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1 **Exploiting single-marker and haplotype-based genome-wide association studies to**  
2 **identify QTL for the number of teats in Italian Duroc pigs**

3

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13

14

15 Short title: **Genome scans for the number of teats in Italian Duroc pigs**

16

17 **Abstract**

18       The number of teats is a morphological trait of high economic relevance for the pig  
19 industry. Here, to dissect the genomic architecture of this trait in the Italian Duroc pig  
20 population, we present the results of genome-wide association studies in this Italian heavy  
21 pig breed. A total of 1,162 pigs, for which the number of teats was recorded, was  
22 genotyped with two high-throughput single nucleotide polymorphism (SNP) genotyping  
23 platforms (60K and 70K). Genome-wide association analyses were based on a single-  
24 marker approach and on a haplotype-based approach. Two quantitative trait loci (QTL)  
25 affecting the number of teats were identified. The most significant QTL, identified by the  
26 single-marker analysis and confirmed by the haplotype-based method, was located on *Sus*  
27 *scrofa* chromosome (SSC) 7, in the region of the *vertnin* (*VRTN*) gene. Suggestively  
28 associated markers (SNPs and haplotypes) were located on SSC10, in the region of the  
29 *FERM domain containing 4A* (*FRMD4A*) gene, the second identified QTL. These  
30 findings confirm previous results obtained in a few other Duroc populations. Overall, this  
31 study further supported the important role of variability in the *VRTN* gene region in  
32 affecting the number of teats in pigs. Moreover, the results also indicated that this trait in  
33 the Italian Duroc breed, as in many other pig breeds, is affected by few QTL, with the  
34 contribution of many other genetic factors with small effects, following the classical  
35 theory of quantitative traits.

36

37 **Keywords:** Breed: GWAS; heavy pig; single nucleotide polymorphism; *Sus scrofa*

38

## 39 **1. Introduction**

40       The number of teats is one of the most relevant morphological traits related to the  
41 reproductive performance of the sows. This trait is indirectly associated with the  
42 mothering ability, that, in turn, determines the number of piglets weaned per sow and per  
43 year (Kim et al., 2005; Andersen et al., 2011). To maximize the number of weaned piglets,  
44 selection programs, that have as main objective an increased litter size (a trait with low  
45 heritability), need to select, in parallel, for increased number of functional teats. Selection  
46 to improve the number of teats is, to some extent, facilitated by the medium to high  
47 heritability of the trait itself, as demonstrated by most studies in several pig breeds or  
48 lines that estimated the fraction of the genetic variance over the phenotypic variance for  
49 this morphological feature (e.g., Willham and Whatley 1962; Toro et al., 1986; McKay  
50 and Rahnefeld 1990; Borchers et al., 2002; Chalkias et al., 2013; Felleki and Lundeheim  
51 2015; Balzani et al., 2016).

52       A broad variability in the number of teats (which could range from about 8 to 21)  
53 has been observed both across and within pig breeds and lines (Borchers et al., 2002;  
54 Lopes et al., 2014; Verardo et al., 2016; Rohrer and Nonneman 2017; Dall'Olio et al.,  
55 2018; van Son et al., 2019). Despite being represented by discrete and countable values,  
56 the number of teats is considered a quantitative trait determined by the effect of a high  
57 number of genetic factors. Several studies have however identified a few quantitative trait  
58 loci (QTL) affecting this morphological trait in pigs. The first QTL investigations were  
59 based on reference populations (F2 and backcrosses) constructed using parental animals  
60 of different breeds or lines (some of which with divergent number of teats), including  
61 some hyper-prolific Chinese breeds (e.g., Wada et al., 2000; Hirooka et al., 2001;  
62 Rodríguez et al., 2005; Bidanel et al., 2008; Ding et al., 2009; Hernandez et al., 2014).  
63 Subsequent studies based on high density single nucleotide polymorphism (SNP)

64 genotyping data explored the within breed variability via genome-wide association  
65 studies (GWAS). These studies confirmed the previously reported QTL regions and/or  
66 identified other novel genome regions affecting this trait (e.g., Arakawa et al., 2015;  
67 Rohrer and Nonneman 2017; Tang et al., 2017; Lee et al., 2019; van Son et al., 2019).  
68 One of the most relevant QTL for the number of teats, confirmed by several studies, is  
69 located on *Sus scrofa* chromosome (SSC) 7 (Mikawa et al., 2007, 2011; Duijvesteijn et  
70 al., 2014; Rohrer and Nonneman 2017; Dall’Olio et al., 2018; van Son et al., 2019;  
71 Moscatelli et al., 2020). At this QTL, alleles segregating in many populations are  
72 determined by variability in the *vertnin* gene, also known as *vertebrae development*  
73 *associated gene* (*VRTN*; Mikawa et al., 2011; Arakawa et al., 2015). Originally reported  
74 to have pleiotropic effects on the number of vertebrae (Mikawa *et al.*, 2011), *VRTN*  
75 encodes a novel DNA-binding transcription factor which regulates the transcription of a  
76 set of genes that harbor *VRTN* binding motifs and modulates somite segmentation via the  
77 Notch signaling pathway (Duan et al., 2018). Some studies in a few pig populations  
78 proposed that variability in other genes on SSC7 [*latent transforming growth factor*  
79 *binding protein 2* (*LTBP2*); *BRMS1 like transcriptional repressor* (*BRMS1L*); and *Fos*  
80 *proto-oncogene, AP-1 transcription factor subunit* (*FOS*)], close to the *VRTN* gene, are  
81 involved in affecting the number of teats and the number of vertebrae (Zhang et al., 2016;  
82 Park et al., 2018; Liu et al., 2020).

83         Genome-wide association studies are usually run with a model fitting one marker  
84 at a time. However, to further extract information at the genome-wide level, the use of  
85 haplotypes has been recommended to capture additional marker-phenotype associations  
86 that cannot be detected with the single-marker approach (Lorenz et al., 2010; Barendse  
87 2011). We recently applied haplotype-based analyses in a few GWAS in pigs and  
88 demonstrated that it is possible to improve the identification of genomic regions affecting

89 a targeted phenotype, complementing and completing the results obtained via a single-  
90 marker approach (Bovo et al., 2019, 2020, 2021). The additional information that can be  
91 retrieved is population-dependent, as it is related to the level of linkage disequilibrium  
92 between QTL alleles and the genotyped markers, which may vary among breeds, lines  
93 and populations. An ascertainment bias derived by the SNP composition of the  
94 commercial genotyping panels used in the investigation is also another element that  
95 should be considered in this context.

96 We recently carried out GWAS for the number of teats in two heavy pig breeds,  
97 Italian Large White and Italian Landrace, and identified several genome regions affecting  
98 this trait, not overlapping between the two breeds (Moscatelli et al., 2020; Bovo et al.,  
99 2021). Only in Italian Large White, the *VRTN* gene region was associated with this trait  
100 whereas in Italian Landrace no significant effects of *VRTN* (or other markers of SSC7)  
101 were evidenced (Bovo et al., 2021). For the other heavy pig breed, Italian Duroc, included  
102 in the Italian selection program conducted by the Italian Pig Breeders Association  
103 (ANAS), only few information on the segregation of the *VRTN* gene alleles was reported  
104 thus far (Fontanesi et al., 2014a). This Duroc population is a close nucleus constituted in  
105 the 1990' that is under selection with the main aim to optimize the production of green  
106 legs transformed in Protected Designation of Origin (PDO) dry cured hams. Other  
107 purebred Duroc pig populations (e.g., from USA, Canada, China, Japan and The  
108 Netherlands) have been already investigated with GWAS to identify genome regions  
109 affecting the number of teats. The investigations involving these populations reported  
110 partially overlapping results in terms of QTL regions (Arakawa et al., 2015; Yang et al.,  
111 2016; Tan et al., 2017; van Son et al., 2019; Zhuang et al., 2020; Li et al., 2021).

112 In this study, we carried out two GWAS for the number of teats in Italian Duroc  
113 heavy pigs using single-marker and haplotype-based approaches. Obtained results were

114 compared with the QTL information for the same trait reported in other pure-bred Duroc  
115 populations and in the other two Italian heavy pig breeds.

116

## 117 **2. Materials and methods**

### 118 **2.1. Animals**

119 All animals used in this study were kept according to Italian and European  
120 legislation for pig production and all procedures described were in compliance with  
121 national and European Union regulations for animal care and slaughtering. All animals  
122 were part of the routine Italian pig breeding program and were slaughtered in a  
123 commercial authorized abattoir following standard procedures. Animals were not raised  
124 or sampled for the purpose of this study. As no treatment was given to any animals, no  
125 ethical approval was needed according to the rules of the animal research ethics  
126 committee of the University of Bologna based on the Italian legislation, as reported in the  
127 “DECRETO LEGISLATIVO 4 marzo 2014, n. 26”.

128 The animals included in this study were from the national selection program of  
129 heavy pig breeds that is run by ANAS. A total of 1,164 Italian Duroc pigs, born in the  
130 years 1996-2018, was investigated (727 males and 437 females). Animals were included  
131 in the sib-testing evaluation program run by ANAS, described in previous reports  
132 (Fontanesi et al., 2012, 2014b, 2015). The number of teats on these animals was routinely  
133 recorded by direct counting at the beginning of the testing period and animals having less  
134 than 12 teats were excluded from the herd book of this breed.

135

### 136 **2.2. Genotyping and SNP quality**

137 DNA was extracted by using the Wizard Genomic DNA Purification kit (Promega  
138 Corporation, Madison, WI, USA) from blood samples routinely collected on all pigs

139 included in the national selection program. A total of 606 animals were genotyped with  
140 the GeneSeek 70K GGP Porcine BeadChip (which includes 68,516 SNPs) whereas the  
141 remaining 558 pigs were genotyped with the Illumina PorcineSNP60 BeadChip v.2  
142 (which includes 61,565 SNPs). The two panels presented a total of 41,862 shared genomic  
143 positions. Genotyping followed standard procedures based on the supplier's  
144 recommendations. DNA markers shared between the two SNP platforms were used in the  
145 analysis. BLAST+ v.2.7.1 (Camacho et al., 2009) was used to map SNPs to the  
146 Sscrofa11.1 reference genome and markers assigned to more than one position or  
147 assigned to sex chromosomes were discarded. Genotypes were evaluated with PLINK  
148 v.1.09 (Chang et al., 2015). Individual pigs with a call rate  $>0.90$  and SNPs with a call  
149 rate  $> 0.90$ , a minor allele frequency (MAF)  $> 0.01$  and in Hardy-Weinberg equilibrium  
150 ( $P > 0.0001$ ) were retained for further analyses. The final dataset counted 1,162 animals  
151 (727 males and 435 females) and 29,604 DNA markers.

152

### 153 **2.3. Haplotype estimation**

154 The software SHAPEIT v.2 (Delaneau et al., 2011) was used for genotype phasing  
155 considering: (i) a genomic window size of 2 Mb, (ii) an effective population size ( $N_e$ )  
156 estimated with SNeP v.1.1 (Barbato et al., 2015) and (iii) a chromosome specific  
157 recombination rate given by Tortereau et al., (2012). The R package GHap 1.2.2  
158 (Utsunomiya et al., 2016) was further used to call haplotypes considering a genomic  
159 window of 400 kb with a sliding block of 100 kb (Veroneze et al., 2013). Each haplotype  
160 was treated as bi-allelic DNA variant (genotypes are: NN, NH and HH; H = haplotype  
161 allele and N = NULL = all other N alleles present in the haploblock. PLINK was used to  
162 filter out haplotypes having a MAF  $< 0.02$ . A total of 85590 haplotypes coming from  
163 66,363 overlapping haploblocks were estimated.



164

## 165 2.4. Linear mixed model analyses

166 To assess the association between the DNA markers (SNP or haplotypes) and the  
167 number of teats we used a linear mixed effect model. An additive genetic model assuming  
168 a trend per copy of the minor allele that specify the dependency of the number of teats on  
169 genotype categories was implemented as follows:

$$170 \quad \mathbf{y} = \mathbf{W}\boldsymbol{\alpha} + \mathbf{x}\beta + \mathbf{g} + \mathbf{e} \quad (1)$$

171 where  $\mathbf{y}$  ( $n \times 1$ ) is a vector containing the phenotype (the number of teats) for the  $n^{\text{th}}$   
172 animal,  $\mathbf{W}$  ( $n \times k$ ) is a covariate matrix with  $k = 2$  (a column of 1s and a column for the  
173 sex of the animal) and  $\boldsymbol{\alpha}$  is the  $k$ -dimensional vector of covariates effects,  $\mathbf{x}$  ( $n \times 1$ ) is the  
174 vector containing genotypes for the  $i^{\text{th}}$  DNA marker (SNP or haplotype),  $\beta$  is the additive  
175 fixed effect of the  $i^{\text{th}}$  DNA marker on the phenotype,  $\mathbf{g} \sim \mathbf{N}(\mathbf{0}, \sigma_g^2 \mathbf{K})$  is a multivariate  
176 Gaussian polygenic effect, with covariance matrix proportional to the relatedness matrix  
177  $\mathbf{K}$  ( $n \times n$ ) and  $\mathbf{e} \sim \mathbf{N}(\mathbf{0}, \sigma_e^2 \mathbf{I})$  is a multivariate Gaussian vector of uncorrelated residuals.  
178 Polygenic effect and residuals represented the random effects. The assessment of the  
179 association between each DNA marker and the total number of teats was obtained by  
180 testing the null hypothesis  $H_0: \beta = 0$ . Significance was tested by using the Wald test. All  
181 the models were fitted with GEMMA v.0.98 (Zhou and Stephens 2012) after computing  
182 the relatedness matrices  $\mathbf{K}_1$  and  $\mathbf{K}_2$  as a centered genomic matrices, for SNPs and  
183 haplotypes, respectively. Details about the genomic matrix construction are given in the  
184 manual of GEMMA. Bonferroni correction was applied to account for multiple  
185 comparisons, considering the total number of DNA markers used in each genome scan  
186 and value of  $\alpha = 0.05$ . Markers presenting a  $P < 5.5 \times 10^{-05}$  were considered suggestive for  
187 associations (Wellcome Trust Case Control Consortium 2007). The proportion of

188 variance in phenotype explained (PVE) by each QTL was calculated as reported by Shim  
189 et al., 2015.

190 SNPs and haplotypes that had the lowest  $P$  in chromosome regions separated by  
191 at least 5 Mb were considered as tag DNA markers. GEMMA was also used to estimate  
192 the genomic (chip) heritability ( $h_G^2$ ). Genomic control inflation factor ( $\lambda_{GC}$ ) was computed  
193 in R v.3.6.0 (R Core Team 2018). Quantile-quantile plots (QQplots) and the Miami plot  
194 were generated in R by using the *qqman* package (Turner 2018).

195

## 196 **2.5. Haploblock analysis and genome annotation**

197 HaploView v.4.2 (Barrett et al., 2011) was used to study the structure of the  
198 haplotypes and linkage disequilibrium of the most interesting QTL regions. Each QTL  
199 was annotated considering the protein coding genes spanning the region of  $\pm 500$  kb  
200 around the evaluated DNA marker. The Sscrofa11.1 NCBI's GFF file and Bedtools  
201 v.2.17.0 (Quinlan and Hall 2010) were used for this purpose.

202

## 203 **3. Results**

### 204 **3.1. Descriptive statistics**

205 The numbers of teats in this pig population ranged from 12 to 17. About 42% of the  
206 pigs had 12 teats (Figure 1) as this represents the lower limit considered for including an  
207 animal in the Herd Book of this Italian heavy pig breed (ANAS 2021). Detailed  
208 information on the number of pigs with different numbers of teats are reported in Table  
209 S1. On average, pigs had  $12.95 \pm 0.97$  (mean  $\pm$  standard deviation) teats.

210 Table 1 reports the number of pigs and DNA markers used in GWAS. Assessment  
211 of results via QQplots (Figure S1) and the genomic control inflation factors (Table 1)  
212 indicated a good control of the population stratification.

213 Genomic heritability estimated for the number of teats in Italian Duroc pigs was  
214 moderate, with the value that increased from the estimates based on single-marker to the  
215 value obtained with the haplotype information (Table 1). This suggested that the  
216 haplotype analysis might have captured an additional fraction of heritability, as already  
217 reported for the same trait in other pig populations (Bovo et al., 2020, 2021).

218

### 219 **3.2. Single-marker and haplotype-based association studies**

220 The top associated DNA markers (SNPs and haplotypes) for each QTL region are  
221 reported in Table 2, whereas Table S2 lists all the significant and suggestively associated  
222 markers. Miami plot including SNP and haplotype data is showed in Figure 2.

223 Three SNPs and one haplotype on SSC7 were significantly associated with the  
224 number of teats. The most significant SNP was MARC0038565 (rs80894106;  $P =$   
225  $8.58 \times 10^{-10}$ ), located on SSC7 at position 97,652,632 bp, in the region of the *VRTN* gene  
226 (located on SSC7:97.61-97.62). This marker had a MAF = 0.44 (allele A) and a negative  
227 regression coefficient ( $\beta = -0.29$ ), indicating a decrease in number of teats while  
228 increasing the number of copies of the minor allele. Hence, the Italian Duroc population  
229 had a higher frequency of the favorable allele (allele G) at this SNP position. This marker  
230 had also the highest proportion of variance in phenotype explained (PVE% = 3.22). The  
231 other two significant markers were closely located to the mentioned SNP and had a  
232 medium-high linkage disequilibrium ( $r^2 = 0.64$ , Figure 3) with the most significant  
233 marker. In general, this region did not show a very high level of linkage disequilibrium  
234 spanning the genotyped markers (Figure 3).

235 The peak of association on SSC7 was also highlighted by the haplotype analysis  
236 (SSC7:97.6-97.8 Mb; haplotype GAG;  $P = 2.51 \times 10^{-8}$ ), confirming the involvement of the  
237 *VRTN* gene region in affecting the number of teats in the Italian Duroc pig breed. The

238 DNA marker MARC0038565 was included in this haplotype [first position in the  
239 haplotype sequence AAG; Figure 3]. Hence, the haplotype GAG [higher frequent in the  
240 population with  $f(\text{GAG}) = 0.52$ ; favorable allele] and marker MARC0038565 presented  
241 similar MAF and  $\beta$  values.

242 A suggestively associated chromosome region identified both using the single-  
243 marker and the haplotype-based analyses was detected in the region of the *FERM domain*  
244 *containing 4A (FRMD4A)* gene located on SSC10:47.3-48.1 Mb. The results for the most  
245 significant SNP in this region, located at position 47,947,214 bp (ASGA0090802;  
246 rs81309209;  $P = 4.80 \times 10^{-06}$ ), were confirmed by the haplotype analysis ( $P = 5.08 \times 10^{-06}$ ).  
247 Both regions (SSC7 and SSC10) overlapped with the two QTL regions already reported  
248 in a previous study to affect the number of teats in Duroc pigs (van Son et al., 2019).

249

#### 250 **4. Discussion**

251 Duroc pig populations are well known to have, in general, lower reproduction  
252 performances than other cosmopolitan pig breeds, as they are usually selected to  
253 maximize production performances and meat quality traits for the use of Duroc boars in  
254 terminal crosses (e.g., Cameron 1990; Gaugler et al., 1984; Skorupski et al., 1996; Hoque  
255 et al., 2007; Alam et al., 2021). However, to maintain a sustainable breeding in the  
256 selection nuclei of this breed, it is also needed to improve reproduction efficiency starting  
257 from traits with medium-high heritability.

258 A lower number of teats has been reported in Duroc populations than in the  
259 other two main cosmopolitan breeds, Large White and Landrace, which usually exceed  
260 the threshold of 14 teats. As such, 14 is the lower limit that is applied by ANAS to register  
261 animals to the Italian Large White and Italian Landrace herd books. In a recent survey  
262 that we carried out, the mean number of teats in these two breeds was  $14.88 \pm 0.92$

263 (standard deviation) and  $14.77 \pm 0.87$ , respectively (Bovo et al., 2021), that are larger  
264 than what we observed in Italian Duroc pigs ( $12.95 \pm 0.97$ ). This number is however  
265 larger than what was reported in other Duroc populations. For example, Zhuang et al.,  
266 (2020) reported the mean number of  $10.90 \pm 1.16$  and  $10.92 \pm 1.14$  in Duroc populations  
267 derived from US and Canadian nuclei and Tan et al., (2017) reported an average value of  
268  $10.72 \pm 1.72$  in a Chinese Duroc population. It is worth to mention that our statistics are  
269 biased as, in Italy, pigs with less than 12 teats are not registered in the Italian Duroc herd  
270 book and are therefore not considered for the calculation of the population mean in our  
271 study. Similar mean values to what we reported in the Italian Duroc breed were reported  
272 in a Dutch Duroc line ( $12.93 \pm 1.05$ ; van Son et al., 2019) and in another Chinese Duroc  
273 herd ( $13.17 \pm 1.12$ ; Li et al., 2021). These results suggest that some heterogeneity exists  
274 in Duroc populations, probably due to different selection pressures that have been applied  
275 on this trait in nuclei of this cosmopolitan breed around the world. Genomic heritability  
276 that we estimated in the Italian Duroc breed was close to what we already reported in  
277 Italian Landrace pigs using both single-marker and haplotype-based approaches ( $h_G^2 \pm$   
278 standard error:  $0.25 \pm 0.02$  and  $0.30 \pm 0.03$ , respectively) and a little lower than what we  
279 estimated in Italian Large White pigs ( $0.30 \pm 0.03$  and  $0.43 \pm 0.04$ , respectively; Bovo et  
280 al., 2021). Genomic heritability estimates for the number of teats were also reported in  
281 other Duroc populations. The single-marker heritability in an US nucleus was  $0.19 \pm 0.02$ ,  
282 in a Canadian population was  $0.34 \pm 0.03$  (Zhuang et al., 2020) and in a Chinese nucleus  
283 was  $0.29 \pm 0.05$  (Li et al., 2021). These other studies, however, did not report any  
284 estimation based on haplotypes. Genomic heritability estimated using haplotype  
285 information indicated that this approach can potentially capture additional fractions of the  
286 so called “missed heritability” that would not be disclosed using single-marker  
287 approaches (Bovo et al., 2021). Therefore, the use of haplotypes might be useful to further

288 exploit genomic information for this purpose (Lorenz et al., 2010; Barendse 2011).

289           Only two QTL for the number of teats, one highlighted by significant markers  
290 and one identified by suggestively significant markers, were identified in the Italian  
291 Duroc population. Results obtained by the single-marker analysis were confirmed by the  
292 haplotype-based analysis. To continue the comparison with the results obtained in other  
293 Duroc populations, it is worth to mention that a limited number of segregating QTL for  
294 this trait has been also reported in other Duroc populations (Tan et al., 2017; van Son et  
295 al., 2019; Zhuang et al., 2020; Li et al., 2021). For example, by investigating a Dutch  
296 Duroc population, van Son et al., (2019) reported only the same two QTL regions that we  
297 identified in the Italian Duroc pigs. The larger number of pigs that was investigated in the  
298 Dutch study made it possible to reach significant results even for markers in the region  
299 of the *FRMD4A* gene that, however, remained less significantly associated than what was  
300 reported for the SNPs on *SSC7*, located in the region of *VRTN*. The *VRTN* region also  
301 included the most significant SNPs identified in GWAS carried out in Canadian Duroc  
302 pigs and in two Chinese Duroc populations (Tan et al., 2017; Zhuang et al., 2020; Li et  
303 al., 2021). No significant QTL for the number of teats were however identified in an  
304 American derived nucleus of Duroc pigs (Zhuang et al., 2020). Comparison with the  
305 results obtained in a Japanese Duroc population is not possible due to the different  
306 statistical approaches that were used to detect QTL regions for the number of teats  
307 (BayesC methods; Arakawa et al., 2015). The *VRTN* region, however, emerged as one of  
308 the most significant regions also in this population (Arakawa et al., 2015).

309           The proportion of variance in phenotype explained (PVE) by the most  
310 significant marker in the *VRTN* region that we identified in the Italian Duroc population  
311 was about half the PVE of the most significant marker of this region identified in a Dutch  
312 Duroc line (van Son et al., 2019): 3.2% vs 6.0%. This difference might be due to different

313 levels of linkage disequilibrium between the markers identified in these two Duroc  
314 populations (the Italian and the Dutch populations) and the causative mutation(s) and to  
315 the different experimental power determined by the number of animals analysed in our  
316 study and by van Son et al. (2019). Markers in this region have a much higher PVE for  
317 vertebral numbers are directly related phenotypes (van Son et al., 2019), which are  
318 probably the primary traits affected by *VRTN* variability (Mikawa et al., 2011), with  
319 pleiotropic effects on teat number.

320           A QTL pattern similar to what was identified in Italian Duroc pigs was already  
321 reported in Italian Large White pigs where, in addition to the *VRTN* and *FRMD4A* gene  
322 regions, only another significant haplotype region was identified on SSC12 (Bovo et al.,  
323 2021). The Italian Landrace population was however completely different, in terms of  
324 QTL regions for the number of teats, from both the Italian Duroc and Italian Large White  
325 breeds (Bovo et al., 2021).

326           These comparative analyses, in general, showed that one major QTL for the  
327 number of teats segregates in the most important cosmopolitan pig breeds and derived  
328 lines and populations (like the Italian Duroc breed). This QTL is located on SSC7 for  
329 which the most plausible causative mutation has been reported in the *VRTN* gene  
330 (Mikawa et al., 2011). In Italian Duroc pigs, the frequency of the insertion allele at the  
331 *VRTN* gene suggested to be the putative favorable allele (allele Q; Fontanesi et al., 2014a),  
332 which might increase the number of vertebrae and the number of teats (Mikawa et al.,  
333 2011), is almost equal to the frequency of the positive allele of the most significant SNP  
334 that we identified on SSC7 (allele G of MARC0038565). This information could  
335 indirectly suggest a very high linkage disequilibrium between the two markers.  
336 Additional studies are needed to complete the linkage disequilibrium pattern of this SSC7  
337 genome region in Italian Duroc, including genotyping data of the *VRTN* gene which might

338 confirm the significant results obtained for the MARC0038565 SNP. Additional studies  
339 are also needed to characterize the variability in the *FRMD4A* gene and identify the  
340 causative mutation(s) affecting this trait. This gene encodes a FERM domain-containing  
341 protein that regulates epithelial polarity by connecting ADP ribosylation factor 6 (ARF6),  
342 which is considered an important factor involved in actin cytoskeleton dynamics and  
343 membrane trafficking (Ikenouchi and Umeda 2010). Therefore, the role of this gene in  
344 affecting the number of teats is not obvious. Thus, it will be also important to clarify the  
345 molecular mechanisms that could lead to this effect and eventually evaluate the effect of  
346 other close genes in determining the QTL located on SSC10.

347

#### 348 **4. Conclusion**

349 In terms of genotyped animals, this is the largest GWAS carried out in the Italian  
350 Duroc pig breed thus far. The estimated genomic heritability that we obtained confirmed  
351 that genetic factors affect this trait in a similar way as it was evidenced in other Duroc  
352 populations. The limited number of major QTL influencing this trait and segregating in  
353 the Italian Duroc breed indicates that a marker assisted selection based on *VRTN* alleles  
354 and eventually on *FRMD4A* gene markers, might be effective only in part as it would not  
355 completely exploit the genetic variance related to this trait for which many other genes  
356 are involved. Their effect could be captured including this trait in a genomic selection  
357 program that can take into account genome-wide variability.

358

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369

370 **Conflict of interest statement**

371 The authors declare that there is no conflict of interest regarding the publication of  
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373

374 **References**

375 Alam, M., Chang, H-K., Lee, S-S., Choi, T-J., 2021. Genetic analysis of major production  
376 and reproduction traits of Korean Duroc, Landrace and Yorkshire pigs. *Animals* 11,  
377 1321. <https://doi.org/10.3390/ani11051321>

378 ANAS, 2019. Libro Genealogico. Retrieved on the 2nd September 2019. from  
379 <http://www.anas.it/>.

380 Andersen, I.L., Nævdal, E., Bøe, K.E., 2011. Maternal investment, sibling competition,  
381 and offspring survival with increasing litter size and parity in pigs (*Sus scrofa*).  
382 *Behav. Ecol. Sociobiol.* 65, 1159-1167. [https://doi.org/10.1007/s00265-010-1128-](https://doi.org/10.1007/s00265-010-1128-4)  
383 4

384 Arakawa, A., Okumura, N., Taniguchi, M., Hayashi, T., Hirose, K., Fukawa, K., Ito, T.,  
385 Matsumoto, T., Uenishi, H., Mikawa, S., 2015. Genome-wide association QTL  
386 mapping for teat number in a purebred population of Duroc pigs. *Anim. Genet.* 46,  
387 571-575. <https://doi.org/10.1111/age.12331>

388 Balzani, A., Cordell, H.J., Sutcliffe, E., Edwards, S.A., 2016. Heritability of udder  
389 morphology and colostrum quality traits in swine. *J. Anim. Sci.* 94, 3636-3644.  
390 <https://doi.org/10.2527/jas.2016-0458>

391 Barbato, M., Orozco-terWengel, P., Tapio, M., Bruford, M.W., 2015. SNeP: a tool to  
392 estimate trends in recent effective population size trajectories using genome-wide  
393 SNP data. *Front. Genet.* 6, 109. <https://doi.org/10.3389/fgene.2015.00109>

394 Barendse, W., 2011. Haplotype analysis improved evidence for candidate genes for  
395 intramuscular fat percentage from a genome wide association study of cattle. *PLoS*  
396 *One* 6, e29601. <https://doi.org/10.1371/journal.pone.0029601>

397 Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization  
398 of LD and haplotype maps. *Bioinformatics* 21, 263-265.  
399 <https://doi.org/10.1093/bioinformatics/bth457>

400 Bidanel, J.P., Rosendo, A., Iannuccelli, N., Riquet, J., Gilbert, H., Caritez, J.C., Billon,  
401 Y., Amigues, Y., Prunier, A., Milan, D., 2008. Detection of quantitative trait loci  
402 for teat number and female reproductive traits in Meishan × Large White F2 pigs.  
403 *Animal* 2, 813-820. <https://doi.org/10.1017/S1751731108002097>

404 Borchers, N., Reinsch, N., Kalm, E., 2002. Teat number, hairiness and set of ears in a  
405 Piétrain cross: variation and effects on performance traits. *Arch. Anim. Breed.* 45,  
406 465-480. <https://doi.org/10.5194/AAB-45-465-2002>

407 Bovo, S., Ballan, M., Schiavo, G., Gallo, M., Dall'Olio, S., Fontanesi, L., 2020.  
408 Haplotype-based genome-wide association studies reveal new loci for  
409 haematological and clinical-biochemical parameters in Large White pigs. *Anim.*  
410 *Genet.* 51, 601-606. <https://doi.org/10.1111/age.12959>

411 Bovo, S., Ballan, M., Schiavo, G., Ribani, A., Tinarelli, S., Utzeri, V.J., Dall'Olio, S.,  
412 Gallo, M., Fontanesi, L., 2021. Single-marker and haplotype-based genome-wide

413 association studies for the number of teats in two heavy pig breeds. *Anim. Genet.*  
414 52, 440-450. <https://doi.org/10.1111/age.13095>

415 Bovo, S., Mazzoni, G., Bertolini, F., Schiavo, G., Galimberti, G., Gallo, M., Dall'Olio,  
416 S., Fontanesi, L., 2019. Genome-wide association studies for 30 haematological  
417 and blood clinical-biochemical traits in Large White pigs reveal genomic regions  
418 affecting intermediate phenotypes. *Sci Rep.* 9, 7003.  
419 <https://doi.org/10.1038/s41598-019-43297-1>

420 Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden,  
421 T.L., 2009. BLAST+: architecture and applications. *BMC Bioinformatics* 10, 421.  
422 <https://doi.org/10.1186/1471-2105-10-421>

423 Cameron, N.D., 1990. Comparison of Duroc and British Landrace pigs and the estimation  
424 of genetic and phenotypic parameters for growth and carcass traits. *Anim. Sci.* 50,  
425 141-153. doi:10.1017/S0003356100004542

426 Chalkias, H., Rydhmer, L., Lundeheim, N., 2013. Genetic analysis of functional and non-  
427 functional teats in a population of Yorkshire pigs. *Livest. Sci.* 152, 127-134.  
428 <https://doi.org/10.1016/j.livsci.2013.01.003>

429 Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., Lee, J.J., 2015.  
430 Second-generation PLINK: rising to the challenge of larger and richer datasets.  
431 *Gigascience* 4, 7. <https://doi.org/10.1186/s13742-015-0047-8>

432 Dall'Olio, S., Ribani, A., Moscatelli, G., Zambonelli, P., Gallo, M., Nanni Costa, L.,  
433 Fontanesi, L., 2018. Teat number parameters in Italian Large White pigs:  
434 phenotypic analysis and association with *vertnin* (*VRTN*) gene allele variants.  
435 *Livest. Sci.* 210, 68-72. <https://doi.org/10.1016/j.livsci.2018.01.020>

436 Delaneau, O., Marchini, J., Zagury, J-F., 2011. A linear complexity phasing method for  
437 thousands of genomes. *Nat. Methods* 9, 179-181.  
438 <https://doi.org/10.1038/nmeth.1785>

439 Ding, N., Guo, Y., Knorr, C., Ma, J., Mao, H., Lan, L., Xiao, S., Ai, H., Haley, C.S.,  
440 Brenig, B., Huang, L., 2009. Genome-wide QTL mapping for three traits related to  
441 teat number in a White Duroc x Erhualian pig resource population. *BMC Genet.*  
442 10, 6. <https://doi.org/10.1186/1471-2156-10-6>

443 Duan, Y., Zhang, H., Zhang, Z., Gao, J., Yang, J., Wu, Z., Fan, Y., Xing, Y., Li, L., Xiao,  
444 S., Hou, T., Ren, J., Huang, L., 2018. *VRTN* is required for the development of  
445 thoracic vertebrae in mammals. *Int. J. Biol. Sci.* 14, 667-681.  
446 <https://doi.org/10.7150/ijbs.23815>

447 Duijvesteijn, N., Veltmaat, J.M., Knol, E.F., Harlizius, B., 2014. High-resolution  
448 association mapping of number of teats in pigs reveals regions controlling vertebral  
449 development. *BMC Genomics* 15, 542. <https://doi.org/10.1186/1471-2164-15-542>

450 Felleki, M., Lundeheim, N., 2015. Genetic heteroscedasticity of teat count in pigs. *J.*  
451 *Anim. Breed. Genet.* 132, 392-398. <https://doi.org/10.1111/jbg.12134>

452 Fontanesi, L., Schiavo, G., Galimberti, G., Calò, D.G., Scotti, E., Martelli, P.L.,  
453 Buttazzoni, L., Casadio, R., Russo, V., 2012. A genome wide association study for  
454 backfat thickness in Italian Large White pigs highlights new regions affecting fat  
455 deposition including neuronal genes. *BMC Genomics.* 13, 583.  
456 <https://doi.org/10.1186/1471-2164-13-583>

457 Fontanesi, L., Scotti, E., Buttazzoni, L., Dall'Olio, S., Russo, V., 2014a. Investigation of  
458 a Short Interspersed Nuclear Element polymorphic site in the porcine *vertnin* gene:  
459 allele frequencies and association study with meat quality, carcass and production

460 traits in Italian Large White pigs. *Ital. J Anim. Sci.* 13, 3090.  
461 <https://doi.org/10.4081/ijas.2014.3090>

462 Fontanesi, L., Schiavo, G., Galimberti, G., Calò, D.G., Russo, V., 2014b. A genomewide  
463 association study for average daily gain in Italian Large White pigs. *J. Anim. Sci.*  
464 92, 1385-1394. <https://doi.org/10.2527/jas.2013-7059>

465 Fontanesi, L., Schiavo, G., Scotti, E., Galimberti, G., Calò, D.G., Samorè, A.B., Gallo,  
466 M., Russo, V., Buttazzoni, L., 2015. A retrospective analysis of allele frequency  
467 changes of major genes during 20 years of selection in the Italian Large White pig  
468 breed. *J. Anim. Breed. Genet.* 132, 239-246. <https://doi.org/10.1111/jbg.12127>

469 Gaugler, H.R., Buchanan, D.S., Hintz, R.L., Johnson, R.K. 1984., Sow productivity  
470 comparisons for four breeds of swine: purebred and crossbred litters. *J. Anim. Sci.*  
471 59, 941-947. <https://doi.org/10.2527/jas1984.594941x>

472 Hernandez, S.C., Finlayson, H.A., Ashworth, C.J., Haley, C.S., Archibald, A.L., 2014. A  
473 genome-wide linkage analysis for reproductive traits in F2 Large White × Meishan  
474 cross gilts. *Anim. Genet.* 45, 191–197. <https://doi.org/10.1111/age.12123>

475 Hirooka, H., de Koning, DJ., Harlizius, B., van Arendonk, JA., Rattink, A.P., Groenen,  
476 M.A., Brascamp, E.W., Bovenhuis, H., 2001. A whole-genome scan for  
477 quantitative trait loci affecting teat number in pigs. *J. Anim. Sci.* 79, 2320-2326.  
478 <https://doi.org/10.2527/2001.7992320x>

479 Hoque, M.A., Suzuki, K., Kadowaki, H., Shibata, T., Oikawa, T., 2007. Genetic  
480 parameters for feed efficiency traits and their relationships with growth and carcass  
481 traits in Duroc pigs. *J. Anim. Breed. Genet.* 124, 108-116.  
482 <https://doi.org/10.1111/j.1439-0388.2007.00650.x>

483 Ikenouchi, J., Umeda, M., 2010. *FRMD4A* regulates epithelial polarity by connecting  
484 Arf6 activation with the PAR complex. *Proc. Natl. Acad. Sci. USA* 107, 748-753.  
485 <https://doi.org/10.1073/pnas.0908423107>

486 Kim, J.S., Jin, D.I., Lee, J.H., Son, D.S., Lee, S.H., Yi, Y.J., Park, C.S., 2005. Effects of  
487 teat number on litter size in gilts. *Anim. Reprod. Sci.* 90, 111-116.  
488 <https://doi.org/10.1016/j.anireprosci.2005.01.013>

489 Lee, J., Lee, S., Park, J-E., Moon, S-H., Choi, S-W., Go, G-W., Lim, D., Kim, J-M., 2019.  
490 Genome-wide association study and genomic predictions for exterior traits in  
491 Yorkshire pigs. *J. Anim. Sci.* 97, 2793-2802. <https://doi.org/10.1093/jas/skz158>

492 Li, Y., Pu, L., Shi, L., Gao, H., Zhang, P., Wang, L., Zhao, F., 2021. Revealing new  
493 candidate genes for teat number relevant traits in Duroc pigs using genome-wide  
494 association studies. *Animals* 11, 806. <https://doi.org/10.3390/ani11030806>

495 Liu, Q., Yue, J., Niu, N., Liu, X., Yan, H., Zhao, F., Hou, X., Gao, H., Shi, L., Wang, L.,  
496 Wang, L., Zhang, L., 2020. Genome-wide association analysis identified *BMPRIA*  
497 as a novel candidate gene affecting the number of thoracic vertebrae in a Large  
498 White × Minzhu intercross pig population. *Animals* 10, E2186.  
499 <https://doi.org/10.3390/ani10112186>

500 Lopes, M.S., Bastiaansen, J.W.M., Harlizius, B., Knol, E.F., Bovenhuis, H., 2014. A  
501 Genome-wide association study reveals dominance effects on number of teats in  
502 pigs. *PLoS One* 9, e105867. <https://doi.org/10.1371/journal.pone.0105867>

503 Lorenz, A.J., Hamblin, M.T., Jannink, J-L., 2010. Performance of single nucleotide  
504 polymorphisms versus haplotypes for genome-wide association analysis in barley.  
505 *PLoS One* 5, e14079. <https://doi.org/10.1371/journal.pone.0014079>

506 McKay, R.M., Rahnefeld, G.W., 1990. Heritability of teat number in swine. *Can. J. Anim.*  
507 *Sci.* 70, 425-430. <https://doi.org/10.4141/cjas90-054>

508 Mikawa, S., Morozumi, T., Shimanuki, S-I., Hayashi, T., Uenishi, H., Domukai, M.,  
509 Okumura, N., Awata, T., 2007. Fine mapping of a swine quantitative trait locus for  
510 number of vertebrae and analysis of an orphan nuclear receptor, germ cell nuclear  
511 factor (*NR6A1*). *Genome Res.* 17, 586-593. <https://doi.org/10.1101/gr.6085507>

512 Mikawa, S., Sato, S., Nii, M., Morozumi, T., Yoshioka, G., Imaeda, N., Yamaguchi, T.,  
513 Hayashi, T., Awata, T., 2011. Identification of a second gene associated with  
514 variation in vertebral number in domestic pigs. *BMC Genet.* 12, 5.  
515 <https://doi.org/10.1186/1471-2156-12-5>

516 Moscatelli, G., Dall'Olio, S., Bovo, S., Schiavo, G., Kazemi, H., Ribani, A., Zambonelli,  
517 P., Tinarelli, S., Gallo, M., Bertolini, F., Fontanesi, L., 2020. Genome-wide  
518 association studies for the number of teats and teat asymmetry patterns in Large  
519 White pigs. *Anim. Genet.* 51, 595-600. <https://doi.org/10.1111/age.12947>

520 Park, H-B., Lee, J-B., Han, S-H., Kim, S-G., Shin, H-S., Shin, M-C., Kang, Y-J., Cho, I-  
521 C., 2018. Joint linkage and linkage disequilibrium mapping reveals association of  
522 *BRMS1L* with total teat number in a large intercross between Landrace and Korean  
523 native pigs. *Livest. Sci.* 218, 2–96. <https://doi.org/10.1016/j.livsci.2018.09.014>

524 Quinlan, A.R., Hall, I.M., 2010. BEDTools: a flexible suite of utilities for comparing  
525 genomic features. *Bioinformatics* 26, 841-842.  
526 <https://doi.org/10.1093/bioinformatics/btq033>

527 R Core Team. 2018. R: A language and environment for statistical computing. R  
528 Foundation for Statistical Computing, Vienna, Austria.

529 Rodríguez, C., Tomás, A., Alves, E., Ramirez, O., Arqué, M., Muñoz, G., Barragán, C.,  
530 Varona, L., Silió, L., Amills, M., Noguera, J.L., 2005. QTL mapping for teat  
531 number in an Iberian-by-Meishan pig intercross. *Anim. Genet.* 36, 490-496.  
532 <https://doi.org/10.1111/j.1365-2052.2005.01358.x>

533 Rohrer, G.A., Nonneman, D.J., 2017. Genetic analysis of teat number in pigs reveals  
534 some developmental pathways independent of vertebra number and several loci  
535 which only affect a specific side. *Genet. Sel. Evol.* 49, 4. [https://doi.org/](https://doi.org/10.1186/s12711-016-0282-1)  
536 10.1186/s12711-016-0282-1

537 Shim, H., Chasman, D.I., Smith, J.D., Mora, S., Ridker, P.M., Nickerson, D.A., Krauss,  
538 R.M., Stephens, M., 2015. A multivariate genome-wide association analysis of 10  
539 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. *PLoS*  
540 *One* 10, e0120758. <https://doi.org/10.1371/journal.pone.0120758>

541 Skorupski, M.T., Garrick, D.J., Blair, H.T., 1996. Estimates of genetic parameters for  
542 production and reproduction traits in three breeds of pigs. *New Zealand J. Agric.*  
543 *Res.* 39, 387-395. <https://doi.org/10.1080/00288233.1996.9513198>

544 Tan, C., Wu, Z., Ren, J., Huang, Z., Liu, D., He, X., Prakapenka, D., Zhang, R., Li, N.,  
545 Da, Y., Hu, X., 2017. Genome-wide association study and accuracy of genomic  
546 prediction for teat number in Duroc pigs using genotyping-by-sequencing. *Genet.*  
547 *Sel. Evol.* 49, 35. <https://doi.org/10.1186/s12711-017-0311-8>

548 Tang, J., Zhang, Z., Yang, B., Guo, Y., Ai, H., Long, Y., Su, Y., Cui, L., Zhou, L., Wang,  
549 X., Zhang, H., Wang, C., Ren, J., Huang, K., Ding, N., 2017. Identification of loci  
550 affecting teat number by genome-wide association studies on three pig populations.  
551 *Asian-Australas J. Anim. Sci.* 30, 1-7. <https://doi.org/10.5713/ajas.15.0980>

552 Toro, M., Dobao, M.T., Rodríguez, J., Silió, L., 1986. Heritability of a canalized trait:  
553 teat number in Iberian pigs. *Genet. Sel. Evol.* 18, 173-184. [https://doi.org/](https://doi.org/10.1186/1297-9686-18-2-173)  
554 10.1186/1297-9686-18-2-173

555 Tortereau, F., Servin, B., Frantz, L., Megens, H-J., Milan, D., Rohrer, G., Wiedmann, R.,  
556 Beever, J., Archibald, AL., Schook, L.B., Groenen, M.A., 2012. A high density  
557 recombination map of the pig reveals a correlation between sex-specific



558 recombination and GC content. *BMC Genomics* 13, 586.  
559 <https://doi.org/10.1186/1471-2164-13-586>

560 Turner, S., 2018. qqman: an R package for visualizing GWAS results using Q-Q and  
561 manhattan plots. *J. Open Source Softw.* 3, 731. <https://doi.org/10.21105/joss.00731>

562 Utsunomiya, Y.T., Milanese, M., Utsunomiya, A.T.H., Ajmone-Marsan, P., Garcia, J.F.,  
563 2016. GHap: an R package for genome-wide haplotyping. *Bioinformatics* 32, 2861-  
564 2862. <https://doi.org/10.21105/joss.00731>

565 van Son, M., Lopes, M.S., Martell, H.J., Derks, M.F.L., Gangsei, L.E., Kongsro, J., Wass,  
566 M.N., Grindflek, E.H., Harlizius, B., 2019. A QTL for number of teats shows breed  
567 specific effects on number of vertebrae in pigs: bridging the gap between molecular  
568 and quantitative genetics. *Front. Genet.* 10, 272.  
569 <https://doi.org/10.3389/fgene.2019.00272>

570 Verardo, L.L., Silva, F.F., Lopes, M.S., Madsen, O., Bastiaansen, J.W.M., Knol, E.F.,  
571 Kelly, M., Varona, L., Lopes, P.S., Guimarães, S.E.F., 2016. Revealing new  
572 candidate genes for reproductive traits in pigs: combining Bayesian GWAS and  
573 functional pathways. *Genet. Sel. Evol.* 48, 9. [https://doi.org/10.1186/s12711-016-](https://doi.org/10.1186/s12711-016-0189-x)  
574 [0189-x](https://doi.org/10.1186/s12711-016-0189-x)

575 Veroneze, R., Lopes, P.S., Guimarães, S.E.F., Silva, F.F., Lopes, M.S., Harlizius, B.,  
576 Knol, E.F., 2013. Linkage disequilibrium and haplotype block structure in six  
577 commercial pig lines. *J. Anim. Sci.* 91, :3493-3501.  
578 <https://doi.org/10.2527/jas.2012-6052>

579 Wada, Y., Akita, T., Awata, T., Furukawa, T., Sugai, N., Inage, Y., Ishii, K., Ito, Y.,  
580 Kobayashi, E., Kusumoto, H., Matsumoto, T., Mikawa, S., Miyake, M., Murase,  
581 A., Shimanuki, S., Sugiyama, T., Uchida, Y., Yanai, S., Yasue, H., 2000.

582 Quantitative trait loci (QTL) analysis in a Meishan x Göttingen cross population.  
583 Anim. Genet. 31, 376-384. <https://doi.org/10.1046/j.1365-2052.2000.00696.x>

584 Wellcome Trust Case Control Consortium. 2007. Genome-wide association study of  
585 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447,  
586 661-678. <https://doi.org/10.1038/nature05911>

587 Willham, R.L., Whatley, J.A., 1962. Genetic variation in nipple number in swine.  
588 Zeitschrift für Tierzucht und Züchtungsbiologie 78, 350-363.  
589 <https://doi.org/10.1111/j.1439-0388.1962.tb01289.x>

590 Yang, J., Huang, L., Yang, M., Fan, Y., Li, L., Fang, S., Deng, W., Cui, L., Zhang, Z.,  
591 Ai, H., Wu, Z., Gao, J., Ren, J., 2016. Possible introgression of the *VRTN* mutation  
592 increasing vertebral number, carcass length and teat number from Chinese pigs into  
593 European pigs. Sci. Rep. 6, 19240. <https://doi.org/10.1038/srep19240>

594 Zhang, L.-C., Yue, J.-W., Pu, L., Wang, L.-G., Liu, X., Liang, J., Yan, H., Zhao, K.-B.,  
595 Li, N., Shi, H.-B., Zhang, Y.-B., Wang, L.-X., 2016. Genome-wide study refines  
596 the quantitative trait locus for number of ribs in a Large White × Minzhu intercross  
597 pig population and reveals a new candidate gene. Mol. Genet. Genomics 291, 1885-  
598 1890. <https://doi.org/10.1007/s00438-016-1220-1>

599 Zhou, X., Stephens, M., 2012. Genome-wide efficient mixed-model analysis for  
600 association studies. Nat. Genet. 44, 821-824. <https://doi.org/10.1038/ng.2310>

601 Zhuang, Z., Ding, R., Peng, L., Wu, J., Ye, Y., Zhou, S., Wang, X., Quan, J., Zheng, E.,  
602 Cai, G., Huang, W., Yang, J., Wu, Z., 2020. Genome-wide association analyses  
603 identify known and novel loci for teat number in Duroc pigs using single-locus and  
604 multi-locus models. BMC Genomics 21, 344. [https://doi.org/10.1186/s12864-020-  
6742-6](https://doi.org/10.1186/s12864-020-<br/>605 6742-6)

606 **Table 1.** Datasets used in the single-marker and haplotype-based genome-wide  
607 association studies carried out in Italian Duroc pigs.

608

<b>Parameter</b>	<b>Single-marker</b>	<b>Haplotype-based</b>
Animals used in GWAS ( <i>n</i> )	1162	1162
Genotyped DNA markers used in GWAS ( <i>n</i> )	29604	85590
Genomic heritability $h_G^2$ (standard error)	0.25 (0.05)	0.30 (0.06)
Inflation factor ( $\lambda_{GC}$ )	1.015	1.010

609

**Table 2.** Genomic regions associated and significantly associated with the number of teats in Italian Duroc pigs. Results are stratified by marker position.

Genome scan <sup>1</sup>	SSC <sup>2</sup>	Pos <sup>3</sup>	Marker <sup>4</sup>	Min/Maj <sup>5</sup>	MAF <sup>6</sup>	<i>B</i> <sup>7</sup>	<i>s.e.</i> <sup>8</sup>	<i>P</i> <sup>9</sup>	PVE(%) <sup>10</sup>	Candidate gene
Haplotype-based	7	97600001	CHR7_B920_GAG	N/H	0.480	-0.256	0.046	2.51×10 <sup>-8</sup>	2.60	<i>VRTN</i>
Single-marker	7	97652632	MARC0038565	A/G	0.442	-0.286	0.046	8.58×10 <sup>-10</sup>	3.22	<i>VRTN</i>
	7	98066911	H3GA0022664	A/G	0.442	-0.233	0.047	8.92×10 <sup>-7</sup>	2.07	<i>VRTN</i>
	7	98089286	ASGA0035527	G/A	0.442	-0.233	0.047	8.96×10 <sup>-7</sup>	2.07	<i>VRTN</i>
Haplotype-based	10	47000001	CHR10_B437_AGA	N/H	0.460	0.211	0.046	5.08×10 <sup>-6</sup>	1.78	<i>FRMD4A</i>
Single-marker	10	47947214	ASGA0090802	A/G	0.450	-0.209	0.045	4.80×10 <sup>-6</sup>	1.82	<i>FRMD4A</i>

<sup>1</sup> Genome scans performed in the Italian Duroc population.

<sup>2</sup> *Sus scrofa* chromosome.

<sup>3</sup> Position, in base pairs, on the *Sus scrofa* reference genome (version Sscrofa1.1).

<sup>4</sup> DNA marker identifier reported in the chip panel. For haplotypes, the haploblock identifier (chromosome specific) and the allele is reported.

<sup>5</sup> Minor/Major alleles. Haplotypes have been treated as bi-allelic variants (H = haplotype allele and N = other *N* alleles).

<sup>6</sup> Minor allele frequency.

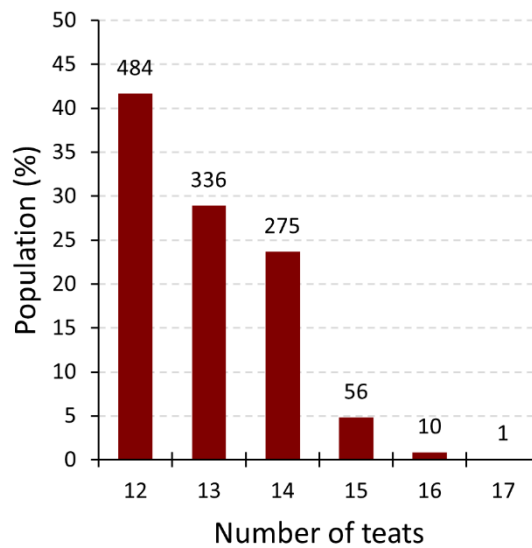
<sup>7</sup> Regression coefficient. A positive value indicates that the no. of teats increases with the increasing of the number of copies of the minor allele. A negative value indicates that the no. of teats decreases with the increasing of the number of copies of the minor allele.

<sup>8</sup> Standard error of the regression coefficient.

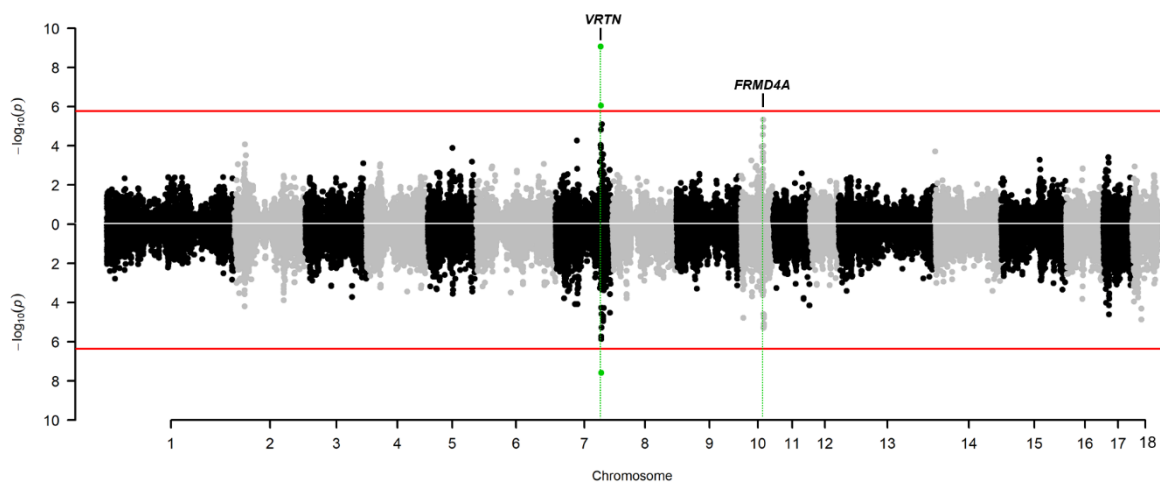
<sup>9</sup> *P* at the Wald test of GEMMA.

<sup>10</sup> Proportion of variance in phenotype explained (%) by the DNA marker.

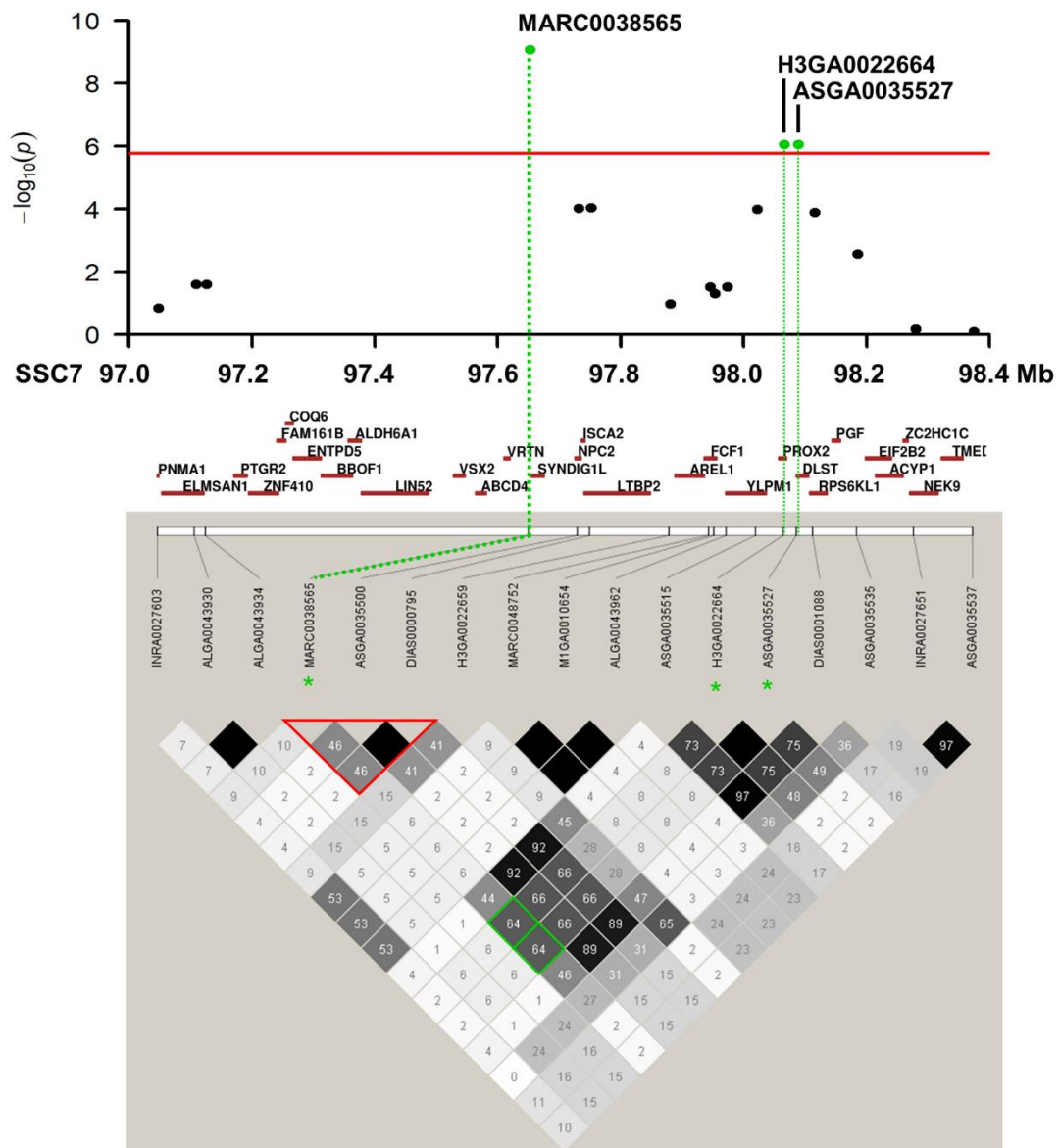
**Figure 1.** Distribution plot of the number of teats in the Italian Duroc population. The number of animals is reported at the top of each bar.



**Figure 2.** Miami plot of the number of teats in the Italian Duroc population. Results of the single-marker GWAS are on the top part of the plot whereas results of the haplotype-based analysis are on the bottom. Dots represent the single nucleotide polymorphism (SNP) or the haplotype markers. The red lines identify the significance thresholds (Bonferroni correction;  $\alpha = 0.05$ ). Statistically associated SNPs are highlighted in green.



**Figure 3.** Regional association plot (single-marker analysis) for the SSC7:97.0-98.4 Mb genomic region. Linkage disequilibrium (LD) was measured between SNP pairs as  $r^2$  and it is reported/showed in each box coloured in relation to its magnitude. The associated SNPs are marked with a green star symbol whereas DNA markers within the top associated haplotype (CHR7\_B92\_97400001\_97800001\_GAG) are marked with a red triangle.



## Supplementary Material

**Table S1.** Distribution of the number of teats in the Italian Duroc pigs.

<b>Teat number class</b>	<b>Total no. of pigs (%)<sup>§</sup></b>	<b>No. of females (%)</b>	<b>No. of males (%)</b>
12	484 (41.65)	177 (40.69)	307 (42.23)
13	336 (28.92)	130 (29.89)	206 (28.34)
14	275 (23.67)	106 (24.37)	169 (23.25)
15	56 (4.82)	20 (4.60)	36 (4.95)
16	10 (0.86)	2 (0.46)	8 (1.10)
17	1 (0.09)	0 (0)	1 (0.14)
<b>TOTAL</b>	<b>1162</b>	<b>435</b>	<b>727</b>

<sup>§</sup> Percentages are given within class (population, males, females).

**Table S2.** Genomic regions affecting the number of teats ( $P < 5.5 \times 10^{-05}$ ) in Italian Duroc pigs. Results are sorted by chromosome and  $P$ .

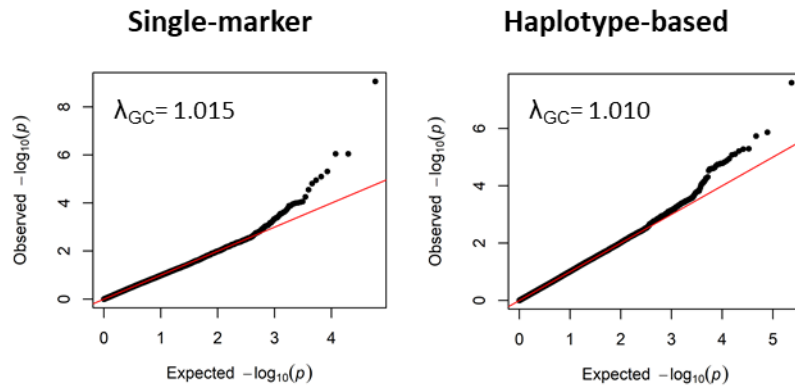
Genome scan <sup>1</sup>	SSC <sup>2</sup>	Pos <sup>3</sup>	Marker <sup>4</sup>	Min/Maj <sup>5</sup>	MAF <sup>6</sup>	$\beta^7$	<i>s.e.</i> <sup>8</sup>	$P^9$
Single-marker	7	97652632	MARC0038565	A/G	0.44	-0.286	0.046	8.58E-10
Haplotype-based	7	97600001	CHR7_B920_97400001_97800001_GAG	N/H	0.48	-0.256	0.046	2.51E-08
Single-marker	7	98066911	H3GA0022664	A/G	0.43	-0.233	0.047	8.92E-07
Single-marker	7	98089286	ASGA0035527	G/A	0.43	-0.233	0.047	8.96E-07
Haplotype-based	7	97600001	CHR7_B920_97400001_97800001_AGA	H/N	0.33	-0.237	0.049	1.36E-06
Haplotype-based	7	97500001	CHR7_B919_97300001_97700001_AA	H/N	0.35	-0.231	0.048	1.84E-06
Haplotype-based	7	98600001	CHR7_B930_98400001_98800001_ACG	N/H	0.41	-0.210	0.046	5.23E-06
Single-marker	7	100073102	ALGA0044076	A/G	0.41	-0.220	0.049	7.96E-06
Haplotype-based	7	101900001	CHR7_B963_101700001_102100001_GAAAAGC	H/N	0.39	-0.211	0.048	1.13E-05
Haplotype-based	7	101800001	CHR7_B962_101600001_102000001_GGGAAAAG	H/N	0.39	-0.209	0.048	1.47E-05
Single-marker	7	98469335	ASGA0035543	G/A	0.41	-0.205	0.047	1.54E-05
Haplotype-based	7	101700001	CHR7_B961_101500001_101900001_AGAGGGGAAAA	H/N	0.39	-0.205	0.048	2.02E-05
Haplotype-based	7	98700001	CHR7_B931_98500001_98900001_GG	N/H	0.38	-0.211	0.050	2.53E-05
Haplotype-based	7	116400001	CHR7_B1106_116200001_116600001_AGAAGGA	H/N	0.04	0.424	0.101	2.93E-05
Single-marker	10	47947214	ASGA0090802	A/G	0.45	-0.209	0.045	4.80E-06
Haplotype-based	10	47000001	CHR10_B437_46800001_47200001_AGA	N/H	0.46	0.211	0.046	5.08E-06
Haplotype-based	10	47200001	CHR10_B439_47000001_47400001_AGAAAGGA	N/H	0.50	0.209	0.046	6.14E-06
Haplotype-based	10	47100001	CHR10_B438_46900001_47300001_AGAAAG	N/H	0.46	0.208	0.046	7.83E-06
Haplotype-based	10	47400001	CHR10_B441_47200001_47600001_AAGGAGGCA	N/H	0.49	0.212	0.047	8.37E-06
Single-marker	10	47963773	ASGA0094083	A/G	0.46	-0.201	0.046	1.12E-05
Haplotype-based	10	3200001	CHR10_B31_3000001_3400001_GAGGA	H/N	0.04	0.475	0.110	1.64E-05
Haplotype-based	10	47500001	CHR10_B442_47300001_47700001_GAGGCAGC	H/N	0.48	-0.201	0.046	1.65E-05
Haplotype-based	10	47600001	CHR10_B443_47400001_47800001_GGCAGCACG	H/N	0.49	-0.192	0.045	1.77E-05
Haplotype-based	10	47300001	CHR10_B440_47100001_47500001_AAAGGAG	N/H	0.49	0.201	0.047	1.79E-05
Haplotype-based	10	47700001	CHR10_B444_47500001_47900001_GCAGCACGG	H/N	0.46	-0.194	0.046	2.30E-05
Haplotype-based	10	46900001	CHR10_B436_46700001_47100001_AG	N/H	0.31	0.211	0.050	2.59E-05
Single-marker	10	47026618	ALGA0059028	G/A	0.31	0.209	0.050	2.80E-05

<sup>1</sup> Genome scans. SNPs and Haplotypes indicates which DNA markers have been used to carry out the genome scans.



- <sup>2</sup> *Sus scrofa* chromosome.
- <sup>3</sup> Position, in base pairs, on the *Sus scrofa* reference genome (Sscrofa11.1).
- <sup>4</sup> DNA marker identifier reported in the chip panels. For haplotypes, we report; chromosome, haploblock, start, end, haplotype.
- <sup>5</sup> Minor/Major alleles. Haplotypes have been treated as bi-allelic variants (H = haplotype allele and N = other *N* alleles).
- <sup>6</sup> Minor allele frequency.
- <sup>7</sup> Regression coefficient. A positive value indicates that the no. of teats increases with the increasing of the number of copies of the minor allele. A negative value indicates that the no. of teats decreases with the increasing of the number of copies of the minor allele.
- <sup>8</sup> Standard error of the regression coefficient.
- <sup>9</sup> *P* at the Wald test of GEMMA.

- 1 **Figure S1.** Quantile-quantile plots of the genome-wide association studies carried out in the Italian
- 2 Duroc population. Inflation factor ( $\lambda_{GC}$ ) is reported.



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