts introduction (hundreds of years instead of thousand).

Future Proposal – In order to enhance the knowledge on the genetic structure of Protostrongylidae, we are working on sequencing the complete mithocondrial DNA and to increase sampling from other mainland areas.

References

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- Mengoni C., Trocchi V., Mucci N., Gotti C., Giannini F., Mallia E., Geminiani C., Baccetti N., (2018). The secret of Pianosa island: an Italian native population of European brown hare (*Lepus europaeus meridiei* Hilzheimer, 1906). Conservation Genetics. **19**; 1513–1518.