

SHORT COMMUNICATION



## Microsatellite DNA markers applied to the classification of the Podenco Valenciano canine breed

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### ABSTRACT

Traditional morphology studies form the basis for the classification of canine breeds as established by the Fédération Cynologique Internationale (FCI). The FCI recommendations for the acceptance of a new breed include both the differentiation and the identification of close phylogenetic relationships with the previously accepted breeds. These requirements can be met in an affordable way by using the information provided by routine genetic identification procedures. In this report, a set of 21 microsatellite molecular markers is applied to the study of three non-achondroplastic Spanish 'Podenco' breeds, currently included in FCI's group five (section 7). For this purpose, factorial correspondence analysis (FCA), genetic distances and Bayesian population structure are applied to the multilocus genotypes obtained from a sample of 'Podenco' individuals of the different breeds, while German Shepherd Dogs are used as the outgroup. Molecular analysis confirms the existing classification and supports the inclusion of 'Podenco Valenciano' as a new breed in the same FCI group and section. Therefore, the FCI requirements for its acceptance are met in a cost efficient way. A similar approach is suitable for other canine populations, supplementing the traditional morphological criteria.

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### Introduction

As of January 2017, the Fédération Cynologique Internationale (FCI) recognises 360 breeds (www.fci.be, accessed 16 January 2017). For over one century, FCI has been mainly based on the definition of both groups and sections on morphology and aptitude. Breeds are classified into 10 groups. Group five is named 'Spitz and Primitive types' and is divided into seven sections. Section 7 (Primitive type-Hunting Dogs) includes the two Spanish breeds 'Podenco Canario' (PC) and 'Podenco Ibicenco' (PI) and the 'Podengo Português' (Portugal), the 'Cirneco dell'Etna' (Italy), the 'Taiwan Dog' and the 'Thai Ridgeback Dog'. The Royal Spanish Canine Society (www.rsce.es, accessed 16 January 2017) also recognises and includes in the National Origins Book the 'Podenco Andaluz' (PA, standard 401, not accepted by the FCI) and the 'Podenco Maneto', an achondroplastic breed from the south province of Cádiz (standard 408, not accepted by the FCI).

The growing interest for a regional canine population traditionally specialised in rabbit hunting in the Valencia region (Eastern Spain) has attracted the attention of local breeders; the so called 'Podenco Valenciano' (PV) is presumably related with the rest of non-achondroplastic Spanish 'Podencos'. PV is mainly found in Valencia and Alicante, and in the bordering Province of Albacete (San José 2013). As of January 2017, the Valencia Register of Animal Identification (RIVIA) includes 953 adult PV, even if this is only a partial census.

Zoo-ethnology measures indicate the existence of significant morphological differences among the PV, PA, PC and PI (for a detailed description and a gallery of images, see San José 2013). Obviously, such differences result from the genetic selection for a particular function in a defined geographical area, even if no detailed records are available on the procedures implemented for this purpose (pedigrees, etc.). Hypothetically, reproductive isolation, together with

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selection, should also result in the increase of genetic distance indices to the other breeds, as estimated by means of molecular genetic markers. In practice, the estimation of such indices obtained from these markers within canine breeds is usually higher than those calculated from morphological similarities, providing useful parentage testing (Altet et al. 2001; Irion et al. 2003).

The possible use of such markers for the purpose of recognition of breeds by the FCI is mentioned in a document containing scientific reflections on this procedure (Denis 2017). In fact, the molecular genetics methodology is providing new tools to analyse the differences and relationships existing among accepted canine breeds and varieties (Bigi et al. 2015; Dreger et al. 2016; La Manna et al. 2016).

In the present investigation, we apply this methodology to the study of the relationships among the three previously accepted non-achondroplastic Spanish Podenco breeds (PI, PA and PC) and the PV, using the German Shepherd Dog (GSD) as an outgroup. Our results support the recognition of PV as a breed belonging to section 7 of group 5 of breeds.

## Materials and methods

A total of 61 PV individuals, from 48 different owners, 37 PI (eight owners), 30 PC (six owners), 30 PA (13 owners) and an outgroup consisting of 27 GSDs (FCI group 1, section 1) were investigated. DNA was purified from hair roots following standard procedures. The following 21 microsatellite markers of the 2005 International Society for Animal Genetics Canine Panel for Parentage Verification were amplified in four multiplexed Polymerase Chain Reactions: *AHTk253*, *AHT121*, *FH2054*, *CXX279*, *INRA21*, *AHTk211*, *REN54P11*, *REN162C04*, *AHTH260*, *AHTH171*, *REN105L03*, *AHTH130*, *REN169O18*, *REN64E19*, *REN169D01*, *FH2848*, *AHT137*, *REN247M23*, *INU005*, *INU030*, *INU055* (<http://www.isag.us/Docs/consignmentforms/2005ISAGPanelDOG.pdf>, accessed 16 January 2017). Basic variability data were obtained by means of the CERVUS 2.0 software (Marshall et al. 1998). The study of genetic distances and factorial correspondence analysis (FCA) were carried out using the GENETIX software (Belkhir et al. 2004). Bayesian structure analysis was performed using the STRUCTURE software (Pritchard et al. 2000). For each  $k$  value (number of genetic clusters), 10,000 burn-in iterations followed by a run of 100,000 Markov chain Monte Carlo repetitions were performed, and four repeats were done for each calculation. The most

accurate  $k$  value was identified according to Evanno et al. (2005).

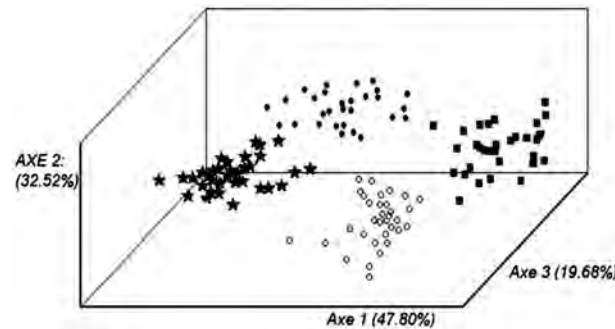
## Results and discussion

The mean number of alleles/locus in the ensemble of 'Podenco' populations was 9.14 (range 6–15) while the mean polymorphic information content (PIC) value was 0.718 (range 0.509–0.824). Therefore, the total exclusionary power (first parent) of the panel of markers is 0.999960 (over 0.9999999 when the second parent is known).

Genetic distances among the different breeds, as estimated by  $F_{st}$  indices are highly significant ( $p < .01$ ). As expected, the highest values are those found among the GSD and the 'Podenco' breeds (Table 1). Figure 1 graphically illustrates the differences among the PC, the PA, the PI and the PV, as observed via FCA analysis. Moreover, Supplementary Figure 1 shows the FCA results of these four breeds and the GSD outgroup. Both FCA and  $F_{st}$  confirm the differences existing among the four non-achondroplastic Spanish Podenco populations. All of them appear in different clouds in the FCA vector analysis, confirming the  $F_{st}$  results. When the GSD outgroup is added to the FCA analysis, PV is clearly shown to belong to the group including PI, PA and PC.

**Table 1.**  $F_{st}$  values estimated among the different populations. PV (Podenco Valenciano); PI (Podenco Ibicenco); PC (Podenco Canario); PA (Podenco Andaluz); GSD (German Shepherd Dog). All the values are highly statistically significant ( $p < .01$ ).

	$F_{st}$ values			
	PI	GSD	PC	PA
PV	0.06081	0.14651	0.05350	0.01811
PI	–	0.23248	0.07565	0.10166
GSD	–	–	0.21546	0.15498
PC	–	–	–	0.07025



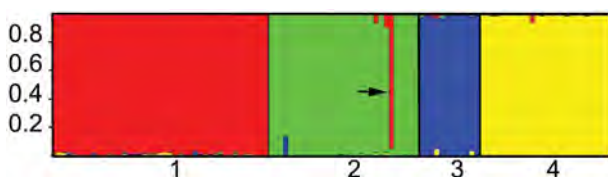
**Figure 1.** FCA results for the four Podenco populations. White circles: Podenco Valenciano; squares: Podenco Andaluz; black circles: Podenco Canario; stars: Podenco Ibicenco.

Table 2 shows the results obtained in the Bayesian population structure analysis using the STRUCTURE software (Pritchard et al. 2000), for the same four 'Podencos'. The most probable number of clusters is  $k=4$ . In all cases, the proportion of membership of each pre-defined populations to a single inferred cluster is very high, supporting both genetic differences among populations and homogeneity in each of them. The probability of belonging to a particular cluster is also estimated for each dog. The usual procedure assigns to a given cluster any animal showing a probability over 80%, while those not reaching 80% are considered admixed. In the present case, the cluster assignation for all but one of the 206 analysed 'Podencos' (99.51%) is coincident with that obtained for their populations of origin (Figure 2): in fact, any individual clusters with the rest of the animals of the same population with the single exception of a dog classified as PI in the database but clearly assigned to the cluster containing the PV dogs ( $p > .92$ ). Further research verified that this individual belonged to a proprietor owning both PI and PV, pointing to a possible error in the data collection and registration during sampling. No admixed animals were detected. The inclusion of the GSD outgroup results in a fifth cluster isolated from the other four. In all, this clustering highlights the differences among the four considered Podenco populations (and among these and the GSD).

Molecular genetic markers analysis provides a tool to meet the two requirements in the FCI statement on

**Table 2.** Proportion of membership of each pre-defined Podenco population in each of the four clusters inferred by means of the genetic structure analysis.

	Inferred clusters			
	1	2	3	4
PV	0.988	0.003	0.004	0.006
PI	0.038	0.953	0.007	0.002
PC	0.007	0.006	0.979	0.008
PA	0.006	0.001	0.003	0.990



**Figure 2.** Genetic clustering obtained by means of the STRUCTURE software. Podenco Valenciano (PV), Podenco Ibicenco (PI), Podenco Canario (PC) and Podenco Andaluz (PA) are denominated 1, 2, 3 and 4, respectively and separated by vertical black lines. Clusters are represented by different colours. The animals from each population cluster together with a single exception (arrow): an individual initially classified as PI is clustered to PV.

the admission of new breeds and varieties (Denis 2017): it indicates significant genetic distances of the PV with the previously accepted non-achondroplastic Spanish Podenco breeds while supporting its inclusion in the same group and section according to morphological and phylogenetic evidence when outgroups are used in the genetic analysis for comparative purposes. A similar Bayesian approach was applied to the analysis of four Italian shepherd dog breeds and their possible relationships with other shepherd dogs from the rest of Europe (Bigi et al. 2015). The data obtained suggested that the Border Collie are genetically closer than GSD to three of the four Italian breeds.

The genetic clustering of different breeds based on multilocus microsatellite typing was previously reported to be infrequent (Irion et al. 2003). These authors found only two significant groupings in a set of 28 breeds (not including Podencos): Bull terrier grouped with Mini Bull terrier while Australian shepherds were separated from the rest of breeds. In contrast, genetic clustering confirmed later the well-known historical relationships existing for 18 pairs of breeds (from a total of 8646 possible pairs) in a set of 132 canine breeds not including any Podenco (Parker et al. 2007). In the same report,  $k=5$  was proposed as the most accurate number of clusters by means of STRUCTURE analysis when four or five individuals are sampled for each breed. However, when  $k=132$  was applied, 114 among the 132 breeds formed distinct single clusters composed of dogs from only one breed. Our results (limited to a smaller set of breeds but including more individuals from each breed) provide a more accurate  $k$  value equivalent to the number of supposed breeds, while each cluster contains animals from a single breed too (with the single exception discussed earlier). In contrast, a recent report (La Manna et al. 2016) provides a clear example of the power of the Bayesian analysis to detect the existence of introgression into two Italian dog breeds (Segugio dell'Appennino and Segugio Maremmano) since the STRUCTURE analysis highlighted a third population sub-structure probably due to a genetic introgression.

The present cost of microsatellite DNA markers is affordable for both breeders and organisations. In practice, valuable individuals have been routinely genotyped for a panel of microsatellites for the purpose of identity and parentage control for years (Altet et al. 2001). Such a service is offered at low prices by different companies and charities: a genetic markers profile like the one described in this paper is currently offered in Spain for collective samples for around 15€ (USD 16) plus taxes per individual. Data obtained from this routine practice can be directly submitted to statistical

analysis in order to obtain additional information on the phylogenetic relationships existing among the different populations.

Whole genome sequencing and single nucleotide polymorphism arrays will provide a rich source of data about genetic variability and relationships among the different canine breeds. A recent report using these methodologies on 168 animals has established the relationship among the Sardinian Fonni's dog and 27 other breeds originating mostly from the Mediterranean Area (Dreger et al. 2016). Several introgressions among these breeds were observed: for instance, a 17.43% contribution of Fonni's Dog to the ancestors of the Portuguese Water Dog and Cane Paratore was estimated. However, at its present cost, this methodology is not applicable to routine individual analysis yet.

## Conclusions

The analysis of multilocus microsatellite genotypes confirms the correctness of the existing classification of the Spanish 'Podenco' breeds. It also supports the inclusion of the PV in the same group and section by the FCI and other institutions.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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