

## **Supporting information**

### **Study area**

The Italian territory considered includes 5 provinces (Udine, Trieste, Belluno, Bolzano and Trento) of three Italian north-eastern regions (from east to west): Friuli Venezia Giulia, Veneto and Trentino Alto Adige (Fig 1). Elevation ranges from sea level up to approximately 3900 m (Ortles-Cevedale, Trentino Alto Adige), the total area is roughly 39,850 km<sup>2</sup> and the regions are characterized by very different ecosystems, such as a) forested areas in the Pre-Alpine and Alpine regions (Eastern Alps), b) rural farmlands, c) flatlands with cultivated fields and d) scattered farms and coastlands, both characterized by a more urbanized and industrial environment. This area shares its borders with Slovenia to the east and Austria to the north and is crossed by a number of highways (A22, A23), rivers (i.e. the Tagliamento, the Piave, the Brenta and the Adige rivers), canals and lakes (i.e. Lake Garda).

The Austrian territory covers an area of 83,879 km<sup>2</sup>, it includes part of the Rhaetian Alps and the whole group of the Eastern Alps (Austrian Alps, Tauern, Carnic and Noric Alps). Three-fifths (3/5) of the country are occupied by the Alpine region; moving eastward, the Eastern Alps descend gradually towards the Carpathians. One third (1/3) of the country located further west consists of a corridor between Germany and Italy, 32-60 km wide. The Alps (Julian Alps, Kamnik-Savinja Alps, Karavanke, Pohorje) also dominate Northern Slovenia along its border with Austria and Italy.

Slovenia covers 20,273 km<sup>2</sup> and most of the country is hilly or mountainous, with around 90% of the surface located at 200 meters or more above sea level.

Croatia's territory covers 56,594 km<sup>2</sup> and the morphology varies from the plains along the Hungarian border to the low mountains and islands. The area of the Black Sea drainage basin covers 62% of the Croatian territory and includes the largest rivers flowing across the country, the Danube, the Sava and the Drava.

### **Detection of rabies and canine distemper viruses**

Field samples were screened for rabies infection through the Fluorescent Antibody Test (FAT), as elsewhere described [1]. All positive samples were confirmed in parallel through viral isolation [1] as well as a one-step RT-PCR amplification, as previously described [2].

The detection of canine distemper virus was performed by using the QuantiTect Multiplex RT-PCR kit (Qiagen, Hilden, Germany), following a real-time RT-PCR protocol which amplifies a small region of the N gene coding for the viral nucleoprotein. Primers sequences and protocols are reported by Elia et al., 2006 [3].

**Table A. Sampling frequencies according to Nation.**

A. Italy									
	Period			Disease status			Region		
	2006-07	2008-09	2010-11	CDV POS	Rabies POS		Friuli VG*	Veneto	Trentino AA**
					(Italy1+Italy2)				
379 Samples	39	106	234	148	95(25+70)	136	96	97	186
B. Austria									
	Period			Disease status					
	2010	2011	2012	CDV POS	Rabies POS	NEG			
	98 Samples	-	51	47	8	-	90		
C. Slovenia									
	Period			Disease status					
	2010	2011	2012	CDV POS	Rabies POS	NEG			
	86 Samples	53	33	-	10	-	76		
D. Croatia									
	Period			Disease status					
	2010	2011	2012	CDV POS	Rabies POS	NEG			
	64 Samples	-	-	64	7	-	57		
627 Samples (Total)									

\*Friuli Venezia Giulia; \*\*Trentino Alto Adige

Sampling frequency according to period, region and disease status for Italy (**A**); sampling frequency according to period and disease status for Austria (**B**), Slovenia (**C**), Croatia (**D**).

**Table B. Genetic differentiation between pairs of red fox clusters (membership probability  $Q \geq 0.8$ ).**

	Cluster1	Cluster2	Cluster3	Cluster4
Cluster1		0.001	0.001	0.001
Cluster2	0.042		0.001	0.001
Cluster3	0.045	0.039		0.001
Cluster4	0.042	0.039	0.047	

Pairwise values of  $F_{st}$  in the lower triangle of the matrix and p-values in the upper triangle, among four clusters composed by individuals with membership probability  $Q \geq 0.8$  (GenALEx).

**Table C. Alleles and Heterozygosity: complete dataset.**

Locus	N	Na	Ne	Ho	He	F
FH2010	626	4.000	2.817	0.599	0.645	0.071
C04-140	625	13.000	6.025	0.810	0.834	0.029
C01-424	624	7.000	3.293	0.647	0.696	0.070
FH2001	624	23.000	5.931	0.796	0.831	0.042
RF-CPH2	625	5.000	2.143	0.509	0.533	0.046
FH2328	612	24.000	4.999	0.735	0.800	0.081
CPH18	627	14.000	4.238	0.675	0.764	0.117
RF-CXX468	627	10.000	6.226	0.796	0.834	0.045
FH2848	626	16.000	7.246	0.823	0.862	0.046
RF-CPH3	625	16.000	9.838	0.875	0.898	0.026
RF-INU055	627	10.000	4.922	0.767	0.797	0.037
RF-REN169O18	627	16.000	8.726	0.843	0.885	0.047
AHT-137	627	12.000	6.872	0.813	0.854	0.048
RF-REN162C04	624	12.000	7.548	0.856	0.868	0.014
RF-CXX402	626	8.000	5.034	0.738	0.801	0.079
AHT-121	625	16.000	7.619	0.829	0.869	0.046
RF-REN105L03	626	13.000	7.476	0.834	0.866	0.037
C08-618	627	11.000	5.835	0.790	0.829	0.047
RF-CPH11	626	8.000	2.236	0.548	0.553	0.009
RF-CXX-279	627	13.000	4.774	0.756	0.791	0.044
FH2088	626	15.000	6.368	0.762	0.843	0.096

N = No. of Samples; Na = No. of Different Alleles; Ne = No. of Effective Alleles =  $1 / (\sum p_i^2)$ ; Ho = Observed Heterozygosity = No. of Hets / N; He = Expected Heterozygosity =  $1 - \sum p_i^2$ ; F =  $(He - Ho) / He$

**Table D. Alleles and Heterozygosity: groups A and B (membership probability  $Q \geq 0.7$ ).**

Group		N	Na	Ne	Ho	He	F
Group A	Mean	262.095	11.905	5.593	0.762	0.787	0.032
	SE	0.168	0.971	0.448	0.024	0.023	0.008
Group B	Mean	285.238	10.571	5.453	0.745	0.784	0.049
	SE	0.478	0.782	0.427	0.023	0.022	0.008

N = No. of Samples; Na = No. of Different Alleles; Ne = No. of Effective Alleles =  $1 / (\sum \pi^2)$ ; Ho = Observed Heterozygosity = No. of Hets / N; He = Expected Heterozygosity =  $1 - \sum \pi^2$ ; F =  $(He - Ho) / He$

**Table E. Alleles and Heterozygosity: clusters 1, 2, 3, 4 (membership probability  $Q \geq 0.7$ ).**

Cluster		N	Na	Ne	Ho	He	F
Cluster 1	Mean	38.952	8.286	4.904	0.732	0.756	0.035
	SE	0.048	0.598	0.428	0.030	0.025	0.016
Cluster 2	Mean	57.571	8.333	4.946	0.753	0.769	0.020
	SE	0.289	0.607	0.352	0.023	0.021	0.015
Cluster 3	Mean	54.952	8.667	4.725	0.718	0.756	0.050
	SE	0.048	0.630	0.357	0.031	0.023	0.018
Cluster 4	Mean	57.952	9.190	5.437	0.750	0.779	0.035
	SE	0.048	0.639	0.423	0.027	0.026	0.017

N = No. of Samples; Na = No. of Different Alleles; Ne = No. of Effective Alleles =  $1 / (\sum \pi^2)$ ; Ho = Observed Heterozygosity = No. of Hets / N; He = Expected Heterozygosity =  $1 - \sum \pi^2$ ; F =  $(He - Ho) / He$

## References

1. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018. Chapter 2.1.17, Rabies (infection with rabies virus and other lyssaviruses). 2018. Available at: [http://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/2.01.17\\_RABIES.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.17_RABIES.pdf).
2. De Benedictis P, De Battisti C, Dacheux L, Marciano S, Ormelli S, Salomoni A, Caenazzo ST, Lepelletier A, Bourhy H, Capua I, Cattoli G. Lyssavirus detection and typing using pyrosequencing. J Clin Microbiol. 2011 May;49(5):1932-8. doi:10.1128/JCM.02015-10.
3. Elia G, Decaro N, Martella V, Cirone F, Lucente MS, Lorusso E, Di Trani L, Buonavoglia C. Detection of canine distemper virus in dogs by real-time RT-PCR. J Virol Methods. 2006 Sep;136(1-2):171-6.