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Genome-wide association analyses for several exterior traits in the autochthonous Casertana pig breed

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Running title: Mining the genome of Casertana pigs

Highlights

- Casertana is an autochthonous pig genetic resource reared in Central-South of Italy.
- Genome-wide association studies and F_{ST} analyses were carried out in this breed.
- Two ear conformation traits, presence/absence of wattles and coat colours were studied.
- Markers associated with the presence of wattles were identified on SSC1 and SSC11.
- Markers associated with coat colours were identified on SSC6, SSC8, SSC14 and SSC15.

Abstract

Casertana is an autochthonous pig breed reared in the Central-South of Italy, mainly in extensive or semi-extensive production systems. A small number of animals are registered to the breed herd-book. The population of this breed is not completely fixed for several exterior traits, including ear size (small vs large), ear bearing (forward vs floppy), presence or absence of wattles and coat colour (black vs slate-grey) that we recorded in a total of 101 pigs from six different farms. All these pigs were also genotyped with the Illumina PorcineSNP60 BeadChip. Then, with the aim to identify DNA markers associated with these traits, we run four genome-wide association studies (GWAS) comparing pigs having different phenotypes for each of the four external traits. Genome-wide fixation index (F_{ST}) analyses were run to confirm the results. GEMMA software was used in the GWAS. Single nucleotide polymorphisms (SNPs) associated to the wattle trait were identified on porcine chromosome (SSC) 1 and SSC11. Markers associated to the coat colour trait were located on SSC6, SSC8, SSC14 and SSC15. These chromosome regions were confirmed by the independent F_{ST} analyses. F_{ST} did not confirm the signals detected for the two ear conformation traits. Candidate genes were identified according to the function of the annotated genes in the detected genome regions. Despite the small number of pigs that could be investigated, this study showed that autochthonous animal genetic resources might be useful to disclose genetic factors affecting traits that are not fixed in a population.

Keywords: Animal genetic resource; coat colour; ear conformation; single nucleotide polymorphism; *Sus scrofa*; wattle.

1. Introduction

Autochthonous breeds are important animal reservoirs of genetic diversity. These genetic resources are usually characterized by associated inheritable phenotypes, in several cases not completely fixed, that define the uniqueness of these populations and that could be explored to understand their adaptation to different production systems and their history and origin (Leroy et al., 2016).

Casertana is a local pig breed mainly raised in Central-South regions of the Italian peninsula. This breed is considered an endangered pig breed as its herd book currently accounts only for about 100 registered boars and sows (ANAS, 2019). Casertana pigs are regarded as the descendant of the ancient Neapolitan pigs that were used to shape the genetic pool of the British pig populations over the 19th century (Porter, 1993). Animals of the Casertana breed have a characteristic slate-grey or black coat colour, curtly tail, wrinkled skin, forward-bending ears of generally medium-size and a typical hairless phenotype. Pigs could have wattles, their head is usually of medium size with a truncated conical-shape and a rectilinear or slightly concave profile with long and thin snout (Bozzi et al., 2019). However, Casertana population is not completely fixed for most of these traits, thus phenotypic variability exists within the breed. This is probably due i) to recent introgression from European wild boars, which are in close contacts to this domesticated population, usually raised in extensive or semi-extensive production systems and ii) to crossbreeding with other pig breeds, which occurred in the past to recover this small population.

Variability related to a few morphological traits in Casertana made it possible to design effective genome wide association studies (GWAS) that already identified gene markers associated with the targeted traits. Schiavo et al. (2018) carried out a GWAS in this breed to compare the genome of pigs without hairs (the majority) against that of few pigs with hairs that were identified in this population. Bertolini et al. (2018) did a similar GWAS in this breed comparing genotyping data of animals with curly tail *vs* animals of straight tail and identified a single nucleotide polymorphism (SNP) associated with the shape of the tail in pigs.

Studies in other pig populations have already contributed to understand the genetic factors affecting a few other morphological traits which are variable in Casertana. For example, QTL and GWAS and selection signature analyses identified chromosome regions and gene markers associated to ear morphology in several pig breeds and crossbred reference families (Wei et al., 2007; Ma et al., 2009, 2015; Ren et al., 2011; Li et al., 2012; Wilkinson et al., 2013; Zhang et al., 2014; Zhang et al., 2015; Chen et al., 2018; Liang et al., 2019). The presence of wattles in pigs, which are defined as appendages suspended from the mandibular contiguous region of the neck, has been studied by few authors so far. These works proposed that in pigs this phenotype could be controlled by a single locus, with a dominant mode of inheritance based on two alleles (Wa^W, dominat for the presence; Wa^+, for the absence; Kronacher, 1924; Roberts and Morrill, 1944; Sabbioni et al., 2011).

The genetics of coat colours has been largely studied in pigs (reviewed in Fontanesi and Russo, 2013). The black coat colour (a morph allowed in Casertana) is mainly determined by dominant alleles at the *Extension* locus, which is encoded by the melanocortin 1 receptor (*MC1R*) gene (Kijas et al., 1998). Casertana is not fixed at this locus as recently reported by Muñoz et al. (2018) and Ribani et al. (2019) and at least four *MC1R* alleles segregate in this breed. The genetic determinism of the other colour morph allowed in Casertana (slate grey) is not well defined in pigs yet. A few studies reported the effect of *KIT* gene alleles on roan or grey phenotypes and QTL studies suggested the presence of a few chromosome regions that might contribute to define greyish coat colours in *Sus scrofa* (Hirooka et al., 2002; Fontanesi et al., 2010).

In this study, we took advantage from the phenotypic variability of several exterior traits (two ear morphological traits, presence or absence of wattles and different coat colours) that we recorded in a Casertana pig population to carry out GWAS and comparative genome-wide F_{ST} analyses with the final objectives to identify genomic regions that could affect the observed heterogeneity in this local pig breed.

2. Materials and methods

2.1. Animals and traits

This study did not have any ethical implications as animals were not treated in any way. Operations on the animals were carried out under routine veterinary inspections. All animals were raised according to national and European legislation and not ethical permit was therefore needed.

A total of 101 Casertana pigs (of about 7 to 20 months old) from six different farms were included in this study. Photographic records and subjective evaluations of each animal were obtained to capture information on several traits: ear size (small or large); ear bending (forward or floppy); presence or absence of wattles. Ear size was defined according to an approximation based on the ratio between the ear length (EL) and the snout length (SL): small, when EL/SL $\leq 1/3$; large when EL/SL $\geq 2/3$. A few pigs with intermediate EL/SL ratio were not considered in this study that attempted to capture extreme differences considering the small population size that was analysed. Ear size was based on a relative parameter calculated from photographic records, as precise measures could not be obtained due to the difficulties to immobilize the head of the animals in the routine operating conditions of the extensive production systems in which animals were kept. In addition, this relative measure could overcome the problem derived by the age and size effect of the animals that could not be determined. Table 1 summaries the number of animals that were included in the different classes for the four mentioned traits. Figure 1 reports a few examples of the recorded phenotype variability in Casertana pigs.

2.2. Genotyping of single nucleotide polymorphisms

Hair roots, collected from the studied Casertana pigs, were used for DNA extraction that was obtained with the Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA) following the manufacturer's instructions. The Illumina PorcineSNP60 BeadChip v.2 (Illumina, Inc., San Diego, CA, USA) was used for the genotyping of 61,565 single nucleotide polymorphisms (SNPs). The positions of the markers were assigned by applying the mapping method described in Fontanesi et al. (2012) and using the Sscrofa11.1 genome version as reference.

Genotyping data were filtered using PLINK 1.9 software (Chang et al., 2015) using the following criteria already used in similar studies (Bertolini et al., 2018; Schiavo et al., 2018): genotyping call rate >0.9, minor allele frequency (MAF) >0.01 and Hardy-Weinberg equilibrium P>0.001. After filtering, the number of SNPs in the GWAS for ear size, ear bearing, presence/absence of wattles and coat colours was 39,732, 41,027, 38,423 and 41,230, respectively.

2.3. Genome-wide analyses

Multidimensional scaling (MDS) plots were obtained with the PLINK 1.9 software (Chang et al., 2015) to evaluate distance relationships among the animals of the investigated cohort included in the different comparative genome-wide analyses for the considered exterior traits. In another work, aimed to the selection of a SNP panel for breed classification (Schiavo et al., 2020), the same individuals have been correctly assigned to Casertana breed without any classification errors to other breeds. This indicates that the eventual clusters in MDS plot are only due to within breed stratification problems. Genome-wide association studies were carried out by applying the univariate mixed model of GEMMA (Zhou and Stephens, 2012) that can accommodate the centered relatedness matrix calculated from SNP genotypes to correct for population stratification in a case and control analysis. The model applied for the different exterior traits included the farm and the sex as fixed effects. To be able to identify markers affecting exterior traits in this experiment that could include a low number of animals and considering the fact that the used SNP chip might have an ascertain bias (derived by the fact that local breeds were not used for the selection of the informative markers), the significant threshold was defined at the $P_{nominal\ value}$<5.00E-05 level, as already applied in several other GWAS in livestock (e.g. Fontanesi et al., 2012; Sanchez et al., 2014) and in similar analyses for the Casertana breed (Bertolini et al., 2018; Schiavo et al., 2018). GenABEL (Aulchenko et al., 2007) was used to obtain genomic inflation factors (λ) and quantile–quantile (Q–Q) plots for the GWAS.

To confirm the results of the GWAS, F_{ST} analyses were performed on the same dataset and for each trait using PLINK 1.9 software (Chang et al., 2015), by comparing single markers between the

groups of pigs with alternative phenotypes for each analysed exterior trait. Relevant F_{ST} differences were defined considering the SNPs over the 99.9th percentile distribution.

Gene annotation of the genomic regions identified by both genome-wide association and genome-wide F_{ST} analyses was retrieved from the Sscrofa11.1 genome version available at the Ensembl database (http://www.ensembl.org/Sus_scrofa/Info/Index), release 97 (June 2019), considering 500 kbp from both sides of the significant markers.

3. Results and discussion

A recent phenotypic characterization of the autochthonous Casertana pig population that we carried out evidenced several morphological differences, including the presence or absence of hairs and the shape of the tail, among the animals of this local breed. Despite the number of animals involved was limited, this diversity made it possible to successfully identify SNPs associated to these exterior traits using genome-wide analyses and, in turn, to identify candidate genes based on the annotations in the corresponding genome regions (Bertolini et al., 2018; Schiavo et al., 2018).

In this phenotypic survey of the Casertana population, we also reported differences for two ear phenotypes (size and position), presence or absence of wattles and coat colours (Figure 1; Table 1), that we explored in genome-wide analyses to identify markers associated with the observed phenotypic variability.

Figure 2 shows the Manhattan plots obtained in the GWAS and genome-wide F_{ST} analyses for the four investigated exterior traits. Q-Q plots obtained for the GWAS are reported in Figure S1. Table 2 lists the markers that were confirmed by the two genome-wide approaches, as they passed the thresholds in both analyses for the different considered traits.

Ear size was classified into two categories (small and large, 51% and 49% of the pigs, respectively). Phenotypic characterization of ear size was opportunistic for practical reasons. Therefore, only a total of 37 animals that could be classified for this phenotype were included in the genome-wide analyses. Multidimensional scaling plot based on these two ear size categories

identified potential substructures in the analysed sub-populations, defined according to this approximated phenotype (Figure S2). The GWAS, that according to the λ value (1.044) was able to correct most of the observed stratification in this small sample of pigs, identified two significant markers (P=2.52E-6) on porcine chromosome (SSC) 9 (MARC0047658, at position 130,362,191; ASGA0085666, at position 130,366,477). This result was however not confirmed by the F_{ST} analysis that identified an outlier signal on SSC9 (ALGA0118777, at position 125,868,947), about 5 Mbp apart from the GWAS significant SNPs. Using an F2 population established by crossing European Large White with Chinese Meishan pigs, Wei et al. (2007) reported a large QTL region for ear size on SSC9 that might also include the positions we identified in Casertana pigs.

Ear bearing included two clearly distinguished conformations in Casertana pigs, forward (28%) and floppy (72%; Table 1). The MDS plot of the animals classified with these two ear morphological classes (Figure S2) indicated some potential stratification in the analysed population that was possible to partially correct in the GWAS that had a λ equal to 1.124. The F_{ST} analysis indicated several signals in different chromosomes (Figure 2) that however were not supported by any results in the GWAS, which did not identify any significant markers.

Weak or absence of significant and confirmed genomic regions for the two ear conformation traits might be due to the quantitative genetic architecture that might control these traits in pigs (Wei et al., 2007; Ma et al., 2009) and that could not be dissected by the low number of analysed animals. Simplified phenotypic categories were also used for ear size that might not completely explain the phenotypic variability for this ear trait. Previous studies that investigated ear size and shape in pigs included hundreds of phenotyped animals and the most significant markers identified on a few chromosomes (i.e. SSC5 and SSC7) explained just a fraction of the total genetic variability (Wei et al., 2007; Ma et al., 2009). Therefore, the absence of meaningful results obtained in our study may mean that also in Casertana these ear conformation traits cannot be explained by simple Mendelian inherited genes.

About 23% of the Casertana pigs considered in this study had wattles. Animals with one (n. = 1) or two wattles (n. = 10) were considered as having wattles without any distinction, according to previous studies that defined the genetic transmission model of this morphological trait in pigs (Kronacher, 1924; Roberts and Morrill, 1944; Sabbioni et al., 2011). The remaining animals for which this phenotype was collected (77%) did not have any appendages suspended from the mandibular contiguous region of the neck (Table 1; Figure 1). The MDS plot of the pigs with or without wattles showed some potential sub-structures of the analysed population (Figure S2) that however were corrected in the GWAS, that had a genomic inflation factor equal to 1.005. This association study identified two significant markers on SSC11 (MARC0021800, at position 14,148,820, P = 1.40E-06; and DRGA0010900, at position 14,357,170, P = 3.27E-05) and two markers on SSC1 (H3GA005056, at position 268,698,293, P = 3.27E-05; and INRA0007668, at position 268,102,753, P = 4.51E-05) that were confirmed by the F_{ST} analysis (Figure 2 and Table 2). The SSC11 region including the significant markers harbors seven annotated genes (Table S1). Among them, the FRAS1 related extracellular matrix protein 2 (*FREM2*) gene (positions 13,959,865-14,154,754) seems an interesting candidate for this phenotype. In humans, mutations in this gene cause the Fraser syndrome, which is a multisystem disorder characterized by epidermal blistering, syndactyly and a range of other developmental abnormalities (Jadeja et al., 2005; Smyth and Scambler, 2005). The other two significant markers located on SSC1 identified a region harbouring 35 annotated genes. This SSC1 gene rich region includes the nuclear receptor subfamily 6 group A member 1 (*NR6A1*) gene, encoding for a DNA-binding transcription factor involved in embryonal developmental stages and affecting vertebral numbers in pigs (Mikawa et al., 2007; Duan et al., 2018). The causative mutation having an effect on the number of vertebrae (p.P192L; Mikawa et al., 2007) was genotyped in these Casertana pigs by Ribani et al. (2019) but no significant association could be observed between this *NR6A1* missense mutation and the presence of wattles (data not shown). Therefore, other mutations in this gene (or other close genes) could affect, at least in part, the wattle formation in some animals. Partial penetrance or oligogenic models should be also considered to explain the genetic architecture

of this trait, considering that we identified significant markers in at least two different chromosomes, despite classical genetic studies indicated that only one locus might be involved in pigs to determine the presence or absence of wattles (Kronacher, 1924; Roberts and Morrill, 1944; Sabbioni et al., 2011).

Two coat colours were reported in the investigated Casertana population. About 17.5% of the pigs were black whereas 82.5% had a solid slate grey coat colour (Table 1; Figure 1). Stratification of the population was present when these two groups of pigs were plotted in an MDS representation (Figure S2). Genome-wide association analysis, that was not completely able to correct this bias (λ = 1.235), identified several significant SNPs, some of which (most on SSC6, one on SSC8, one on SSC14 and two on SSC15; Table 2) were confirmed in the F_{ST} analysis. The confirmed SNPs of SSC6 were not located in the *MC1R* region, excluding variability in this gene as being the main driver of this coat colour diversity. Other positions on SSC6 harbored confirmed SNPs, namely regions at about 80-81 Mbp, 86.5 Mbp, 125-127 Mbp and 136-137 Mbp in which no obvious candidate genes could be located (Table S1). The same might be applied for the signals on SSC14 and SSC15, in which no candidate genes could be identified according to the functions and roles of the annotated genes in these regions (Table S1). Among the annotated genes around the signal on SSC8, the fibroblast growth factor receptor 3 (*FGFR3*) gene (positions 879,207-895,912) plays different roles in several skin processes, which might be relevant for the pigmentation in the hairless Casertana pigs. In humans, mutations in this gene cause acanthosis nigricans, a pigmentary skin disorder associated with obesity (Fukuchi et al., 2018), that also might match the high adipogenic potential of the Casertana pigs.

Taking together all results reported in this study for the four considered morphological traits and what we previously provided in the same Casertana population for the hairless and tail shape phenotypes (Bertolini et al., 2018; Schiavo et al., 2018), it is interesting to note that in most cases it was possible to obtain hints that could explain the genetic mechanisms controlling the expression of simple traits, despite the limits that derived by the small population size. As expected, complex

quantitative traits could not be clearly dissected in this simple experimental design and local pig populations might not be useful for these purposes.

Conclusion

The experimental design, based on a small number of phenotyped and genotyped pigs, posed a few challenging questions that we tried to solve by combining two different genome-wide approaches and by adding a candidate gene analysis based on the functions of the genes located in the genomic regions that were confirmed by the two genomic methods (i.e. GWAS and genome-wide F_{ST} approaches).

Critically evaluating the obtained results, the applied strategy made it possible to identify candidate genes that could be involved in affecting expected monogenic (or oligogenic) traits, like presence of wattles or coat colours. Potential stratifications that could be in some cases not solved might have introduced false positive signals (i.e. for the coat colour GWAS) that, in part, could be disentangled considering the function of the underlying annotated genes and could be improved by additional annotation and functional characterization of these genes. It is also clear that it would be important to support these results by increasing the number of involved pigs, that however, in the case of this local pig breed, is a challenging solution, considering the low number of available Casertana pigs and the difficulties in collecting data and biological materials in extensive systems. The parallel addition to the study of animals of other breeds (in a multibreed analysis) with similar phenotypes might strengthen the power in detecting genomic regions associated with peculiar exterior (potentially monogenic) traits. This strategy could provide additional values to local and untapped animal genetic resources that might be useful to dissect phenotypic traits that cannot be genetically characterized using commercial or cosmopolitan populations.

Declaration of competing interests

The authors declare no conflict of interest

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409 **Table 1.** Phenotypic descriptions and numbers of the Casertana pigs included in this study.

Trait	Condition/description	N. of males	N. of females	Total number[†]
Ear size	Small	4	15	19
	Large	10	8	18
Ear bearing	Forward	10	14	24
	Floppy	32	29	61
Wattles	Presence	5	6	11
	Absence	13	24	37
Coat colour	Black	5	12	17
	Slate-grey	31	49	80

410

411 [†] The phenotypic description of a few animal/trait combinations was not obtained.

Table 2. List of single nucleotide polymorphisms (SNPs) associated with the investigated traits that were confirmed by the F_{ST} analysis. Annotation of the different chromosome regions is reported in Table S1.

Traits	SSC[†]	Position[‡]	SNP	P-value GWAS	F_{ST} value	Alleles[§]	AF[¶]
Presence/absence of wattles	1	268102753	INRA0007668	4.51E-05	0.325	G/A	0.932
	1	268698293	H3GA0005056	3.27E-05	0.340	A/C	0.450
	11	14148820	MARC0021800	1.40E-06	0.397	G/A	0.920
	11	14357170	DRGA0010900	3.27E-05	0.340	G/A	0.955
Coat colours	6	79999211	MARC0056786	6.56E-07	0.266	C/A	0.219
	6	80108499	MARC0028970	1.59E-06	0.235	A/G	0.214
	6	80241553	ASGA0028642	1.59E-06	0.235	A/G	0.214
	6	80459834	ASGA0093075	1.59E-06	0.235	A/G	0.214
	6	80491095	MARC0045097	4.53E-06	0.237	A/G	0.672
	6	80778880	ASGA0028671	1.59E-06	0.235	A/G	0.214
	6	80873393	H3GA0018314	1.59E-06	0.235	G/A	0.214
	6	81130094	ASGA0101946	1.59E-06	0.235	G/A	0.214
	6	81326625	ASGA0099275	1.59E-06	0.235	A/G	0.214
	6	86543912	ALGA0108236	6.76E-07	0.246	G/A	0.755
	6	125700603	ALGA0036595	2.15E-06	0.331	A/G	0.359
	6	126122455	ALGA0036616	2.47E-06	0.331	A/C	0.359
	6	127514190	ALGA0011111	1.71E-06	0.340	A/C	0.354
	6	136414674	ASGA0029542	3.20E-06	0.283	A/G	0.344
	6	136634162	MARC0070940	5.31E-06	0.267	C/A	0.604
	6	136701217	CASI0009826	9.55E-07	0.323	G/A	0.323
	6	136921345	ASGA0029563	1.20E-06	0.312	A/G	0.651
	8	1186987	ALGA0046044	1.88E-05	0.259	G/A	0.802
	14	72379700	ALGA0079568	5.12E-06	0.302	A/G	0.333
	15	27126842	ASGA0069071	3.02E-05	0.262	A/C	0.505
	15	29094014	MARC0105811	5.34E-06	0.292	G/A	0.661

[†] *Sus scrofa* chromosome.

[‡] Position on the indicated chromosome retrieved from Sscrofa11.1 genome version.

§ Alleles. The first nucleotide is that of the reference genome.

⁋ Frequency of the first reported nucleotide in the “allele” column.

Figure 1. Examples of Casertana pigs with different exterior traits such as ear size (small or large), ear bearing (forward or floppy), presence or absence of wattles and coat colour (slate-grey or black).

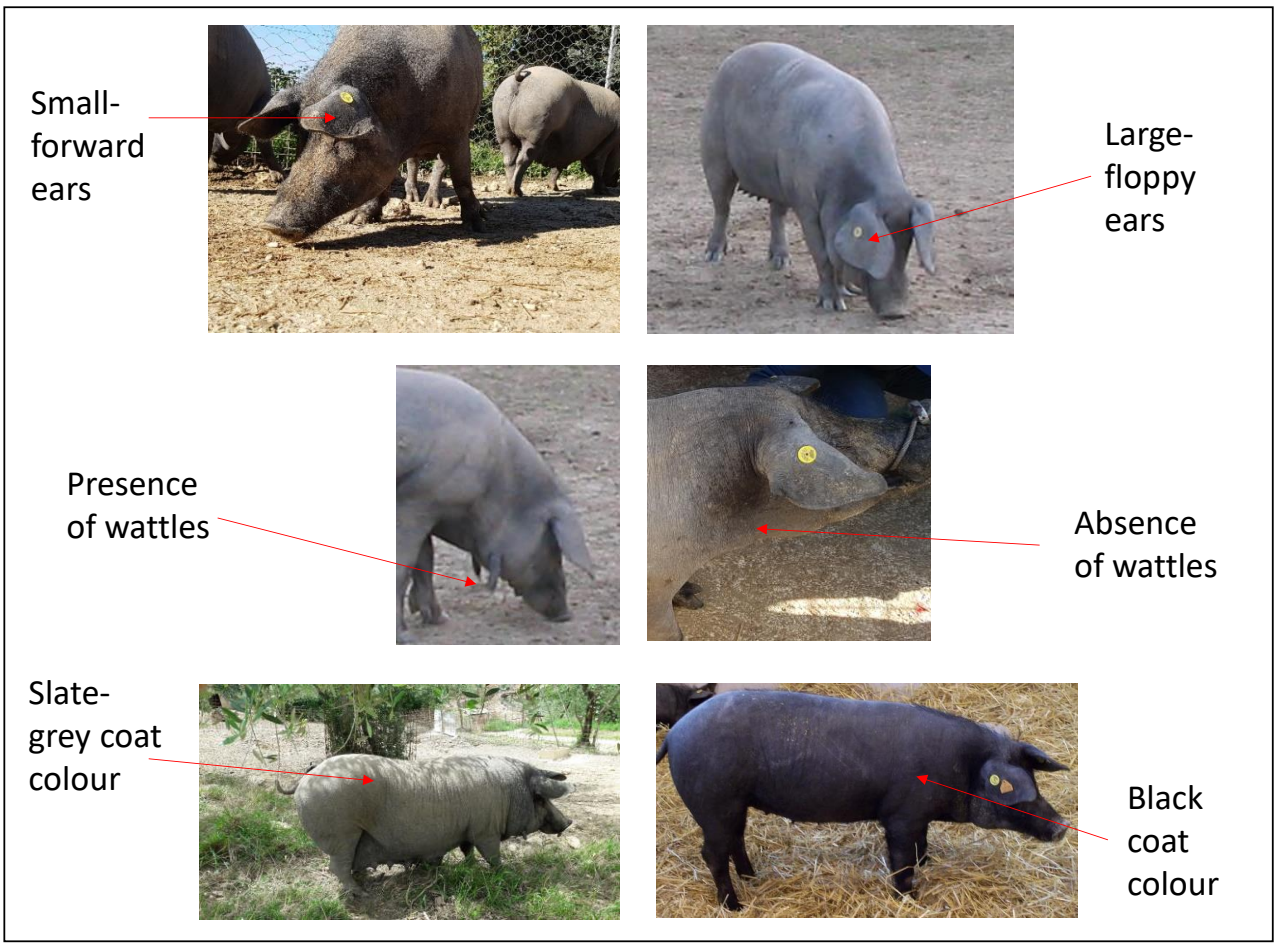
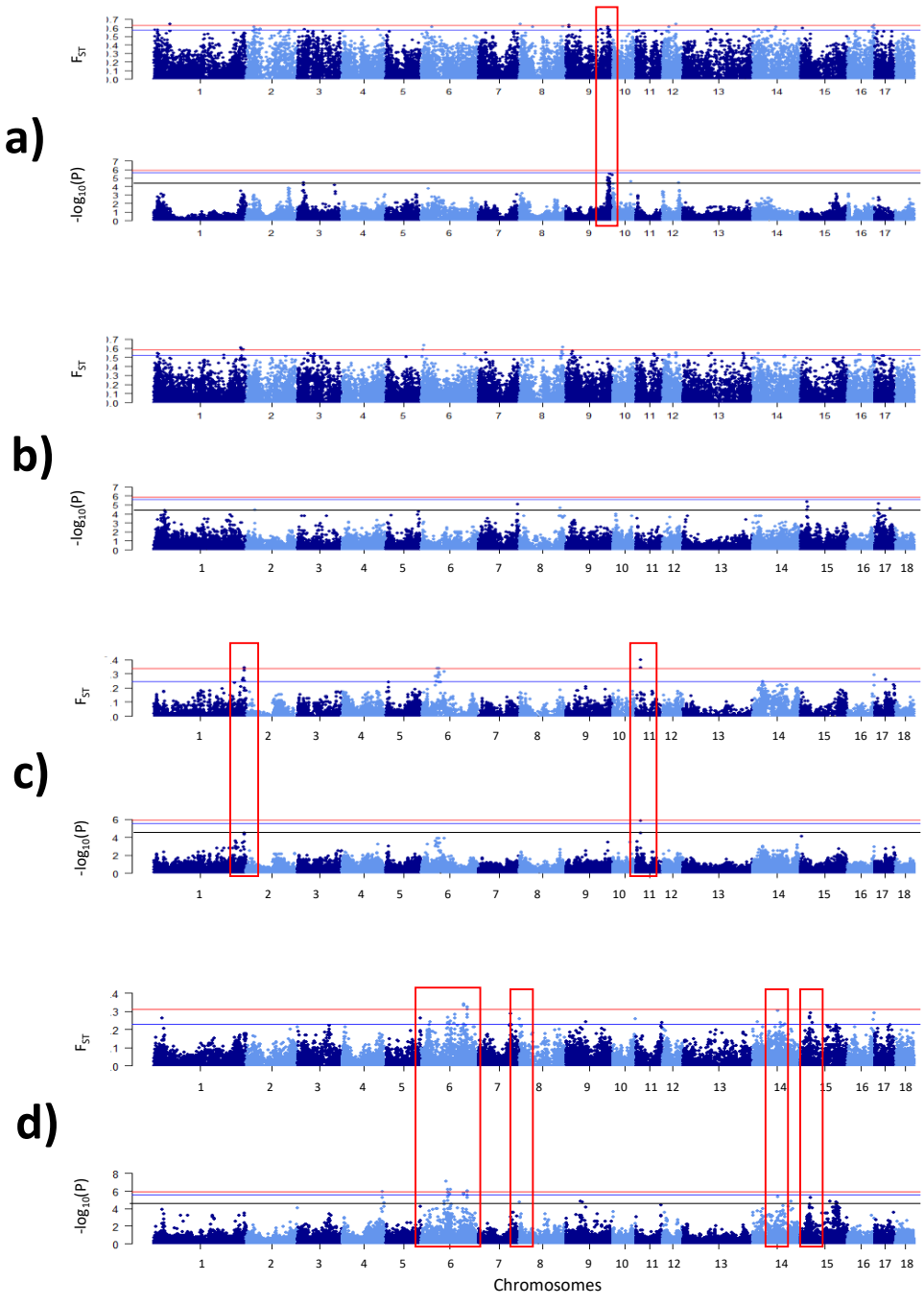


Figure 2. Manhattan plots of the genome-wide F_{ST} analyses and genome-wide association studies for the four investigated exterior traits: a) ear size; b) ear bearing; c) presence or absence of wattles; d) coat colours. Thresholds in the F_{ST} plots are for the 99.99th percentile (red line) or for the 99.9th percentile (blue line). Thresholds in the genome-wide association studies are for p-value = 0.05 Bonferroni corrected (red line), 0.10 Bonferroni corrected (blue line) or for p-value = 5.0E-05 (black line). Significant single nucleotide polymorphism regions described in the text are evidenced in red.



Supplementary material

Table S1. Genes included in the chromosome regions harboring single nucleotide polymorphisms (SNPs) associated with the presence or absence of wattle and coat colours (slate-grey or black) in Casertana pigs. Chromosome regions discussed in the text were defined considering contiguous SNPs and ± 500 kbp from the first and last SNPs in the detected regions.

Trait/Chromosome region[†]	SSC[‡]	Gene start (bp) [§]	Gene end (bp)[¶]	Strand	Gene symbol	Gene Ensembl ID
Wattle/SSC1_region1	1	267480768	267770444	1	*RALGPS1*	ENSSSCG00000005607
	1	267639638	267672923	-1	*ANGPTL2*	ENSSSCG00000005608
	1	267771636	267923390	1	*GARNL3*	ENSSSCG00000005609
	1	267833052	267833154	-1	*RF00026*	ENSSSCG00000020000
	1	267925189	267934094	1	*SLC2A8*	ENSSSCG00000005610
	1	267941953	267959747	1	*ZNF79*	ENSSSCG00000005611
	1	267958456	267965555	-1		ENSSSCG00000033019
	1	267962230	267962359	-1	*RF00302*	ENSSSCG00000018580
	1	267964929	268008830	1	*LRSAM1*	ENSSSCG00000005613
	1	268010307	268066016	-1	*FAM129B*	ENSSSCG00000005614
	1	268116124	268205801	1	*STXBP1*	ENSSSCG00000005617
	1	268209443	268213894	1	*CFAP157*	ENSSSCG00000005618
	1	268213686	268215523	-1	*PTRH1*	ENSSSCG00000005615
	1	268216049	268237839	1	*TTC16*	ENSSSCG00000005616
	1	268233661	268237404	-1	*TOR2A*	ENSSSCG00000005619
	1	268237679	268270361	-1	*SH2D3C*	ENSSSCG00000005620
	1	268276841	268283860	1	*CDK9*	ENSSSCG00000005621
	1	268287729	268309406	1	*FPGS*	ENSSSCG00000005626
	1	268306120	268343936	-1	*ENG*	ENSSSCG00000005625
	1	268355035	268365598	-1	*AK1*	ENSSSCG00000005627
	1	268372867	268383544	-1		ENSSSCG00000005623
	1	268389483	268402517	-1	*ST6GALNAC4*	ENSSSCG00000005628
	1	268408129	268439109	1		ENSSSCG00000031500

1	268409449	268417562	-1	*PIP5KL1*	ENSSSCG00000005629
1	268418445	268423560	-1	*DPM2*	ENSSSCG00000032481
1	268427708	268465802	-1	*FAM102A*	ENSSSCG00000005631
1	268527327	268530563	-1	*NAIF1*	ENSSSCG00000005630
1	268531752	268576994	1	*SLC25A25*	ENSSSCG00000005636
1	268581835	268590836	-1	*PTGES2*	ENSSSCG00000022655
1	268609633	268621997	1	*LCN2*	ENSSSCG00000005638
1	268616034	268619637	1		ENSSSCG00000022295
1	268620965	268640934	-1	*CIZ1*	ENSSSCG00000005640
1	268653174	268701674	1	*DNM1*	ENSSSCG00000005641
1	268691502	268691610	-1	*MIR199B*	ENSSSCG00000019744
1	268701511	268721176	-1		ENSSSCG00000005643
1	268720306	268727670	1		ENSSSCG00000032748
1	268746329	268757458	-1	*TRUB2*	ENSSSCG00000005645
1	268756129	268767931	1	*COQ4*	ENSSSCG00000005646
1	268773861	268788207	1	*SLC27A4*	ENSSSCG00000005648
1	268798425	268816348	1	*URM1*	ENSSSCG00000005649
1	268816961	268817057	-1	*MIR219B*	ENSSSCG00000027037
1	268827445	268827664	1	*RF00012*	ENSSSCG00000019510
1	268838055	268847233	1	*CERCAM*	ENSSSCG00000005650
1	268856766	268894210	1	*ODF2*	ENSSSCG00000005651
1	268894276	268922750	1	*GLE1*	ENSSSCG00000005652
1	268924948	268927232	1		ENSSSCG00000035449
1	268936026	269003036	1	*SPTAN1*	ENSSSCG00000005654
1	269002245	269030719	-1	*WDR34*	ENSSSCG00000005655
1	269008363	269009987	1		ENSSSCG00000033804
1	269056717	269064634	1		ENSSSCG00000005656
1	269069388	269081016	1	*PKN3*	ENSSSCG00000005657
1	269081272	269084849	-1		ENSSSCG00000005658
1	269090333	269122156	-1	*ZER1*	ENSSSCG00000005659
1	269134527	269153066	1	*TBC1D13*	ENSSSCG00000005660
1	269154090	269157788	1	*ENDOG*	ENSSSCG00000005661
1	269154639	269167196	-1	*SPOUT1*	ENSSSCG00000005662
1	269172628	269209494	-1		ENSSSCG00000005663

Wattle/SSC11_region1	11	13765501	13774791	1	*UFM1*	ENSSSCG00000031224
	11	13959865	14154754	1	*FREM2*	ENSSSCG00000009364
	11	14131211	14147122	-1	*STOML3*	ENSSSCG00000009366
	11	14151151	14183851	-1	*PROSER1*	ENSSSCG00000040697
	11	14184134	14370915	1	*NHLRC3*	ENSSSCG00000037746
	11	14319425	14576135	-1	*LHFPL6*	ENSSSCG00000029855
	11	14576214	14723157	1	*COG6*	ENSSSCG00000009369
Coat colour/SSC6_region1	6	79589691	79649644	1	*ALPL*	ENSSSCG00000032607
	6	79662137	79735361	-1	*RAP1GAP*	ENSSSCG00000022029
	6	79744936	79810724	-1	*USP48*	ENSSSCG00000024197
	6	79850757	79921097	-1	*HSPG2*	ENSSSCG00000003514
	6	79968501	79972061	-1		ENSSSCG00000033553
	6	79995339	80002723	1		ENSSSCG00000037400
	6	80012212	80017669	1		ENSSSCG00000030015
	6	80035048	80090934	1		ENSSSCG00000003520
	6	80068259	80088564	-1		ENSSSCG00000035899
	6	80112277	80139670	-1		ENSSSCG00000003521
	6	80419826	80485378	1	*ZBTB40*	ENSSSCG00000026276
	6	80521572	80548696	1	*EPHA8*	ENSSSCG00000003523
	6	80586183	80591269	1	*C1QA*	ENSSSCG00000003524
	6	80592198	80599440	1	*C1QC*	ENSSSCG00000038706
	6	80601303	80608011	1	*C1QB*	ENSSSCG00000033512
	6	80649143	80843567	1	*EPHB2*	ENSSSCG00000003527
	6	80870866	80882391	-1	*LACTBL1*	ENSSSCG00000003529
	6	80896656	80898939	-1	*TEX46*	ENSSSCG00000003530
	6	80903358	80973632	1	*KDM1A*	ENSSSCG00000003531
	6	80931318	80931385	1	*MIR3115*	ENSSSCG00000029242
	6	80973261	81112327	-1	*LUZP1*	ENSSSCG00000003532
	6	81089662	81112347	-1		ENSSSCG00000025500
	6	81196062	81239387	-1	*HNRNPR*	ENSSSCG00000023761
	6	81250123	81261853	-1	*ZNF436*	ENSSSCG00000035332
	6	81268514	81304581	-1	*TCEA3*	ENSSSCG00000032674
	6	81309748	81360248	-1	*ASAP3*	ENSSSCG00000028108
	6	81376652	81398346	-1	*E2F2*	ENSSSCG00000032710

	6	81422546	81424169	-1	*ID3*	ENSSSCG00000039514
	6	81509567	81519072	1		ENSSSCG00000038035
	6	81522760	81526880	1	*RPL11*	ENSSSCG00000024260
	6	81566801	81582028	1	*ELOA*	ENSSSCG00000025440
	6	81590951	81598872	1	*PITHD1*	ENSSSCG00000023495
	6	81601486	81605912	1	*LYPLA2*	ENSSSCG00000023191
	6	81605918	81630365	-1	*GALE*	ENSSSCG00000027827
	6	81610427	81630470	-1	*HMGCL*	ENSSSCG00000026025
	6	81637698	81723231	-1	*FUCA1*	ENSSSCG00000027659
	6	81659186	81692550	-1	*CNR2*	ENSSSCG00000027849
	6	81706663	81741526	-1	*SRSF10*	ENSSSCG00000036293
	6	81722026	81726489	1	*PNRC2*	ENSSSCG00000023326
	6	81780869	81780977	1	*RF00026*	ENSSSCG00000024834
	6	81799570	81903971	-1	*MYOM3*	ENSSSCG00000036534
Coat colour/SSC6_region2	6	85891364	86131721	-1	*SRSF4*	ENSSSCG00000003589
	6	86085281	86085423	-1	*RF02271*	ENSSSCG00000033491
	6	86130111	86130217	-1	*RF00026*	ENSSSCG00000031401
	6	86137754	86228145	1	*PTPRU*	ENSSSCG00000003590
Coat colour/SSC6_region3	6	125354757	125493965	-1		ENSSSCG00000038382
	6	125890532	126043141	1	*PIK3C3*	ENSSSCG00000003749
	6	126083379	126083698	-1		ENSSSCG00000037233
	6	127382248	127392164	-1	*SYT4*	ENSSSCG00000037079
	6	127866246	127890915	1	*KCNG2*	ENSSSCG00000005780
	6	127881306	127952556	-1	*PQLC1*	ENSSSCG00000023171
	6	127954766	127991170	-1	*TXNL4A*	ENSSSCG00000005777
	6	127960266	127973191	1	*HSBP1L1*	ENSSSCG00000005778
	6	128004528	128030296	1		ENSSSCG00000038475
Coat colour/SSC6_region4	6	136695884	136696193	-1	*RF00100*	ENSSSCG00000030678
	6	137263585	137263683	1	*RF00026*	ENSSSCG00000024120
	6	137410919	137453959	-1		ENSSSCG00000003774
	6	136633437	137201213	-1	*ST6GALNAC3*	ENSSSCG00000024494
	6	136057959	136176334	1	*PIGK*	ENSSSCG00000038329
	6	135745879	136020581	-1	*AK5*	ENSSSCG00000003773
	6	136203309	136391034	-1	*ST6GALNAC5*	ENSSSCG00000033425

	6	137383193	137400783	1	*ASB17*	ENSSSCG00000031522
Coat colour/SSC8_region1	8	796389	773556	-1	*FAM53A*	ENSSSCG00000032633
	8	818217	802940	-1	*SLBP*	ENSSSCG00000008676
	8	825182	818588	-1	*TMEM129*	ENSSSCG00000008678
	8	837322	825339	1		ENSSSCG00000008677
	8	895912	879207	1	*FGFR3*	ENSSSCG00000030827
	8	928019	898181	-1	*LETM1*	ENSSSCG00000008675
	8	1019326	964893	1	*NSD2*	ENSSSCG00000008682
	8	1013071	1012948	1	*RF00427*	ENSSSCG00000020173
	8	1038993	1018910	-1	*NELFA*	ENSSSCG00000008681
	8	1058148	1056750	1	*C4orf48*	ENSSSCG00000008686
Coat colour/SSC14_region1	14	71827631	71894754	1	*CCAR1*	ENSSSCG00000010243
	14	71928395	71983778	1	*STOX1*	ENSSSCG00000010245
	14	71970814	71970946	-1	*RF00156*	ENSSSCG00000019527
	14	71989533	72030993	1	*DDX50*	ENSSSCG00000010246
	14	72040083	72061392	1	*DDX21*	ENSSSCG00000010247
	14	72071874	72099801	1	*KIF1BP*	ENSSSCG00000010248
	14	72183363	72199259	1	*SRGN*	ENSSSCG00000023374
	14	72210483	72241657	1	*VPS26A*	ENSSSCG00000010250
	14	72245532	72274827	1	*SUPV3L1*	ENSSSCG00000010251
	14	72282016	72336533	1	*HKDC1*	ENSSSCG00000010252
	14	72382912	72460924	1	*HK1*	ENSSSCG00000010253
	14	72462004	72475662	-1	*TACR2*	ENSSSCG00000010254
	14	72501289	72555081	1	*TSPAN15*	ENSSSCG00000036152
	14	72604468	72606363	-1	*NEUROG3*	ENSSSCG00000034488
	14	72637075	72649799	1	*FAM241B*	ENSSSCG00000040498
	14	72787602	72953095	1	*COL13A1*	ENSSSCG00000010256
Coat colour/SSC15_region1	15	26882562	27362814	-1	*CNTNAP5*	ENSSSCG00000015726
	15	26998479	27356862	1		ENSSSCG00000036368
	15	28530166	28530733	-1		ENSSSCG00000030079
	15	28834319	28837216	-1		ENSSSCG00000039209

435

† Chromosome regions associated with the presence or absence of wattles (wattles) and slate-grey or black colours (coat colours).

‡ *Sus scrofa* chromosome.

§ Starting position of the annotated gene on Sscrofa11.1 genome version.

⁋ End position of the annotated gene on Sscrofa11.1 genome version.

Figure S1. Genomic inflation factors (λ) and quantile–quantile (Q–Q) plots for the genome-wide association studies applied for the four exterior traits investigated: a) ear size (39,732 filtered SNPs); b) ear bearing (41,027 filtered SNPs; c) presence or absence of wattles (38,423 filtered SNPs); d) coat colours (41,230 filtered SNPs).

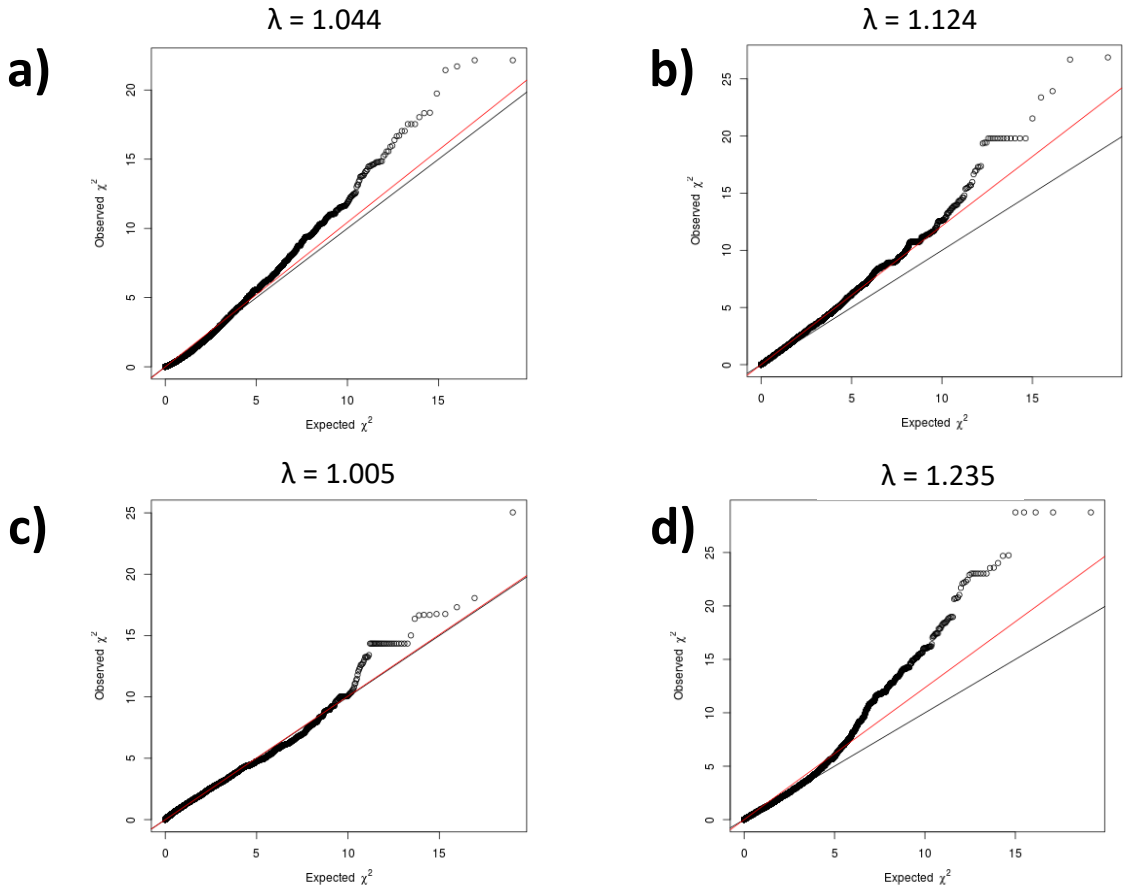
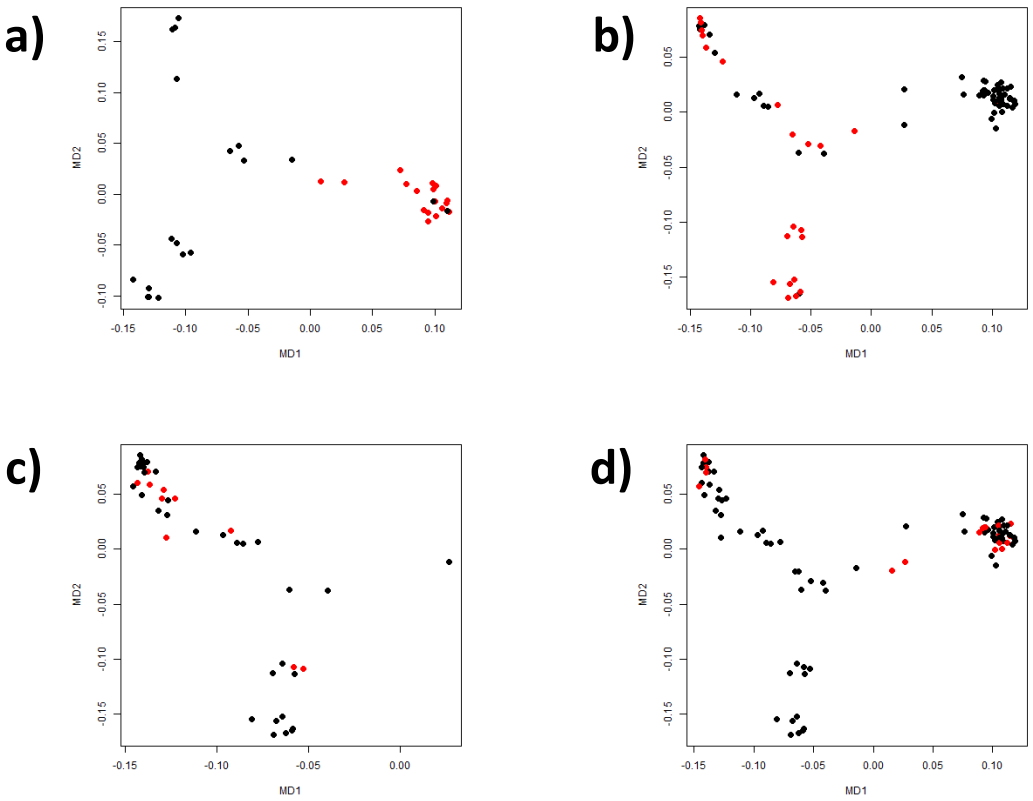



Figure S2. Multidimensional scaling (MDS) plots obtained for the pigs classified according to the alternative phenotypes for the investigated traits: a) ear size (small, black dots; large, red dots); b) ear bearing (floppy, black dots; forward, red dots); c) absence (black dots) or presence (red dots) of wattles ; d) coat colours (slate-grey, black dots; black, red dots). For each trait, the first (MD1) and the second (MD2) coordinates are reported.