ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Genome-wide detection of copy number variants in European autochthonous and commercial pig breeds by whole-genome sequencing of DNA pools identified breed-characterising copy number states

This is the submitted version (pre peer-review, preprint) of the following publication:

This version is available at: https://hdl.handle.net/11585/805594 since: 2021-02-24

Published:

DOI: http://doi.org/10.1111/age.12954

Terms of use:

(Article begins on next page)

13

14

Genome-wide detection of copy number variants in European autochthonous and commercial pig breeds by whole genome sequencing of DNA pools identified breed-characterising copy number states

18

Samuele Bovo[1], Anisa Ribani[1], Maria Muñoz[2], Estefania Alves[2], Jose P. Araujo[3], Riccardo Bozzi[4], Rui Charneca[5], Federica Di Palma[6], Graham Etherington[6], Ana I. Fernandez[2], Fabián García[2], Juan García-Casco[2], Danijel Karolyi[7], Maurizio Gallo[8], Kristina Gvozdanović[9], José Manuel Martins[5], Marie-José Mercat[10], Yolanda Núñez[2], Raquel Quintanilla[11], Čedomir Radović[12], Violeta Razmaite[13], Juliette Riquet[14], Radomir Savić[15], Giuseppina Schiavo[1], Martin Škrlep[16], Graziano Usai[17], Valerio J. Utzeri[1], Christoph Zimmer[18], Cristina Ovilo[2], Luca Fontanesi[1]

25

[1] Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Viale Fanin 46, 40127 Bologna, Italy.

[2] Departamento Mejora Genética Animal, INIA, Crta. de la Coruña, km. 7,5, 28040, Madrid, Spain.

[3] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Viana do Castelo, Escola Superior Agrária, Refóios do Lima, 4990-706 Ponte de Lima Portugal.

[4] DAGRI – Animal Science Section, Università di Firenze, Via delle Cascine 5, 50144 Firenze, Italy.

[5] MED – Mediterranean Institute for Agriculture, Environment and Development & Universidade de Évora, Pólo da Mitra, Apartado 94, 7006-554 Évora, Portugal.

[6] Earlham Institute, Norwich Research Park, Colney Lane, Norwich, NR47UZ, United Kingdom

[7] Department of Animal Science, Faculty of Agriculture, University of Zagreb, Svetošimunska c. 25, 10000 Zagreb, Croatia.

[8] Associazione Nazionale Allevatori Suini (ANAS), Via Nizza 53, 00198 Roma, Italy.

38   [9] Faculty of Agrobiotechnical Sciences Osijek, University of Osijek, Vladimira Preloga 1, 31000,

39   Osijek, Croatia.

40   [10] IFIP Institut du porc, La Motte au Vicomte, BP 35104, 35651, Le Rheu Cedex, France.

41   [11] Programa de Genética y Mejora Animal, IRTA, Torre Marimon, 08140 Caldes de Montbui,

42   Barcelona, Spain.

43   [12] Department of Pig Breeding and Genetics, Institute for Animal Husbandry, 11080 Belgrade-

44   Zemun, Serbia.

45   [13] Animal Science Institute, Lithuanian University of Health Sciences, Baisogala, Lithuania.

46   [14] GenPhySE, Université de Toulouse, INRA, Chemin de Borde-Rouge 24, Auzeville Tolosane,

47   31326 Castanet Tolosan, France.

48   [15] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Serbia.

49   [16] Kmetijski Inštitut Slovenije, Hacquetova 17, SI-1000 Ljubljana.

50   [17] AGRIS SARDEGNA, Loc. Bonassai, 07100 Sassari, Italy.

51   [18] Bäuerliche Erzeugergemeinschaft Schwäbisch Hall, Schwäbisch Hall, Germany.

52

53   * Corresponding author

54   E-mail addresses:

55   LF: luca.fontanesi@unibo.it

56

57   **Short title:** CNV in European pig breeds

58   **Summary**

59   In this study we identified copy number variants (CNVs) in 19 European autochthonous pig breeds

60   and in two commercial breeds (Italian Large White and Italian Duroc) that represent important genetic

61   resources for this species. The genome of 725 pigs was sequenced using a breed specific DNA pooling

62   approach (30-35 animals per pool) obtaining an average depth per pool of 42×. This approach

63   maximized CNV discovery as well as the related copy number states characterizing, on average, the

64   analysed breeds. By mining more than 17.5 billion reads, we identified a total of 9592 CNVs (~683

65   CNVs per breed) and 3710 CNV regions (CNVRs; 1.15% of the reference pig genome), with an

66   average of 77 CNVRs per breed that was considered as private. A few CNVRs were analysed in more

67   details, together with other information derived from sequencing data. For example, the CNVR

68   encompassing the *KIT* gene was associated with coat colour phenotypes in the analysed breeds,

69   confirming the role of the multiple copies in determining breed specific coat colours. The CNVR

70   covering the *MSRB3* gene was associated with ear size in most breeds. The CNVRs affecting the

71   *ELOV6* and *ZNF622* genes were private feature observed in the Lithuanian Indigenous Wattle and in

72   the Turopolje pig breeds, respectively. Overall, genome variability here unravelled can explain part

73   of the genetic diversity among breeds and might contribute to explain their origin, history and

74   adaptation to a variety of production systems.

75

76   **Keywords:** CNV; *ELOV6*; Genetic resource; *KIT*; *MSRB3*; Next generation sequencing; *Sus scrofa*;

77   *ZNF622*.

Introduction

Livestock genomes have been shaped by natural and artificial selection, leading to the accumulation of a broad range of phenotypic and genetic variability that have largely contributed to differentiate populations and constitute modern breeds. As a result, livestock populations and breeds represent a reservoir of genetic diversity, harbouring genetic variants that span from single nucleotide polymorphisms (SNPs) to more complex structural variants, some of which with small to large phenotypic effects on a variety of exterior and economically relevant traits (Andersen *et al.* 2011). Copy number variants (CNVs) are a type of structural variants in the form of large DNA segments, usually more than 1kb of length, which are present in a variable copy number within a species as compared to its reference genome (Feuk *et al.* 2006).

CNVs represent an important source of genetic variability, by influencing phenotypes through a variety of molecular mechanisms such as gene dosage effect, disruption or alteration of coding and regulatory regions among several other modifications (Redon *et al.* 2006, Zhang *et al.* 2006, Bickhart & Liu 2014). Detection of CNVs is technically challenging when applied on genome-wide scale and different technologies have been applied to this aim. Among them, the most commonly used are array comparative genome hybridization (aCGH), high density SNP chip high-throughput sequencing (HTS) platforms (Winchester *et al.* 2009; Alkan *et al.* 2011; Pirooznia *et al.* 2015; Pollard *et al.* 2018). However, due to the decreased cost of HTS analyses and the advantage that this approach has to obtain more precise information on CNVs, whole genome resequencing is becoming a standard approach to discover and characterize CNVs in complex genomes.

Genetic diversity described by CNVs and CNV regions (CNVRs; i.e. CNVs present in different individuals in the same or overlapping genome regions) has been extensively studied in livestock, including, for example, cattle (Fadista *et al.* 2010; Bickhart *et al.* 2012), sheep (Fontanesi *et al.* 2011; Yang *et al.* 2018), goats (Fontanesi *et al.* 2010b; Liu *et al.* 2019a), rabbits (Fontanesi *et al.* 2012) and chickens (Yi *et al.* 2014), among other species. Several studies investigating CNVs and CNVRs have been also reported in pigs, including also an interspecies survey within the genus *Sus* (Paudel *et al.*

5

104   2015). Studies have been focused on the main commercial European breeds (i.e. Duroc, Landrace,

105   Large White, Hampshire, Yorkshire, Piétrain) (e.g. Fadista *et al.* 2008; Li *et al.* 2012; Chen *et al.*

106   2012; Fowler *et al.* 2013; Wang *et al.* 2014, 2015a, c, 2019b; Jiang *et al.* 2014; Wiedmann *et al.* 2015;

107   Revay *et al.* 2015; Long *et al.* 2016; Revilla *et al.* 2017; Stafuzza *et al.* 2019) and Asian breeds

108   (Meishan, Erhualian) (Wang *et al.* 2012, 2014, 2015b, c; Li *et al.* 2012; Chen *et al.* 2012; Jiang *et al.*

109   2014). Other studies screened commercial pig populations in the attempt to capture part of the missing

110   heritability (expected to be explained by CNVs) on economically important traits, including number

111   of piglets born alive (Stafuzza *et al.* 2019), fertility (Revay *et al.* 2015), meat quality traits (Wang *et*

112   *al.* 2015c), fatty acid composition and growth traits (Revilla *et al.* 2017), fat deposition (Fowler *et al.*

113   2013; Schiavo *et al.* 2014), among other traits.

114   Although the modern pig industry relies on few commercial pig breeds, autochthonous pig

115   populations subsist in many different regions, mainly associated with local and traditional niche

116   markets (Čandek-Potokar and Nieto 2019). These breeds represent genetic resources adapted to local

117   agro-climatic and environmental conditions. Up to date, the genome architecture of CNVs has been

118   studied mainly in Asian autochthonous populations/breeds (Li *et al.* 2012; Wang *et al.* 2014, 2015b,

119   2019a; Jiang *et al.* 2014; Dong *et al.* 2015; Xie *et al.* 2016). European autochthonous pig breeds have

120   been mainly investigated by exploring their genetic variability using SNP data (e.g. Ovilo *et al.* 2002;

121   Tomás *et al.* 2011; Wilkinson *et al.* 2013; Silió *et al.* 2016; Yang *et al.* 2017; Muñoz *et al.* 2018,

122   2019; Schiavo *et al.* 2018, 2019, 2020a, b; Ribani *et al.* 2019). A few studies, using SNP arrays,

123   analysed CNVs in European autochthonous pig breeds (e.g. Iberian, Swallow-Bellied Mangalitsa)

124   (Ramayo-Caldas *et al.* 2010; Fernández *et al.* 2014; Molnár *et al.* 2014).

125   Results of CNV studies in pigs showed a limited degree of agreement in terms of CNVRs

126   number and size ranges. Even if part of these discrepancies may be attributed to breed-specific

127   genome features, the remaining discrepancies may derive from the different technologies and

128   algorithms used to unravel CNVs, which mainly used aCGH and SNP arrays. Few other studies

129   analysed CNV and CNVRs in the pig genome using HTS platforms (e.g. Rubin *et al.* 2012; Jiang *et*

130   *al.* 2014; Paudel *et al.* 2015; Wang *et al.* 2015c, 2019b; Long *et al.* 2016; Revilla *et al.* 2017; Keel *et*

131   *al.* 2019).

132   In this study, we provide a detailed survey of CNVs and CNVRs in the pig genome by whole

133   genome resequencing of DNA pools constituted from 21 European pig breeds: 19 autochthonous

134   breeds and belonging to nine different countries and two Italian commercial breeds. These breeds,

135   some of them untapped, stem from different production systems and breeding programmes in Europe.

136   Therefore, dissection of their genome architecture at the level of CNVs could provide new insights

137   into their histories, origin, potential selection signatures and adaptation to different local agro-climatic

138   and environmental conditions.

139

140   **Materials and methods**

141   **Animals**

142   Blood samples were collected from a total of 30 or 35 animals from each of the 21 pig breeds

143   included in the study, distributed in nine European countries (from West to East and then North; Fig.

144   1): Portugal (Alentejana and Bísara); Spain (Majorcan Black); France (Basque and Gascon); Italy

145   (autochthonous: Apulo-Calabrese, Casertana, Cinta Senese, Mora Romagnola, Nero Siciliano and

146   Sarda; and commercial breeds: Italian Large White and Italian Duroc); Slovenia (Krškopolje pig,

147   hereafter indicated as Krškopolje); Croatia (Black Slavonian and Turopolje); Serbia (Moravka and

148   Swallow-Bellied Mangalitsa); Germany (Schwäbisch-Hällisches Schwein); and Lithuania

149   (Lithuanian indigenous wattle and Lithuanian White old type). Selection of individuals for sampling

150   was performed by avoiding highly related animals (no full- or half-sibs), balancing between sexes,

151   and prioritizing adult individuals or at least animals with adult morphology. All animals were

152   registered to their respective Herd Books and presented standard breed characteristics. Details on the

153   analysed animals and investigated breeds, including geographical distribution and phenotypic

154   description, are reported in Table S1.

155

7

DNA samples and sequencing

Genomic DNA was extracted from 8–15 mL of peripheral blood for each pig, collected in Vacutainer tubes containing 10% 0.5 M EDTA (ethylenediaminetetraacetic acid, disodium dihydrate salt) at pH 8.0. The extraction was performed using either a standardized phenol-chloroform (Sambrook *et al*. 1989) or the NucleoSpin® Tissue commercial kit (Macherey-Nagel, Düren, Germany). A total of 21 DNA pools were constructed, including in each pool 30 or 35 individual DNA samples pooled at equimolar concentration (Table S2).

A sequencing library was generated for each DNA pool by using the Truseq® Nano DNA HT Sample preparation Kit (Illumina, CA, USA), following the manufacturer's recommendations. Briefly, DNA was randomly sheared to obtain 350 bp fragments which were end polished, A-tailed, and ligated with the full-length adapter for Illumina sequencing with further PCR amplification. PCR products were purified (AMPure XP system) and libraries were analysed for size distribution by Agilent 2100 Bioanalyzer and quantified using real-time PCR. The qualified libraries were then fed into an Illumina Hi-Seq sequencer for paired-end sequencing, obtaining 150 bp length reads.

Quality controls and sequence alignment

Obtained reads underwent several cleaning and filtering steps including removal of (i) adapters, (ii) reads containing more than 10% unknown bases (N) and (iii) reads containing low quality bases ($Q \leq 5$) over 50% of the total sequenced bases. FASTQ files were sub-sequentially inspected with FASTQC v.0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) that highlighted very high-quality reads.

Reads were mapped on the latest version of the *Sus scrofa* reference genome (Sscrofa11.1) with BWA tool 0.7.17 (Li & Durbin, 2009) (function: MEM) and the parameters for paired-end data. Picard v.2.1.1 (https://broadinstitute.github.io/picard/) was used to remove duplicated reads. Whole genome sequencing data statistics are reported in Table S2.

Detection of CNVs and CNVRs from sequencing data

The cn.Mops v.1.32 tool (Klambauer *et al.* 2012) was used to identify autosomal CNVs. cn.Mops was run with default parameters except for the window size that was lowered to 750 bp. Since three consecutive genome windows positive for copy number are required by cn.Mops to assert the presence of a CNV, the minimum size of a detected CNV was 2250 bp. The 750 bp window size allowed us to detect short CNVs (CNV ≥ 3 kbp with default parameters) with a length fitting the definition of CNV (usually more than 1 kbp). Smaller window sizes were tested resulting in longer computational times without any specific indication on their reliability. CNVs identified in the different breeds were merged into CNVRs with Bedtools v.2.17.0 (Quinlan & Hall 2010) (function: merge) whenever overlapping genome windows, constituting the different CNVs, were encountered.

CNVRs were then compared with previous studies. The comparison was carried out remapping CNVRs on the Sscrofa11.1 using the NCBI genome remapping tool (https://www.ncbi.nlm.nih.gov/genome/tools/remap) looking for CNVRs sharing at least one nucleotide, as proposed by Keel *et al.* (2019).

Cluster analysis of breeds based on CNVRs

Pig breeds were clustered based on the read count ratio of each genome window covered by a CNVR. This ratio was defined as $\frac{RC}{RC_g}$, where RC and RC_g indicate the exact number and the average number of reads in a genome window for a specific pig breed, respectively. Hierarchical clustering was computed in R v.3.6 (R Core Team, 2018) (function: hclust) using the Ward.D2 distance (we excluded genome windows presenting a ratio ≥ 50 in at least one pig breed).

Genomic analysis of repeated elements in CNVs/CNVRs and flaking regions

The GFF file reporting the location of repeated elements interspersed in the *S. scrofa* genome was downloaded from the UCSC Genome Browser (https://genome.ucsc.edu/). For CNVs/CNVRs

9

207 and the related 1-kb flanking regions, we counted the number of bases overlapping each repeated

208 element (Bedtools; function: intersect), assessing their enrichment via Fisher's exact test as

209 implemented in Python 2.6 (Scipy library; function: stats.fisher_exact; alternative hypothesis:

210 greater). We considered statistically enriched classes of repeated elements presenting a $P < 0.05$,

211 Bonferroni corrected.

212

213 **Annotation of CNVRs**

214 Annotated genes overlapping the identified CNVRs were retrieved from the Sscrofa11.1

215 NCBI's GFF file by using Bedtools (function: intersect). Functional analysis was carried out with

216 PANTHER (Mi *et al*. 2019) via Fisher's exact test. Analyses were run over a subset of the Gene

217 Ontology – Biological Process resource (PANTHER GO-slim v.14.1; release 2019-03-12; no. = 2004

218 biological processes) and the Reactome database (Reactome v.65; release 2019-03-12; no. = 1569

219 pathways). We made use of pig specific gene annotations. We considered statistically enriched terms

220 presenting a $P < 0.05$, FDR corrected.

221 The presence of QTLs in CNVRs was evaluated and tested via Fisher's exact test. QTLs were

222 downloaded from the Pig Quantitative Trait Locus Database (Pig QTLdb; release 39) (Hu *et al*. 2019)

223 and checked. Distribution of QTL size pointed out a fraction of long QTLs (> 2 Mbp) probably due

224 lack of resolution derived by the information retrieved from several QTL studies. These QTLs were

225 discarded. We noted that for a given QTL class (i.e. trait) several DNA markers, defining the QTL in

226 different breeds, were close to each other. Thus, QTLs that were less than 500 kbp of distance were

227 merged with Bedtools (function: merge) to obtain QTL regions. The final dataset presented a total of

228 295 traits and 1978 QTL regions. For each trait, the fraction of CNVR nucleotides overlapping QTLs

229 was retrieved with Bedtools (function: intersect). Fisher's exact test was run in Python, retrieving

230 statistically enriched traits presenting a $P < 0.05$, Bonferroni corrected.

231

232 **Results**

Sequenced reads and genome wide identification of CNVs

About 17.5 billion reads were produced from the sequencing of the 21 pig DNA pools. On average, each DNA pool presented about 417.7 million of mapped reads spanning 98.5% of the *S. scrofa* reference genome, with an average read depth of about 42×. Summary statistics of sequencing data are reported in Table S2.

Using cn.Mops we identified a total of 9592 CNVs (14344 events) across the 21 analysed breeds. On average, each pig breed had 683 CNVs (median = 601; min. = 209, Sarda; max. = 1440 Turopolje) covering 0.18% (s.d. = 0.09%) of the reference genome, with the smallest fraction in Sarda (0.04%) and the largest coverage in Turopolje (0.40%), reflecting the lowest and highest number of CNVs, respectively (Table 1). For each pig breed, CNVs were divided in losses (copy number < 2, as inferred by cn.Mops) and gains (copy number > 2, as inferred by cn.Mops) that represented the most frequent copy number (CN) state characterizing the animals analysed in the pools. On the whole, we identified a total of 3492 losses, 5012 gains and 638 showing a mix of copy number loss and gain. The losses/gains ratio was around 0.79. Stratified by chromosome, this value ranged from 0.57 to 1.02, for SSC12 and SSC1, respectively (Table S3). Considering the CNVs detected in each breed, the number of losses and gains strongly correlated (r = 0.93). CNV length ranged from 2250 to 560250 bp. The longest CNV (560250 Mbp) was detected on SSC8 in the Italian Large White and Lithuanian White Old Type pig breeds (Table 1). The number of CNVs and the chromosome length had a medium-high Pearson's correlation coefficient (r = 0.69; P < 0.05).

Identification of CNVRs

CNVs were merged across breeds resulting in a total of 3710 CNVRs (Table S4). The distribution of CNVRs along each chromosome is presented in Fig. 2. SSC1, SSC2 and SSC3 had the largest number of detected CNVRs (no. = 359, no. = 361 and no. = 307, respectively; Table 2). The number of CNVRs and the chromosome length highly correlated (r = 0.87; P < 0.05). Positive correlation (r = 0.92, P < 0.05) was observed also between the number of CNVRs and their total

11

259   length. On average, each pig breed had 586 CNVRs (min. = 180 in Sarda; max. = 1257 in Turopolje;

260   Table S5). Among the 3710 CNVRs, 1615 (43.5%) were breed specific (and indicated as private

261   CNVRs; Table S5). Size of CNVRs ranged from 2250 bp up to 560250 bp (the same of CNVs), with

262   an average length of 7038 bp and a median value of 3750 bp (Table 2). Distribution of CNVR size

263   showed a decrease in CNVR counts while increasing their size. CNVRs occupied a total of 26.1 Mbp,

264   equal to 1.15% of the Sscrofa11.1 reference genome. Among the CNVRs, based on the copy number

265   state (i.e. the number of copies; CN state) provided by cn.MOPS, 1305 (35.2%) had only copy number

266   gains (duplication), 1323 (35.6%) had only copy number losses (deletion), and 1082 (29.2%) showed

267   a mix of copy number losses and gains from different pig breeds.

268   The 3710 detected CNVRs encompassed a total of 34821 genome windows. After filtering, the

269   read count ratio of each genome window was used to cluster pig breeds (Fig. 3), which grouped breeds

270   in agreement to their main specific phenotypes or their geographic origin. A first group encompassed

271   breeds that have a coat colour with white background or white patterns (Lithuanian Indigenous

272   Wattle, Italian Large White, Krškopolje, Bísara and Lithuanian White Old Type). This may be due to

273   the strong signals of genome windows encompassing the *KIT* gene, that accounts for ~15% of the

274   total positive windows for CNVs. The two reddish brown coloured breeds (Mora Romagnola and

275   Italian Duroc) were on the same branch. Three autochthonous Italian breeds (Casertana, Nero

276   Siciliano and Sarda) constituted a cluster whereas one Portuguese and one Spanish breed (Alentejana

277   and Majorcan Black, respectively) constituted another cluster. The Turopolje pig breed was the only

278   one that clustered apart from all other breeds.

279

280   **Repeated elements within and flanking CNVs and CNVRs**

281   Highly repetitive sequences were investigated for their co-occurrence with CNVs and CNVRs

282   (Table S6). The following classes of repeated elements were statistically over-represented within

283   CNVs: long interspersed nuclear elements (LINE), long terminal repeats (LTR), satellites, rolling-

284   circle (RC/Helitron) and pseudogenes (tRNAs, snRNAs, srpRNAs, and rRNAs). Additionally, CNV

12

285 flanking regions (1-kbp per side) were enriched for the following classes: short interspersed nuclear

286 elements (SINE), simple repeat and low complexity. CNVRs differed for the absence of RC elements

287 and the absence of SINE and srpRNAs in the 1-kbp flanking regions. However, SINE were over-

288 represented when the flanking region size was extended to 10-kbp.

289

290 **QTLs in CNVRs**

291 A total of 1978 QTL regions, associated to 554 phenotypic traits, were retrieved from the pig

292 QTL database. CNVRs overlapped a total of 336 QTL regions representing 295 phenotypic traits.

293 Enrichment analysis identified 126 traits ($\sim 43\%$) significantly over-represented ($P < 0.05$, Bonferroni

294 corrected). These traits spanned different classes, including meat quality, body shape and

295 conformation, reproduction, disease susceptibility, haematological and metabolism related traits

296 (Table S7).

297

298 **Functional annotation of CNVRs and detailed analysis of selected genes**

299 A total of 1571 genes overlapped the identified CNVRs, including 1296 protein coding genes,

300 261 lncRNAs, 3 miRNAs and 11 tRNAs. The number of overlapped genes correlated with the number

301 of CNVRs ($r = 0.99$). A total of 993 protein-coding genes were annotated by PANTHER and used

302 for functional enrichment over the GO slim Biological process resource. A total of 17 terms were

303 over-represented (Table S8), encompassing different biological processes such as sensory perception,

304 nervous system process, fatty acid metabolic process, gene expression and biological adhesion. Over

305 the Reactome database, PANTHER over-represented the olfactory signalling pathway and the related

306 mechanism of transduction mediated by G protein-coupled receptors (Table S8). Analysis of genes

307 located in private CNVRs did not identify any over-represented process/pathway.

308 The *v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog* (*KIT*) and the *methionine*

309 *sulfoxide reductase B3* (*MSRB3*) genes were two important genes presenting variable copies among

310 breeds. CNVs affecting the *KIT* gene are responsible for different coat colour phenotypes (Johansson

13

311 Moller *et al.* 1996; Marklund *et al.* 1998; Johansson *et al.* 2005; Rubin *et al.* 2012) whereas variable

312 copies of the *MSRB3* have been associated with ear size in pigs (Chen *et al.* 2018).

313   The detailed analysis of the *KIT* gene indicated the presence of the four duplicated regions

314 (DUP1-4; Fig. 4a) previously described by Rubin *et al.* (2012). Structural variants as well as the

315 presence of the splice mutation at the first base in intron 17 (g.41486012G>A, rs345599765) are all

316 required for manifesting a solid white coat colour (Marklund *et al.* 1998). Using sequence data, we

317 estimated the allele frequencies of this SNP (Fig. 4a; Table S9) to complement CNV results. Pools

318 from colored pigs did not show any CNV and the splice mutation. White pigs (Italian Large White

319 and Lithuanian White Old Type) had DUP1-4 and the splice mutation (allele A). However, allele

320 frequencies were divergent (Table S9) suggesting a different structure of the CNV (different gene

321 copies with the the A or G nucleotides). The Sarda (not fixed for any coat colour and including many

322 spotted animals) and Lithuanian Indigenous Wattle breeds presented DUP1, did not have DUP2-4

323 and had allele A (the only two other breeds having the splice mutation). Bísara, another spotted breed,

324 had also DUP2-3. The piebald breed Basque and the belted breed Cinta Senese had DUP2-4, whereas

325 the other two belted breeds (Krškopolje and Schwäbisch-Hällisches Schwein) had only DUP2 and

326 DUP4.

327   The detailed analysis of the *MSRB3* gene region revealed the presence of the 38.4-kbp

328 duplication (SSC5:29826981-29865653; Fig. 4b) previously described by Chen *et al.* (2018). Copy

329 number gains encompassing the *MSRB3* exons 6 and 7 have been associated with large ear size in

330 Chinese pig breeds and with half-floppy ears in Landrace pigs (Chen *et al.* 2018). Alentejana, Cinta

331 Senese, Mora Romagnola, Italian Duroc and Italian Large White that are breeds characterized by

332 small/medium ear size, had a normal copy number state (that means no gain of copies). The remaining

333 pig breeds showed variable copy number which seems to be correlated to ear size (Fig. 4c).

334 Regression analysis between the average CN state and the ear size (coded as follows: small = 1,

335 medium = 1.5, medium/large = 1.75 and large = 2) resulted in a positive association ($P = 0.0001$).

336 However, other breeds characterized by small ears (i.e. Nero Siciliano and Sarda) had variable copy

14

numbers. Variability in ear size was also analysed by estimating the allele frequency of two SNP in the 5' flanking region (g.29695369C>T; rs340841870) and in the 3'-untranslated region (g.29862412C>T; rs326411202) of the *MSRB3* gene, that Zhang *et al.* (2015) reported to be associated with ear size. These SNP positions are not included in the CNVR of this gene. For each SNP, the regression analysis pointed out a significant association between allele frequencies and ear size ($P < 0.0001$). Additionally, allele frequencies of these two SNPs (Table S9) correlated with the average CN state ($|r| > 0.8$; $P < 0.0001$; Fig. 4d).

We further explored genomic regions harbouring private information on CNVRs. Among them, we identified two interesting examples. The first one, characterizing Lithuanian Indigenous Wattle pigs, encompassed the intron 10 of the *ELOVL fatty acid elongase 6* (*ELOVL6*) gene (Fig. 5a). Variants in this gene has been associated with fatty acid composition in pigs (Corominas *et al.* 2013). The second one, characterizing Turopolje pigs, was the *Zinc finger protein 622* (*ZNF622*) gene, a regulator of early embryonic development (Hasegawa *et al.* 2015). The CNV affecting this gene was quite complex. Copy number gains were in the correspondence of the exonic regions but also included the complete intron 1, intron 2 and intron 5. Most of introns 3 and 4 were not affected by CN gains (only small and contiguous intronic segments to the exonic regions were included in the CN gains) (Fig. 5b). The regions with CN gains were clearly evidenced in all breeds except Turopolje, which did not have any copy number and, in part, in Krškopolje and Italian Duroc, that had CN higher than that of Turopolje but lower than that of all other breeds (Fig. 5b).

Comparison with other studies

The positions of CNVRs we detected were compared with the CNVRs reported by previous studies, which analysed different pig breeds and other species of the *Sus* genus using whole genome sequencing. A total of five datasets, which investigated Asian pig breeds, commercial and European pig breeds, and five species of the genus *Sus*, were considered for this comparison (Table S4). The

362   overlap ranged from about 10 to 25% (Table S10). Overall, a total of 595 CNVRs detected in our

363   work (16%) overlapped with CNVRs reported by the considered studies (Table S4).

364

365   **Discussion**

366   In this study we carried out a genome-wide CNV/CNVR analysis in 19 European

367   autochthonous and two Italian commercial pig breeds. Breeds were analysed by using a whole

368   genome sequencing strategy from breed specific DNA pools to maximize CNV discovery. CNVs

369   were detected via cn.MOPS, a tool that implements a Bayesian approach that models depth of

370   coverage across samples by decomposing its variability in a part coming from copy numbers and the

371   remaining part due to noise, in order to reduce false discoveries (Klambauer *et al.* 2012). Other

372   software based on different assumptions have been also developed and used for CNV detection from

373   HTS datasets. However, there is no consensus in the literature on the strategy and methodology that

374   might be applied for this purpose.

375   As our study was based on DNA pools from a large number of populations, we maximized the

376   power of cn.MOPS in reducing the false discovery rate, as this tool is specifically designed to deal

377   with multiple samples.

378   Even if this design could not precisely define the exact number of copy gains or losses for all

379   animals in the sequenced pools, the obtained results made it possible to capture within breed averaged

380   states. This was supported by the agreement among the different coat colour phenotypes and the

381   expected CN states, rightly detected at the *KIT* locus which indirectly confirmed and validated CNV

382   calls from cn.MOPS. This approach demonstrated that CNVs detected using whole genome

383   sequencing can be useful to identify breed specific features (including in this definition the most

384   frequent breed features) and describe genetic diversity across pig breeds, complementing SNP based

385   studies.

386   We confirmed a high correspondence between CNV data detected from sequenced DNA pools

387   and SNP information using Pearson's correlation calculated considering the fraction of the pig

16

genome covered by CNVRs detected for each breed (Table 1) and SNP based diversity measured on the same animals genotyped with the GeneSeek ® GGP Porcine HD Genomic Profiler (Muñoz *et al*. 2019). Among these SNP averaged variability parameters, correlation with the above mentioned CNVR parameter was highly negative with both the minor allele frequency (MAF; r = -0.90) and expected heterozygosity (r = -0.90), whereas highly positive correlation with the Fixation Index (F_{ST}; r = 0.96) values. These correlations mean that when within breed variability was low, it increased the possibility to identify losses/gains at variable CN state and that the fraction of the genome covered by CNVRs detected in DNA pools is a good indicator of the diversity among breeds.

With few differences, these breeds were clustered resembling the relationships that we already reported using array SNP datasets obtained from individually genotyped pigs and SNPs detected from whole genome sequencing (Muñoz *et al*. 2018, 2019; Bovo *et al*, in preparation). Geographical and some major morphological features (i.e. coat colour) mainly determined breed clusters obtained from CN states. Turopolje, the breed that accounted for the largest number of CNVs (with the largest fraction of the genome covered by CNVRs), was clustered apart, as also reported with SNP data (Muñoz *et al*. 2018, 2019; Bovo *et al*, in preparation).

Some CNVRs were considered as breed specific or identified in a few breeds, suggesting that this variability might contribute to determine several phenotypic characteristics that distinguish autochthonous and commercial European breeds. In addition, considering the whole patterns of CNVRs that we detected, a quite high frequency of these events was classified as mixed CNVs (including both gains and losses). This indicates that despite breeds share genome regions affected by CNV, the single breed carries a gain or a loss specific for the breed itself.

In the current study, an average of 77 CNVRs (~16% of all breed reported CNVRs) was considered as private for each analysed breed, highlighting the power of the DNA pooling strategy in capturing distinctive breed features. However, as the sequencing depth is not so high, for a given private CNVR we cannot completely exclude the possibility that few animals of the other investigated breeds could carry the same alleles in these regions. The remaining CNVRs were shared among two

17

414   or more breeds, indicating that admixture and crossbreeding events or a common origin might have

415   contributed to spread this variability. However, further studies are needed to clarify their allelic status

416   or their common origin, as in our first survey we did not characterize into detail the precise breakdown

417   positions and structure of all identified CNVRs.

418   CNVRs we detected overlapped genes involved in different biological processes including

419   nervous system and sensory perception such as olfactory signalling. Brain functions control several

420   behaviours, including feeding, habitat selection, reproduction and social interaction that strongly

421   depend on the genetics architecture of an individual (Bendesky & Bargmann 2011). Several studies

422   in mammals including pigs reported CNVs in genes involved in the olfactory signalling pathway,

423   linking gene variability to food foraging and mate recognition abilities (Paudel *et al*. 2015; Keel *et*

424   *al*. 2019). In addition, considering the overlapping of CNVRs and QTL regions, the main traits

425   associated with changes in CN state were meat quality, body shape and conformation, reproduction

426   and metabolism. Variability in chromosome regions harbouring functionally relevant genes or QTL

427   may reflect the adaptation of these breeds to different production systems and environments.

428   The impact of this type of variability on exterior characteristics of the pigs has been already

429   demonstrated for the CNVs in the *KIT* gene region affecting coat colours and patterns, which

430   characterize the *Dominant white* phenotype (Rubin *et al*. 2012). Other evidences came for the CNVs

431   in the *MS3B3* gene region, involved in ear size as mainly reported in Chinese breeds (Chen *et al*.

432   2018). These CNVRs were also detected in our study with some interesting new information for some

433   of the analysed breeds.

434   The complexity of the *Dominant white KIT* locus has been explained by the presence of six

435   main allele groups (in addition to a few other potential variants; Fontanesi & Russo 2013): (i) a

436   recessive wild-type allele *i* (that is carried by wild boar and coloured pigs), (ii) the *Patch* allele I^P

437   (determining spotted patterns), (iii) the *Belt* allele I^{Be} (determining the belted phenotype), (iv) the

438   *Roan/Gray* allele I^{Rn} or I^d (causing the grey-roan phenotype), (v) the dominant white alleles *I*,

439   comprising several forms (e.g. I^1, I^2 and I^3) and causing the white solid phenotype that mainly

18

440   characterize Large White and Landrace breeds and (vi) the I^L allele, a null and lethal allele (Johansson

441   Moller *et al*. 1996; Marklund *et al*. 1998; Johansson *et al*. 2005; Rubin *et al*. 2012). Variants in this

442   chromosome region are mainly associated with a 450-kbp duplication encompassing the entire *KIT*

443   gene (DUP1; the only CN of the I^P allele), including also another 4.3-kbp duplication (DUP2) located

444   ∼100 kbp upstream of *KIT* gene, and a 23-kbp duplication (DUP3) ∼100 kbp downstream from *KIT*,

445   which in turn resulted to contain another 4.3-kbp duplication (DUP4; Rubin *et al*. 2012). The *I* alleles

446   presented variable copy numbers of DUP1/2/3/4, whereas DUP2/3/4 were identified in pigs with the

447   I^{Be} allele (Rubin *et al*. 2012). Moreover, a recent whole genome resequencing study uncovered new

448   *KIT* alleles conferring different coat colour phenotypes (Wu *et al*. 2019). The CN state states that we

449   identified in our study encompassed all four duplicated regions, describing for the first time the

450   structure of the *KIT* gene in several autochthonous pig breeds (Fig. 4a).

451   In addition, analysis of sequencing data let us to estimate the frequency of the splice mutation

452   g.41486012G>A (rs345599765) that distinguish the CN state of the I^P from the *I Dominant white*

453   allele series (Marklund *et al*. 1998). As expected, all breeds that did not show any duplicated regions

454   are characterized by solid coat colours and did not have the splice mutation. They are considered to

455   carry only the *i* wild-type at the *Dominat white* locus. Sarda, which is a breed not fixed for any coat

456   colours and that includes also white and white spotted pigs, showed the presence of DUP1, with some

457   faint signs at the DUP4 position (with a low frequency of the splice mutation). Several alleles at the

458   *KIT* gene might be present in this breed, including I^P, *I* variants and I^{Be} forms. A similar pattern was

459   observed in the Lithuanian Indigenous Wattle breed, which includes mainly spotted pigs. According

460   to the CN state observed in this breed, I^P might be the most frequent allele, even if other and I^{Be} and

461   *I* forms (including also DUP4) might be present. A more marked copy number pattern was evidenced

462   for the Bísara breed (which has mainly heterogeneous coats: grey or black and white or spotted) that

463   reported DUP1 copy number status similar to Sarda and Lithuanian Indigenous Wattle) in addition to

464   DUP2-3 (without signals indicating the presence of DUP4).

465   The analysis of the *KIT* gene region in breeds characterized by a belted phenotype, even if not

466   homogeneous, indicated that more alleles at this locus might produce belted pigs even if with some

467   different phenotypic effects. Cinta Senese and Basque had equal CN state at DUP2-3 but differed in

468   DUP4 (higher in Basque and lower in Cinta Senese). Cinta Senese is a classical belted breed whereas

469   Basque pigs are usually black and white with heterogenous patterns but usually with black head and

470   rump. Other breeds having white belts of varying size and shape (Krškopolje and Schwäbisch-

471   Hällisches Schwein) showed only DUP2 and DUP4. The connection between the two breeds might

472   be derived by ancestral origins (not clearly defined), that preserved the same structure at the *Dominant*

473   *white* locus. Wu *et al*. (2019) observed that the presence of DUP2 together with DUP4 can produce

474   a belted phenotype in Duroc × (Landrace × Large White) hybrid pigs. The presence of multiple alleles

475   conferring a belted phenotype is also confirmed by the results of the analysis of the rs328592739 SNP

476   in the *KIT* gene that was associated with the belted pattern in Cinta Senese pigs (Fontanesi *et al*. 2016)

477   but not in Krškopolje and Schwäbisch-Hällisches Schwein pigs (Ogorevc *et al*. 2017).

478   White breeds (Italian Large White and Lithuanian White Old Type) had a classical copy number

479   pattern in DUP1-4 and the splice mutation already described for completely white pigs carrying *I*

480   alleles (Fontanesi *et al.* 2010a). Heterogeneity on the presence of the splice mutation suggested that

481   *Dominant white* alleles having different G/A ratios at this position. In Lithuanian White Old Type,

482   gene copies at this position carried G only in 1 out of 5 copies (as estimated from its 0.20 frequency).

483   In Italian Large White, about 2 out of 3 gene copies carried the G nucleotide (G = 0.68), suggesting

484   that the CNV structure in this breed might be determined by different *Dominant white* alleles than

485   those frequently present in the Lithuanian White Old Type breed.

486   Interesting copy number patterns were also observed in the region of SSC5 encompassing the

487   last exons of the *MSRB3* gene (Fig. 4b), which is associated with ear size (Chen *et al*. 2018). These

488   authors proposed that large ear size is due to the increased CN state in this region, which affects the

489   expression of the nearby miR-584-5p that in turn inhibits the expression of its target gene *MSRB3*.

490   Our CNV analysis for the *MSRB3* gene across autochthonous European pig breeds indicated, with

491   the exception of some breeds, a significant correlation between ear size and the average CN state

492   (Fig. 4c). The latter also correlated with allele frequencies estimated for the rs340841870 and

493   rs326411202 SNPs (outside this CNVR), which suggested the presence of linkage between these two

494   types of variants: allele C at both positions is associated with a normal copy state whereas the

495   alternative allele at both sides (T) is associated with the presence of 5 or 6 copies (of the linked

496   multiple copy region), as estimated from the sequencing data in the CNVR. Even if pigs of the studied

497   breeds were in general described to have breed-specific traits, heterogeneity for ear size has been

498   already reported in some breeds which might not actually have fixed ear size shape (Schiavo *et al*.

499   2019). Therefore, correlation between CN state and ear size might not precisely estimated by the

500   DNA pooling approach (Fig. 4b). It is also worth mentioning that ear size and position have been

501   already shown to be under polygenic control with a few major genes affecting these traits (e.g. Wei

502   *et al.* 2007; Ma *et al.* 2009; Ren *et al.* 2011). Thus, other genomic regions and polymorphisms could

503   be responsible for the ear size phenotype in some of the analysed breeds.

504   The CNV in the *ELOVL6* gene might interesting to explain economically relevant traits,

505   considering the role of this gene in affecting fatty acid composition in pigs (Corominas *et al.* 2013).

506   Other studies reported that variability in this gene or variability in its expression level might explain,

507   at least in part, differences of intramuscular fat accumulation and lipid metabolism among breeds,

508   which are relevant for meat quality, considering also genotype-feeding interactions to design

509   appropriate fatty-acid diets in pigs to maximize this aspect (e.g. Benítez *et al.* 2016; Muñoz *et al.*

510   2018; Revilla *et al.* 2018). Association studies and functional analysis of the CNV in this gene are

511   needed to understand if this variability could be involved in affecting meat quality traits in pigs.

512   Targeted analyses are also needed to detect with more precision if this variability segregates within

513   the analysed breeds as well as in other breeds in which meat quality parameters are important factors

514   determining the quality of their products.

515   Detailed analyses of CN states of some chromosome regions can also identify (or suppose) the

516   occurrence of other or more complex mutational events that might not be properly considered as

517 derived by CNVs. The case of the *ZNF622* gene that reported three distinct copy number gains

518 (mainly in the correspondence of exonic regions) might raise a few hypotheses on the occurrence of

519 this strange pattern. The three divided copy number gains might be due to the presence of a

520 pseudogene derived by the *ZNF622* gene (inserted somewhere into the genome) or that the

521 duplication of the gene subsequently underwent other mutational events that eliminated most of the

522 sequence of introns 3 and 4 (Fig. 5b). Other studies are needed to clarify these hypotheses. After a

523 preliminary analysis, CN states reported in the correspondence of this gene appeared to produce a

524 private condition in the Turopolje breed that did not have any copy number gain (common in all other

525 breeds). Inspection of the clustering analysis for the CN at this gene in all breeds, indicated that two

526 other breeds (Krškopolje and Italian Duroc) might not have fixed copy number gains, mainly in the

527 correspondence of the annotated exons of the *ZNF622* gene.

528 On the whole, our survey on European pig breeds reported that CNVRs occupy 26.1 Mbp,

529 representing 1.15% of the reference genome size. Compared to other whole genome sequencing based

530 studies, this genome fraction is similar to what was reported by Paudel *et al*. (2015) and Keel *et al*.

531 (2019) (17.83 and 22.9 Mbp, respectively). Other two studies (Paudel *et al*. 2013; Jiang *et al*. 2014)

532 identified larger fractions of the pig genome covered by CNVRs (39.2 and 102.8 Mbp, respectively).

533 Although this divergence could be attributed in part to the algorithms used to detect CNVs and the

534 sequencing approaches (single pigs *vs* pools of individuals), it might be also due to differences among

535 the studied pig populations. Distribution of CNVR sizes showed a decrease in CNVR counts while

536 increasing their size, as also described by Jiang *et al*. (2014). Differences among breeds were also

537 clearly shown in our study, as detailed above. Some of the CNVRs we detected in our study

538 overlapped with CNV events reported by the other whole genome sequencing mentioned studies (on

539 average, ~13% of overlap), pointing out that they could exist also in other breeds that we did not

540 survey. However, they represent just fraction a small fraction, strengthening the evidence that CNV

541 are breed-specific genome features. Additional studies are needed to obtain a global overview of

542 CNVs segregating in the *Sus scrofa* species, by comparing more breeds and populations.

543   As CNVs mutate about 2-3 times faster than SNPs, some of the CNVRs that we detected across

544   several breeds could eventually also be derived from recurrent mutational events through nonallelic

545   homologous recombination, potentially driven by the presence of repeated regions within or in

546   flanking positions (Liu *et al.* 2012). Analyses of CNVRs and their flanking regions identified

547   enrichments of different classes of repeated elements, confirming what other studies reported this

548   species (e.g. Paudel *et al*. 2013; Wang *et al*. 2015b). This further suggest that these sequence features

549   might contribute to chromosome instability and mutational mechanisms promoting these structural

550   changes also in *Sus scrofa.*

551   Our study investigated CNVs in the porcine genome over a large number of pig breeds that

552   represent important European genetic resources for this species. This variability can explain part of

553   the genetic diversity among breeds and might contribute to explain their origin, history and adaptation

554   to a variety of production systems. Further studies are needed to better understand how CNVs could

555   be considered in defining conservation programmes of these autochthonous genetic resources.

556

565

Availability of data

567 Sequence data generated and analysed in the current study are available in the EMBL-EBI European

568 Nucleotide Archive (ENA) repository (http://www.ebi.ac.uk/ena), under the study accession

569 PRJEB36830. CNVRs are available as Supplementary Table S4 and from the corresponding author

570 on reasonable request.

571

572

573

574 **Competing interests**

575 The authors declare they do not have any competing interests.

576

577 **References**

578 Alkan C., Coe B.P. & Eichler E.E. (2011) Genome structural variation discovery and genotyping.

579   *Nature Reviews Genetics* **12**, 363–76.

580 Andersen I.L., Nævdal E. & Bøe K.E. (2011) Maternal investment, sibling competition, and offspring

581   survival with increasing litter size and parity in pigs (*Sus scrofa*). *Behavioral Ecology and*

582   *Sociobiology* **65**, 1159–67.

583 Bendesky A. & Bargmann C.I. (2011) Genetic contributions to behavioural diversity at the gene-

584   environment interface. *Nature Reviews Genetics* **12**, 809–20.

585 Benítez R., Núñez Y., Fernández A., Isabel B., Rodríguez C., Daza A., López-Bote C., Silió L. &

586   Óvilo C. (2016) Adipose tissue transcriptional response of lipid metabolism genes in growing

587   Iberian pigs fed oleic acid v. carbohydrate enriched diets. *Animal* **10**, 939–46.

588 Bickhart D.M., Hou Y., Schroeder S.G., Alkan C., Cardone M.F., Matukumalli L.K., Song J.,

589   Schnabel R.D., Ventura M., Taylor J.F., Garcia J.F., Van Tassell C.P., Sonstegard T.S., Eichler

590   E.E. & Liu G.E. (2012) Copy number variation of individual cattle genomes using next-

591   generation sequencing. *Genome Research* **22**, 778–90.

592 Bickhart D.M. & Liu G.E. (2014) The challenges and importance of structural variation detection in

593   livestock. *Frontiers in Genetics* **5**, 37.

594 Čandek-Potokar M. & Nieto Liñan R.M. (2019) European Local Pig Breeds - Diversity and
595   Performance. A study of project TREASURE. IntechOpen, doi:10.5772/intechopen.83749.

596 Chen C., Liu C., Xiong X., Fang S., Yang H., Zhang Z., Ren J., Guo Y. & Huang L. (2018) Copy
597   number variation in the *MSRB3* gene enlarges porcine ear size through a mechanism involving
598   miR-584-5p. *Genetics Selection Evolution* **50**, 72.

599 Chen C., Qiao R., Wei R., Guo Y., Ai H., Ma J., Ren J. & Huang L. (2012) A comprehensive survey
600   of copy number variation in 18 diverse pig populations and identification of candidate copy
601   number variable genes associated with complex traits. *BMC Genomics* **13**, 733.

602 Corominas J., Ramayo-Caldas Y., Puig-Oliveras A., Pérez-Montarelo D., Noguera J.L., Folch J.M.
603   & Ballester M. (2013) Polymorphism in the *ELOVL6* gene is associated with a major QTL
604   effect on fatty acid composition in pigs. *PLoS One* **8**, e53687.

605 Dong K., Pu Y., Yao N., Shu G., Liu X., He X., Zhao Q., Guan W. & Ma Y. (2015) Copy number
606   variation detection using SNP genotyping arrays in three Chinese pig breeds. *Animal Genetics*
607   **46**, 101–9.

608 Fadista J., Nygaard M., Holm L.E., Thomsen B. & Bendixen C. (2008) A snapshot of CNVs in the
609   pig genome. *PLoS One* **3**, e3916.

610 Fadista J., Thomsen B., Holm L.-E. & Bendixen C. (2010) Copy number variation in the bovine
611   genome. *BMC Genomics* **11**, 284.

612 Fernández A.I., Barragán C., Fernández A., Rodríguez M.C. & Villanueva B. (2014) Copy number
613   variants in a highly inbred Iberian porcine strain. *Animal Genetics* **45**, 357–66.

614 Feuk L., Carson A.R. & Scherer S.W. (2006) Structural variation in the human genome. *Nature*
615   *Reviews Genetics* **7**, 85–97.

616 Fontanesi L., Beretti F., Martelli P.L., Colombo M., Dall'olio S., Occidente M., Portolano B., Casadio
617   R., Matassino D. & Russo V. (2011) A first comparative map of copy number variations in the
618   sheep genome. *Genomics* **97**, 158–65.

619   Fontanesi L., D'Alessandro E., Scotti E., Liotta L., Crovetti A., Chiofalo V. & Russo V. (2010a)

620   Genetic heterogeneity and selection signature at the *KIT* gene in pigs showing different coat

621   colours and patterns. *Animal Genetics* **41**, 478–92.

622   Fontanesi L., Martelli P.L., Beretti F., Riggio V., Dall'Olio S., Colombo M., Casadio R., Russo V. &

623   Portolano B. (2010b) An initial comparative map of copy number variations in the goat (*Capra*

624   *hircus*) genome. *BMC Genomics* **11**, 639.

625   Fontanesi L., Martelli P.L., Scotti E., Russo V., Rogel-Gaillard C., Casadio R. & Vernesi C. (2012)

626   Exploring copy number variation in the rabbit (*Oryctolagus cuniculus*) genome by array

627   comparative genome hybridization. *Genomics* **100**, 245–51.

628   Fontanesi L. & Russo V. (2013) Molecular genetics of coat colour in pigs. *Acta Agriculturae*

629   *Slovenica* **4**, 16.

630   Fontanesi L., Scotti E., Gallo M., Nanni Costa L. & Dall'Olio S. (2016) Authentication of "mono-

631   breed" pork products: Identification of a coat colour gene marker in Cinta Senese pigs useful

632   to this purpose. *Livestock Science* **184**, 71–7.

633   Fowler K.E., Pong-Wong R., Bauer J., Clemente E.J., Reitter C.P., Affara N.A., Waite S., Walling

634   G.A. & Griffin D.K. (2013) Genome wide analysis reveals single nucleotide polymorphisms

635   associated with fatness and putative novel copy number variants in three pig breeds. *BMC*

636   *Genomics* **14**, 784.

637   Hasegawa Y., Taylor D., Ovchinnikov D.A., Wolvetang E.J., de Torrenté L., Mar J.C. (2015)

638   Variability of gene expression identifies transcriptional regulators of early human embryonic

639   development. *PLoS Genetics* **11**, e1005428.

640   Hay E.H.A., Choi I., Xu L., Zhou Y., Rowland R.R.R., Lunney J.K. & Liu G.E. (2017) CNV Analysis

641   of host responses to Porcine Reproductive and Respiratory Syndrome Virus infection. *Journal*

642   *of Genomics* **5**, 58–63.

643 Hu Z.L., Park C.A. & Reecy J.M. (2019) Building a livestock genetic and genomic information

644   knowledgebase through integrative developments of Animal QTLdb and CorrDB. *Nucleic*

645   *Acids Research* **47**, D701–10.

646 Jiang J., Wang J., Wang H., Zhang Y., Kang H., Feng X., Wang J., Yin Z., Bao W., Zhang Q. & Liu

647   J.F. (2014) Global copy number analyses by next generation sequencing provide insight into

648   pig genome variation. *BMC Genomics* **15**, 593.

649 Johansson Moller M., Chaudhary R., Hellmén E., Höyheim B., Chowdhary B. & Andersson L. (1996)

650   Pigs with the dominant white coat color phenotype carry a duplication of the KIT gene encoding

651   the mast/stem cell growth factor receptor. *Mammalian Genome* **7**, 822–30.

652 Johansson A., Pielberg G., Andersson L. & Edfors-Lilja I. (2005) Polymorphism at the porcine

653   Dominant white/KIT locus influence coat colour and peripheral blood cell measures. *Animal*

654   *Genetics* **36**, 288–96.

655 Keel B.N., Lindholm-Perry A.K. & Snelling W.M. (2016) Evolutionary and Functional Features of

656   Copy Number Variation in the Cattle Genome. *Frontiers in Genetics* **7**, 207.

657 Keel B.N., Nonneman D.J., Lindholm-Perry A.K., Oliver W.T. & Rohrer G.A. (2019) A survey of

658   copy number variation in the porcine genome detected from whole-genome sequence. *Frontiers*

659   *in Genetics* **10**, 737.

660 Klambauer G., Schwarzbauer K., Mayr A., Clevert D.A., Mitterecker A., Bodenhofer U. & Hochreiter

661   S. (2012) cn.MOPS: mixture of Poissons for discovering copy number variations in next-

662   generation sequencing data with a low false discovery rate. *Nucleic Acids Research* **40**, e69.

663 Li H. & Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform.

664   *Bioinformatics* **25**, 1754–60.

665 Li Y., Mei S., Zhang X., Peng X., Liu G., Tao H., Wu H., Jiang S., Xiong Y. & Li F. (2012)

666   Identification of genome-wide copy number variations among diverse pig breeds by array CGH.

667   *BMC Genomics* **13**, 725.

668   Liu D., Chen Z., Zhang Z., Sun H., Ma P., Zhu K., Liu G., Wang Q. & Pan Y. (2019) Detection of
669   genome-wide structural variations in the Shanghai Holstein cattle population using next-
670   generation sequencing. *Asian-Australasian journal of animal sciences* **32**, 320–33.

671   Liu P., Carvalho C.M.B., Hastings P.J. & Lupski J.R. (2012) Mechanisms for recurrent and complex
672   human genomic rearrangements. *Current Opinion in Genetics & Development* **22**, 211–20.

673   Liu M., Zhou Y., Rosen B.D., Van Tassell C.P., Stella A., Tosser-Klopp G., Rupp R., Palhière I.,
674   Colli L., Sayre B., Crepaldi P., Fang L., Mészáros G., Chen H., Liu G.E. & ADAPTmap
675   Consortium. (2019a) Diversity of copy number variation in the worldwide goat population.
676   *Heredity* **122**, 636–46.

677   Long Y., Su Y., Ai H., Zhang Z., Yang B., Ruan G., Xiao S., Liao X., Ren J., Huang L. & Ding N.
678   (2016) A genome-wide association study of copy number variations with umbilical hernia in
679   swine. *Animal Genetics* **47**, 298–305.

680   Ma J., Qi W., Ren D., Duan Y., Qiao R., Guo Y., Yang Z., Li L., Milan D., Ren J. & Huang L. (2009)
681   A genome scan for quantitative trait loci affecting three ear traits in a White Duroc x Chinese
682   Erhualian resource population. *Animal Genetics* **40**, 463–7.

683   Marklund S., Kijas J., Rodriguez-Martinez H., Rönnstrand L., Funa K., Moller M., Lange D., Edfors-
684   Lilja I. & Andersson L. (1998) Molecular basis for the dominant white phenotype in the
685   domestic pig. *Genome Research* **8**, 826–33.

686   Mi H., Muruganujan A., Ebert D., Huang X. & Thomas P.D. (2019) PANTHER version 14: more
687   genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic
688   Acids Research* **47**, D419–26.

689   Molnár J., Nagy T., Stéger V., Tóth G., Marincs F. & Barta E. (2014) Genome sequencing and
690   analysis of Mangalica, a fatty local pig of Hungary. *BMC Genomics* **15**, 761.

691   Muñoz M., Bozzi R., García F., Núñez Y., Geraci C., Crovetti A., García-Casco J., Alves E., Škrlep
692   M., Charneca R., Martins J.M., Quintanilla R., Tibau J., Kušec G., Djurkin-Kušec I., Mercat
693   M.J., Riquet J., Estellé J., Zimmer C., Razmaite V., Araujo J.P., Radović Č., Savić R., Karolyi

694   D., Gallo M., Čandek-Potokar M., Fontanesi L., Fernández A.I. & Óvilo C. (2018a) Diversity

695   across major and candidate genes in European local pig breeds. *PLoS One* **13**, e0207475.

696 Muñoz M., Bozzi R., García-Casco J., Núñez Y., Ribani A., Franci O., García F., Škrlep M., Schiavo

697   G., Bovo S., Utzeri V.J., Charneca R., Martins J.M., Quintanilla R., Tibau J., Margeta V.,

698   Djurkin-Kušec I., Mercat M.J., Riquet J., Estellé J., Zimmer C., Razmaite V., Araujo J.P.,

699   Radović Č., Savić R., Karolyi D., Gallo M., Čandek-Potokar M., Fernández A.I., Fontanesi L.

700   & Óvilo C. (2019) Genomic diversity, linkage disequilibrium and selection signatures in

701   European local pig breeds assessed with a high density SNP chip. *Scientific Reports* **9**, 13546.

702 Muñoz M., García-Casco J.M., Caraballo C., Fernández-Barroso M.Á., Sánchez-Esquiliche F.,

703   Gómez F., Rodríguez M.D.C. & Silió L. (2018b) Identification of candidate genes and

704   regulatory factors underlying intramuscular fat content through *longissimus dorsi* transcriptome

705   analyses in heavy Iberian pigs. *Frontiers in Genetics* **9**, 608.

706 Ogorevc J., Zorc M., Škrlep M., Bozzi R., Petig M., Fontanesi L., Čandek-Potokar M. & Dovč P.

707   (2017) Is KIT locus polymorphism rs328592739 related to white belt phenotype in Krškopolje

708   pig? *Agriculturae Conspectus Scientificus* **82**, 155–61.

709 Ovilo C., Clop A., Noguera J.L., Oliver M.A., Barragán C., Rodriguez C., Silió L., Toro M.A., Coll

710   A., Folch J.M., Sánchez A., Babot D., Varona L. & Pérez-Enciso M. (2002) Quantitative trait

711   locus mapping for meat quality traits in an Iberian x Landrace F2 pig population. *Journal of*

712   *Animal Science* **80**, 2801–8.

713 Paudel Y., Madsen O., Megens H.J., Frantz L.A.F., Bosse M., Bastiaansen J.W.M., Crooijmans

714   R.P.M.A. & Groenen M.A.M. (2013) Evolutionary dynamics of copy number variation in pig

715   genomes in the context of adaptation and domestication. *BMC Genomics* **14**, 449.

716 Paudel Y., Madsen O., Megens H.J., Frantz L.A.F., Bosse M., Crooijmans R.P.M.A. & Groenen

717   M.A.M. (2015) Copy number variation in the speciation of pigs: a possible prominent role for

718   olfactory receptors. *BMC Genomics* **16**, 330.

719 Pirooznia M., Goes F.S. & Zandi P.P. (2015) Whole-genome CNV analysis: advances in

720   computational approaches. *Frontiers in Genetics* **6**, 138.

721 Pollard M.O., Gurdasani D., Mentzer A.J., Porter T. & Sandhu M.S. (2018) Long reads: their purpose

722   and place. *Human Molecular Genetics* **27**, R234–41.

723 Quinlan A.R. & Hall I.M. (2010) BEDTools: a flexible suite of utilities for comparing genomic

724   features. *Bioinformatics* **26**, 841–2.

725 R Core Team. (2018) R: A language and environment for statistical computing. R Foundation for

726   Statistical Computing, Vienna, Austria.

727 Ramayo-Caldas Y., Castelló A., Pena R.N., Alves E., Mercadé A., Souza C.A., Fernández A.I., Perez-

728   Enciso M. & Folch J.M. (2010) Copy number variation in the porcine genome inferred from a

729   60 k SNP BeadChip. *BMC Genomics* **11**, 593.

730 Redon R., Ishikawa S., Fitch K.R., Feuk L., Perry G.H., Andrews T.D., Fiegler H., Shapero M.H.,

731   Carson A.R., Chen W., Cho E.K., Dallaire S., Freeman J.L., Gonzalez J.R., Gratacos M., Huang

732   J., Kalaitzopoulos D., Komura D., MacDonald J.R., Marshall C.R., Mei R., Montgomery L.,

733   Nishimura K., Okamura K., Shen F., Somerville M.J., Tchinda J., Valsesia A., Woodwark C.,

734   Yang F., Zhang J., Zerjal T., Zhang J., Armengol L., Conrad D.F., Estivill X., Tyler-Smith C.,

735   Carter N.P., Aburatani H., Lee C., Jones K.W., Scherer S.W. & Hurles M.E. (2006) Global

736   variation in copy number in the human genome. *Nature* **444**, 444–54.

737 Ren J., Duan Y., Qiao R., Yao F., Zhang Z., Yang B., Guo Y., Xiao S., Wei R., Ouyang Z., Ding N.,

738   Ai H. & Huang L. (2011) A missense mutation in PPARD causes a major QTL effect on ear

739   size in pigs. *PLoS Genetics* **7**, e1002043.

740 Revay T., Quach A.T., Maignel L., Sullivan B. & King W.A. (2015) Copy number variations in high

741   and low fertility breeding boars. *BMC Genomics* **16**, 280.

742 Revilla M., Puig-Oliveras A., Castelló A., Crespo-Piazuelo D., Paludo E., Fernández A.I., Ballester

743   M. & Folch J.M. (2017) A global analysis of CNVs in swine using whole genome sequence

744   data and association analysis with fatty acid composition and growth traits. *PLoS One* **12**,

745   e0177014.

746 Revilla M., Puig-Oliveras A., Crespo-Piazuelo D., Criado-Mesas L., Castelló A., Fernández A.I.,

747   Ballester M. & Folch J.M. (2018) Expression analysis of candidate genes for fatty acid

748   composition in adipose tissue and identification of regulatory regions. *Scientific Reports* **8**,

749   2045.

750 Ribani A., Utzeri V.J., Geraci C., Tinarelli S., Djan M., Veličković N., Doneva R., Dall'Olio S., Costa

751   L.N., Schiavo G., Bovo S., Usai G., Gallo M., Radović Č., Savić R., Karolyi D., Salajpal K.,

752   Gvozdanović K., Djurkin-Kušec I., Škrlep M., Čandek-Potokar M., Ovilo C. & Fontanesi L.

753   (2019) Signatures of de-domestication in autochthonous pig breeds and of domestication in

754   wild boar populations from *MC1R* and *NR6A1* allele distribution. *Animal Genetics* **50**, 166–71.

755 Rubin C.J., Megens H.J., Martinez Barrio A., Maqbool K., Sayyab S., Schwochow D., Wang C.,

756   Carlborg Ö., Jern P., Jørgensen C.B., Archibald A.L., Fredholm M., Groenen M.A.M. &

757   Andersson L. (2012) Strong signatures of selection in the domestic pig genome. *Proceedings*

758   *of the National Academy of Sciences of the United States of America* **109**, 19529–36.

759 Sambrook J., Fritsch E.F. & Maniatis T. (1989) Molecular cloning: a laboratory manual. 2nd Ed. Cold

760   Spring Harbor Laboratory Press, Cold Spring Harbor, USA.

761 Schiavo G., Bertolini F., Galimberti G., Bovo S., Dall'Olio S., Nanni Costa L., Gallo M. & Fontanesi

762   L. (2020a) A machine learning approach for the identification of population-informative

763   markers from high-throughput genotyping data: application to several pig breeds. *Animal* **14**,

764   223-32.

765 Schiavo G., Bovo S., Bertolini F., Tinarelli S., Dall'Olio S., Nanni Costa L., Gallo M. & Fontanesi L.

766   (2020b) Comparative evaluation of genomic inbreeding parameters in seven commercial and

767   autochthonous pig breeds. *Animal*, 1–11, doi:10.1017/S175173111900332X.

768 Schiavo G., Bertolini F., Utzeri V.J., Ribani A., Geraci C., Santoro L., Óvilo C., Fernández A.I., Gallo

769   M. & Fontanesi L. (2018) Taking advantage from phenotype variability in a local animal

genetic resource: identification of genomic regions associated with the hairless phenotype in Casertana pigs. *Animal Genetics* **49**, 321–5.

Schiavo G., Bovo S., Tinarelli S., Bertolini F., Dall'Olio S., Gallo M. & Fontanesi L. (2019) Genome-wide association analyses for several exterior traits in the autochthonous Casertana pig breed. *Livestock Science* **230**, 103842.

Schiavo G., Dolezal M.A., Scotti E., Bertolini F., Calò D.G., Galimberti G., Russo V. & Fontanesi L. (2014) Copy number variants in Italian Large White pigs detected using high-density single nucleotide polymorphisms and their association with back fat thickness. *Animal Genetics* **45**, 745–9.

Silió L., Barragán C., Fernández A.I., García-Casco J. & Rodríguez M.C. (2016) Assessing effective population size, coancestry and inbreeding effects on litter size using the pedigree and SNP data in closed lines of the Iberian pig breed. *Journal of Animal Breeding and Genetics* **133**, 145–54.

Stafuzza N.B., Silva R.M. de O., Fragomeni B. de O., Masuda Y., Huang Y., Gray K. & Lourenco D.A.L. (2019) A genome-wide single nucleotide polymorphism and copy number variation analysis for number of piglets born alive. *BMC Genomics* **20**, 321.

Tomás A., Ramírez O., Casellas J., Muñoz G., Sánchez A., Barragán C., Arqué M., Riart I., Óvilo C., Noguera J.L., Amills M. & Rodríguez C. (2011) Quantitative trait loci for fatness at growing and reproductive stages in Iberian × Meishan F(2) sows. *Animal Genetics* **42**, 548–51.

Wang J., Jiang J., Fu W., Jiang L., Ding X., Liu J.F. & Zhang Q. (2012) A genome-wide detection of copy number variations using SNP genotyping arrays in swine. *BMC Genomics* **13**, 273.

Wang J., Jiang J., Wang H., Kang H., Zhang Q. & Liu J.-F. (2014) Enhancing genome-wide copy number variation identification by high density array CGH using diverse resources of pig breeds. *PLoS One* **9**, e87571.

Wang J., Jiang J., Wang H., Kang H., Zhang Q. & Liu J.-F. (2015c) Improved detection and characterization of copy number variations among diverse pig breeds by array CGH. *G3* **5**, 1253–61.

796   Wang Z., Sun H., Chen Q., Zhang X., Wang Q. & Pan Y. (2019a) A genome scan for selection

797   signatures in Taihu pig breeds using next-generation sequencing. *Animal* **13**, 683–93.

798   Wang H., Wang C., Yang K., Liu J., Zhang Y., Wang Y., Xu X., Michal J.J., Jiang Z. & Liu B.

799   (2015a) Genome wide distributions and functional characterization of copy number variations

800   between Chinese and Western pigs. *PLoS One* **10**, e0131522.

801   Wang L., Xu L., Liu X., Zhang T., Li N., Hay E.H., Zhang Y., Yan H., Zhao K., Liu G.E., Zhang L.

802   & Wang L. (2015b) Copy number variation-based genome wide association study reveals

803   additional variants contributing to meat quality in Swine. *Scientific Reports* **5**, 12535.

804   Wang Y., Zhang T. & Wang C. (2019b) Detection and analysis of genome-wide copy number

805   variation in the pig genome using an 80 K SNP Beadchip. *Journal of Animal Breeding and*

806   *Genetics* doi: 10.1111/jbg.12435.

807   Wei W.H., Koning D.J. de, Penman J.C., Finlayson H.A., Archibald A.L. & Haley C.S. (2007) QTL

808   modulating ear size and erectness in pigs. *Animal Genetics* **38**, 222–6.

809   Wiedmann R.T., Nonneman D.J. & Rohrer G.A. (2015) Genome-Wide Copy Number Variations

810   Using SNP Genotyping in a Mixed Breed Swine Population. *PLoS One* **10**, e0133529.

811   Wilkinson S., Lu Z.H., Megens H.J., Archibald A.L., Haley C., Jackson I.J., Groenen M.A.M.,

812   Crooijmans R.P.M.A., Ogden R. & Wiener P. (2013) Signatures of diversifying selection in

813   European pig breeds. *PLoS Genetics* **9**, e1003453.

814   Winchester L., Yau C. & Ragoussis J. (2009) Comparing CNV detection methods for SNP arrays.

815   *Briefings in Functional Genomics & Proteomics* **8**, 353–66.

816   Wu Z., Deng Z., Huang M., Hou Y., Zhang H., Chen H. & Ren J. (2019) Whole-genome resequencing

817   identifies KIT new alleles that affect coat color phenotypes in pigs. *Frontiers in Genetics* **10**,

818   218.

819   Xie J., Li R., Li S., Ran X., Wang J., Jiang J. & Zhao P. (2016) Identification of copy number

820   variations in Xiang and Kele pigs. *PLoS One* **11**, e0148565.

821 Yang B., Cui L., Perez-Enciso M., Traspov A., Crooijmans R.P.M.A., Zinovieva N., Schook L.B.,

822   Archibald A., Gatphayak K., Knorr C., Triantafyllidis A., Alexandri P., Semiadi G., Hanotte

823   O., Dias D., Dovč P., Uimari P., Iacolina L., Scandura M., Groenen M.A.M., Huang L. &

824   Megens H.-J. (2017) Genome-wide SNP data unveils the globalization of domesticated pigs.

825   *Genetics Selection Evolution* **49**, 71.

826 Yang L., Xu L., Zhou Y., Liu M., Wang L., Kijas J.W., Zhang H., Li L. & Liu G.E. (2018) Diversity

827   of copy number variation in a worldwide population of sheep. *Genomics* **110**, 143–8.

828 Zhang F., Gu W., Hurles M.E. & Lupski J.R. (2009) Copy number variation in human health, disease,

829   and evolution. *Annual Review of Genomics and Human Genetics* **10**, 451-81.

830 Zhang Y., Liang J., Zhang L., Wang L., Liu X., Yan H., Zhao K., Shi H., Zhang T., Li N., Pu L. &

831   Wang L (2015) Porcine *methionine sulfoxide reductase B3*: molecular cloning, tissue-specific

832   expression profiles, and polymorphisms associated with ear size in *Sus scrofa*. *Journal of*

833   *Animal Science and Biotechnology* **6**, 60.

834

Figures

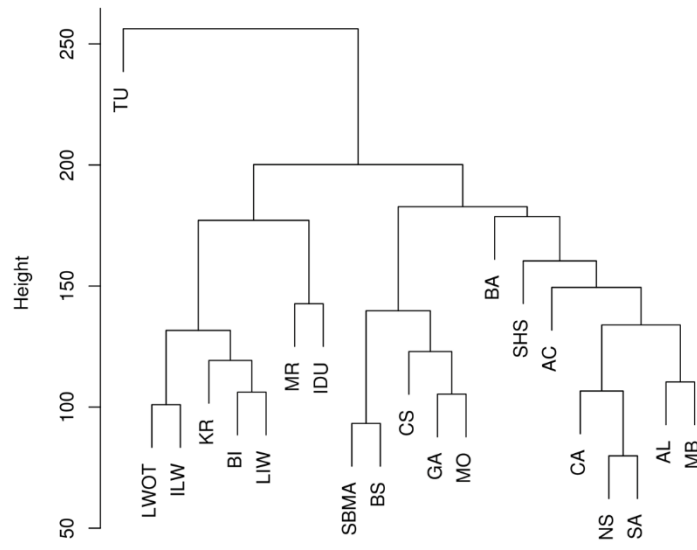**Fig. 1.** Phenotypes and geographical origin of the 21 analysed pig breeds.
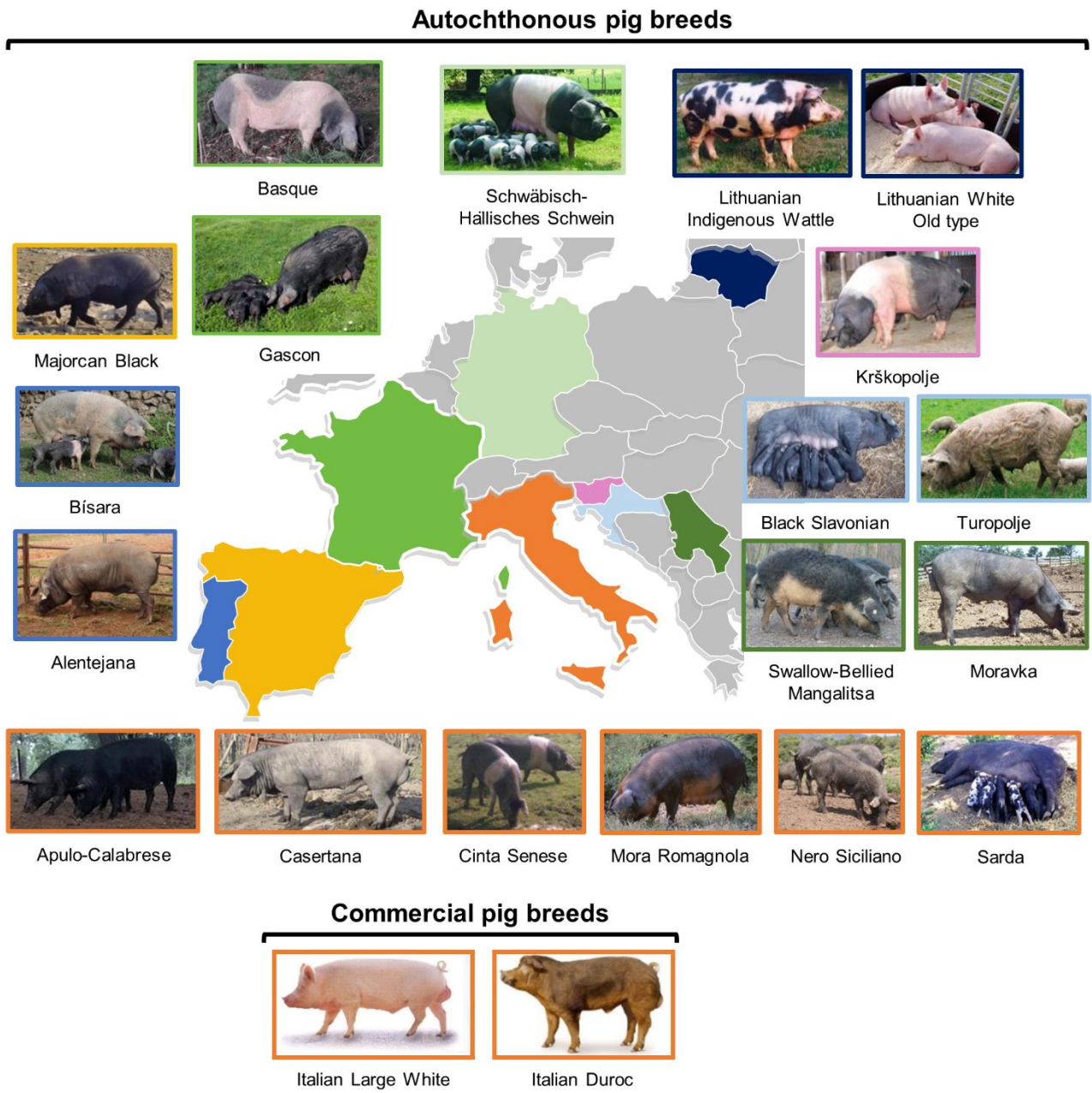
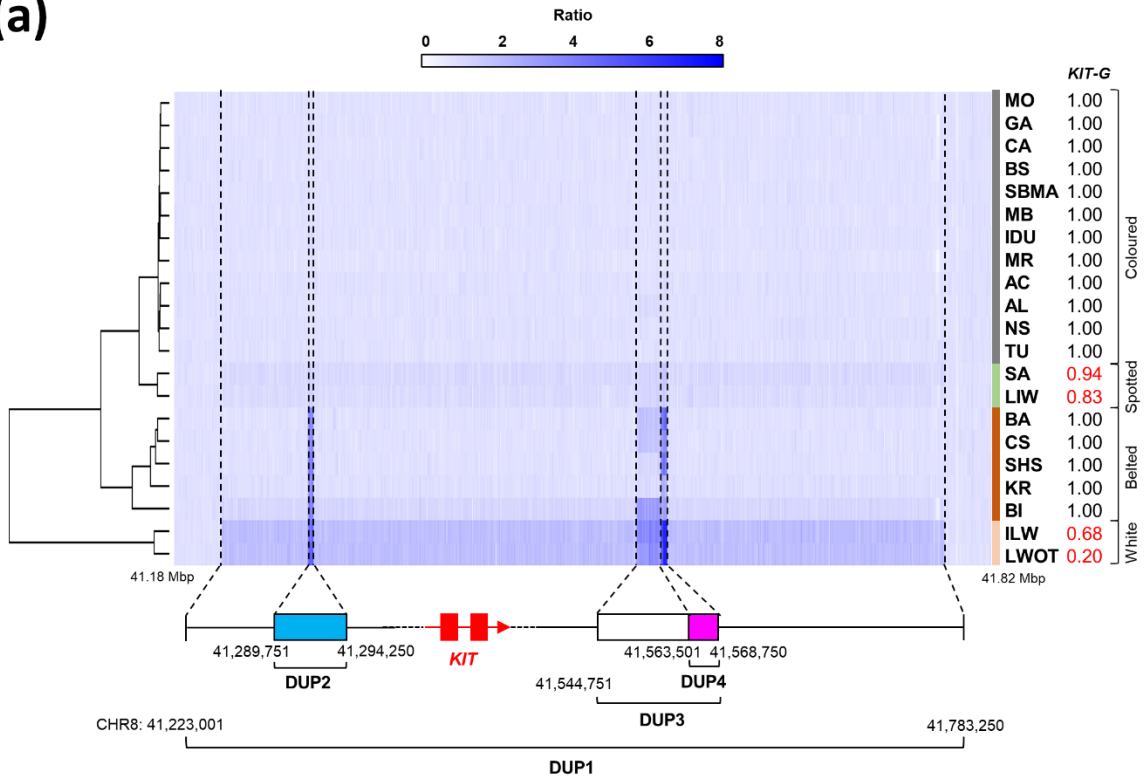**Fig. 2.** Distribution of CNVRs along each autosomal chromosome.

Fig. 3. Dendrogram representing the hierarchical clustering of the copy number state. Acronyms of
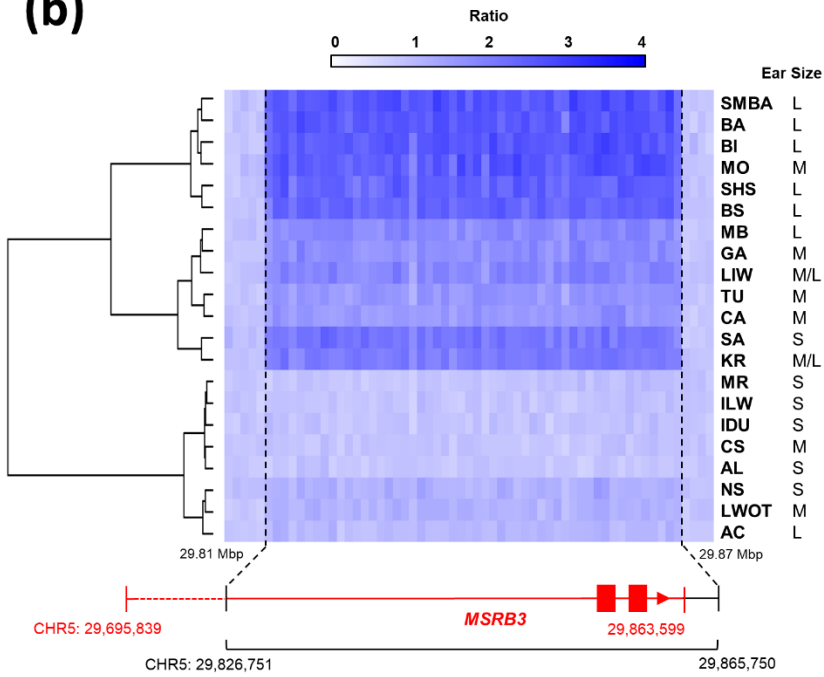
the breed name are explained in Table 1.

843   **Fig. 4.** (**a**) Heatmap of the read count ratios over the *KIT* gene. Coat colour reported in the

844   correspondence of the breeds indicates breed main characteristics. SA (Sarda) has heterogeneous and

845   not-fixed patterns. It was included among the spotted based on the frequency of this phenotype in the

846   breed and according to the copy number (CN) state at this locus. Basque (BA) has spotted/belted

847   heterogeneous patterns but was included among the belted breeds according to the CN state at this

848   locus – (see text and Table S1 for details). KIT-G: frequency of the allele G of the single nucleotide

849   polymorphism (SNP) rs345599765 (splice mutation of the intron 17; Marklund *et al.* 1998). (**b**) Heatmap

850   of the read count ratios over the *MSRB3* gene. Ear size indicated in (b): L = large; M = medium; S =

851   small (see text and Table S1 for details). The light-dark blue bar at the top of (a) and (b) indicates the

852   CN ratio (1 = normal state without any gain or loss). For each breed, the read count ratio was

853   computed in 750-bp consecutive genome windows. Acronyms of the breed name are explained in

854   Table 1. (**c**) Average CN state of the *MSRB3* gene in relation to ear size. (**d**) Relationship between

855   the average CN state of the *MSRB3* gene and the SNPs rs340841870 (green) and rs326411202 (blue).

856   Pearson's correlation coefficient (*r*) are reported.
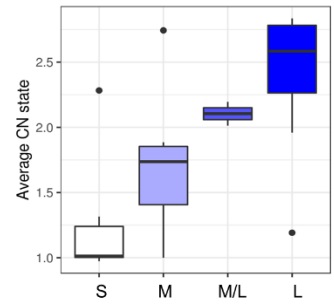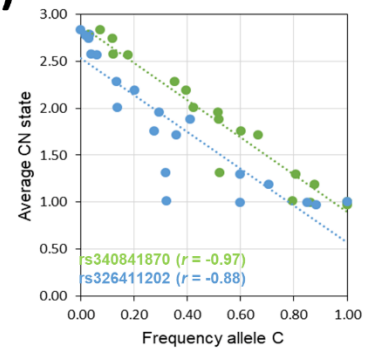
857

858

859 **Fig. 5.** Heatmap of the read count ratios over the *ELOVL6* (**a**) and *ZNF622* (**b**) genes. Exons below

860 the heatmap for the *ZNF622* gene are numbered (E1-E6) according to the annotation in the

861 Sscrofa11.1 genome version. Untranslated regions (UTR) are also reported. The light-dark blue bar

862 at the top of (A) and (B) indicates the copy number (CN) ratio (1 = normal state without any gain or

863 loss). For each breed, the read count ratio was computed in 750-bp consecutive genome windows.

864 Acronyms of the breed name are explained in Table 1.

(a)

Ratio

MB
GA
TU
CA
CS
SA
KR
BA
IDU
SHS
AC
SBMA
MR
BS
NS
ILW
MO
BI
AL
LWOT
LIW

112.06 Mbp   112.09 Mbp

CHR8: 111,750,960   *ELOVL6* (intron)   112,191,274

CHR8: 112,070,251   112,075,500

(b)

Ratio

ILW
SHS
LIW
NS
AC
CA
BI
BA
SBMA
MB
GA
LWOT
AL
MO
SA
CS
BS
MR
KR
IDU
TU

5.68 Mbp   5.72 Mbp

UTR-E6 E5   E4   E3 E2 E1-UTR

CHR16: 5,699,744   *ZNF622*   5,714,516

CHR16: 5.699.251   5.715.750

865

866

41
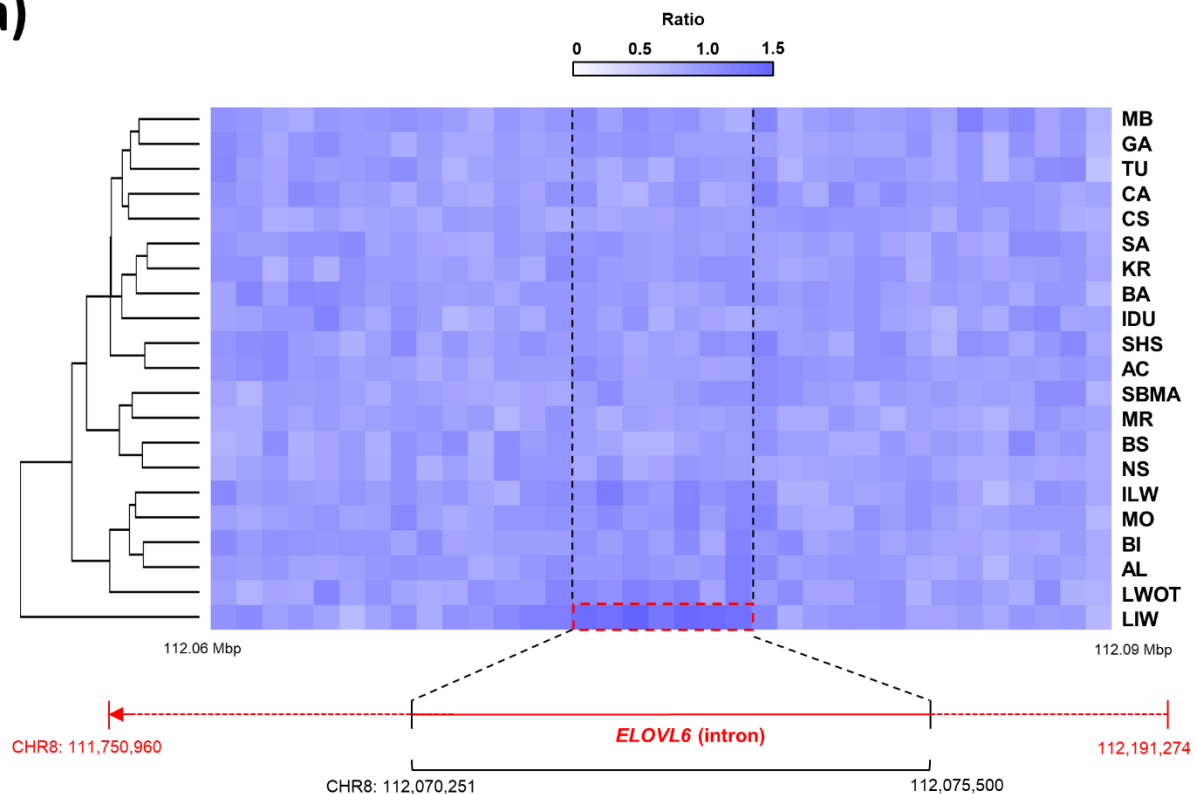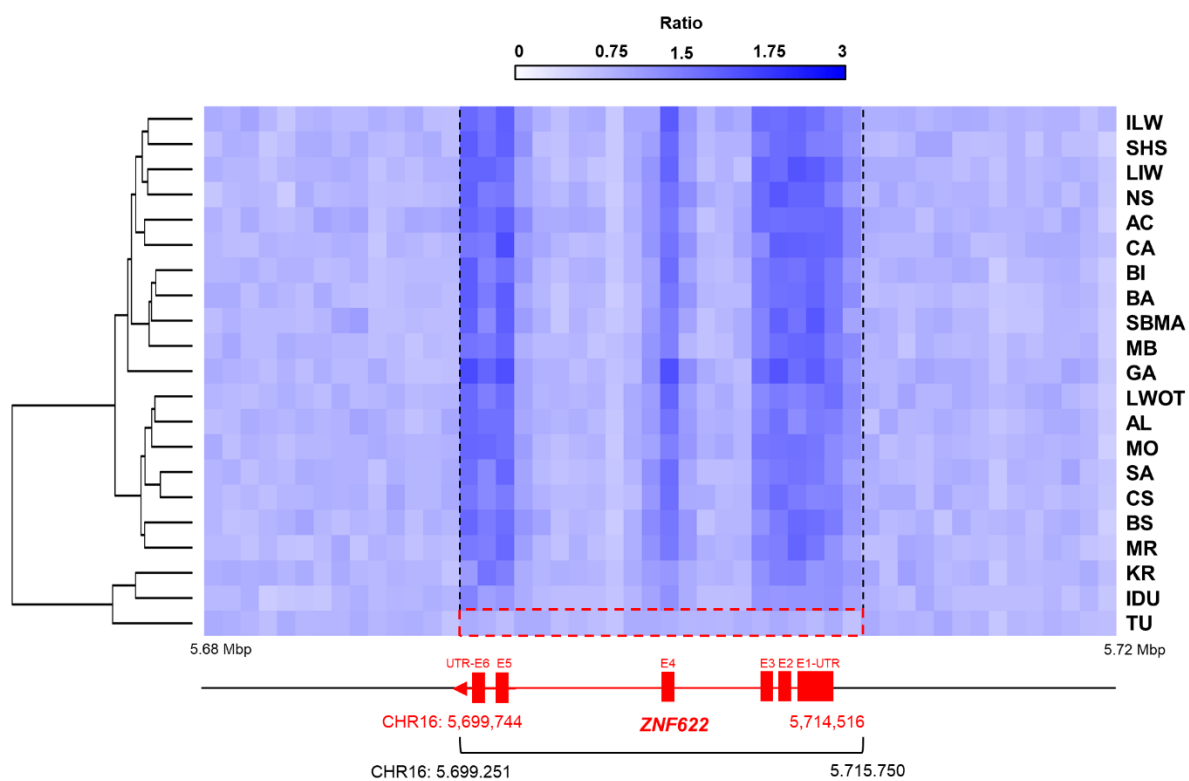
Tables

Table 1. Summary of CNVs of the 21 analysed pig breeds. Data are stratified by breed.

Breed	Short name	CNV[1]	CNL[2]	CNG[3]	Length$_{Min}$[4]	Length$_{Max}$[5]	Length$_{Median}$[6]	% length in CNV[7]
Autochthonous								
Alentejana	AL	601	345	256	2250	69750	3000	0.17
Apulo-Calabrese	AC	676	313	363	2250	142500	3000	0.18
Basque	BA	1122	626	496	2250	99750	3750	0.29
Bísara	BI	437	162	275	2250	63000	3000	0.11
Black Slavonian	BS	504	225	279	2250	142500	3000	0.13
Casertana	CA	596	272	324	2250	113250	3000	0.16
Cinta Senese	CS	662	352	310	2250	89250	3750	0.19
Gascon	GA	781	379	402	2250	126000	3000	0.20
Krškopolje	KR	510	152	358	2250	101250	3000	0.13
Lithuanian Indigenous Wattle	LIW	710	295	415	2250	90750	3750	0.19
Lithuanian White Old Type	LWOT	711	308	403	2250	560250	3750	0.21
Majorcan Black	MB	546	328	218	2250	101250	3750	0.15
Mora Romagnola	MR	1255	647	608	2250	137250	3000	0.34
Moravka	MO	391	159	232	2250	100500	3000	0.10
Nero Siciliano	NS	298	149	149	2250	42750	3000	0.07
Sarda	SA	209	72	137	2250	38250	3000	0.04
Schwäbisch-Hällisches Schwein	SHS	576	277	299	2250	147000	3000	0.15
Swallow-Bellied Mangalitsa	SBMA	757	433	324	2250	121500	3000	0.22
Turopolje	TU	1440	845	595	2250	99750	3750	0.40
Commercial								
Italian Duroc	IDU	1111	249	862	2250	116250	3000	0.28
Italian Large White	ILW	451	148	303	2250	560250	3000	0.14

[1] Total no. of copy number variants; [2] Total no. of copy number losses; [3] Total no. of copy number gains; [4] Minimum length (bp) of CNVs; [5] Maximum
length (bp) of CNVs; [6] Median length (bp) of CNVs; [7] Percentage of the *S. scrofa* genome occupied by CNVs.

871   **Table 2.** Summary of CNVRs of the 21 analysed pig breeds stratified by chromosome.

Chromosome	CNVR[1]	Length$_{Min}$[2]	Length$_{Max}$[3]	Length$_{Median}$[4]	% length in CNVR[5]
SSC1	359	2250	137250	3760	0.88
SSC2	361	2250	43500	3760	1.54
SSC3	162	2250	147750	3010	0.82
SSC4	227	2250	81000	3760	0.99
SSC5	215	2250	46500	3760	1.45
SSC6	302	2250	120750	3760	1.07
SSC7	167	2250	96750	3760	1.16
SSC8	244	2250	560250	3760	1.37
SSC9	259	2250	159000	3760	1.86
SSC10	114	2250	85500	3010	0.96
SSC11	159	2250	153750	3760	1.54
SSC12	110	2250	108000	3385	1.33
SSC13	307	2250	91500	3760	1.05
SSC14	212	2250	195750	3760	1.34
SSC15	196	2250	63000	3760	0.86
SSC16	138	2250	41250	3010	0.88
SSC17	132	2250	80250	3010	1.27
SSC18	46	2250	16500	3010	0.35

872   [1] Total no. of copy number variant regions; [2] Minimum length (bp) of CNVs; [3] Maximum length (bp)
873   of CNVs; [4] Median length (bp) of CNVs; [5] Percentage of the chromosome occupied by CNVRs.
874

Supporting information

876 **Table S1.** Details on the analysed animals and investigated breeds, including geographical

877 distribution and phenotypic description.

878 **Table S2.** Summary statistics of whole-genome sequencing.

879 **Table S3.** Summary statistics of detected CNVs stratified by chromosome.

880 **Table S4.** CNVRs detected over all analysed breeds.

881 **Table S5.** Summary statistics of detected CNVRs, stratified by pig breed.

882 **Table S6.** Over-represented repeated element classes.

883 **Table S7.** Within CNVRs over-represented QTLs.

884 **Table S8.** Within CNVRs over-represented biological functions.

885 **Table S9.** Allele frequency of the single nucleotide polymorphisms at the *KIT* and *MSRB*3 genes

886 estimated from sequencing data.

887 **Table S10.** Summary statistics of CNVRs previously identified in other studies.