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PIG-TYPICAL GENE POOL CHARACTERISTICS IN WILD BOARS FROM VOJVODINA, SERBIA: A STUDY OF GENETIC DIVERSITY, DIFFERENTIATION, ASSIGNMENT, AND ADMIXTURE

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SUMMARY

The aim of this study was to examine whether and to what extent wild boars, *Sus scrofa*, from Vojvodina, Serbia, exhibited pig-typical gene pool characteristics. We used 16 microsatellite markers that have already been proven to distinguish between wild boars from eastern Austria and pigs. We screened genotypes of 21 wild boars from Vojvodina, 20 Mangulica (Serbian Mangalica) and 4 Hungarian Mangalica as well as 48 commercial slaughter pig (CSP) samples. The wild boars exhibited relatively high genetic diversity, but no significant spatial structuring across Vojvodina. Our analyses of factorial correspondence, Bayesian assignment, as well as genetic structure and admixture demonstrated a clear distinction between wild boars, Mangulica, Hungarian Mangalica, and CSPs. The latter were characterized by admixture of variable portions of six genetic clusters, while wild boars, Mangulica, and Hungarian Mangalica were characterized largely by single genetic clusters, respectively; that finding was in strong contrast to earlier results showing presence of several genetic clusters in Austrian wild boars but only one cluster in CSPs. Two (9.5%) of the wild boar samples were massively introgressed by CSPs (or represented pig samples, possibly due to sample confusion). All wild boars harbored at least very small portions of pig-typical gene pool characteristics, supposedly representing signals of historical introgressions or incomplete gene pool differentiation during domestication. Mangulica-typical signals were also found in the wild boars, but at a significantly lower level than CSP-signals. A more comprehensive data set may reveal possible hot spots of introgression by Mangulica or CSPs in wild boars from Vojvodina, particularly when accompanied by other molecular markers, such as mitochondrial and nuclear DNA sequences or SNPs.

Key words:

wild boar, domestic pigs, genetic diversity, introgression, Vojvodina, Serbia

Abbreviations:

DNA - deoxyribonucleic acid; PCR - polymerase chain reaction; CSP - commercial slaughter pig; HWE - Hardy Weinberg expectation; SNP - single nucleotide polymorphism

INTRODUCTION

In Europe, natural populations of wild boar, *Sus scrofa*, may harbor pig-typical gene pool characteristics at varying levels, resulting from recent or historic introgressive hybridization or/and incomplete gene pool separation in the domestication history of pigs (e.g., Scandura et al., 2011; Nikolov et al., 2017; Iacolina et al., 2018).

A recent microsatellite-based study (Böheim et al., 2023) revealed, principally in good accordance with the SNPs-based study of Iacolina et al. (2018), pig-typical gene pool characteristics in all wild boars studied in eastern Austria, but generally at a very low level. Those microsatellite-based signals of introgression were interpreted as largely indicating shared allelic polymorphism due to a shallow gene pool divergence between wild boars and pigs during domestication (see e.g., Frantz et al., 2015) on the one hand and as possible remaining signals of historic introgressive hybridizations on the other, rather than of ongoing introgression.

Pig-typical gene pool characteristics have also been found at variable degrees in southeastern Europe (e.g., Nikolov et al., 2017; de Jong et al., 2023) where semi-free-range pig farming was carried out at least occasionally and regionally until very recently, possibly even before implementation of the regulations related to African Swine Fever (personal observations by F.S. in Romania). This could indicate that free-living wild boars in the Balkans and other parts of southeastern Europe are particularly rich in pig-typical gene pool characteristics, especially of Mangulica. However, so far a Europe-wide SNP-based study has confirmed only regional introgression in Balkan populations, with a low to slightly increased level of introgression in some Serbian wild boars (de Jong et al., 2023). Even though SNPs provide a clearly better resolution of genetic variability, we used the same set of microsatellite markers as in the study on eastern Austrian wild boars (Böheim et al., 2023) to analyze introgression in wild boars from Vojvodina by specifically considering Mangulica (Serbian Mangaliza) in addition to commercial slaughter pigs (CSPs) for a representative sample of whole Vojvodina. Until relatively recently – in evolutionary terms – free-ranging or semi-free ranging farming practices were not uncommon for Mangulica (cf. e.g., Nikolić et al., 2017) and perhaps also for other pig breeds of small farm holdings and this might have provided gene flow between free-ranging wild boars and pigs. According to Böheim et al. (2023), we expected relative genetic uniformity of CSPs in comparison to the wild boars from Vojvodina. We checked for significant spatial variation of genetic variability as well as possible introgression signals in the wild boars. At least in a few regions (Srem, northern Vojvodina), we expected higher introgression levels by Mangulica than by CSPs, due to supposedly more free-ranging Mangulica farming than semi-free-ranging farming of local pig breeds, which are probably similar genetically to CSPs.

MATERIAL AND METHODS

Wild boar and pig samples

We used tissue samples of 21 wild boars, *Sus scrofa* L., shot during regular hunts in 2008-2011, i.e., well before the appearance of African Swine Fever at various locations across Vojvodina, Serbia, in the course of regular hunting management. While collection date and location were available for most of the samples, no specific information was provided on the phenotypic appearance of individual wild boars, such as suspicious fur coloration, that could have given an indication of introgression by pigs. In addition, we used tissue samples from twenty Mangulica, i.e., Serbian Mangaliza, collected in 2014 at Ravno Selo (Bačka, Vojvodina), and of four Hungarian Mangaliza pigs, purchased from a butcher in eastern Austria (at Pamhagen, Province of Burgenland), as well as 48 commercial slaughter pigs (CSP), purchased as pork packages in 2017 and 2018 from supermarkets in Austria, Germany, the Czech Republic, Hungary, Slovenia, and Bulgaria, all of which have already been used in a study for comparison with Austrian wild boars (Böheim et al., 2023).

Genetic markers and laboratory procedures

We used the same sixteen microsatellite markers, i.e., S0002, S0097, S0101, S0155, S0215, SW24, SW72, SW122, SW240, SW461, SW857, SW936, SW1492, SW2021, SW2496, SW2532, as in Böheim et al. (2023) to determine allelic variation and individual overall genotypes for population genetic statistics and as a basis for estimating individual introgression of wild boars by pigs. For DNA extraction, purification, and PCR specifications we followed the procedures detailed in Vetter et al. (2014; 2016) and applied also by Böheim et al. (2023). Marker individuals of the latter study were used to synonymize the currently identified alleles and genotypes with those of the latter study, which enabled a direct comparison of the population genetic results.

Population genetic statistics and estimates of individual introgression levels

We used IDENTIX vers. 1.1.5 (Belkhir et al., 2002) to calculate pairwise genetic identity for any two individual overall genotypes to identify possible sample confusion or identical twins, and to evaluate the power of our marker system for individual sample identification. An increased number of genetically identical samples would make them statistically dependent and could lead to an undesired bias of the population genetic statistics; it would also indicate a reduced level of resolution of genetic differentiation of our marker system. We further used GENETIX 4.05.2 (Belkhir, 2004) to calculate allele frequencies, observed (H_o) and expected (H_e) heterozygosity, mean number of alleles per locus (A), population (group)-specific inbreeding coefficients (F_{is}), and pairwise relative (F_{st}) and absolute (CSE distances - Cavalli-Sforza & Edwards distances) genetic differentiation. Moreover, we used the latter software to test for linkage disequilibrium (LD) and to run a ten-factorial correspondence analysis (FCA). Group-specific allelic richness (R_s) that accounted for different sample sizes by rarefaction was calculated in FSTAT (Goudet, 1995). In addition, we estimated the likelihood of every individual being assigned to either wild boar, Mangulica, Hungarian Mangaliza, or CSPs according to its overall genotype with the Bayesian approach of Rannala & Mountain (1997) implemented in GeneClass2 vers. 2.0.h (Piry et al., 2004, see also Cornuet et al. 1999). Specifically, we used the resampling algorithm of Paetkau et al. (2004) for 1.000 simulated individuals, an assignment threshold of scores of 0.01 and a type-1 error (α) of 0.01.

We used STRUCTURE 2.3.4 (Pritchard et al., 2000; Falush et al., 2003) to determine the number of genetic clusters (K , i.e., theoretical Hardy-Weinberg populations) inherent to the total data set of overall individual genotypes and the composition of K s for each individual and respective percentages (Q). Specifically, we used admixture models of correlated allele frequencies without population priors (i.e., no information of assignment of individuals to either wild boar, Mangulica, Hungarian Mangaliza, or CSPs), with 250.000 Monte-Carlo Markov chain (MCMC) repetitions after 100.000 MCMC burn-in, with an initial $\alpha=1$, for $K=1-10$ and ten iterations per K . We used the STRUCTURE HARVESTER on-line platform (Earl & von Holdt et al., 2012) to calculate mean, maximum, minimum, and standard deviation of $\ln [Pr(X_jK)]$ for each K and Evanno's (see Evanno et al., 2005) ad hoc statistics of the second order rate of change of the likelihood function with respect to K . In determining the number of K we were predominately guided by the likelihood values of each K (means and variation over iterations) as well as the specific increase of biological information with each additional K (see Pritchard et al., 2000; Falush et al., 2003), but less by Evanno's ΔK (which often suggests $K=2$, due to an often very low variance of $K=1$ in the denominator in the formula, which consequently inflates ΔK for $K=2$).

Following Böheim et al. (2023), we calculated levels of introgression by pig-typical K for each individual wild boar as the sum of all pig-typical K (i.e., eight K s, as resulting from our STRUCTURE analysis, see "Results and Discussion"), averaged over all iterations per K . In addition, we used BAPS v.6.0 (Corander et al., 2008) for spatially explicit Bayesian clustering of overall individual wild boar genotypes, based on individual geographical coordinates (without a priori population membership) for $K=1-10$, with ten replications per K , and averaging the results based on the likelihood scores. We run BAPS initially with the full set of 21 wild boars and afterwards for only 19 wild boars, i.e., without two individuals that showed very high levels of introgression by pigs (see "Results and Discussion"), to specifically check for those very high introgression signals on numbers and distribution of spatial clusters. Additional statistical tests stated in the "Results and Discussion" section were performed in IBM SPSS 29.0.1.0.

RESULTS AND DISCUSSION

Genetic variability and differentiation

No identical overall genotypes were detected in pairwise comparisons, which suggested a high resolution of our marker system in detecting individual genetic differences. Also, no significant LD was revealed in any of the four studied groups, when accounting for multiple testing. Overall, we detected 122 alleles, of which 30 (24.6%) were private to Serbian wild boars (14=11.48%, average frequency=6.6%), or CSPs (12=9.84%, average frequency=4.51%), or Mangulica (4=3.28%, average frequency=21.25%). On the other hand, 24 (19.7%) of all alleles were ubiquitous, i.e., they occurred in all four studied groups with a relatively high overall frequency of 34.9% (unweighted mean) and a range between 13.3–88.0% (2.5–95.8% for values in single groups). Allelic richness (R_s) that accounted for different sample sizes varied significantly ($p<0.001$, $n=16$ loci, Friedmann test) across the four groups, with the highest value in wild boars, intermediate values in CSPs and Mangulica, and the lowest value in Hungarian Mangaliza. However, when computing and comparing R_s values separately for Serbian wild boars on the one hand and collectively all three pig groups on the other, the numerical difference between those two groups (i.e., R_s of wild boars=6.25 vs. R_s of pigs=5.68) could not be confirmed statistically ($p=0.07$, Wilcoxon matched pairs signed rank test). Group-specific values of A , H_o , H_e , R_s , and F_{is} as well as significant deviations of HWE are listed in Table 1.

Table 1. Indices of population genetic variability and Wright's inbreeding coefficients (*F_{is}*) of Vojvodina wild boars (WB), Mangulica (MA), Hungarian Mangaliza (HM), and commercial slaughter pigs (CSP)

Index	WB	MA	HM	CSP
			pigs	
mean number of alleles/locus (A)	6.31	3.56	2.56	5.69
observed heterozygosity (<i>H_o</i>)	0.72	0.54	0.41	0.51
expected heterozygosity (<i>H_e</i>)	0.72	0.54	0.51	0.66
allelic richness (<i>R_s</i>)	2.73	2.52	2.06	2.15
inbreeding coefficient (<i>F_{is}</i>)	0.003	-0.007	0.232	0.232*
Legend: * - significantly different from zero at $p < 0.05$ (strict Bonferroni corrections for multiple testing), as tested by 1000 permutations over alleles				

A somewhat higher level of genetic variability was reported for the Vojvodina and neighboring regions in the West Balkans by Veličković, et al. (2012), based, however, on only four microsatellite markers that were already known for their relatively high allelic variation. The presently detected level of genetic variability was also slightly lower than the one found in eastern Austrian wild boars from a somewhat smaller geographical range, but the same set of markers. The study of Veličković, et al. (2012) also found levels of effective migrants per generation enough to counteract in principle potential differentiation of (neutral) gene pool elements in wild boars from Vojvodina, Slavonia in Croatia, and Bosnia in the West Balkans. That indicated relatively unrestricted gene exchange and favored maintenance of genetic variability over a larger geographical area. The currently detected low to slightly increased genetic differentiation (Tab. 2) between wild boars and CSPs corresponded to that previously determined between wild boars from eastern Austria and CSPs (Böheim et al., 2023). It was also within the range found between several wild boar populations from East and Southeast Asia, also based on 16 microsatellites (Choi et al., 2014), and principally in line with that observed among various populations of many terrestrial mammal species, when a mixture of loci with high, moderate, and little allelic diversity was examined, like in our study. It corresponded to a long-standing merely shallow genetic divergence between pigs and wild boars throughout the domestication of pigs, with increasing separation of modern breeds from free-ranging wild boars only recently in evolutionary terms (Frantz et al., 2015). The presently found clearly elevated level of genetic differentiation between wild boars and Mangulica as well as between wild boars and Hungarian Mangaliza is a bit astonishing, in the face of their supposedly relatively old origin compared to the modern standard commercial breeds (i.e., CSPs) used nowadays in indoor breeding systems for pork production (see e.g., Böheim et al., 2023 for a short discussion of the breeding history of Mangaliza/Mangulica). But the strong population decline of Mangaliza/Mangulica in their recent history might have resulted in increased genetic drift (stochastic effects) of allele frequencies and thereby in an elevated level of differentiation from the wild boars (comp. Addo & Jung, 2022). Alternatively or additionally, the increased genetic differentiation observed between Mangulica and Serbian wild boars and the Hungarian Mangaliza might be due to stochastic sampling effects as Mangulica were sampled exclusively from a single location in Serbia (see Material & Methods) and the Hungarian Mangaliza were purchased from a single butcher. A larger geographically more dispersed sample of both Mangulica and Hungarian Mangaliza might help to reach better inferences.

Table 2. Indices of pairwise relative (*F_{st}* – above diagonal) and absolute (CSE distances – below diagonal) genetic differentiation between the studied groups (populations)

Population/group	WB	MA	HM	CSP
			pigs	
Vojvodina wild boars (WB)	-	0.176*	0.137*	0.110*
Mangulica (MA)	0.202*	-	0.224*	0.174*
Hungarian Mangaliza (HM)	0.210*	0.207	-	0.179*
commercial slaughter pigs (CSP)	0.122*	0.190*	0.233*	-
Legend: WB - Vojvodina wild boars (WB); MA – Mangulica; HM - Hungarian Mangaliza; CSP - commercial slaughter pigs; * - significantly different from zero at $p < 0.05$ after accounting for multiple testing (strict Bonferroni corrections), as tested by 1000 permutations of individuals for each population pair				

Our FCA (Fig. 1) conveyed 39.13% of the allelic variation and already the first three dimensions (factors) that reflected 17.67% of the currently revealed allelic variation resulted in a clear separation of the four studied groups, except for two wild boar individuals that clustered with the CSPs.

Genetic identification and assignment of wild boars and pigs

Corresponding to our FCA results, our Bayesian assignment analysis returned highest individual probabilities for assignment to the group (“population”) where the respective individuals were collected from, except for one wild boar that was assigned to CSPs (probability of CSP=0.391, probability of wild boar=0.305); that latter individual was one of the two wild boars that clustered with the CSPs in our FCA. However, the second wild boar that grouped with the CSPs in our FCA was undoubtedly (“correctly”) “identified” as wild boars, with a probability of 0.282 compared to a probability of 0.034 for CSPs.

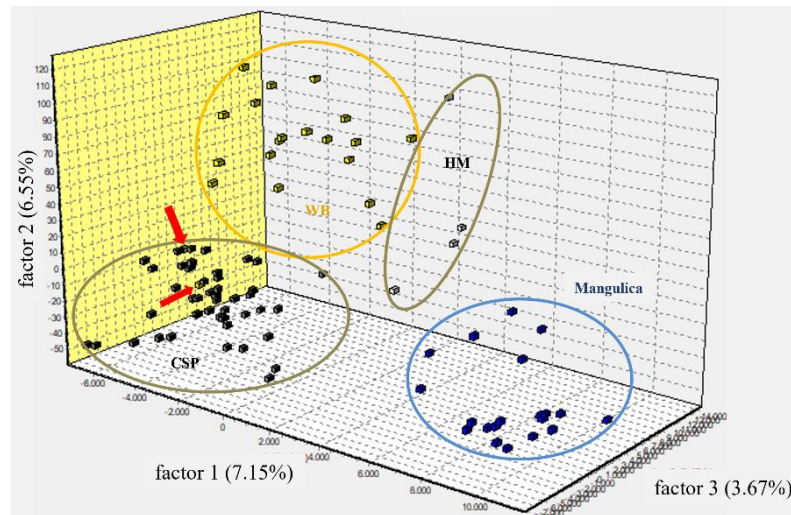


Figure 1. Distribution of individual overall genotypes in the three-dimensional space according to our ten-factorial corresponding analysis. CSP – commercial slaughter pigs, HM – Hungarian Mangalica, WB – Serbian wild boars from Vojvodina; percentages of allelic variation per factor are in parentheses; the two red arrows indicate the positions of two wild boars that clustered with CSPs (see also STRUCTURE results in Fig.2).

Genetic structure and admixture

The likelihood distribution of K and the associated variation over iterations suggested nine genetic clusters inherent to the total data set, whereas Evanno's ΔK ad-hoc statistics suggested only three genetic clusters (Fig. 2). Since our inspection of the $K=1-9$ plots suggested biological information increased as K increased, and according to the likelihood distribution and variances for the different numbers of K , we accepted $K=9$ for our introgression analysis (also in line with the recommendations of Pritchard et al., 2000; Falush et al., 2003). For $K=3$ we obtained a few clear introgression signals of wild boars and Mangulica in CSPs, which disappeared in almost all cases for $K=9$, and that suggested as well that $K=3$ was not sufficient. The two wild boars that clustered with CSPs in our FCA showed strong introgression by CSP-typical K s in all our plots for $K=2-9$. Remarkably, despite the high number of $K=9$ there was only one genetic cluster typical for wild boars from Vojvodina and by far predominating (very high Q) in each wild boar, whereas one K was typical for Mangulica, one for Hungarian Mangalica, and six K s were present in CSPs. This indicated on the one hand the largely absence of genetic admixture of the wild boars from the Vojvodina, representing one Hardy-Weinberg population, apart from the two individuals that were identified as CSPs or at least as wild boars massively introgressed by CSP-typical K s, or represented possibly backcrosses with pigs. On the other hand, both the Mangulica and the Hungarian Mangalica were genetically clearly separate from each other and the wild boars, with one (25%) Hungarian Mangalica individual showing admixture with Mangulica and wild boar gene pool characteristics (see also Böheim et al., 2023).

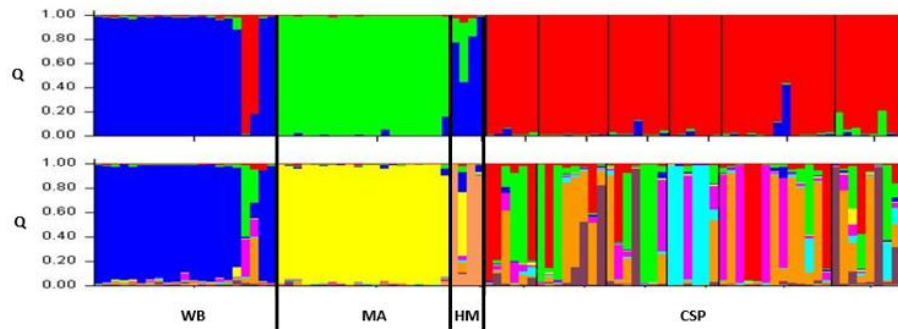


Figure 2. STRUCTURE analysis. Exemplary plots of genetic structure and admixture (without population priors) for $K=3$ (upper plot) and $K=9$ (lower plot). Q – percentage of genetic cluster (K) for each individual (i.e., column); WB – Vojvodina wild boars, MA – Mangulica, HM – Hungarian Mangaliza, CSP – commercial slaughter pigs (from Austria, Slovenia, Hungary, Bulgaria, Czech Republic, Germany according to upper plot CSP segments).

Notably, our CSP individuals that were all genetically rather similar and almost not admixed in the study of Böheim et al. (2023) showed in the present STRUCTURE analysis very heterogenous and quite admixed individual gene pools. This remarkably different result for the same individuals used by Böheim et al. (2023) in comparison with wild boars from eastern Austria and in the present study strongly recommended considering genetic structure and admixture results primarily in the context of the simultaneously studied groups/populations; generalizing findings of genetically homogenous, little structured, or unstructured/not admixed groups or populations in one specific data set may be misleading. In fact, the currently studied CSPs that were rather uniform and represented merely one K in the study of Böheim et al. (2013), where they were compared to eastern Austrian wild boars, contained quite some genetic variability, even though their allelic richness amounted to only 78.8% of that of wild boars from Vojvodina. Moreover, the CSPs were currently significantly partitioned into more than one Hardy-Weinberg population, whereas the wild boars from Vojvodina represented only a single Hardy-Weinberg population (Tab. 1). The absence of any significant genetic sub-structuring of the Vojvodina wild boars was also supported by our BAPS analysis, when removing the two individuals that were identified as introgressed both by our FCA and STRUCTURE analyses or were possibly even pigs and erroneously considered wild boars due to sample confusion (Fig. 3).

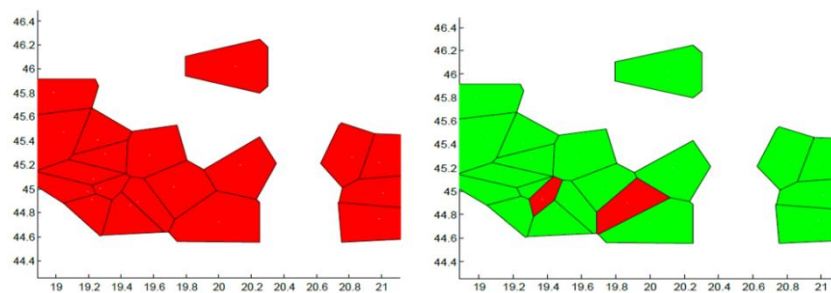


Figure 3. BAPS analysis. Left plot – spatial distribution of genetic clusters of Vojvodina wild boar individuals, without the two individuals identified as “introgressed” (or even confused with pig samples) by our FCA and STRUCTURE analyses ($n=19$); right plot – all Vojvodina wild boars ($n=21$) including the two “introgressed” samples; different colors indicate different genetic clusters (K).

Introgression analysis

When estimating the extent of wild boar introgression by pigs by calculating the summed individual Q values of the eight pig-typical K s (i.e., characteristic for Mangulica, Hungarian Mangaliza, and CSPs), averaged over the iterations (Böheim et al. 2023), we found introgression signals in all wild boars, but mostly of low to very low magnitude, except for two individuals (Tab. 3). Many Mangulica and all CSPs also showed at least a very small amount (Q) of wild boar-typical K ; but averaged summed Q values of pig-typical K s in wild boars were significantly higher than the Q values of wild boar-typical K in Mangulica and CSPs, respectively ($H=41.32$, $p<0.001$, $d.f.=2$; Kruskal-Wallis test; Hungarian Manguliza were not considered). Those generally small or very small Q values of introgression may indicate basic allelic characteristics (shared alleles) common to both wild boars and pigs and/or historic introgressions (see also Böheim et al. 2023). Remarkably, mean Mangulica-typical Q (0.01%, 0.0-0.09%) in wild boars was significantly ($p<0.001$, $d.f.=2$; Mann-Whitney U-test) smaller than mean CSP-typical Q (0.051, 0.02-

0.09%), which may suggest more historical introgression by CSPs (or the local breeds that have partly formed the basis of the current CSP breeds and that were likely in partly free-ranging systems on small family farms) rather than by Mangulica. In any case, the general level of introgressed gene pool characteristics of the Vojvodina wild boars was very similar to that found among eastern Austrian wild boars with the same marker system. According to our BAPS results, the two (9.5%) wild boars with very high pig-typical gene pool characteristics (as revealed by our FCA and STRUCTURE results) might indeed indicate the presence of locally massively introgressed wild boars or originate from confusions with pig samples. At least the regional position (Srem) of those two latter wild boars corresponded to free-ranging Mangulica farming before the registration of African Swine Fever (Prof. M. Beuković, Novi Sad, pers. comm.). Notably, those two massively introgressed wild boars were not introgressed by Mangulica but by CSP-typical gene pool characteristics. In any case, that signal confirmed our above conclusion of our marker system being sensitive to distinguish between Vojvodina wild boars, Mangulica, and CSPs and possible recent hybrids. Our FCA, genetic assignment and STRUCTURE results also suggested the chance of (secure) genetic identification of Hungarian Mangaliza with our marker system, but that needs to be based on a bigger sample size.

Table 3. Percentages (Q) of pig-typical genetic clusters (Ks) in wild boars (WB) and of the wild boar-typical genetic cluster in Mangulica (MA), Hungarian Mangaliza (HM), and commercial slaughter pigs (CSP). Means/medians/ranges are given for sums of pig-typical Ks and of the wild boar-typical K are given, as averages over all iterations for STRUCTURE models of K=9

Q (averaged over iterations)	WB	MA	HM	CSP
			pigs	
sum of pig-typical Ks				
mean/median	0.151/0.061	0.008/0.003	0.053/0.027	0.019/0.01
Range	0.03-0.99	0.00-0.06	0.00-0.16	0.01-0.03

Nevertheless, introgressions by Mangulica or pigs in wild boars stemming from the more distant past may not be detected by the exclusive use of our (or any other) microsatellite marker system, as suggested e.g., by studies on wild boar introgression in northwestern continental Europe (Frantz et al. 2013) and Bulgaria (Nikolov et al. 2017). Both studies found higher levels of introgression with other molecular markers, such as mtDNA sequences and coat color genes (e.g. MC1-R). Phenotypically suspicious wild boars were observed to some extent in the wild boars from Vojvodina, and free-ranging pig/Mangulica farming was not uncommon in parts of Vojvodina, before African Swine Fever regulations have been implemented (Prof. M. Beuković, Novi Sad, pers. comm.) Even though wild boars have presently most likely no or very little chance of contact with Mangulica or pigs, pig-typical gene pool elements from hybridizations in earlier generations would not be perched unless under strong selective pressure and disappearance of allelic introgression signals would depend on genetic drift effects (unless microsatellites were linked to genes under selection). Given the rather uniform population structure (i.e., one Hardy-Weinberg population) of the wild boars from Vojvodina, which translates into genetic homogenization and rather unhindered gene flow across the whole study area (see also Veličković, et al. 2012), we may expect introgressed wild boars in all parts of our study region and possibly beyond (albeit mostly at very low individual introgression levels).

CONCLUSION

Overall, our results indicated quite a rich genetic diversity of wild boars from Vojvodina, which was, however, a bit lower than that found among eastern Austrian wild boars from a somewhat smaller area and that were studied by using exactly the same molecular marker system. Also, in contrast to the eastern Austrian wild boars allelic variability was rather uniformly distributed across whole Vojvodina, without any significant subdifferentiation, i.e., gene flow was sufficient to counteract potential gene pool differentiation in Vojvodina. As in the earlier study on eastern Austrian wild boars, our microsatellite marker system successfully discriminated wild boars from Mangulica, Hungarian Mangaliza, and commercial slaughter pigs purchased as pork samples at various supermarkets in different countries of central and eastern/southeastern Europe. All wild boars showed mostly very little traces of pig-typical gene pool characteristics; this may indicate at least occasional gene flow between wild boars and pigs/Mangulica under partly free-ranging farming practices in the past or/and indicate incomplete gene pool differentiation between wild boars and pigs. In addition, a low level (9.5%) of very recent introgression by pigs (not by Mangulica) was detected among the wild boars from Vojvodina. However, we could not fully exclude unrecognized sample confusion with pigs in those latter cases. Our findings represent a base line of the status of introgression of pigs/Mangulica in Vojvodina wild boars and recommend a complementary study on mtDNA- and nuclear (e.g., coat color gene) markers or/and SNPs to better understand the full extent of introgression in Vojvodina wild boars. Particularly a SNP-based study may provide insight into selection processes of introgressed gene pool compartments in wild boars.

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Conflict of interest: The authors declare that they have no conflict of interest.

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