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Genomic characterization of Croatian local pig breeds

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Content

1. Diversity of modern pigs
2. Structure of pig populations/breeds in Croatia
3. Methodologies of genomic characterization
4. Croatian local pigs in world/European genetic context
5. Conclusion

Diversity of modern pigs

- The worldwide pig population is dominated by five breeds or, generally by their hybrids
  - Large White
  - Duroc
  - Landrace
  - Piétrain
  - Hampshire
- However, across the world, nearly 400 breeds have been exploited, with the largest number of breeds being found in Asia and Europe (Groeneveld et al., 2010).

PIC®

Hypor

Topigs Norsvin

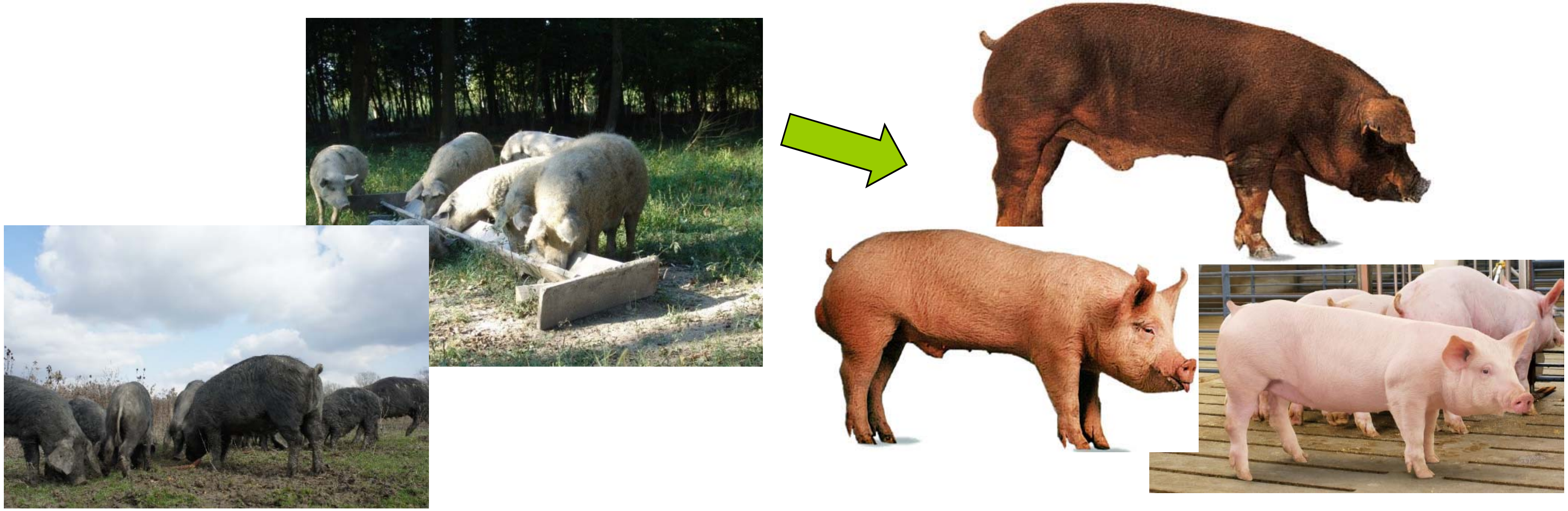
CHOICE
Caring for life

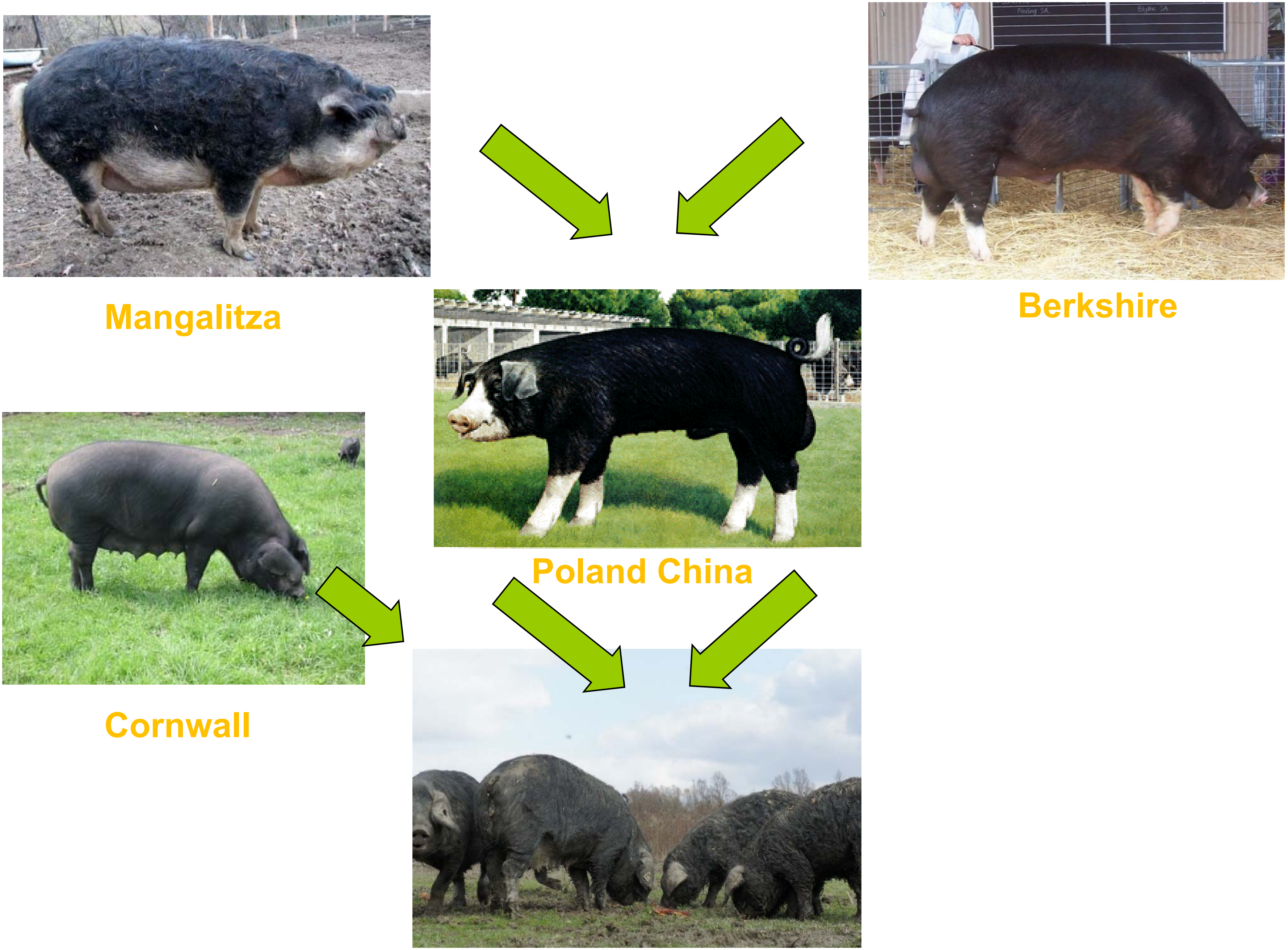
RA • SE GENETICS
EASY2MANAGE

DANBRED

CONSERVATION

In pig breeding during the last decades, highly productive breeds developed mainly by breeding companies have replaced the local breeds which are essential resources of genetic diversity (FAO, 2007).





Mangalitza

Berkshire

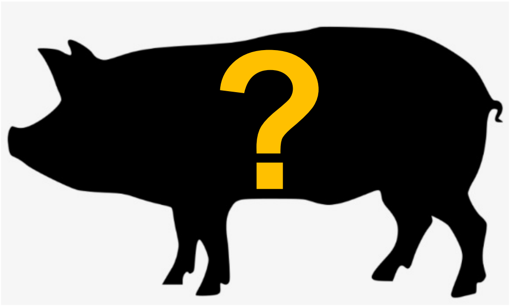
Poland China

Cornwall

BLACK SLAVONIAN

1.

Siska



Local pigs

TUROPOLJE PIG

2.

Krskopolje pig

TUROPOLJE PIG

3.

Siska

Berkshire

Krskopolje pig

TUROPOLJE PIG

Regions of breeding

Breeding program

1. Destiny for both breeds is not bright
2. Black Slavonian breed is better positioned in that context as their carcass traits better suits today’s market demands

BLACK SLAVONIAN

3. Turopolje breed is a typical lard type of pig which is no longer profitable for farmers

GOAL – Explore genomic diversity

- Utilize high throughput genomic information
- Calculate shared genetic coancestry using multidimensional scaling (MDS)
- Estimate runs of homozygosity (ROH) based inbreeding level and admixture
- Estimate genetic admixture using STRUCTURE
- Estimate population structure using fixation index
- Identify specific genome regions

Material and methods

1. A total of **32, 16 Black Slavonian** (six boars and 10 sows) and **16 Turopolje** (four boars and 12 sows)
2. **Illumina PorcineSNP60** v2 **Genotyping** BeadChip with **64,232 SNPs**
3. Autosomal SNPs were used for analyzes.
4. SNPs where more than **10% of genotypes were missing and SNPs** with Illumina GenCall score ≤0.7 or Illumina GenTrain score ≤0.4 were excluded from analyzes.
5. Pigs with **> 5% of missing genotypes** were also excluded from further analysis.
6. **Sscrofa 10.2** (EnsEMBL db version 83).

Material and methods

- We used additional dataset **in order to compare with ours** (Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.30tk6); Yang et al., 2017.
- This data set was then merged with ours, counting **45,000 SNPs** in total and **931 animals** from **48 breeds (nine wild).**
- SNP genotypes were used to calculate **shared genetic coancestry** between all individuals of all breeds included in the analyzes in **R 3.4.3 Software** by computing all **pairwise proportion of alleles identical-by-state (IBS).**

Material and methods

- The obtained matrix was transformed to a **distant matrix**, on which classical **Multidimensional Scaling (MDS)** was applied in the principal component analysis (PCA).
- The remaining **"final data set"** consisted of **556 animals sampled from 30 breeds**, including six wild boar populations.

ABBRIVIATION	COUNTRY	POPULATION
DEAS	Germany	Angler Sattleschwein
ESCM	Spain	Chato Murciano
ESIB	Spain	Iberian
HRWB	Croatia	Croatia WildBoar
HUMA	Hungary	Hungary Mangalica
IBWB	Spain	Iberian WildBoar
ITCA	Italy	Italy Calabrese
ITCS	Italy	Italy Cinta_Senese
ITCT	Italy	Italy Casertana
ITNS	Italy	Italy Nera Siciliana
ITWB1	Italy	Italy WildBoar
ITWB2	Italy	Italy Sardinia WildBoar
LDR1	Commercial	Landrace 1
LDR2	Commercial	Landrace 2
NEWB	Europe	NW European WildBoar
PIT1	Commercial	Pietrain 1
PIT2	Commercial	Pietrain 2
PLPS	Poland	Poland Pulawska Spot
PTBI	Portugal	Portugal Bisaro
SBWB	Euope Med	South Balkan WildBoar
TRPR	Czech Republic	Czech Prestice
UKBK	UK	UK Berkshire
UKBS	UK	UK British Saddleback
UKGO	UK	UK Gloucester Old Spot
UKHS	UK	UK Hampshire
UKLB	UK	UK Large Black
UKTA	UK	UK Tamworth
USBK	USA	USA Berkshire
USHS	USA	USA Hampshire
USPC	USA	USA Poland China
CROCS	Croatia	Black Slavonian
CROTS	Croatia	Turopolje

Material and methods

GENETIC ADMIXTURE

- Performed on the "final data set", with a reduced number of 15,000 SNP genotypes, using a Bayesian approach implemented in the STRUCTURE 2.3.4 (Pritchard et al., 2000), without prior information on the population.
- We used a model with assumed admixture and correlated allele frequencies, as this provides a greater power to reveal populations that are closely related
- We performed analyses for the assumed K number of populations from 1 to 34, with 20 independent runs and a burn-in period of 10,000 followed by 100,000 MCMC repetitions.

Material and methods

GENETIC ADMIXTURE

- The choice of the most likely number of clusters (K) was determined according to recommendations provided in Pritchard et al. (2000) as well as according to visual presentations showing the rate of change in the ln Pr(G|K) between successive K values as recommended by Evanno et al. (2005). CLUMPAK (Kopelman et al., 2015) was used to estimate the maximum probability for K = 1 to 30 and average individual results among the 20 runs for each K (Jakobsson and Rosenberg, 2007) and over different K-s.
- Obtained results were visualized with pophelper 2.2.7 (Francis, 2017) package for R 3.5 (R Core Team, 2018).

Material and methods

RUNS OF HOMOZYGOSITY AND GENOMIC INBREEDING

- **ROH based genomic inbreeding coefficient** (F_{ROH}) was calculated following the concept described in McQuillan et al. (2008) and Curik et al., (2014) where F_{ROH} = Genome length in ROH / Autosomal genome length covered by SNP chip (here 2,444,159,506,432 bp spread over 18 chromosomes).
- The ROH were called if **15 or more consecutive homozygous SNP were present at a density of at least one SNP every 100 kb, with gaps of no more than 1000 kb between them.**
- One, two, and four missing calls per window were allowed for **ROH > 4 Mb, ROH > 8 Mb, and ROH > 16** Mb respectively, resulting in the identification of ROHs according to the classes of different length size.

Material and methods

RUNS OF HOMOZYGOSITY AND GENOMIC INBREEDING

- Subsequently, by merging the information related to each class we were able to calculate defined genomic inbreeding coefficients ($F_{ROH>4Mb}$ and $F_{ROH>8Mb}$).
- Additionally, we have calculated $F_{ROH4to8Mb}$ as a difference between $F_{ROH>4Mb}$ and $F_{ROH>8Mb}$.
- In this way, we were able to distinguish **$F_{ROH>4Mb}$ from the "remote" ($F_{ROH4to8Mb}$) inbreeding** arising from ancestors that are approximately from six to 13 generations remote, and "**recent" ($F_{ROH>8Mb}$) inbreeding, arising approximately within the last seven generations**

Material and methods

POPULATION STRUCTURE USING FIXATION INDEX

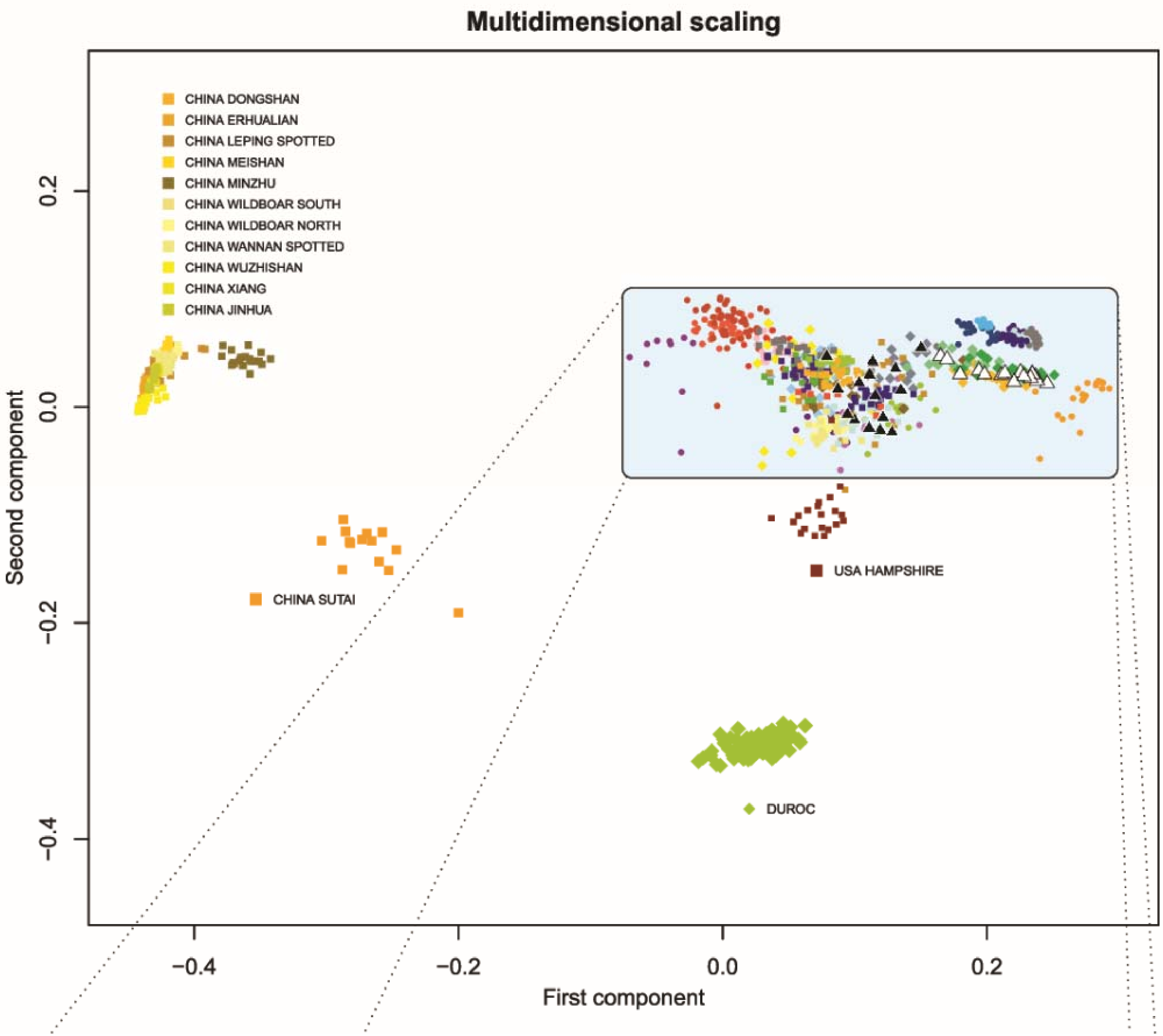
- The global genetic differentiation between the Croatian local breeds as well as all other world populations was assessed by the genome wide F_{ST} (according Weir and Cockerham, 1984) values for each SNP pair, calculated in PLINK (Purcell et al. 2007) and GenePop Version 4.7.0. (Rousset, F. 2008).

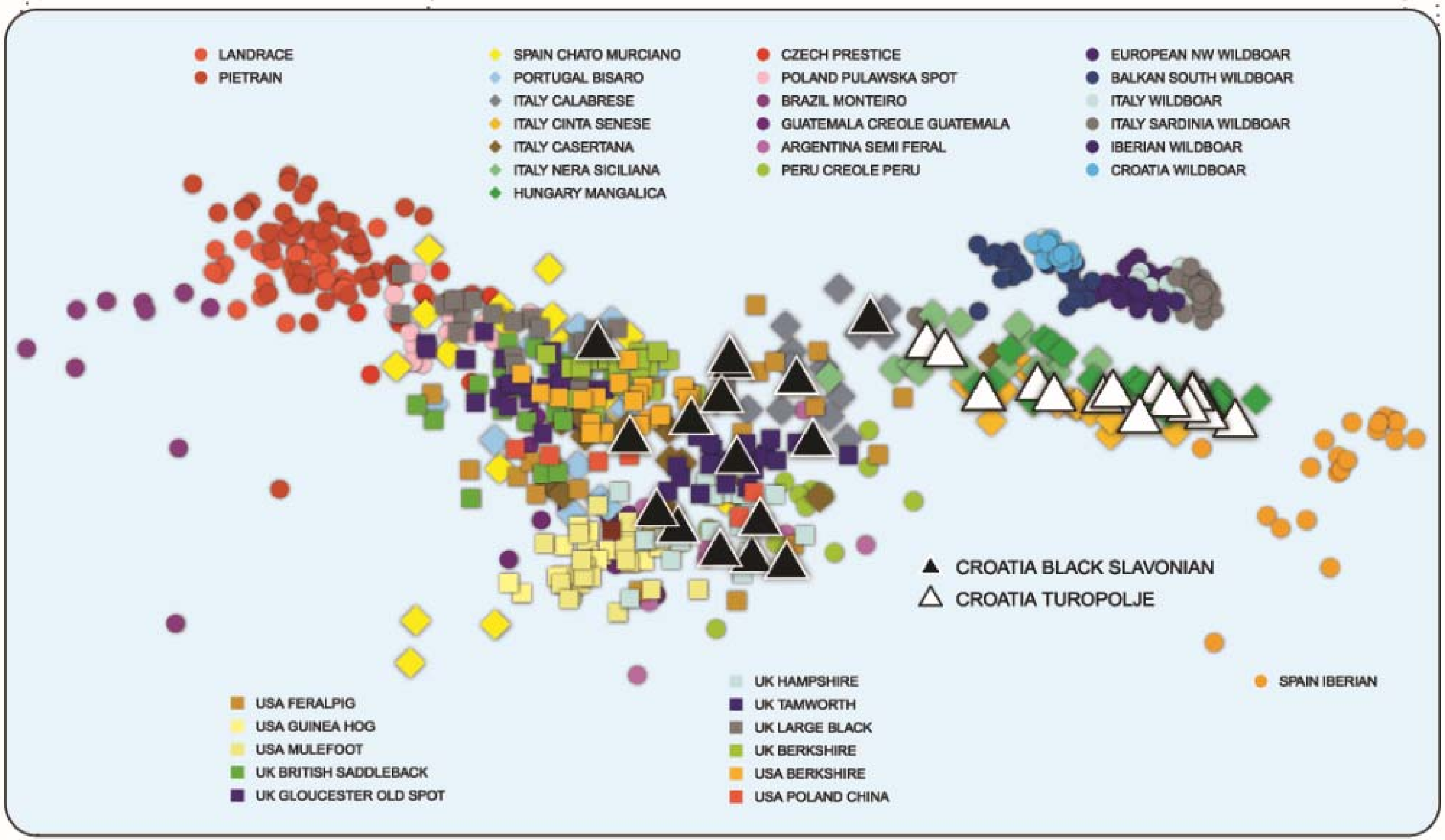
Material and methods

IDENTIFICATION OF SPECIFIC GENOMIC REGIONS

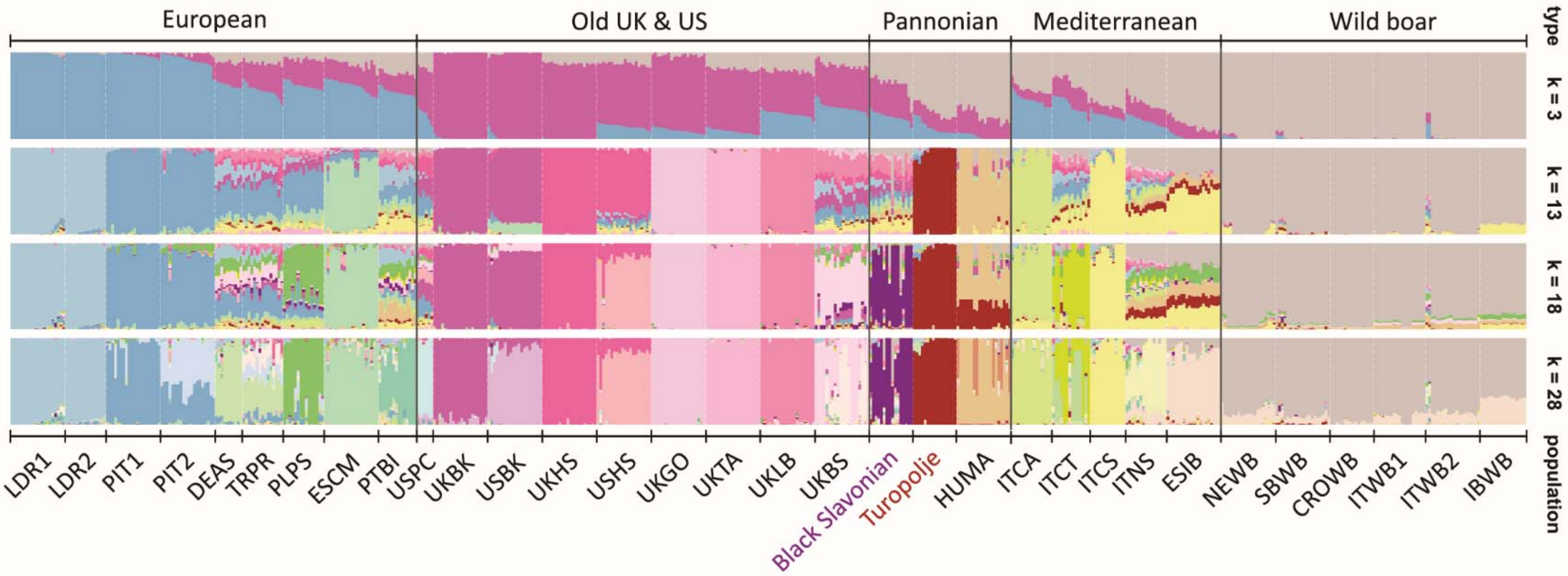
- In order to identify SNP alleles with high FST values specific to Croatian local breeds, we have created two additional datasets, one composed of Black Slavonian and modern commercial breeds (Landrace and Pietrain), and other with Turopolje pig and the same modern commercial breeds, respectively.
- We selected 30 genome-wide SNPs with the highest FST values for Black Slavonian and for Turopolje pig

RESULTS

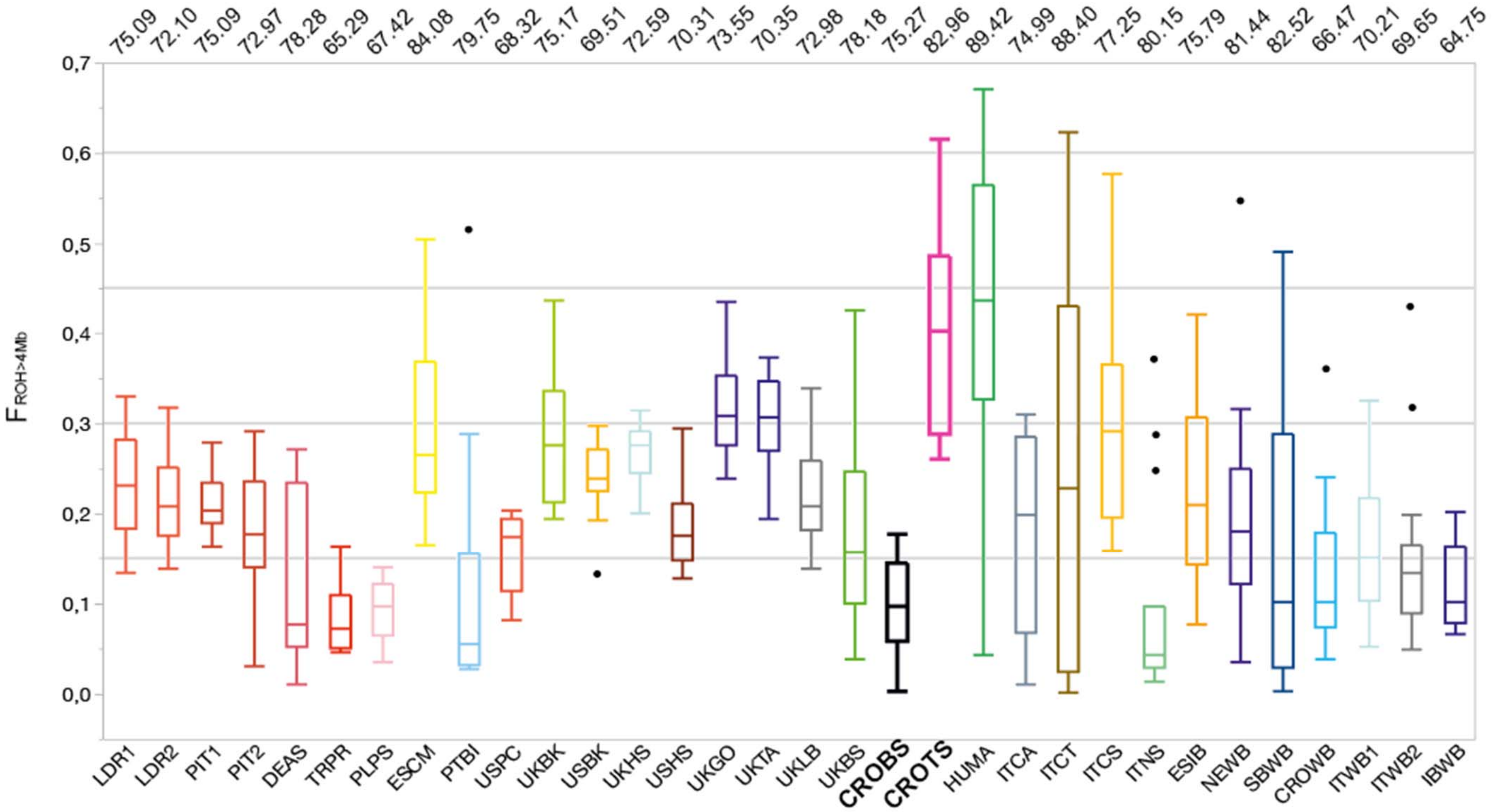

Multidimensional scaling
CHINA DONGSHAN
CHINA ERHUALIAN
CHINA LEPING SPOTTED
CHINA MEISHAN
CHINA MINZHU
CHINA WILDBOAR SOUTH
CHINA WILDBOAR NORTH
CHINA WANNAN SPOTTED
CHINA WUZHISHAN
CHINA XIANG
CHINA JINHUA
CHINA SUTAI
USA HAMPSHIRE
DUROC
Second component
0.2
0.0
−0.2
−0.4
First component
−0.4
−0.2
0.0
0.2


LANDRACE
PIETRAIN
SPAIN CHATO MURCIANO
PORTUGAL BISARO
ITALY CALABRESE
ITALY CINTA SENESE
ITALY CASERTANA
ITALY NERA SICILIANA
HUNGARY MANGALICA
CZECH PRESTICE
POLAND PULAWSKA SPOT
BRAZIL MONTEIRO
GUATEMALA CREOLE GUATEMALA
ARGENTINA SEMI FERAL
PERU CREOLE PERU
EUROPEAN NW WILDBOAR
BALKAN SOUTH WILDBOAR
ITALY WILDBOAR
ITALY SARDINIA WILDBOAR
IBERIAN WILDBOAR
CROATIA WILDBOAR
CROATIA BLACK SLAVONIAN
CROATIA TUROPOLJE
SPAIN IBERIAN
USA FERALPIG
USA GUINEA HOG
USA MULEFOOT
UK BRITISH SADDLEBACK
UK GLOUCESTER OLD SPOT
UK HAMPSHIRE
UK TAMWORTH
UK LARGE BLACK
UK BERKSHIRE
USA BERKSHIRE
USA POLAND CHINA

GENETIC ADMIXTURE



RUNS OF HOMOZYGOSITY AND GENOMIC INBREEDING



RUNS OF HOMOZYGOSITY AND GENOMIC INBREEDING

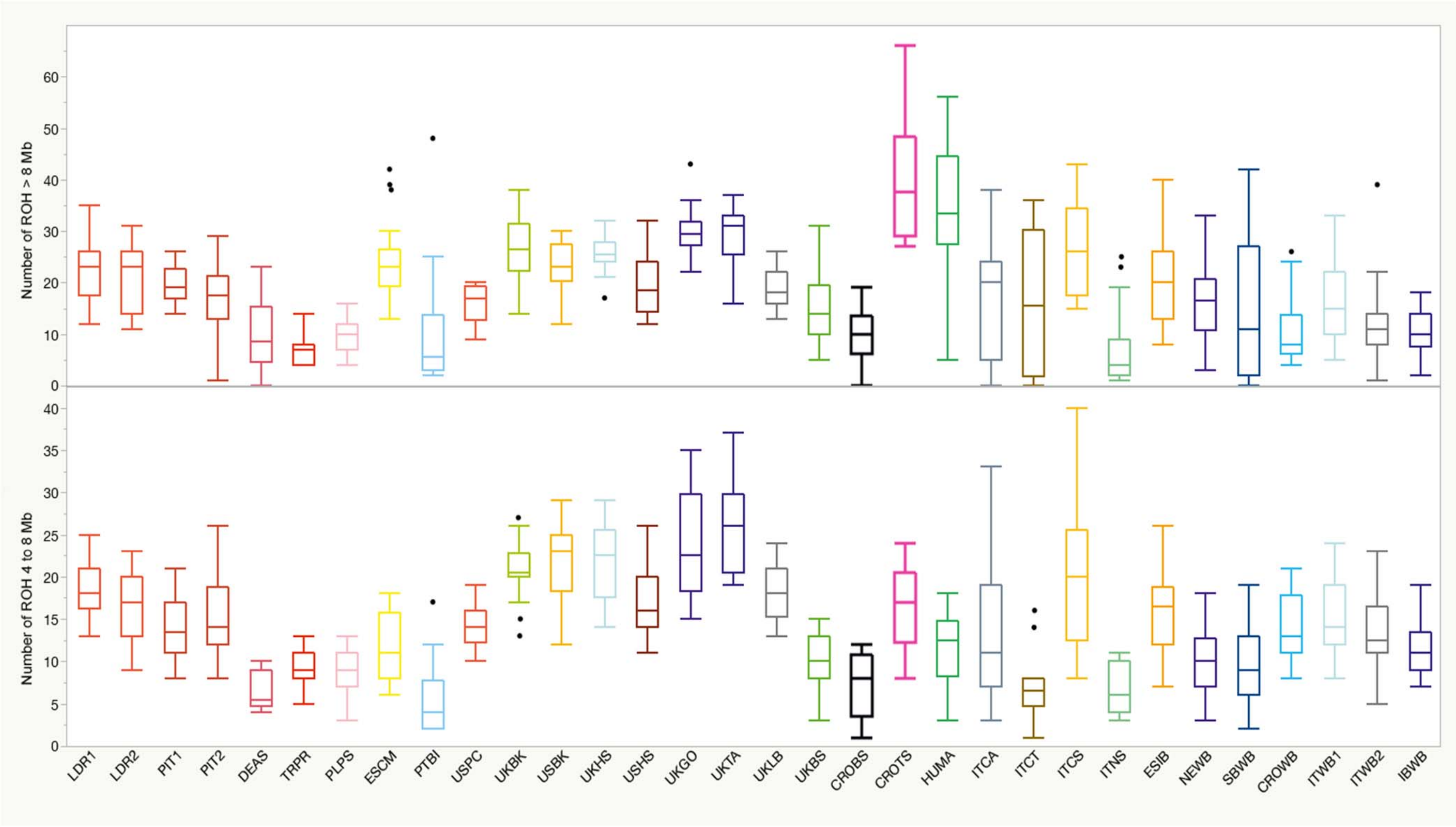
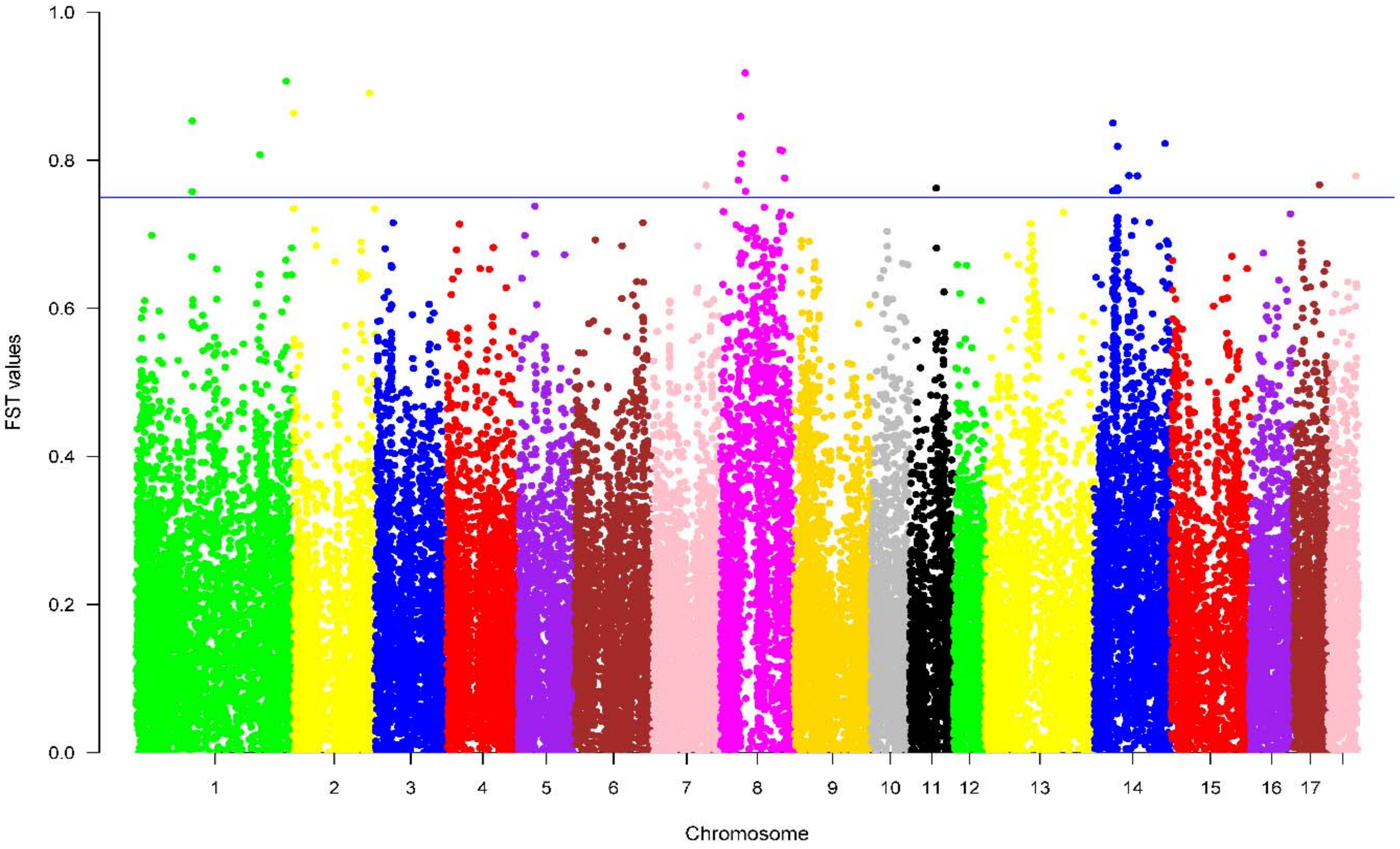


Table 1. Genetic differentiation among pig breeds/populations based on F_{ST} estimates

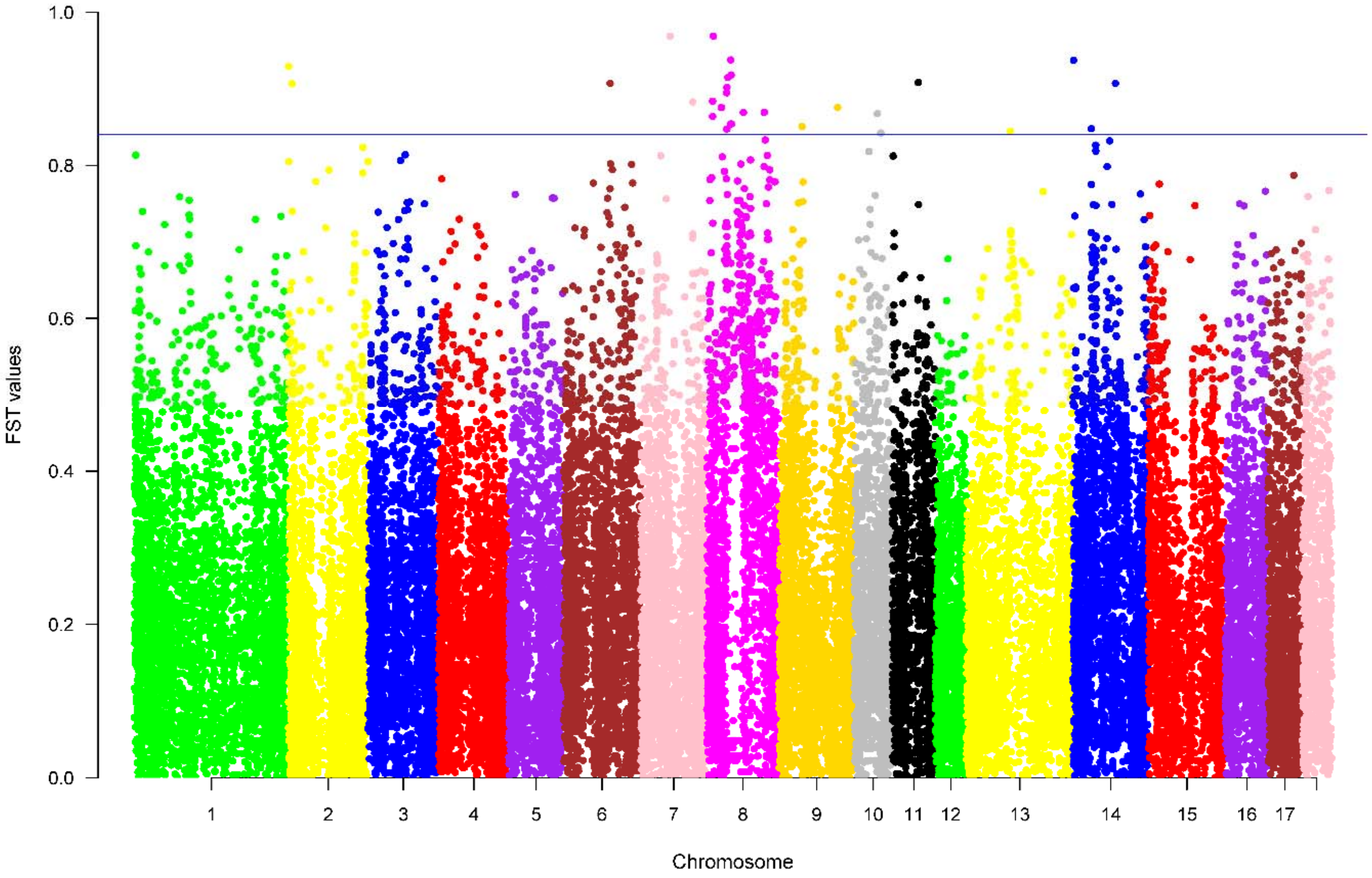
Breed/Population	DEAS	ITCS	ITCT	ESIB	UKLB	LDR1	HUMA	NEWB	SBWB	CROWB	TRPR	USPC	UKBK	CROBS	F_{ST}
Italy Cinta Senese - ITCS	0.25														0.29
Italy Casertana - ITCT	0.18	0.25													0.23
Spain Iberian - ESIB	0.21	0.22	0.19												0.23
UK Large Black - UKLB	0.21	0.30	0.23	0.25											0.26
Landrace - LDR1	0.19	0.30	0.23	0.28	0.27										0.27
Hungary Mangalica - HUMA	0.28	0.31	0.27	0.22	0.30	0.33									0.29
NW European Wild Boar - NEWB	0.26	0.28	0.25	0.19	0.29	0.31	0.28								0.26
South Balkan Wild Boar - SBWB	0.24	0.27	0.22	0.18	0.27	0.30	0.26	0.13							0.24
Croatia Wild Boar - CROWB	0.27	0.30	0.25	0.21	0.29	0.32	0.29	0.15	0.10						0.26
Czech Prestice - TRPR	0.08	0.21	0.15	0.17	0.17	0.18	0.23	0.22	0.20	0.22					0.18
USA Poland China - USPC	0.19	0.30	0.22	0.24	0.25	0.26	0.32	0.29	0.27	0.30	0.15				0.25
UK Berkshire - UKBK	0.25	0.35	0.27	0.29	0.29	0.30	0.34	0.33	0.31	0.34	0.21	0.28			0.29
Black Slavonian pig - CROBS	0.17	0.24	0.18	0.18	0.20	0.23	0.24	0.22	0.20	0.23	0.13	0.21	0.24		0.21
Turopolje pig - CROTS	0.30	0.35	0.29	0.26	0.33	0.34	0.34	0.32	0.30	0.33	0.25	0.35	0.37	0.28	0.32

*F_{ST} values (Weir and Cockerham 1984) of the pairwise genetic differentiation among selected breeds, on the lower diagonal

**F_{ST} average values among breeds, on the far-right column



Genome-wide analysis of global F_{ST} between Black Slavonian pig and modern pig breeds (Landrace and Pietrain). Manhattan plot of genome-wide F_{ST} values between the Black Slavonian pigs and modern pig breeds.



Genome-wide analysis of global F_{ST} between Turopolje pig and modern pig breeds (Landrace and Pietrain). Manhattan plot of genome-wide F_{ST} values between Turopolje pig and modern pig breeds.

IDENTIFICATION OF SPECIFIC GENOMIC REGIONS

For Black Slavonian pig, we identified important genes associated with:

- Steroid receptor activity - CYP-40 on SSC 8 (Ratajczak et al. 2015);
- Meat to fat ratio in pigs - DEAF1 on SSC2 (Falker-Gieske et al. 2019);
- Growth traits in cattle - KSR2 on SSC14 (Puig-Oliveras et al. 2014); animal organ and system development in pigs - SEZ6L on SSC 14 (Kwon et al. 2019),
- Haematological parameters in pigs - RHOBTB1 on SSC 14 (Bovo et al. 2019);
- Female reproduction in mice - CDK1 on SSC 14 (Adhikari et al. 2016);
- Salivary secretion in pigs - KCNMA1 gene on SSC14 (Li et al. 2013); milk fat percentage in buffaloes - KCTD8 on SSC8 – (de Camargo et al. 2015);
- Back-fat thickness in pigs - RIMS4 on SSC17 (Lee et al. 2018);
- Carcass length in pigs - SPTLC2 on SSC7 (Falker-Gieske et al. 2019)
- Muscle fiber types in pigs - MYO18B on SSC14 (Ropka-Molik et al. 2018) etc.

IDENTIFICATION OF SPECIFIC GENOMIC REGIONS

For Turopolje pig, we identified important genes associated with:

- fatty acid metabolism in pigs - PEX11A on SSC7 (Huang et al. 2017);
- carcass traits in cattle - WDR93 on SSC7 (Medeiros de Oliveira Silva et al. 2017);
- number of ribs in pigs - MESP1 on SSC7 (Zhu et al. 2015);
- meat to fat ratio in pigs - DEAF1 on SSC2 (Falker-Gieske et al. 2019);
- pregnancy rate in pigs - PPID on SSC8 (Gu et al. 2014);
- steroid receptor activity - CYP-40 on SSC 8 (Ratajczak et al. 2015);
- brain development in horses - DLGAP1 on SSC6 (Schubert et al. 2014);
- salivary secretion in pigs - KCNMA1 gene on SSC14 (Li et al. 2013);
- reproduction in pigs - CWH43 on SSC8 (He et al. 2016);
- spermiogenesis in mouse - AMPH on SSC9; boar taint - NWD2 on SSC8 (Drag et al. 2018);
- female pregnancy in pigs - RAPGEF2 on SSC8 (Pérez-Enciso et al. 2009);
- back-fat fatty acid composition - APBB1IP on SSC10 (Zappaterra et al. 2018) etc.

CONCLUSION

- Results show that **Black Slavonian and Turopolje pigs** are **distinct breeds** **with genetic relatedness within other European pig breeds**.
- Thus, an **uncontrolled breeding will certainly impoverish genomic diversity** of the overall European pig breeding capacity and hamper the cultural heritage of these breeds.
- The **conservation status of the Turopolje pig is alarming** and an urgent conservation plan is needed.
- **For the Black Slavonian pig, results are generally positive**, while actions toward consolidation and management of the admixture status are also required.
- **The establishment of good conservation plans is important for the long term perspectives of all pig breeds.**
- While the current contribution of the two analyzed breeds to the commercial pig production is marginal, **we need to start protecting the genetic variability of local breeds** to guarantee the necessary genetic diversity for the future.

Hvala na pozornosti

☺