e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 73 (2025), No 3
https://doi.org/doi.org/10.3409/fb_73-3.12

Application of microsatellite DNA markers for the genetic identification of selected hybrid dog breeds

Anna Radko, Magdalena Pieszka and Agnieszka Szumiec

Accepted September 09, 2025 *Published online September 30, 2025* *Issue online September 30, 2025*

Original article

Radko A., Pieszka M., Szumiec A. 2025. Application of microsatellite DNA markers for the genetic identification of selected hybrid dog breeds. Folia Biologica (Kraków) **73**: 118-128.

The aim of the study was to assess the effectiveness of a microsatellite DNA analysis to genetically identify dog breeds and crossbreeds, using reference populations of purebred individuals and Bayesian clustering methods. The study was conducted based on 21 microsatellite markers (STR) that are recommended by the International Society for Animal Genetics (ISAG) for routine canine pedigree verification. The genetic diversity and population structure were assessed for 4 selected breeds: Golden Retriever, Bernese Mountain Dog, Poodle and Chinese Crested Dog. These reference populations were then used to analyse two case studies involving breed verification. Key genetic parameters were calculated, including the observed (H_O) and expected heterozygosity (H_E), degree of inbreeding (F_{IS}) and the polymorphism information content (PIC). The probability of exclusion was estimated in cases of knowing the genotype of one of the parents (CPE_1) and both parents (CPE_2). A relatively high level of genetic diversity among the studied breeds, constituting reference populations, was found to be above 50% for H_O and PIC, with no inbreeding. The probability of CPE_1 and CPE_2 was obtained at the level of 99% and 99.99%, respectively, which allowed the use of these markers for a parentage analysis. Bayesian clustering (STRUCTURE) and a Principal Coordinates Analysis (PCoA) were applied to assess the genetic structure and to identify the admixture in genotypes of the crossbred individuals. In both cases, the analyses successfully confirmed the breed's pedigree or detected mixed ancestry, which was further supported by the parentage testing. The results confirm that the applied STR marker panel is suitable for breed identification and the detection of hybrid ancestries in dogs. While further research is needed to validate the method across a wider range of breeds, the study provides a valuable foundation for the development of DNA-based tests to support breed verification and the assessment of breed compositions in designer and hybrid dogs.

Key words: designer dogs, breed verification, STR markers, genetic structure.

Anna Radko, Agnieszka Szumiec, National Research Institute of Animal Production, Department of Animal Molecular Biology, Balice, Poland.
Magdalena Pieszka, University of Agriculture in Krakow, Department of Animal Genetics, Breeding and Ethology, Kraków, Poland.
E-mail: magdalena.pieszka@urk.edu.pl

The dog population can be conventionally divided into three groups: purebred dogs; mixed-breed dogs (mongrels – without a known share of parent breeds); and hybrid dogs, sometimes called 'designer' breeds, where the share of the parent breeds is known or even desired (Ackerman *et al.* 2021). The term 'purebred dog' refers to an individual whose parents belong to the same breed and they are characterised by the same exterior and interior, i.e. they meet the requirements of the breed standard and have a documented origin. Many of today's dog breeds were created by crossing other breeds with each other, in order to achieve the effect of cumulating the positive traits of the parent breeds. Currently, we are coming

across so-called 'designer' dog breeds, such as the increasingly popular Labradoodle, Goldendoodle and Cockapoo, as well as the lesser known Bernendoodle, Chorkie and the excellent, working sled dog Greyster. Designer breeds are currently causing a great deal of controversy due to their growing popularity, but also because of the threats they pose to established breeds and their breeders. However, it cannot be forgotten that in the case of descriptions of the origins of the vast majority of dog breeds recorded and registered today by kennel clubs or cynological organisations, other groups or dog breeds known at the time participated in their creation. Therefore, it can be argued that many of today's recorded breeds originate from so-called designer or hybrid breeds, which were created based on the crossing of various original breeds. For example, it is believed that the modern Doberman was bred by a German tax collector of the same name by crossing old-type German Shepherds, Rottweilers and German Pinschers, also known as German Terriers (Flaim 2024, www.akc.org/expert-advice/dog-breeds/doberman-pinscher-history).

Another excellent example is the Kromfohländer registered in Group IX of Toy and Companion Dogs (as the only one in its section), which is derived from a crossing of the Fox Terrier and the Vendeen Griffon (https://www.fci.be/nomenclature/Standards/192g09-en.pdf). Mixed dogs, often called mongrels, unlike purebred dogs, are usually the result of accidental, unplanned mating, and may result from the crossbreeding of different purebred dogs or other mixed breed dogs. Mixed breed dogs do not have described and registered breed standards, i.e. a detailed description of the interior and exterior features required for purebred dogs (Ackerman *et al.* 2021). Their appearance and also their psychological traits are very diverse and, in the case of a puppy, we cannot predict what it will look like in the future or how it will behave. On the other hand, the deliberate mixing of breeds, as a result of which so-called 'hybrid' or 'designer' dogs are obtained, aims to create new, previously unseen individuals with certain desired traits, usually related to a specific appearance, but also taking into account desirable behavioural traits. The best example of this would be the Labradoodle breed, which was intentionally created by Wally Conron in 1989 to combine the coat characteristics of the Poodle (no or very little shedding, with less allergenic properties) with the temperament, intelligence and personality of the Labrador, known as an excellent service dog. Since Poodles come in three sizes, Labradoodle puppies also vary in both their size and coat quality – sometimes they may look more like a Poodle and sometimes more like a Labrador.

Since the end of the 20th century, we have been observing a growing interest in dogs originating from the crossbreeding of different breeds. In addition to the Labradoodle, the Maltipoo, Cavapoo and Cockapoo are also popular breeds, which originated from crossbreeding Poodles with the Maltese, Cavalier and Cocker Spaniel, respectively. They are not only considered to be non-shedding, hypoallergenic dogs, but are also attractive as their coat is wavy or curly, they come in different coat colours, and they are known for their friendly and cheerful disposition. 'Hybrid' dogs often command higher prices than their purebred parents. This results in a fairly rapid increase in the number of breeders that are focused on breeding dog hybrids. These breeders try to perpetuate the desired traits in subsequent generations, so that the 'hybrid variety' can be recognised as a true breed in the future. An example is the Maltipoo breed, which has been recognized in the USA for 30 years. The great popularity and demand for such dogs has also caused an increase in the number of so-called 'pseudo-breeding', 'puppy mills' or 'puppy farms' that are focused on financial profits and that operate similarly to factories producing saleable goods. Such a 'production' process is burdened with a high degree of risk, as the puppies from parents of two different breeds may differ significantly in size, type of coat and body structure. There is no guarantee that after crossing the individuals of two different breeds the desired phenotype will occur in the first generation. We are also not able to predict in any way which genes will be revealed in the offspring. In addition, dogs from 'puppy farms' are often not tested and treated, so they may be burdened with genetic defects, be ill or be carriers of undesirable genes characteristic of the parent's breeds. Often, such puppies are not dewormed or vaccinated. Therefore, it seems vital to be able to genetically control 'hybrid' dogs, which may prove important for the protection of purebred dogs in order to maintain the purity of breeds.

Another problem that can be observed in the breeding of hybrid or mixed-breed dogs is double mating. This involves mating a given female dog in the same heat with two different sires, in order to obtain puppies from two fathers (i.e. two different 'hybrids') in one litter. This can be planned by the breeder or may happen randomly, without the breeder's knowledge. However, even planned double mating is not in line with the breeding regulations of most respectable kennel clubs. This widespread practice has led to the creation of different rapid identification tests

for breeds or mixed breeds, while also leading to the need of developing DNA tests to confirm the genetic affiliation to a given breed or the identification of breeds in hybrids and mixed breeds. These tests are becoming increasingly popular, but unfortunately, they are not always reliable and effective (Cowley & Dhanraj 2023). Only the use of validated genetic tests provides a basis for conducting a reliable analysis of the breed identification of a dog.

Short Tandem Repeat (STR) markers have been widely used for individual identification and parentage testing in animals for many years. Moreover, breed identification based on STR markers is currently the most commonly applied genetic method in various animal species (Radko & Podbielska 2021; Parker *et al.* 2004; Pires *et al.* 2009; Berger *et al.* 2018; Garcia *et al.* 2022; Perfilyeva *et al.* 2023).

In the present work, we chose a panel of 21 STR markers recommended by the International Society for Animal Genetics (ISAG 2014) for routine pedigree tests of dogs (Goleman *et al.*2019; Radko & Podbielska 2021) in order to evaluate whether this set, combined with the Bayesian clustering algorithm implemented in STRUCTURE, can distinguish genetically purebred dogs from crossbreeds and can statistically assign individuals to specific breeds (Radko & Podbielska 2021; Parker *et al.* 2004; Pires *et al.* 2009; Berger *et al.* 2018; Garcia *et al.* 2022; Perfilyeva *et al.* 2023). In addition, we applied a Principal Coordinate Analysis (PCoA) to visualise the genetic relationships among individuals, enabling a graphical representation of the clustering patterns by breed (Palotti *et al.* 2017; Vychodilova *et al.* 2018). Both STRUCTURE and PCoA were used to analyse two case studies: 1) A litter of 11 Golden Retriever (GR) puppies, nearly half of which were born with black coats – a colour disallowed by the GR breed standard – suggesting the possible involvement of an additional sire during fertilisation; and 2) A puppy purchased as a Chinese Crested Dog (CC) that, during its development, began to display unexpected coat characteristics consistent with those of the Poodle (PD) breed.

The objective of this study was to determine whether a microsatellite DNA polymorphism analysis using 21 STR markers – commonly used for dog identification and pedigree verification – can also be effectively applied to the genetic identification of dog breeds in cases involving mixed-breed ancestry, specifically two-breed crosses, through a comparison with reference populations of purebred individuals.

Materials and Methods

Materials

For the population studies, calculation of statistical parameters and the analysis of the genetic structures of the selected breeds of dogs, the results of pedigree studies collected in the DNA database National Research Institute of Animal Production (NRIAP) in 2018-2023 were used. Blood samples were collected from dogs undergoing routine parentage testing at NRIAP. All of the sampled animals were registered with the Polish Kennel Club. A total of 473 unrelated dogs were chosen to collect reference groups, as representative samples of the Polish population, including a Poodle (PD, n = 85), Chinese Crested Dog (CC, n = 84), Bernese Mountain Dog (BM, n = 114) and a Golden Retriever (GR, n = 190).

The analysis of two cases was carried out at the request of the dog owners.

Case 1 involved a confirmation of the paternity in a litter suspected of double mating.

Fourteen samples were analysed: eleven from the puppies, one from the mother (Golden Retriever) and one each from the two potential fathers – a Golden Retriever and a Bernese Mountain Shepherd Dog (Fig. 1).

Case 2 concerned the verification of the breed of a puppy purchased as a Chinese Crested Dog (CC), which did not phenotypically match the breed standard.

Two samples were analysed: one from the puppy and one from its father, also a Chinese Crested Dog. (Fig. 2).

Methods

DNA was extracted from swabs and blood samples using the Sherlock AX Kit (A&A Biotechnology, Gdynia, Poland), following the manufacturer's protocol. The extracted DNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). In the analysis, we selected 21 loci from the recommended ISAG core panel for the identification of individuals and parentage testing in the dogs: AHTk211, CXX279, REN169O18, INU055, REN54P11, INRA21, AHT137, REN169D01, AHTh260, AHTk253, INU005, INU030, FH2848, AHT121, FH2054, REN162C04, AHTh171, REN247M23, AHTH130, REN105L03, REN64E19 and the Amel locus. The markers and used primer sequences are presented by Goleman *et al*. (Goleman *et al.* 2021). The STR loci were amplified using Phusion U Hot Start DNA Polymerase

Fig. 1. Alleged fathers of the puppies from the mixed litter: Father No.1 (male of the Bernese Mountain breed) and Father No. 2 (male of the Golden Retriever breed).

Fig. 2. The dog was purchased as a Chinese Crested Dog but showing characteristics specific to the Poodle.

(Thermo Scientific, Wilmington, DE, USA). The PCR reaction was performed on the Veriti® Thermal Cycler amplifier (Applied Biosystems, Foster City, CA, USA), using the following thermal profile: 5 min of initial DNA denaturation at 98°C, followed by 30 cycles of denaturation at 98°C for 15 s, annealing at 58°C for 75 s, elongation of the starters at 72°C for 30 s, and a final elongation of the starters at 72°C for 5 min. The obtained PCR products were analysed using an ABI 3130xl capillary sequencer (Applied Biosystems, Foster City, CA, USA). The amplified DNA fragments were subjected to electrophoresis in 7% denaturing POP-7 polyacrylamide gel in the presence of a standard length of 500 Liz and a reference sample. The results of the electrophoretic separation were analysed automatically using GeneMapper® Software 4.0 (Applied Biosystems, Foster City, CA, USA).

Data Analysis

The statistical analyses of the obtained results were carried out based on the genetics parameters: observed heterozygosity – H_O, expected heterozygosity – H_E, and inbreeding coefficient – F_{IS} for each marker was calculated according to Nei and Roychoudhury (Nei & Roychoudhury 1974), and Wright (Wright 1978). The polymorphic information content – PIC was estimated by Botstein (Botstein *et al.* 1980). The probability of parentage exclusion was calculated for two cases, when the genotypes of one and both of the parents were known – CPE_1 and CPE_2 (Jamieson & Taylor 1997). The statistical analysis was carried out using IMGSTAT software, ver. 2.10.1 (2009), which supports the laboratory of the National Research Institute of Animal Production.

The population Structure was analysed using a Bayesian clustering algorithm implemented in STRUCTURE software version 2.3.4 (Pritchard *et al.* 2000), considering an admixture model with correlated allele frequencies between breeds. The lengths of the burn-in and Monte Carlo Markov Chain (MCMC) simulations were 100,000 and 500,000, respectively, in 5 runs for each number of clusters (K) ranging between 2 and 6. The population relationships based on a principal coordinate analysis (PCoA) were obtained using GenAlEx ver. 6.51 software (Peakall & Smouse 2012).

Results and Discussion

Mixed-breed dogs that do not have any purebred ancestors within several generations are often difficult or impossible to identify. The inherited variants of DNA that are unique to specific breeds get further lost with each generation of mixed-breed progeny, which is why mixed-breed dogs that do not have any purebred ancestors within 2-3 generations are often difficult or impossible to identify, in contrast to first-generation crosses between two purebred parents which are relatively easy to identify. However, to carry out such breed identification it is necessary to have a reference population that represents these breeds.

In the first stage of the study, the polymorphism of 21 STR markers was evaluated within the established reference populations of the studied breeds. Estimates of the within-breeds genetic diversity are summarised in Table 1. The highest average heterozygosity was found for the Chinese Crested Dog ($H_O = 0.56$ and $H_E = 0.61$). Similar H_O and $H_E > 0.5$ values were obtained in the rest of the breeds. The similar H_O and H_E values had low F_{IS} values, ruling out the occurrence of inbreeding in these populations. The population inbreeding coefficient – F_{IS} was low and ranged from 0.003 (BM and PD) to 0.053 (CC). Mean PIC values for the studied breeds were at the same level of PIC > 0.5. The degree of polymorphism and heterozygosity observed and expected at a level above 50% is similar to the variability observed in many other dog breeds throughout the world (Goleman *et al.* 2019; Radko & Podbielska 2021; Perfilyeva *et al.* 2023; Radko & Słota 2009; Ciampolini *et al.* 2011; Mellanby *et al.* 2013; Tahir *et al.* 2015; Bigi *et al.* 2015; Bigi *et al.* 2018; Radko *et al.* 2017), which indicates the usefulness of this panel for further research.

To confirm the parentage of the dogs from the indicated parents, a comparative analysis of the DNA profiles was performed; additionally, the probability of exclusion was calculated. The genetic structure of the examined breeds was assessed using two complementary approaches: Bayesian clustering (STRUCTURE) and a Principal Coordinate Analysis (PCoA).

The probability of exclusion was calculated for two situations: when the genotype of one parent was available (CPE_1) and when the genotypes of both parents were available (CPE_2). The cumulative exclusion probability for CPE_1 and CPE_2 was higher than 0.99 and 0.9999, respectively, which indicates that in the studied breeds, we can exclude the pedigree of the dog with a 99% probability when we know the genotype of one of the parents, and with over 99.99% when we know the genotypes of both parents (Table 1). Such an exclusion probability allows for a reliable analysis of the verification of the

Table 1

Mean values of the genetic parameters were assessed for 21 STR loci of the study breeds

Breed	H_o	H_E	F_{IS}	PIC	CPE_1	CPE_2
BM (n=114)	0.577	0.581	0.003	0.533	0.993499	0.999937
GR (n=190)	0.560	0.584	0.049	0.5385	0.993244	0.999939
CC (n=54)	0.585	0.614	0.053	0.573	0.996508	0.99998
PD (n=114)	0.578	0.580	0.003	0.533	0.993499	0.999937

presumed/indicated parents (Dodd *et al.* 2001; DeNise *et al.* 2004; Mei *et al*. 2023; Arata *et al*. 2016).

The genetic population structure of each study breed was determined based on the admixture level for each dog using the correlated allele frequencies model implemented within the STRUCTURE software. For some breeds, such as the Pitbull or the abovementioned Doberman, the genotypes do not refer to a single group of individuals in a recognised breed, but to genetically diverse groups, most often depending on the breeding region (Ciampolini *et al.* 2013; Wade *et al.* 2023), which share similar physical features. For such breeds, creating a reference population that results in one coherent cluster is difficult or even impossible, which does not allow for the genetic breed identification of dogs that can be phenotypically classified as a given breed. In addition, it must be borne in mind that when the dog comes from foreign breeding, or its parents come from another, separate population, the test may not confirm that individual as belonging to the expected breed. This is due to genotypic differences between a given individual and a reference population created from the national population. The variants of the genetic markers (alleles) used may be incompatible with the marker variants in our native population. However, it has been shown that canine STRs exhibit breed-specific genotype patterns and that STR panels could be suitable for differentiating dog breeds (Radko & Podbielska 2021; Pires *et al.* 2009; Berger *et al.* 2018; Garcia *et al.* 2022; Perfilyeva *et al.* 2023). In the study by Leroy *et al*. 2009, covering 1514 dogs representing 61 dog breeds, 95.4% of the dogs were correctly assigned to their breed, while in the case of 44 breeds, including the Golden Retriever and Bernese Mountain Dog, the percentage of correct assignments was close to 100% (Leroy *et al.* 2009). Reference populations based on 21 STRs for 4 studied breeds: Golden Retriever, Bernese Mountain Dog, Chinese Crested Dog and Poodle, have allowed for the establishment of genetically uniform clusters composed of individuals with a similar genetic structure for each breed. A Bayesian analysis of the structure of 473 reference dogs showed the existence of 4 genetic clusters (K = 4, Figure 3). All of the reference dogs of each breed were assigned to a separate cluster with an average probability ranging from 97.7% to 99.9%, which is similar to the study by Schelling *et al*. (2005) who, based on the same set of 21 STRs, 311 animals and 7 breeds, obtained a 96.5% correct assignment. This indicates that this method can be successfully applied to further studies.

A principal coordinate analysis (PCoA) was also applied to present genetic relationships among the studied dog breeds. The distribution of genotypes on the plot revealed clear clustering, corresponding to each breed. The PCoA results, showing 4 well-separated groups, were fully consistent with the STRUCTURE analysis, which also identified 4 distinct genetic clusters representing the respective breeds (Figs 3-4).

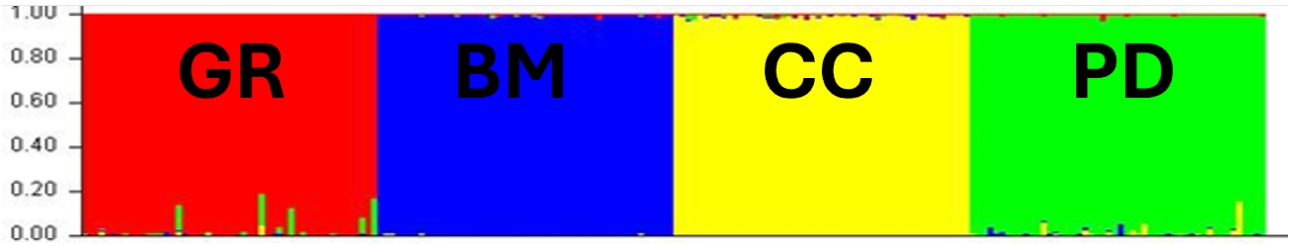

Fig. 3. STRUCTURE analysis of 21 STR genotypes from the dogs studied. The samples were grouped by the 4 breeds (K=4): GR – Golden Retriever; BM – Bernese Mountain Dog; CC – Chinese Crested Dog; and PD – Poodle. The average proportion of the assignment to the cluster (Q) above 97% was found for the GR and PD breeds, and was above 98% and 99% for CC and BM, respectively.

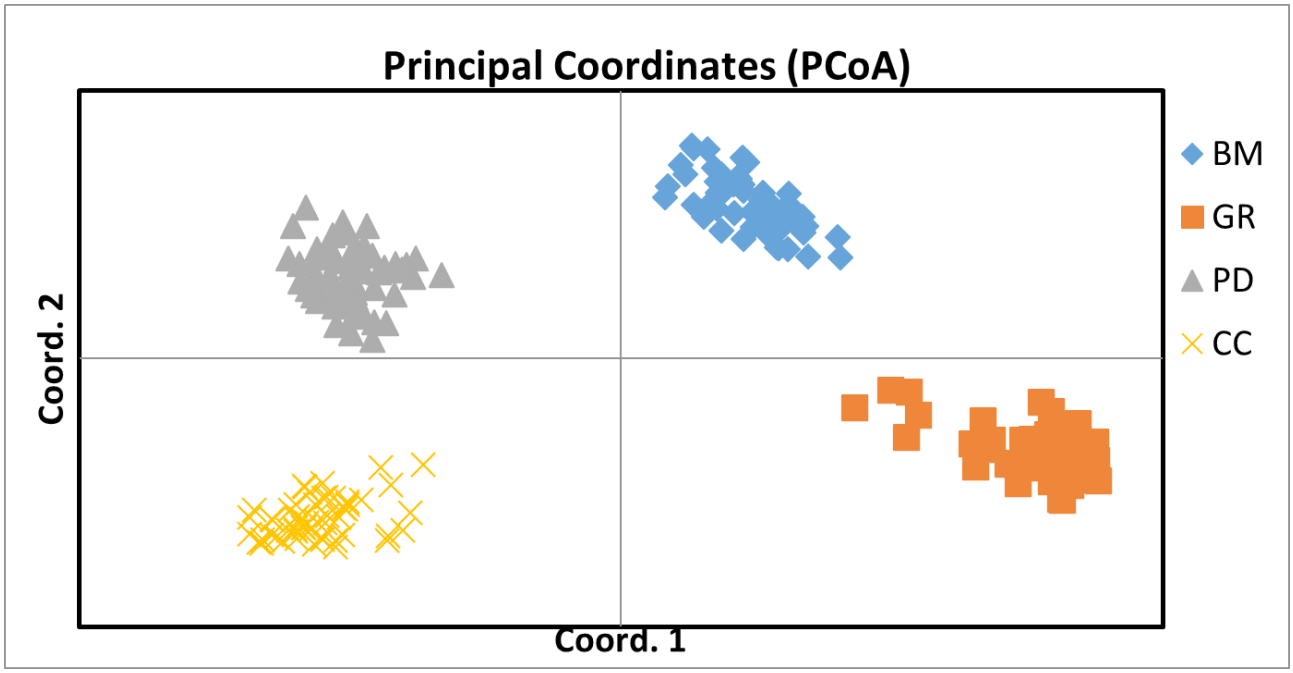

Fig. 4. Principal Coordinates Analysis (PCoA). The PCoA analysis based on genetic distances showed 4 clustered populations corresponding to the dog breeds studied: GR – Golden Retriever; BM – Bernese Mountain Dog; CC – Chinese Crested Dog; and PD – Poodle.

Case Study 1.

The DNA profile analysis at 21 microsatellite loci in 11 puppies from a litter suspected of double mating confirmed the parentage of all puppies from the indicated mother – a GR bitch – and from the two alleged fathers. Of the examined puppies, 6 were consistent with a BM dog and 5 with a GR (Supplementary Material SM.01. – Table 1). In the analysis of the genetic structure of the litter conducted in the STRUCTURE program, the mother (lane 87), one of the fathers (lane 88) and 5 puppies were clearly assigned to the GR breed (lanes 82-86). The mother was assigned with a probability of 97%, while the father and puppies were assigned with a probability of > 98%. The remaining 6 puppies (lanes 76-81) were assigned to both the BM breed and the GR breed, with a probability of 34% to 60%. The second father (lane 89) was clearly clustered with BM individuals with a high – 99.8% probability (Fig. 5).

The differentiation between the GR and GR-BM crossbreed puppies was confirmed by a Principal Coordinate Analysis (PCoA). It separated the littermates into two clusters: a group of 5 individuals that were inferred as GR dogs by the STRUCTURE analysis, and a group of 6 individuals that were inferred as GR-BM crossbreeds, showing a clear differentiation between these groups. The sire and dam identified as GR dogs were clustered together with the GR puppies and were separated by the Coord.1 axis from the crossbreeds and the BM dog sire, while the BM dog was separated from the crossbreed puppies by the Coord.2 axis (Fig. 6).

Case Study 2.

Chinese Crestepoos are a breed that results from crossing a Chinese Crested Dog with a Poodle. They are popular pets because they are appropriate for many different owners, such as singletons, seniors, families with children and people with allergies. They are both ideal lap dogs and animals that enjoy playing. The popularity of Chinese Crestepoos is what causes the possibility of making a breed mistake, that is sometimes also intended. Case 2 concerns a puppy bought as a Chinese Crested Dog; however, with his growth the dog's appearance was different from that of the CC (Figure 2). A DNA profile analysis in 21 microsatellite loci in the puppy and its father excluded its parentage from the indicated father. In 4 loci: AHTh171, Fh2848, INU005 and INU055 (Supplementary Material SM.02. – Table 2), the identified alleles were not consistent in the offspring and its father, which excluded the relationship between these dogs. Material from the mother was unavailable. In addition, the genetic structure analysis conducted in the STRUCTURE program showed that the tested puppy was assigned to both the Chinese Crested breed, with a probability of 51%, and

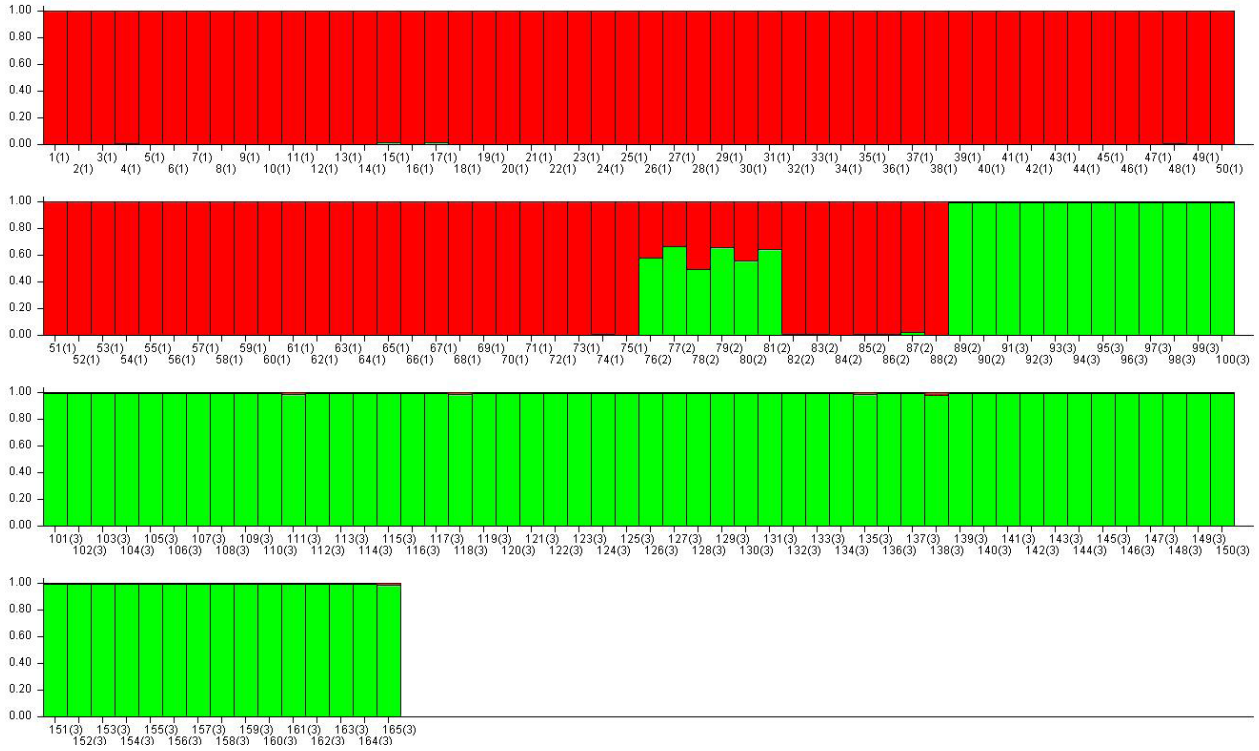

Fig. 5. STRUCTURE analysis of 21 STR genotypes from the Golden Retriever (GR), Bernese Mountain Dog (BM) and mixed dogs. The samples were grouped by K=2.

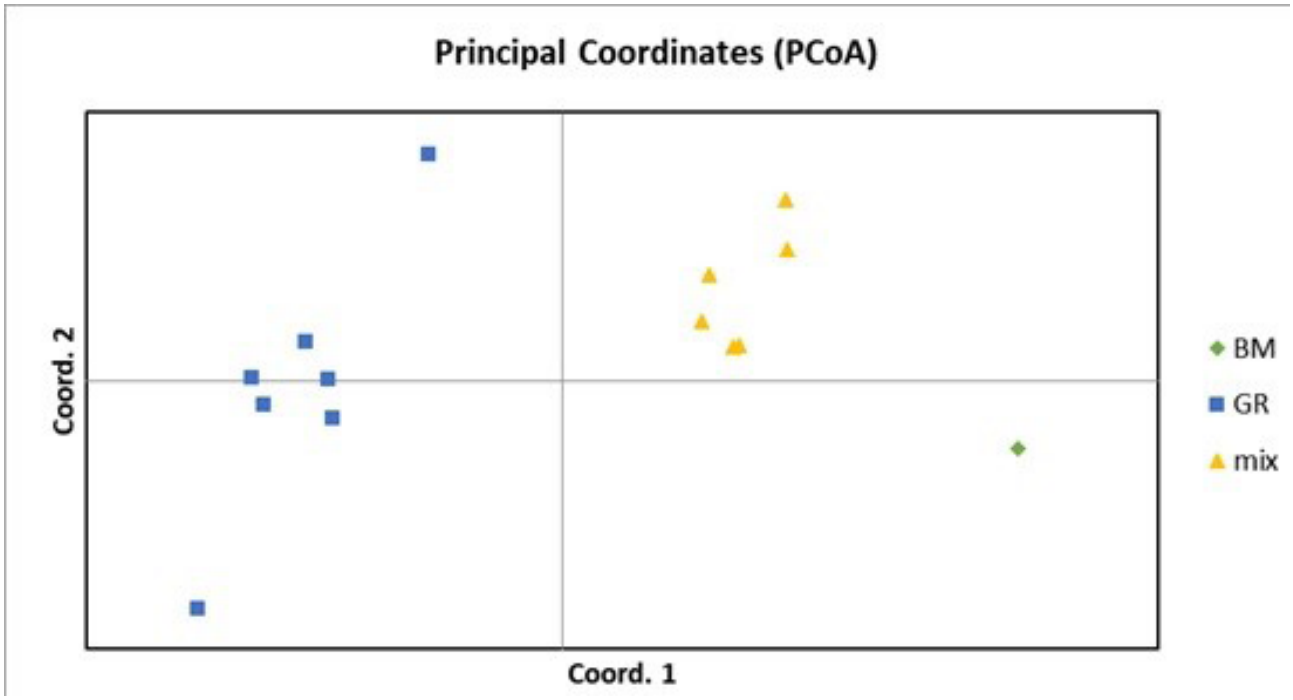

Fig. 6. The Principal Coordinates Analysis (PCoA) showed 2 clustered populations corresponding to the Golden Retriever (GR) breed and mixed dogs. The group of mixed-breed individuals is located between the GR group of dogs and the BM breed father.

to the Poodle breed with a probability of 49% (Figure 7 lane 101). The indicated father of the puppy showed a structure consistent with the Crested breed at a level of > 98% (Fig. 7).

The analysis of the puppy and his alleged father, who on the base DNA profiling was excluded as the father, was confirmed by PCoA (Fig. 8). The PCoA analysis separated the samples from the PD and CC

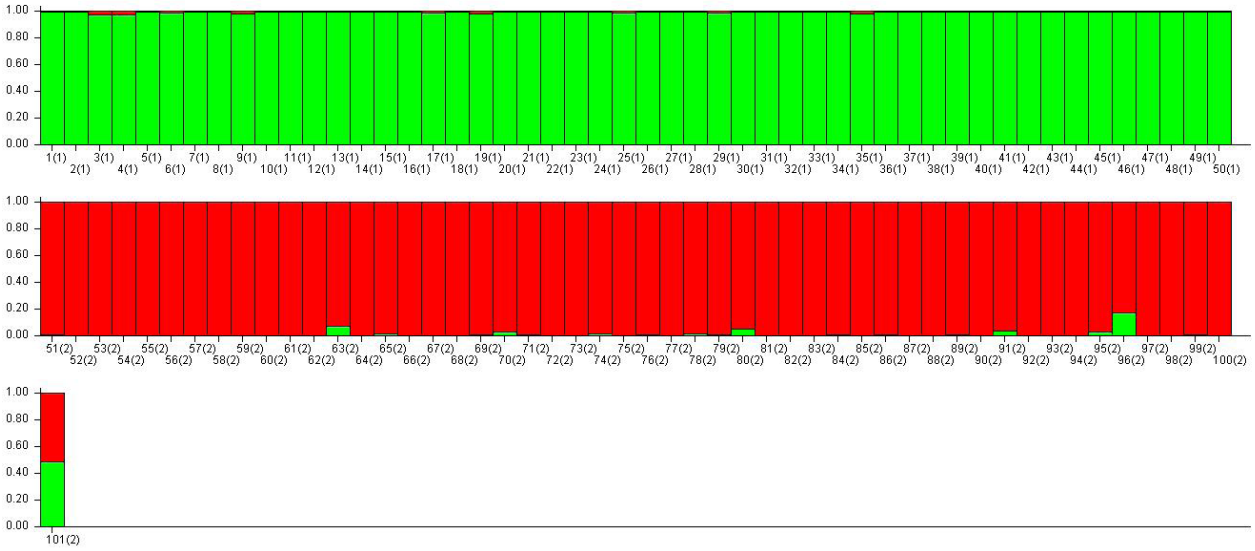

Fig. 7. Structure analysis of 21 STR genotypes from the Chinese Crested Dog (CC) and Poodle (PD) dogs, and the mixed genotype. The samples were grouped into 2 groups by K = 2. Line 101 depicts an alleged hybrid dog – Chinese Crestepoo, which was Chinese Crested Dog (51%) x Poodle (49%).

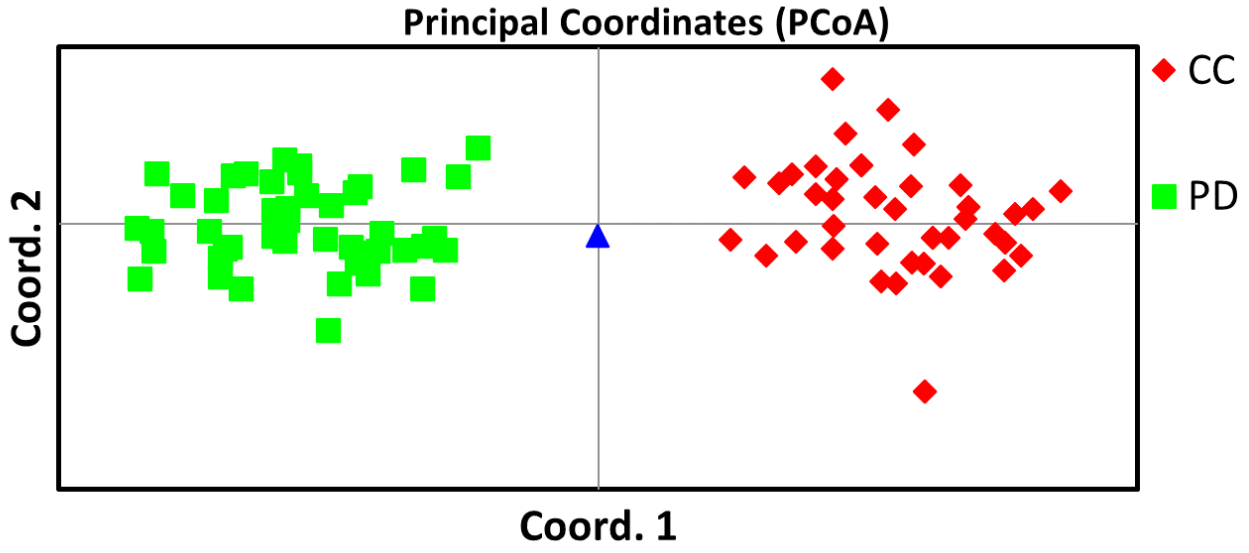

Fig. 8. The Principal Coordinates Analysis (PCoA) plot presents 2 clustered populations corresponding to the Chinese Crested Dog (CC) and Poodle (PD) dog breeds, and the mixed sample (mix) genotype differentiation of the putative hybrid dog.

breeds into two clear clusters separated by Coord.1, while a sample corresponding to the putative hybrid dog – Chinese Crestepoos – was located between them (Fig. 8).

Conclusions

The application of the STR panel and the model-based Bayesian clustering method implemented in the STRUCTURE software allowed for a clear differentiation into four distinct genetic clusters, corresponding to the four analysed dog breeds. This approach may be successfully applied to the identification of a hybrid breed's ancestry. The results were further supported by a Principal Coordinates Analysis (PCoA). Additionally, in both case studies, DNA profiling enabled either the confirmation or exclusion of the paternity, and the obtained results supported the correct assignment of the examined dogs to their respective breeds.

Given that, in both cases, the dogs were correctly assigned to their respective breeds using 21 microsatellite markers (ISAG-recommended) that are routinely used for pedigree testing, our approach proved to be effective for the breeds studied. However, its application to other dog breeds should be approached with caution, as further validation is necessary to confirm the method's reliability.

Acknowledgments

The authors thank the dog owners for their cooperation. Special thanks for sharing the photos.

Author Contributions

Research concept and design: A.R., M.P.; Collection and/or assembly of data: A.R.; Data analysis and interpretation: A.R., A.S.; Writing the article: A.R.; Critical revision of the article: A.R., M.P., A.S.; Final approval of article: A.R., M.P.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Materials

Supplementary Materials to this article can be found online at:
http://www.isez.pan.krakow.pl/en/folia-biologica.html
Supplementary file:
SM.01. Supplementary Material contains Table S1 and Table S2 with the canine DNA profiles obtained for Case 1 and Case 2, respectively.

References

Ackerman L., Milne E.G., Bell J.S., Oberbauer A.M., Nicholas J.C., Boss N., Englar R.E., Grubb T., Dowling P., Burns K.M., Haeussler D.J. Jr. 2021. Hereditary Considerations. In: L. Ackerman (ed.) Pet-Specific Care for the Veterinary Team. John Wiley & Sons, Ltd., Hoboken, NJ, USA: 129-225. https://doi.org/10.1002/9781119540687.ch3

Arata S., Asahi A., Takeuchi Y., Mori Y. 2016. Microsatellite loci analysis for individual identification in Shiba Inu. J. Vet. Med. Sci. **78**: 439-441. https://doi.org/10.1292/jvms.15-0579

Berger B., Berger C., Heinrich J., Niederstätter H., Hecht W., Hellmann A., Parson W. 2018. Dog breed affiliation with a forensically validated canine STR set. Forensic Sci. Int. Genet. **37**: 26-134. https://doi.org/10.1016/j.fsigen.2018.08.005

Bigi D., Marelli S.P., Liotta L., Frattini S., Talenti A., Pagnacco G., Polli M., Crepaldi P. 2018. Investigating the population structure and genetic differentiation of livestock guard dog breeds. Animal. **12**: 2009-2016. https://doi.org/10.1017/S1751731117003573

Bigi D., Marelli S.P., Randi E., Polli M. 2015. Genetic characterization of four native Italian shepherd dog breeds and analysis of their relationship to cosmopolitan dog breeds using microsatellite markers. Animal. **9**: 1921-1928. https://doi.org/10.1017/S1751731115001561

Botstein D., White R.L., Skolnick M., Davis R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. **32**: 314-331.

Ciampolini R., Cecchi F., Bramante A., Casetti F., Presciuttini S. 2011. Genetic variability of the Bracco Italiano dog breed based on microsatellite polymorphism. Ital. J. Anim. Sci. **10**: 267-270. https://doi.org/10.4081/ijas.2011.e59

Ciampolini R., Cecchi F., Paci G., Policardo C., Spaterna A. 2013. Investigation on the genetic variability of the American Pit Bull Terrier dogs belonging to an Italian breeder using microsatellite markers and genealogical data. Cytol. Genet. **47**: 217-221. https://doi.org/10.3103/S009545271304004X

Cowley J., Dhanraj T. 2023. How accurate are dog DNA tests? We unleash the truth. Available online: https://www.cbc.ca/news/business/marketplace-dog-dna-test-1.6763274 (accessed on 22 March 2025).

DeNise S., Johnston E., Halverson J., Marshall K., Rosenfeld D., McKenna S., Sharp T., Edwards J. 2004. Power of exclusion for parentage verification and probability of match for identity in American Kennel Club breeds using 17 canine microsatellite markers. Anim. Genet. **35**: 14-17. https://doi.org/10.1046/j.1365-2052.2003.01074.x

Dodd J.N., Morris B.G., Oliveira D.A.A., Bernoco D. 2001. DNA testing for parentage verification and individual identification in seven breeds of dogs. Rev. Bras. Reprod. Anim. **25**: 35-41.

Flaim D. 2024. Doberman Pinscher History: From Guard Dog to Showman. Available online: https://www.akc.org/expert-advice/dog-breeds/doberman-pinscher-history/ (accessed on 22 March 2025).

García L.S.A., Vergara A.M.C., Herrera P.Z., Puente J.M.A., Barro Á.L.P., Dunner S., Marques C.S.J., Bermejo J.V.D., Martínez A.M. 2022. Genetic Structure of the Ca Rater Mallorquí Dog Breed Inferred by Microsatellite Markers. Animals **12**: 2733. https://doi.org/10.3390/ani12202733

Goleman M., Balicki I., Radko A., Jakubczak A., Fornal A. 2019. Genetic diversity of the Polish Hunting Dog population based on pedigree analyses and molecular studies. Livest. Sci. **229**: 114-117. https://doi.org/10.1016/j.livsci.2019.09.017

Goleman M., Balicki I., Radko A., Rozempolska-Rucinska I., Zieba G. 2021. Pedigree and Molecular Analyses in the Assessment of Genetic Variability of the Polish Greyhound. Animals **11**: 353. https://doi.org/10.3390/ani11020353

ISAG 2014. Proceedings of the 34th International Conference on Animal Genetics, Xi'an, China 2014. Applied Genetics of Companion Animals. Available online: https://www.isag.us/Docs/AppGenCompAnim2014_cor.pdf (accessed on 22 March 2025).

Jamieson A., Taylor S.C.S. 1997. Comparisons of three probability formulae for parentage exclusion. Anim. Genet. **28**: 397-400. https://doi.org/10.1111/j.1365-2052.1997.00186.x

Leroy G., Verrier E., Meriaux J.C., Rognon X. 2009. Genetic diversity of dog breeds: between-breed diversity, breed assignation and conservation approaches. Anim. Genetics **40**: 333-343. https://doi.org/10.1111/j.1365-2052.2008.01843.x

Mei S., Yang J., Li. J., Xiong X., Wang M., Zhao Z., Guo Y., Deng Y. 2023. Genetic Polymorphism and Relationship Analyses of Standard Poodle and Bichon Frise Groups Based on 19 Short Tandem Repeat Loci. J. Forensic Sci. Med. **9**: 331-339. https://doi.org/10.4103/jfsm.jfsm_34_22

Mellanby R.J., Ogden R., Clements D.N., French A.T., Gow A.G., Powell R., Corcoran B., Schoeman J.P., Summers K.M. 2013. Population structure and genetic heterogeneity in popular dog breeds in the UK. Vet. J. **196**: 92-97. https://doi.org/10.1016/j.tvjl.2012.08.009

Nei M., Roychoudhury A.K. 1974. Sampling variances of heterozygosity and genetic distance. Genetics **76**: 379-390. https://doi.org/10.1093/genetics/76.2.379

Pallotti S., La Terza A., De Cosmo A., Pediconi D., Pazzaglia I., Nocelli C., Renieri C. 2017. Genetic variability of the short-haired and rough-haired Segugio Italiano dog breeds and their genetic distance from the other related Segugio breeds. Ital. J. Anim. Sci. **16**: 531-537. https://doi.org/10.1080/1828051X.2017.1317221

Parker H.G., Kim L.V., Sutter N.B., Carlson S., Lorentzen T.D., Malek T.B., Johnson G.S., DeFrance H.B., Ostrander E.A., Kruglyak L. 2004. Genetic structure of the purebred domestic dog. Science **304**: 1160-1164. https://doi.org/10.1126/science.1097406

Peakall R., Smouse P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics **28**: 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Perfilyeva A., Bespalova K., Bespalov S., Begmanova M., Kuzovleva Y., Zhaniyazov Z., Vishnyakova O., Nazarenko I., Perfilyeva Y., Khamdiyeva o., Bekmanov B. 2023. Kazakh national dog breed Tazy: What do we know? PLoS ONE **18**: e0282041. https://doi.org/10.1371/journal.pone.0282041

Pires A.E., Amorim I.R., Ginja C., Gomes M., Godinho I., Simões F., Oom M., Petrucci-Fonseca F., Matos J., Bruford M.W. 2009. Molecular structure in peripheral dog breeds: Portuguese native breeds as a case study. Anim Genet. **40**: 383-392. https://doi.org/10.1111/j.1365-2052.2009.01849.x

Pritchard J.K., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics **155**: 945-959. https://doi.org/10.1093/genetics/155.2.945

Radko A., Podbielska A. 2021. Microsatellite DNA analysis of genetic diversity and parentage testing in the popular dog breeds in Poland. Genes **12**: 485. https://doi.org/10.3390/genes12040485

Radko A., Rubiś D., Szumiec A. 2017. Analysis of microsatellite DNA polymorphism in the Tatra Shepherd Dog. J. Appl. Anim. Res. **46**: 254-256. https://doi.org/10.1080/09712119.2017.1292912

Radko A., Słota E. 2009. Application of 19 microsatellite DNA markers for parentage control in Borzoi dogs. Pol. J. Vet. Sci. **12**: 113-117.

Schelling C., Gaillard C., Dolf G. 2005. Genetic variability of seven dog breeds based on microsatellite markers. J. Anim. Breed. Genet. **122**: 71-77. https://doi.org/10.1111/j.1439-0388.2005.00515.x

Tahir M.S., Hussain T., Babar M.E., Nadeem A., Naseer M., Ullah Z., Intizar M., Hussain S.M. 2015. A panel of microsatellite markers for genetic diversity and parentage analysis of dog breeds in Pakistan. J. Anim. Plant Sci. **25**: 351-356.

Vychodilova L., Necesankova M., Albrechtova K., Hlavac J., Modry D., Janova E., Vyskocil M., Mihalca A.D., Kennedy L.J., Horin P. 2018. Genetic diversity and population structure of African village dogs based on microsatellite and immunity-related molecular markers. PLoS ONE **13**: e0199506. https://doi.org/10.1371/journal.pone.0199506

Wade C. M., Nuttall R., Liu S. 2023. Comprehensive analysis of geographic and breed-purpose influence on genetic diversity and inherited disease risk in the Doberman dog breed. Canine Med. Gen. **10**: 7. https://doi.org/10.1186/s40575-023-00130-3

Wright S. 1978. Evolution and the Genetics of Populations. Vol. 1. University of Chicago Press, Chicago, USA: 300-319.