Genetic estimates of relatedness: Established practices and new opportunities through low coverage whole genome sequencing

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Abstract

Identifying close relatives in wild animal populations is fundamental across many research fields. Genetic estimates of relatedness have expanded rapidly in recent decades, based on various types of genetic data. Here, we review their use and outline opportunities for future studies by combining two complementary approaches. First, we systematically reviewed 2,861 articles to assess how genetic relatedness has been estimated over time. Second, we compare widely used genetic data types for inferring relatedness, conducting computational experiments using data from a rhesus macaque (*Macaca mulatta*) population in Puerto Rico. We compared other methods against precise identity-by-descent segment-based estimates of relatedness. Our results show that most studies of relatedness (89%) continue to rely on short tandem repeat (STR) markers, despite their limited precision. Single-nucleotide polymorphism (SNP)- marker-based relatedness estimates remain underused (8.3% of studies), even though they yield more reliable estimates when sampled in sufficient numbers. Finally, we find that the simple pairwise-mismatch rate (PMR) method for estimating relatedness in low-coverage WGS data (commonly used in human ancient DNA studies) works robustly for low-coverage data, e.g., DNA retrieved from faecal samples or from cost-effective low-coverage whole-genome sequencing (lcWGS). Together, our findings highlight lcWGS combined with PMR-based relatedness estimation as a promising, cost-effective alternative when DNA quality is limited, genomic resources are scarce, or economic efficiency is essential.

Keywords: Microsatellites, paternity assignment, sequencing depth, wildlife genomics, kinship inference, ancient DNA methods

Introduction

Genetic relatedness is the sharing of genetic alleles between two individuals through common ancestry. This genetic relatedness (hereafter relatedness) constitutes a key measure across many disciplines. For example, it is used to study the evolution of kin preferences (Langergraber et al., 2007), to infer dispersal patterns (Aguillon et al., 2017), to understand trait inheritance, or to quantify inbreeding (Widdig et al., 2017).

A wide range of methods has been developed to estimate relatedness (reviewed, e.g., by Speed & Balding, 2015). In the pre-genetic era, relatedness was primarily inferred through observing parent-offspring associations and building pedigrees. This approach requires long-term, detailed demographic data, which may not be available for many wild animal populations (Pemberton, 2008). In mammals, these observational pedigrees can reliably resolve mother-offspring pairs through lactations; however, paternity in group-living animals with promiscuous mating can usually not be inferred from observations alone (e.g., Inoue et al., 1991).

With the advent of genetic markers, researchers gained an alternative to building pedigrees. Several types of genetic variation have been used to infer relatedness. Early studies relied on a small number of markers as genotyping was cumbersome and time-intensive, but advances in more powerful molecular methods have steadily increased resolution and cost-effectiveness. Rapid advances in molecular genotyping methods, however, have made genome-wide data generation accessible (Enbody et al., 2023; Kuderna et al., 2023; Ronco et al., 2021).

Genotyping microsatellites, also known as short tandem repeats (STRs), has been widely used to infer relatives since the 1990s (**Text Box 1**). An alternative to STRs is genotyping single-nucleotide polymorphisms (SNPs, **Text Box 2**). Although SNPs contain less per-marker information than STRs (overall, 1 STR is as informative as ~ 6 SNPs; Städele & Vigilant, 2016), their abundance, the rapid rise of high-throughput sequencing technologies, and the low per-marker sequencing costs (**Tab. 1**) enable the sampling of thousands or even millions of SNPs (Lemopoulos et al., 2019). When genome-wide SNP variation data is available (e.g., through whole-genome sequencing (WGS) or dense genome-wide SNP array data), identity-by-descent (IBD) segments can be inferred, yielding precise estimates of genetic relatedness by identifying exact stretches of shared DNA (**Text Box 3**). However, IBD segment calling requires high-quality genome-wide diploid genotype data (Freudiger et al., 2025), which, as of 2026, is accessible only for humans and a small number of other species.

For many studies estimating genetic relatedness, the quality of available DNA samples is a limiting factor. In particular, some studies on wild populations must employ non-invasive sampling of faeces, shed hair, or feathers. Such samples generally contain only a small fraction of fragmented host DNA and are contaminated with bacterial and environmental DNA. Therefore, researchers need to apply complex enrichment or capture procedures, limiting the feasibility of high-coverage SNP-sequencing

(Alvarez-Estape et al., 2023; Snyder-Mackler et al., 2016; Vullioud et al., 2024; White et al., 2019). Notably, the rapidly growing field of ancient DNA (aDNA), which recovers DNA from fossils or museum specimens, faces similar challenges: the DNA is often fragmented and contaminated by environmental DNA. Therefore, it is frequently not possible to produce sequencing data of sufficient quality for common relatedness estimators based on diploid genotypes (Hämmerle et al., 2024; Ringbauer et al., 2024). To address this challenge, aDNA researchers apply a robust method to estimate pairwise relatedness even for low-coverage WGS (lcWGS) data, by calculating average pairwise-mismatch-rates (PMR) on pseudo-haploid data (**Text Box 4**).

Table 1: Example of costs for whole genome sequencing (WGS) and short tandem repeat (STR) analysis, using DNA extracted from blood samples. (A) Costs per sample for fragment analysis of different numbers of STR markers. The table provides an example for running two repetitions per marker on an ABI PRISM Genetic Analyser, without multiplexing. These costs do not include negative controls, and additional repetitions are often required when working with faecal samples (Taberlet et al., 1996). In well-studied populations, multiple STR markers can often be pooled and genotyped in a single run, reducing the cost per marker, with a pooled run typically costing the same as running a single marker separately. **(B)** Sequencing costs per sample for WGS of different coverages. The price in the brackets includes the library preparation (~80$ per sample). The price for sequencing depends on the number of reads produced. The number of reads required to reach a coverage level depends on the size of the study organism's genome, as well as the quality and percentage of host DNA in the sample. This table provides an example for sequencing rhesus macaques (genome size of ~3Gbp; Warren et al., 2020) on an Illumina NovaSeq sequencer with 2× 150bp paired-end reads.

A)

number STR markers	cost per sample [USD]
1	4.38
5	21.90
10	43.80
25	109.50
50	219
100	438

B)

WGS coverage	cost per sample [USD] (including library prep)
0.1×	1.70 (81.70)
0.5×	8 (88)
1×	16 (96)
5×	80 (160)
10×	160 (240)
20×	319 (399)

The feasibility, costs, and precision of estimates vary across genetic methods for inferring relatedness (Speed & Balding, 2015; Städele & Vigilant, 2016). In this study, we set out to investigate how they have been used, compare them, and outline paths forward. We do so by combining two approaches:

1. By conducting a systematic literature review of 2,861 articles, we quantified how and to what extent relatedness has been assessed with genetic markers during the first quarter of the 21st century.
2. We systematically test popular data types to infer relatedness, using an exceptionally well-studied population of rhesus macaques with high-quality IBD segment data on genetic relatedness as a ground truth benchmark. We aimed to explore how the accuracy and precision

of relatedness estimation vary across marker type, marker number, and estimation method, particularly for STR and SNP data.

Text Box 1: Microsatellites. STRs are stretches of DNA composed of varying numbers of repeats of short base pair motifs (Fan & Chu, 2007). STR markers follow simple Mendelian inheritance patterns (Brockmann et al., 1994) and are assumed to represent neutral variation (Schlötterer, 2004; Zimmerman et al., 2020). The mutation rate within a repeat is up to ten times higher than that of point mutations, resulting in a high per-marker variability and thus information content (Fan & Chu, 2007; Städele & Vigilant, 2016; Zimmerman et al., 2020). STR markers are typically species-specific; hence, primers must be designed for each locus in the study species (Schlötterer & Pemberton, 1998), unless available primers can be used from phylogenetically related species via cross-species amplification (Kayser et al., 1996). Using STR primers developed in another species can bias pairwise relatedness estimates by preferentially amplifying conserved, less variable loci and increasing null alleles in the focal species (Vowles & Amos, 2006). This reduces allelic diversity and can inflate or deflate inferred relatedness. When designed, this may cause a high initial investment to establish STR markers in a study species (Flanagan & Jones, 2019; Lemopoulos et al., 2019) in addition to considerable per-marker sequencing costs (Tab. 1A). Most studies sample relatively few STR loci (often less than 20; Csilléry et al., 2006; van Horn et al., 2008). STR markers can be powerful for identifying parent-offspring dyads by testing the correspondence of mother-offspring genotypes and for paternity testing by comparing non-maternal alleles. However, beyond parent-offspring, their precision for estimating pairwise relatedness usually remains limited (Csilléry et al., 2006; Freudiger et al., 2025; van Horn et al., 2008). STR-based relatedness estimation uses the high allele diversity at STR loci to compare the number of alleles two individuals share with what is expected from population allele frequencies. Method-of-moments estimators compare observed vs expected allele sharing across many loci to estimate a relatedness coefficient (C. Li et al., 1993; Lynch & Ritland, 1999; Queller & Goodnight, 1989; Ritland, 1996; Wang, 2002). At the same time, maximum-likelihood approaches model the probabilities of observing particular genotype pairs given different IBD states and pick the IBD probabilities that maximise this likelihood (Ross et al., 2014; Wagner et al., 2006; Wang, 2011).

Text Box 2: Single nucleotide polymorphisms (SNPs). These single genomic positions differ between individuals due to point mutations (Speed & Balding, 2015). SNPs can be sequenced using various methods. For example, SNP arrays are high-density microarrays designed to detect known SNPs. As SNPs must be identified before sequencing, this requires a high initial investment to develop species-specific panels (i.e., determining a SNP panel from reference genomes) or to validate the applicability of cross-species amplification (Miller et al., 2012; Verlouw et al., 2021). Methods that are not species-specific include restriction-site associated (RAD) sequencing or genotyping-by-sequencing (Torkamaneh et al., 2016). However, these methods usually only cover a small fraction of the genome (typically < 15%; Arnold et al., 2013; Cariou et al., 2016) and the sequenced sites are unevenly distributed (Baird et al., 2008; Dodds et al., 2015). Whole genome sequencing (WGS), on the other hand, produces the most comprehensive data, though sequencing at high coverage can still be cost-intensive (Tab. 1B). One alternative that reduces the costs is low-coverage sequencing (lcWGS; < 10× average coverage) in combination with genotype imputation from a reference panel (**Tab. 2B**; Freudiger et al., 2025; Vi et al., 2025; Watowich et al., 2023).

However, the applicability of this method hinges on the availability of a high-quality reference panel that adequately captures the genetic diversity of the study population (Watowich et al., 2023). This is often the case only for model organisms, though efforts are made to develop panels for an increasing number of species (Enbody et al., 2023; Kuderna et al., 2023; Watowich et al., 2023). SNP-based relatedness estimators quantify how similar the genotypes of two individuals are, relative to expectations under different degrees of shared ancestry, by counting how often they share alleles and adjusting for each allele's population frequency (Purcell et al., 2007). Alternatively, genotype likelihoods (i.e., the probabilities of each possible genotype given the observed reads) can be used to infer the proportions of the genome that are likely IBD through maximum likelihood analysis (Korneliussen & Moltke, 2015).

Text Box 3: Identity-by-descent (IBD) segments. IBD segments are stretches of DNA that two individuals inherited from a common ancestor. They are almost identical (thus identity-by-descent) except for occasional de novo mutations; H. Li et al., 2014. IBD segments can be inferred from dense genome-wide SNP data. The genomes of two individuals are scanned to identify the exact DNA segments that they co-inherited identical haplotypes, using computational methods that account for occasional genotyping errors and leverage phase information (e.g., Browning & Browning, 2011; Ringbauer et al., 2024; Sticca et al., 2021). In principle, IBD segments yield precise estimates of genetic relatedness by identifying the exact stretches of co-inherited DNA (Freudiger et al., 2025; Ringbauer et al., 2024). The number, length, and genomic distribution of IBD segments also provide information beyond a point estimate of average relatedness; for instance, it allows the distinction between kin classes of the same degree, such as full siblings and parent-offspring dyads or maternal and paternal half siblings (Browning & Browning, 2011; Freudiger et al., 2025; Ringbauer et al., 2024; Visscher et al., 2006). However, detecting IBD segments is restricted to high-quality genome-wide data and genomic resources and is therefore currently accessible only for humans and a small number of other species (Freudiger et al., 2025; Ringbauer et al., 2024; Sticca et al., 2021).

Text Box 4: Pairwise Mismatch Rate (PMR) - based detection of relatives using genome-wide data. The human aDNA field has come to rely on a robust method for detecting relatives that works well in the low-coverage regime. First, the genetic data is pseudo-haploidised on a genome-wide set of SNPs (usually the so-called 1240k SNP panel consisting of ~1.2 million human variants), i.e., both alleles of a SNP are set to one of a randomly chosen sequencing read covering the variant, or to missing if no read covers a variant. Using this pseudo-haploid data, a mean pairwise mismatch rate (PMR) is calculated between pairs of individuals (Lipatov et al., 2015; Monroy Kuhn et al., 2018; Ringbauer et al., 2024), averaging over all SNPs covered in both genomes. A specific sample pair's PMR x of a sample dyad then translates into a relatedness estimate *r* as

$$r = 1 - \frac{x - b}{b}$$

with *x* denoting the PMR for a specific dyad and b denoting the expected mismatch rate for two identical individuals from the same population. The value *b* is usually estimated empirically as the median pairwise PMR of a given group, or of all pairs of individuals with similar ancestry. Several

variations and implementations of this approach exist (Alaçamlı et al., 2024; Fowler et al., 2022; Kennett et al., 2017; Rohrlach et al., 2023). This relatively assumption-free PMR method delivers reliable inference of relatedness for lcWGS and error-prone aDNA, distinguishing pairs of identical samples and first- and second-degree relatives (see, e.g., Ringbauer et al., 2024). PMR-based relative detection has become a default tool in human ancient DNA studies and a key tool for reconstructing large ancient DNA pedigrees (e.g., Fowler et al., 2022; Rivollat et al., 2023).

Methods

1. Systematic literature review of genetic relatedness studies

To reach our first aim to quantify the use of different genetic methods to estimate relatedness in 2000-2025, we conducted a systematic literature review inspired by the PRISMA guidelines (Page et al., 2021; **Fig. 1**). To identify studies estimating pairwise relatedness based on genetic data in non-human animals, we included all articles that, based on their abstract and title, met the following two criteria: (1) use newly produced empirical data from non-human animals which bred naturally (i.e., excluding populations in which breeding was managed by humans, such as, but not limited to, commercially bred livestock or laboratory breeding experiments), and (2) estimate pairwise relatedness from genetic data for dyads with known genealogy.

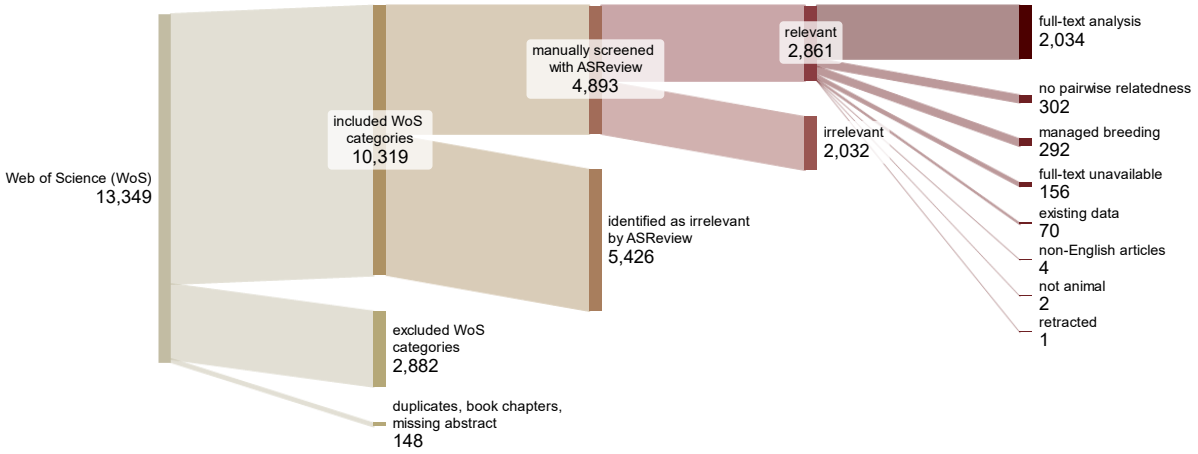

Figure 1: Sankey diagram illustrating the workflow for the systematic literature review. The left edge shows the total number of articles found on Web of Science (WoS) using the keywords specified in Box 1. The total number of articles flows through several filters. The resulting upper node depicts the articles that were kept for further processing, while articles ending in the lower nodes were discarded. Flow widths are proportional to the sample size per node.

To do so, we first searched the Web of Science (WoS) database using a set of keywords related to genetic markers and relatedness (**Text Box 5**). To assess the power of our query to identify target studies, we selected 20 target articles identified manually and independently of our query. Reassuringly, we found that 80% of those articles (16 out of 20) were found by our search query (Tab. S1). We restricted the search to peer-reviewed articles published between January 1, 2000, and July 3, 2025, that included the search terms in their title, abstract, or keywords. Review articles, opinion

pieces, and commentaries were excluded. Based on these criteria, we identified 13,349 potentially relevant articles. Given the large number of articles, we excluded WoS categories (automatically created by WoS) that contained articles on irrelevant topics (e.g., medical studies on humans, veterinary studies, geochemistry, and engineering). To identify irrelevant topics, we screened the titles and abstracts of the first 10 articles in each WoS category. If none of these articles met criteria 1 and 2, we excluded the respective category (see **Text Box S1** for the list of categories). Articles could be assigned to multiple categories. An article was included in our dataset if at least one of its assigned categories was among the included categories, yielding 10,467 articles. After removing duplicates, book chapters, articles without abstracts, and retracted articles, 10,319 articles were retained.

Second, we screened the retained articles using ASReview Lab v2 (de Bruin et al., 2025) with the ELAS Heavy 3 model, which focuses on semantic text comprehension. ASReview utilises active machine learning to iteratively identify relevant literature from large text datasets. The model was initially trained on 120 randomly selected articles manually labelled by a single reviewer (author AF) as relevant (n = 41) or irrelevant (n = 79). Based on this training set, the model classified all remaining articles (n = 10,319) as relevant or irrelevant based on their titles and abstracts. It then repeatedly presented the most relevant studies for human verification in an interactive process. Reviewer feedback from AF was used to continuously update the model's classification. Screening was terminated once at least 33% of records (n = 3,440) had been assessed and 25 consecutive articles were classified as irrelevant, a predefined stopping criterion (de Bruin et al., 2025). This threshold was reached after screening 4,892 articles (n = 2,861 relevant and 2,032 irrelevant).

Third, we manually screened the full texts of the 2,861 articles identified as relevant. For each article, we extracted the information on the (1) study species, (2) study duration (≤ 1 year, 2-3 years, ≥ 4 years), (3) sample size, (4) type of genetic sample (e.g. tissue, blood, faecal), (5) type of genetic marker (e.g., STR, SNP, WGS), (6) number of genetic markers, and (7) which type of kinship was inferred (e.g. paternity analysis, sibling detection).
While manually screening the complete text, we omitted studies (1) that reused previously published genotype data or relatedness values, (2) for which the full text was unavailable, (3) that did not meet our criteria (1. natural breeding and 2. estimation of pairwise relatedness), (4) for which the full text was unavailable, not available in English or retracted, or (5) which did not work on a non-human animal. This filtering resulted in a final sample of 2,034 articles (**Tab. S2**).

We conducted all statistical analyses in *R* version 4.5.0 (R Core Team, 2025) and used the *R* package *scico* (Pedersen & Crameri, 2023) for visualisations.

To identify phylogenetic classifications of the study species, we used the R package *taxize* (Chamberlain et al., 2020). If an article studied multiple species, we treated the markers used for

each species as separate data points. To test whether the number of STR and SNP markers increased over time, we fitted two generalised linear models (GLMs) with a Poisson distribution. In each model, the number of STR or SNP markers was used as the response variable, and the year index was included as the predictor. The year index was defined by setting the year in which each marker type first appeared in the dataset to 0 (STR: 2000; SNP: 2009), with subsequent years numbered sequentially. To test the association strength between phylogenetic class and sequencing method, we calculated the Cramér's V with the *R* package *rstatix* (Kassambara, 2025). To test changes in sample size over time, we fitted a GLM with the same structure as described above, and to assess the association between sample size and phylogenetic class, we calculated Cramér's V.

Text Box 5: Search terms used and categories included to retrieve articles from Web of Science.
Topic = (relatedness OR kin OR kinship OR parentage OR paternity OR maternity)
AND
Topic = (DNA OR mtDNA OR genotyp* OR sequencing OR microsatellite* OR WGS OR "short tandem repeat" OR nucleotide OR SNP OR STR OR genetic)
NOT
All fields = (protist* OR plant OR plants OR bacteria OR forensic* OR microbio* OR "phylogenetic relatedness" OR patient OR patients OR syndrome OR hospital OR tumour OR virus OR "ancient DNA" OR infection* OR infectious OR pollination OR selfing)

2. Comparing relatedness estimation using Cayo macaques

To address our second aim of comparing genetic methods for estimating relatedness, we analysed genomic data from the free-ranging rhesus macaque (*Macaca mulatta*) population on Cayo Santiago. This dataset has the advantage that high-precision relatedness estimates are available, using the IBD-segment data inferred and evaluated in (Freudiger et al., 2025). This data provides us with a high-quality relatedness baseline to compare various widely used methods to.

Study population of rhesus macaques

Cayo Santiago is a 15.2 ha island off the coast of Puerto Rico, USA (Widdig et al., 2017). The population was established in 1938 by introducing 409 wild-born individuals collected from various locations in India (Rawlins & Kessler, 1986). It is managed by the Caribbean Primate Research Center (CPRC), whose staff have routinely collected demographic data through observations since 1956, including date of birth, sex, and matrilineal family per individual. In 1992, a genetic database was established and continuously updated primarily to determine paternity (Widdig et al., 2017). Despite the lack of genetic influx since its foundation, there is a low incidence of inbreeding or a severe genetic bottleneck (Freudiger et al., 2025; Widdig et al., 2016, 2017).

Here, we use data from a subset of 98 individuals of the Cayo macaque population described in Freudiger et al. (2025). The individuals were selected through a three-stage search from the 12,049

individuals included in the 2019 Cayo Santiago pedigree. In the first search stage, a randomly selected individual (born in 1996) was chosen, and all 1st-degree relatives of this individual were included. In the second stage, the search was expanded to include all 1st-degree relatives of the individuals identified in the first stage. In the third stage, all 1st-degree relatives of the individuals identified in the second stage were included. Only individuals for whom genetic samples were available were considered (Freudiger et al., 2025).

Pedigree data

We calculated pedigree-relatedness using the *TRACE* v0.1.0 algorithm (Freudiger et al., 2025; Westphal et al., 2023), using all links within the Cayo pedigree (including 11,805 known mother-offspring and 4,986 known father-offspring dyads, spanning up to 12 generations; Westphal et al., 2023). For any given dyad, we calculated the pedigree relatedness coefficient (r_{PED}). Furthermore, pedigree data were used to group dyads into kin classes. Dyads that were related through more than one kin class within two degrees of their primary kin class were excluded (e.g., dyads simultaneously sharing a 2nd and 4th degree relationship). For this study, we focused on the following primary kin classes: parent-offspring (n = 93), full siblings (n = 16), half-siblings (n = 530), grandparent-offspring (n = 76), half-avuncular (aunt/uncle-niece/nephew; n = 246), 1st-degree half-cousins (n = 267), and nonkin (i.e. dyads without any known link in the pedigree; n = 438).

STR data

We used published STR data for the 98 individuals, which were sequenced using DNA originating mostly from blood samples as described in (Widdig et al., 2017). In brief, only highly polymorphic STR markers, which exhibited similar characteristics in terms of the number of alleles and heterozygosity, were selected. Using the function *HWPerm.mult* with 10,000 permutations from the *R* package *HardyWeinberg* (Graffelman, 2015), we restricted the analysis to markers in Hardy-Weinberg equilibrium (HWE). After filtering, an average of 37 markers (range: 27–41) per individual were available for relatedness analysis. To examine how STR-based relatedness estimates vary with the amount of genetic information used, here we independently subsampled the overall dataset into two additional subsets with a reduced number of markers, one containing an average of 19 markers (range: 12–20), and the other an average of 9 markers (range: 6–10).

We estimated STR-based relatedness (hereafter called r_{STR}) for the full STR-dataset using the *R* package *related* (Pew et al., 2014). This package implements multiple relatedness estimators: *Queller and Goodnight* (Queller & Goodnight, 1989), *Lynch and Li* (C. Li et al., 1993), *Ritland* (Ritland, 1996), *Lynch and Ritland* (Lynch & Ritland, 1999), *Wang* (Wang, 2002), *DyadML* (Milligan, 2003), and *TrioML* (Wang, 2007). Using the best-performing estimators, we additionally calculated r_{STR} for 19 and 9 STR-markers.

SNP data

We used whole genome sequencing (WGS) data, published and described in Freudiger et al. (2025). In brief, individuals were sequenced to a coverage of ~3-38× on an Illumina sequencer (Illumina NovaSeq6000), using paired-end 2×150bp reads. The data was processed after quality control and adapter trimming, by removing PCR and optical duplicates. The data were imputed using GLIMPSE and subsequently filtered for HWE, and a minor allele frequency (MAF) threshold of > 5% was applied. The final dataset contained 6,940,242 SNPs for each individual (Freudiger et al., 2025).

To simulate samples with varying marker counts, we generated exome-capture data. To do so, we downloaded the *Mmul_10 genome assembly* (*GCA_003339765.3*) from *Ensembl* (Warren et al., 2020) and filtered the macaque genome for all known exon positions to obtain the complete exome (i.e., mimicking whole exome sequencing data, n_{SNPs} = 98,833). In addition to the full exome, we generated two smaller subsets by randomly removing exons, retaining only 10% (n_{SNPs} = 9,844) and 1% (n_{SNPs} = 999) of the original exome. Next, we extracted SNPs located within exons for all 98 individuals by aligning their WGS data with the exon positions of each subset using the *BEDTools intersect* function (Quinlan & Hall, 2010), so that only genetic information between the start and end of each exon was retained.

To estimate SNP-based relatedness (hereafter called r_{SNP}) for the full SNP-dataset, we used *ANGSD*'s *NgsRelate* (Korneliussen & Moltke, 2015), which computes two estimators for pairwise relatedness: r_{ab} and two-out-of-three-IBD. Additionally, we estimated r_{SNP} using *PLINK*'s *PI-HAT* (Purcell et al., 2007). Using the best-performing estimators, we additionally calculated r_{SNP} for 100%, 10%, and 1% of the exome.

PMR-based relatedness

Following a standard human aDNA analysis procedure, we calculated a PMR-based pairwise relatedness estimate (hereafter called r_{PMR}) based on first pseudo-haploidizing the genomic data. We started from the filtered SNP set (n = 89) described by Freudiger et al. (2025) based on WGS data (see above), keeping n = 94 genomes with average sequencing coverage > 1×. We first set a genotype for each variant based on one randomly selected sequencing read covering this variant. If no sequencing read covers a relevant genomic position, we set this genotype to "missing". Using these pseudo-haploidised SNP genotypes, we then calculated the average fraction of genotypes that are mismatching in pairs of individuals, using all genotypes that are covered in both genomes, and translated this fraction into r_{PMR} (as described in **Text Box 3**).

To assess PMR-based relatedness estimation for lower sequencing depths, we downsampled the complete genomic data to lower average coverages (1.0×, 0.1×, 0.05×, 0.001×). Starting from the read count data for each variant, we kept each read with a probability such that the expected overall

average sequencing depth matches our targeted value. As described for the full data above, we then set pseudo-haploid genotypes on this down-sampled data and calculated r_{PMR} for all sample pairs.

IBD-segment-based relatedness as the benchmark

To assess different relatedness estimations, we used the quality-controlled IBD-segment data by Freudiger et al. (2025) as a reference, which precisely captures the biological processes underlying relatedness. IBD-segments > 8 cM long were inferred using *ancIBD* (Ringbauer et al., 2024) starting from WGS data. We calculated IBD relatedness (hereafter r_{IBD}) for each dyad by summing the total length of shared IBD segments (accounting also for IBD2, when both haplotypes are IBD) and dividing it by the diploid length of the macaque genome. For details of this pipeline, we refer to Freudiger et al. (2025).

Comparison of genetic relatedness estimation methods

To compare the performance of each relatedness estimator, we investigated the deviance between r_{STR}, r_{SNP}, and r_{PMR} from r_{IBD}, respectively. Additionally, we calculated the coefficient of determination (R^2) by fitting a linear regression between r_{STR} and r_{SNP} against r_{IBD}, respectively, for the entire dataset of STR and SNP markers.

Results

1. Literature review

We identified 2,034 articles that use genetic estimates of relatedness in wild animal populations. The number of published articles overall increased from 2000 to a peak in 2008 (**Fig. S1A**, **Tab. S2**) and then remained generally stable until 2025 with only modest fluctuations. After normalising the number of published articles by the total number of articles indexed in the WoS database per year (identified through the keyword search “the OR a” on WoS), a relative contraction of the field becomes apparent (**Fig. S1B**).

The vast majority of studies (89%) published between 2000 and 2025 used STR markers with a median of 9 markers (range 1–92; **Fig. 2A** and **2B**). The number of STR markers has increased slightly over time ($Intercept_{2000}$ = 2.01, Estimate = 0.029, SE = 0.001, $p < 0.001$). After the initial emergence of SNPs in our dataset in 2009, we observed a steady increase in the use of SNPs in relatedness estimation. In total, 8.3% of studies published in our study period used SNP data (**Fig. 2A**). The median number of SNP markers was 1,038 (range: 33–86,677; **Tab. S2**), which also slightly increased over time ($Intercept_{2009}$ = 8.50, Estimate = 0.013, SE = 0.0003, $p < 0.001$). WGS data only occurred in 8 studies, which makes up 0.4% of the analysed articles (**Fig. 2A**). The studies used a median coverage of 27.5× (range: 0.49–70×; **Tab. S2**).

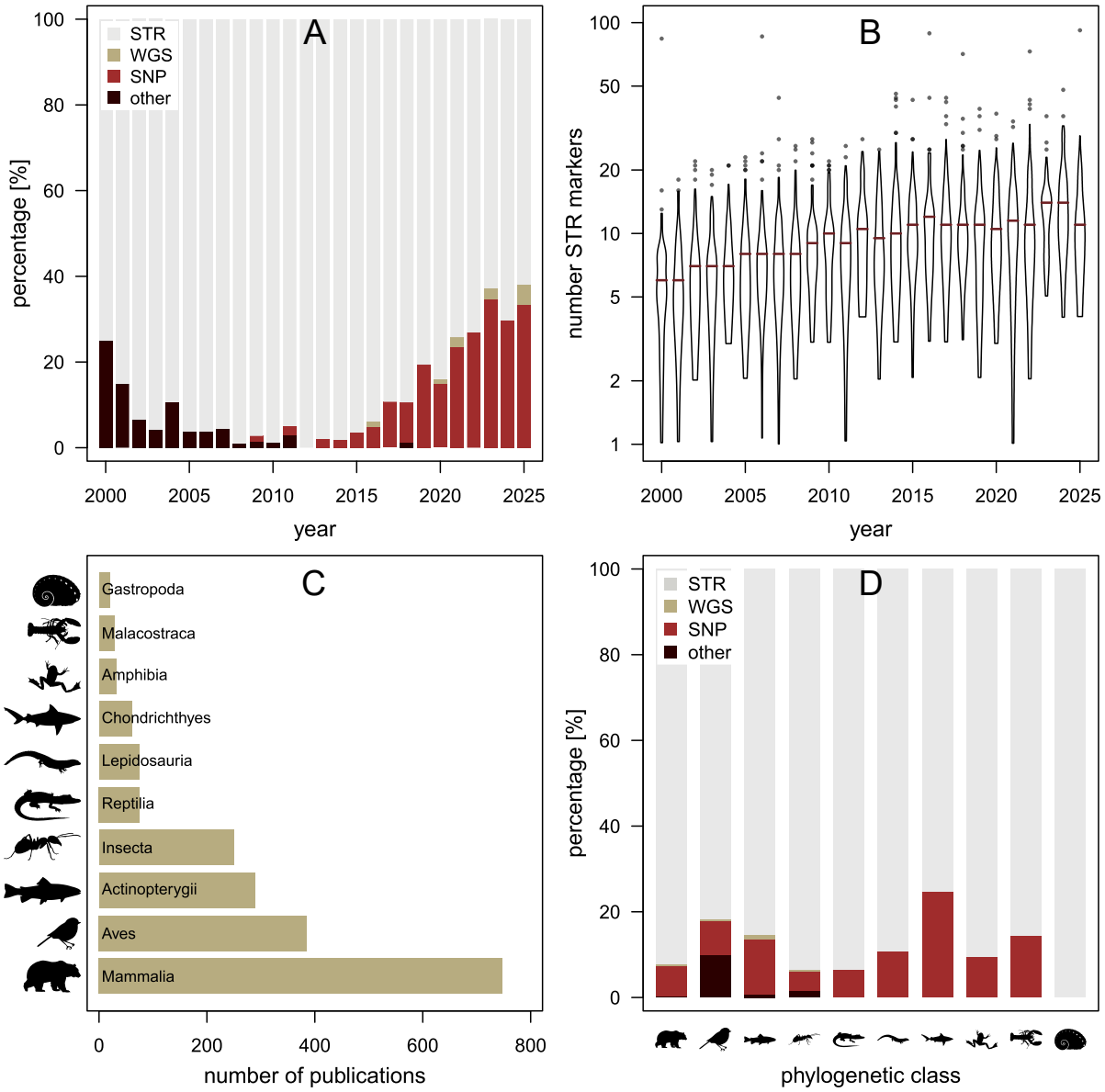

Figure 2: Trends in the genetic relatedness literature 2000-2025. (A) Percentage of marker types used across articles per year. The category *other* includes minisatellites, RFLP (restriction fragment length polymorphism), and RAPD (random amplified polymorphic DNA). **(B)** Number of STR markers used across years, shown on a log scale. The violin extent covers the interquartile range and whiskers (calculated as 1.5 × IQR) following boxplot conventions. Red horizontal lines indicate the median value. Individual points indicate outliers that fall beyond the whisker boundaries. **(C)** Distribution of publications across the ten most commonly represented phylogenetic classes, showing the number of studies that included species from each class. In total, 35 different phylogenetic classes were represented in the dataset. **(D)** Percentage of marker types used across the ten most common phylogenetic classes.

The top ten journals, in which 43% relevant studies (n = 869) have been published, all focus on the topics ecology, behaviour, conservation, heredity, and aquaculture (**Fig. S1C**). Species of 35 phylogenetic classes and 143 orders were represented in the dataset, with Mammalia being by far the most represented class, making up 36.8% of all studies (**Fig. 2C**, **Tab. S2**). We found a weak association between phylogenetic class and sequencing method (Cramér's V = 0.17; **Fig. 2D**).

In total, 19.4% of studies worked at least partly with non-invasively collected samples (i.e., faeces, hair, feathers; note that we did not distinguish between fresh or shed hair and feathers). Of those,

93.5% worked with STR markers, 24 studies used SNP markers, and the remaining two studies used WGS generated partly from feather samples with a mean coverage of 40× and solely from faecal samples with a coverage of 0.49× (**Tab. S2**).

94.8% of the studies inferred parent-offspring relationships, 64.2% inferred full sibling relationships, and 60.0% inferred half-sibling relationships. 44.2% of the studies additionally inferred other kin classes. 6.8% of studies used genetic data to identify identical samples (**Tab. S2**).

The sample size of most studies is on the order of 10-1000 samples. It slightly increased over time ($Intercept_{2000}$: 5.97, Estimate: 0.03, SE: 0.0001, $p < 0.001$; **Fig. S2A**) and shows a substantial association with phylogenetic class (Cramér's V = 0.43; **Fig. S2B**).

2. Comparison of relatedness estimators on the Cayo macaque genomes

An overview of the datasets and applied estimators is presented in **Tab. S3**. Among the STR-based estimators, *DyadML* and *TrioML* performed best with the highest accuracy and precision. *DyadML* had a slightly better accuracy than *TrioML*, so we chose this estimator for the subsequent analyses. For the SNP-based estimators, *PI-HAT* yielded the best results compared to r_{IBD} (see **Tab. S3,** and **Fig. S3** for results of all estimators).

For datasets with the highest number of STR markers, r_{STR} provides a substantially less precise estimate of relatedness than r_{SNP} and r_{PMR}. The comparison between r_{STR} and r_{IBD} yielded a relatively low coefficient of determination ($R^2 = 0.68$), indicating weak agreement between the two sets of genetic relatedness estimates. Overall, r_{STR} underestimates relatedness by -0.045 on average (**Fig. 3A** and **4A**), though the deviation of r_{STR} from r_{IBD} can vary. For some dyads with r_{IBD} up to 0.35 (i.e., 2nd-degree relatives), r_{STR} underestimates the relatedness to be 0 or close to 0 (i.e., unrelated) in many cases, but overestimates up to 0.55 in a few cases (i.e., 1st-degree relatives; **Fig. 3A**, **4A,** and **S4**).

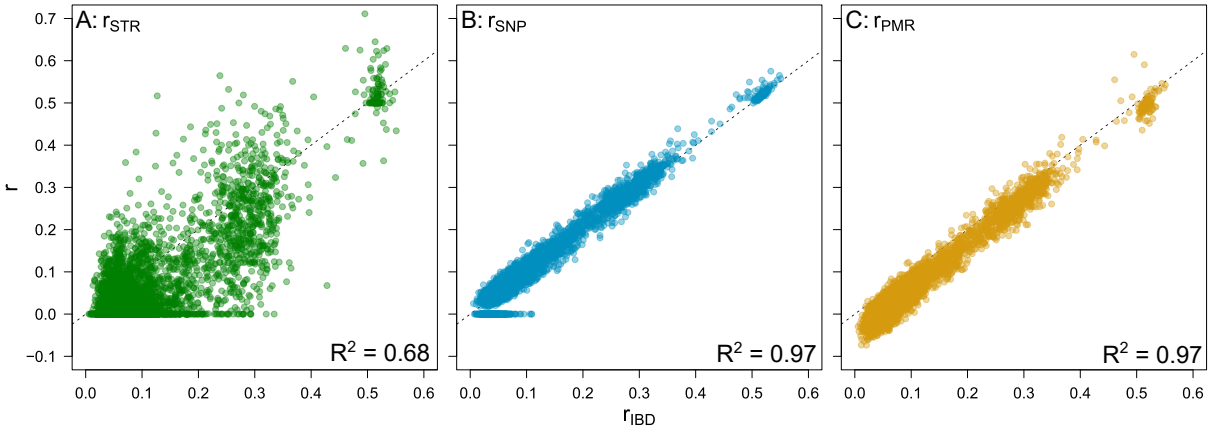

Figure 3: **Correlation between various relatedness estimates and IBD-based relatedness r_{IBD}.** The diagonal dotted line represents perfect correlation, helping to visualise deviations from r_{IBD}. **(A)** Shows the full STR marker set (n_{STR} = 37) using DyadML. **(B)** Shows the SNP-based estimators PI-HAT, based on the WGS dataset (n_{SNP} = 6,940,242). It shows high precision and accuracy, though there is a line of outliers at the bottom. **(C)** Shows the PMR-based estimates using 1× WGS dataset (n_{SNP} = 2,655,057). While the precision is high, it shows a slightly lower accuracy.

On the other hand, r_{SNP} slightly overestimates relatedness by 0.003 (Fig. 3B and 4B) on average, and showed a strong linear relationship with r_{IBD} (R^2 = 0.97). Moreover, r_{SNP} shows a much lower variation in deviance from r_{IBD} (**Fig. 3B** and **4B**), with no severe misclassifications. However, for 365 dyads, r_{SNP} is 0 while r_{IBD} is > 0.007 and < 0.11 (**Fig. 3B**). We identified that the reason for this bias is due to the way default settings of *PLINK* clip intermediate statistics. It first estimates the proportion of the genome that two individuals share in each IBD state: IBD0 (no shared alleles), IBD1 (one shared allele), and IBD2 (two shared alleles), and uses these estimates to compute *PI-HAT.* Each state may fall outside the theoretical [0,1] range because it is derived from method-of-moments calculations that are not constrained to produce valid probabilities. Sampling noise, genotype errors, or allele frequency mismatches can cause minor deviations, particularly among distantly related or unrelated individuals. When IBD0 exceeds 1, *PLINK* by default truncates it to 1, which forces r_{SNP} to 0 regardless of the estimated values of the other IBD states (Purcell et al., 2007).

r_{PMR} in the same dyads showed no noticeable deviations. As with r_{SNP}, r_{PMR} showed a strong linear relationship with r_{IBD} (R^2 = 0.97), while it underestimates relatedness by -0.047 on average. However, this bias is linear, while the precision remains high (**Fig. 3C**). For three 1st-degree relatives with r_{IBD} of 0.45-0.5, r_{PMR} is 0.55-0.62 (**Fig. 4C** and **S4B**). Apart from these three dyads, no substantial deviations from r_{IBD} were found.

Comparison of datasets

As expected, precision decreases with fewer markers used to infer relatedness for all three methods (**Fig. 4**). For r_{STR}, the median deviation from r_{IBD} remains relatively constant across the three subsets, but the strength and variance in deviation increase as the number of markers decreases. This is also reflected in the coefficient of determination: $R^2_{37\ STR}$ = 0.68, $R^2_{19\ STR}$ = 0.49, and $R^2_{9\ STR}$ = 0.28. For the smallest dataset with 9 STR markers, the deviation between r_{STR} and r_{IBD} can reach as high as 0.9. In these cases, r_{STR} treats distantly related pairs as identical individuals. Conversely, the negative deviation of r_{STR} from r_{IBD} can be as low as -0.41 when full siblings are treated as distant kin (i.e., 3rd-degree relatives). For r_{SNP}, the median deviation from r_{IBD} increases with decreasing marker number. This indicates that, in addition to a general reduction of precision when using fewer markers, an overestimation of relatedness intensifies. The concordance between r_{SNP} and r_{IBD} drops with decreasing dataset size: R^2_{WGS} = 0.97, $R^2_{100\%\ exom}$ = 0.94, $R^2_{10\%\ exom}$ = 0.86, and $R^2_{1\%\ exon}$ = 0.53. For r_{PMR}, the accuracy and precision deviations from r_{IBD} remain stable between 1× – 0.05×, but drop for 0.01× coverage (**Fig. 4C**). This is also evident in the coefficients of determination: $R^2_{1\times}$ = 0.97, $R^2_{0.1\times}$ = 0.95, $R^2_{0.05\times}$ = 0.93, and $R^2_{0.01\times}$ = 0.46.

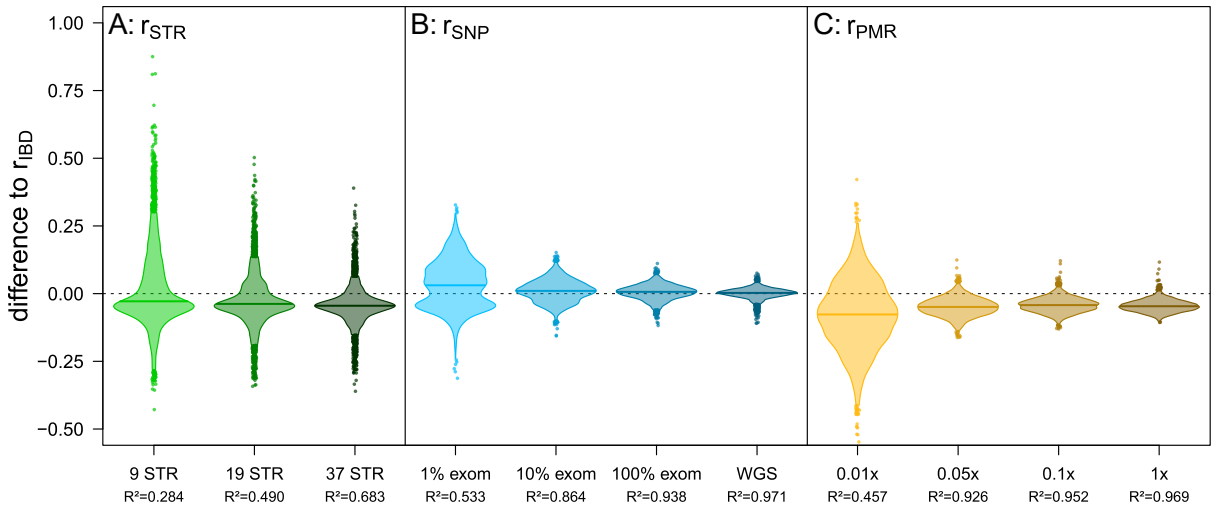

Figure 4: **The precision and accuracy of different tested datasets. (A)** shows STR-based, **(B)** SNP-based, and **(C)** PMR-based relatedness using datasets containing various numbers of markers. The plot shows the deviation of r_{STR}, r_{SNP}, and r_{PMR} from r_{IBD} for each dyad in the data set ($n_{STR/SNP}$ = 4,753, n_{PMR} = 4,371). The violins depict the distribution of data within the 50% interquartile ranges (IQR). All data outside of the IQR are shown as dots. The coloured lines within the violins show the median. The dashed line indicates perfect agreement (i.e., no difference) between r_{IBD} and r_{STR}, r_{SNP}, and r_{PMR}, respectively. r_{SNP} shows the highest precision and accuracy. In r_{STR}, the precision is particularly low with strong deviations from r_{IBD}. While showing high precision, r_{PMR} shows lower accuracy. This linear downward skew occurs because r_{PMR} cannot detect background-relatedness within a population. However, it does not bias the relatedness estimation within a population.

Discussion

In this study, we first conducted a systematic literature review to assess the use of genetic methods and data to estimate relatedness in the first quarter of the 21st century. This allowed us to identify commonly used methods and recent trends. To further evaluate them, we compared results from these different genetic marker types on published data from the well-studied rhesus macaque population of Cayo Santiago. Our results confirm previous studies that STR-based estimates are subject to limitations that can yield misleading results (Csilléry et al., 2006; van Horn et al., 2008). SNP-based estimates, when used with high-quality DNA, produced good results overall, though we observed a potentially problematic behaviour in the PI-HAT estimator for low relatedness. Moving forward, we also presented the potential of a simple PMR estimator, a cost-effective method established in ancient DNA research for inferring relatedness from lcWGS data. We showcase its usefulness on the comparison Cayo dataset in the lcWGS regime, even for as low as 0.1× average sequencing depth.

Confirming that relatedness inference through genetic markers is a central part of many biological studies, our literature compilation identified an extensive publication record of 2,034 relevant articles, concentrated in the fields of ecology, evolution, animal behaviour, and conservation (**Fig. S1C**). The publication count rises consistently from 2000, reaching its peak in 2008, after which it remains stable with moderate fluctuations (**Fig. S1A**). After adjusting for the total number of articles

published per year, we found a relative decrease of the field (**Fig. S1B**). This decline may reflect a saturation of the field. Alternatively, studies that rely on well-established methods for relatedness estimation may no longer explicitly mention the use of genetic markers in their abstracts and are consequently not included in our compilation. The majority of studies to date (89% across all years, and still 72% in 2020-2025) and across all animal classes rely on STR markers with relatively low marker numbers ranging from 1 to 92 (median: 9; **Fig. 2A** and **2B**), which have substantial caveats as we directly demonstrate in our empirical comparison dataset. While for paternity analyses, 10–15 STR markers may be sufficient (depending on how close potential sires are related and on the specifics of the STRs; Costa et al., 2012; K. Li et al., 2010; Vandeputte & Haffray, 2014), our results show that even with 19 typical STR markers (which is above the average number of STRs used; **Fig. 2B**), misclassifications can happen and nonkin or distant kin can be classified as 1st-degree relatives (**Fig. 4A** and **S4A**) or vice versa, confirming earlier studies (Csilléry et al., 2006; van Horn et al., 2008). The problem is even more pronounced when using 9 STR markers (with such a low number of markers still being used in studies published in 2025 (median: 11, range: 1–92; **Tab. S2**, **Fig. 2B**) where some unrelated or distantly related kin are considered identical (**Fig. 4A** and **S4A**). With such small marker numbers, the risk of mistaking loci that are identical-by-state as identical-by-descent is considerably high, and thus, the estimations are unreliable. Furthermore, the error in STR-based relatedness estimation for kin classes beyond parent-offspring is considerable as well. Even with 37 markers, r_{STR} produced wrong estimates across kin classes (**Fig. 3A**). This misclassification could heavily bias the results. While the exact performance of the marker type depends on the heterozygosity (i.e., the information content) of the selected markers and the estimator's performance depends on the kinship structure of the study population (Csilléry et al., 2006; de van Casteele et al., 2001), our specific evaluation of STR-based relatedness in the Cayo population highlights general issues relevant for many studies.

Remarkably, only 8.3% relatedness studies to date have used SNP data (**Fig. 2A**). While there is a steadily increasing trend since the first SNP studies appeared in 2009, even in 2025 only 33.3% of studies use such data. We found that studies used a wide range of SNP markers (33-86,677 SNPs, median = 1,038; **Tab. S2**). WGS only occurred in 8 studies, with coverages of 0.49× to 70× (median = 27.5×; **Tab. S2**). Our empirical results show that common tools to estimate relatedness from high-quality WGS data deliver accurate results overall. However, using the WGS data, r_{SNP} is 0 for 365 dyads, which have an r_{IBD} of > 0 and up to 0.11 (**Fig. 3B**), indicating that *PI-HAT*'s derived r_{SNP} with default settings has limited ability to identify distant relatives.

While r_{SNP} relies on diploid genotypes only accessible through high-coverage sequencing (≥ 10×) or lcWGS combined with imputation, we show that lcWGS data can still be an excellent source for reliable relatedness estimation. Importantly, we find that r_{PMR}, which does not require high-quality diploid genotypes and treats the genotype data as haploid, produces accurate results, even for a

coverage as low as 0.05×. Unlike r_{SNP}, r_{PMR} cannot detect background relatedness within a sample because r_{PMR} is calculated by scaling each dyad's PMR-rate by the population mean. However, this linear skew does not bias the inference of degrees of relatedness within the population, as such relatedness is in addition to background relatedness. As shown in our data, there are no significant deviations between r_{PMR} and r_{IBD} (**Fig. 3C**).

Using lcWG and r_{PMR} is particularly useful for studies that rely on non-invasively collected DNA samples in natural populations. The ability to generate genotyping data from non-invasively collected samples has opened up new perspectives for the study of taxa that are difficult to sample, but the techniques involved have developed only slowly over the past few decades. Our literature research showed that 19.4% studies worked with non-invasive samples. The vast majority of those studies (93.5%) sequenced STR markers, while only 6% used SNPs (**Tab. S2**). Producing high-quality SNP data from non-invasive samples is still challenging and laborious (Alvarez-Estape et al., 2023; Snyder-Mackler et al., 2016; Vullioud et al., 2024). Here, PMR-based relatedness offers a promising alternative, similar to how it is successfully used in human aDNA research with similar challenges of DNA quality and quantity (Hämmerle et al., 2024; Ringbauer et al., 2024).

To conclude, here we catalogued a comprehensive overview of genetic relatedness estimation methods used during the first quarter of the 21st century. Thereby, we showed that the potential offered by contemporary high-throughput sequencing technology is far from fully utilised. Using empirical data, we compared various limitations of existing sequencing and estimation methods. Building on these results, we suggest further establishing PMR-based relatedness estimation in tandem with using lcWGS data. lcWGS has become highly cost-effective, and the success of PMR-based methods in aDNA showcases the potential of lcWGS to robustly identify close relatives, particularly when DNA quality is limited, genomic resources are scarce, or economic efficiency is essential.

Code availability

The code for analysing the literature review data, and for calculating and comparing STR-, SNP-, and PMR-based relatedness is available on GitHub: https://github.com/afreudiger/CayoKinshipComparison/ and https://github.com/hringbauer/cayo_pmr/

Author contributions

Our annotation of author contributions follows the CRediT Taxonomy labels (https://casrai.org/credit/). When multiple authors fulfil the same role, their degree of contribution is identified as either *lead*, *equal*, or *support*. Conceptualisation - lead: HR, AW; support: AF, MMB, KN. Data curation - lead: AF support: VMJ, AVRL, HR, AW. Formal analysis - lead: AF, NK; support: VMJ, MMB, HR. Funding acquisition - HR, AW. Methodology - equal: AF, NK, MMB, HR. Project administration - equal: AF, HR, AW. Resources - equal: AVRL, KN, AW. Supervision - equal: HR, AW. Visualisation - lead: AF; support: NK, MMB, HR. Writing (original draft) - lead: AF; support: MMB, HR, AW. Writing (review & editing) - equal: AF, NK, VMJ, MMB, AVRL, KN, HR, AW.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Material

Text Box S1: Categorisation of articles from our Web of Science (WoS) search. The lists below summarise the categories of articles retrieved by our WoS search. The first list shows the categories we included, and the second list shows those we excluded, with the number of articles in each category indicated in square brackets.

Included: Ecology [3,538], Evolutionary Biology [2,764], Genetics Heredity [2,541], Zoology [2,110], Biochemistry Molecular Biology [1,510], Behavioral Sciences [1,085], Biology [947], Multidisciplinary Sciences [834], Marine Freshwater Biology [785], Fisheries [673], Biodiversity Conservation [666], Ornithology [338], Entomology [332], Environmental Sciences [189], Psychology Biological [123], Oceanography [115], Microbiology [89], Cell Biology [88], Parasitology [57], Infectious Diseases [42], Toxicology [29], Limnology [28], Developmental Biology [27], Geosciences Multidisciplinary [22], Tropical Medicine [22], Geography Physical [19], Water Resources [19], Environmental Studies [16], Engineering Environmental [9], Soil Science [8], Chemistry Medicinal [7], Geography [5], Green Sustainable Science Technology [4], Materials Science Multidisciplinary [3], Urban Studies [3], Engineering Marine [2], Engineering Ocean [2]

Excluded: Agriculture Dairy Animal Science [781], Veterinary Sciences [454], Biotechnology Applied Microbiology [384], Horticulture [358], Plant Sciences [303], Mathematical Computational Biology [258], Agronomy [248], Anthropology [223], Forestry [223], Social Sciences Biomedical [167], Food Science Technology [150], Biochemical Research Methods [135], Public Environmental Occupational Health [114], Statistics Probability [109], Agriculture Multidisciplinary [104], Psychology Multidisciplinary [82], Reproductive Biology [78], Family Studies [70], Medicine Legal [69], Computer Science Interdisciplinary Applications [59], Immunology [58], Psychiatry [55], Social Issues [54], Ethics [50], Neurosciences [49], Pharmacology Pharmacy [48], Sociology [48], Psychology Social [47], Obstetrics Gynecology [46], History Philosophy Of Science [45], Law [41], Medical Ethics [38], Mycology [36], Psychology Experimental [35], Computer Science Artificial Intelligence [33], Medicine Research Experimental [32], Archaeology [31], Medicine General Internal [30], Psychology Developmental [28], Endocrinology Metabolism [26], Hematology [26], Psychology [26], Chemistry Multidisciplinary [25], Social Sciences Interdisciplinary [25], Oncology [24], Chemistry Analytical [22], Computer Science Theory Methods [22], Pathology [21], Language Linguistics [20], Physiology [20], Linguistics [19], Clinical Neurology [18], Economics [18], Psychology Clinical [18], Chemistry Applied [17], Demography [16], Computer Science Information Systems [14], Geochemistry Geophysics [14], Medical Informatics [14], Medical Laboratory Technology [14], Biophysics [13], Criminology Penology [13], Religion [13], Ethnic Studies [12], History [12], Virology [12], Humanities Multidisciplinary [11], Social Work [11], Women S Studies [11], Health Care Sciences Services [10], Mathematics Interdisciplinary Applications [10], Nutrition Dietetics [10], Peripheral Vascular Disease [10], Radiology Nuclear Medicine Medical Imaging [10], Cultural Studies [9], Engineering Electrical Electronic [9], Gerontology [9], Health Policy Services [9], Cardiac Cardiovascular Systems [8], Ophthalmology [8], Education Educational Research [7], Geriatrics Gerontology [7], Literature [7], Mineralogy [7], Neuroimaging [7], Pediatrics [7], Psychology Educational [7], Substance Abuse [7], Anatomy Morphology [6], Business [6], Computer Science Software Engineering [6], Operations Research Management Science [6], Asian Studies [5], Communication [5], Dentistry Oral Surgery Medicine [5], Education Scientific Disciplines [5], Management [5], Mathematics Applied [5], Sport Sciences [5], Audiology Speech Language Pathology [4], Chemistry Inorganic Nuclear [4], Gastroenterology Hepatology [4], History Of Social Sciences [4], Nursing [4], Respiratory System [4], Spectroscopy [4], Area Studies [3], Cell Tissue

Engineering [3], Computer Science Cybernetics [3], Education Special [3], Engineering Chemical [3], Engineering Industrial [3], Information Science Library Science [3], Mechanics [3], Nanoscience Nanotechnology [3], Paleontology [3], Philosophy [3], Physics Applied [3], Psychology Applied [3], Urology Nephrology [3], Acoustics [2], Agricultural Economics Policy [2], Agricultural Engineering [2], Allergy [2], Astronomy Astrophysics [2], Automation Control Systems [2], Chemistry Physical [2], Computer Science Hardware Architecture [2], Engineering Mechanical [2], Engineering Multidisciplinary [2], Film Radio Television [2], Folklore [2], Geology [2], Instruments Instrumentation [2], Integrative Complementary Medicine [2], Meteorology Atmospheric Sciences [2], Otorhinolaryngology [2], Political Science [2], Public Administration [2], Regional Urban Planning [2], Rehabilitation [2], Robotics [2], Surgery [2], Telecommunications [2], Andrology [1], Anesthesiology [1], Chemistry Organic [1], Crystallography [1], Dermatology [1], Development Studies [1], Electrochemistry [1], Energy Fuels [1], Engineering Aerospace [1], Engineering Biomedical [1], Engineering Manufacturing [1], Literary Reviews [1], Literary Theory Criticism [1], Literature German Dutch Scandinavian [1], Materials Science Paper Wood [1], Mathematics [1], Medieval Renaissance Studies [1], Microscopy [1], Music [1]

Table S1: Test paper set used to verify the keyword search on Web of Science (WoS). To assess the power of our search query to find target articles, we selected these 20 articles manually. 16 out of 20 (80%) articles were included in our search criteria (as reported in the last column).

first author	year	journal	title	WoS
Barelli	2013	Am J Prim	Extra-pair paternity confirmed in wild white-handed gibbons	yes
Barth	2014	Plos One	The evolution of extreme polyandry in social insects - insights from army ants	no
Bradley	2004	Curr Biol	Dispersed male networks in western gorillas	yes
Davidian	2016	Sci Adv	Why do some males choose to breed at home when most other males disperse	no
Freudiger	2025	PNAS	Estimating realized relatedness in free-ranging macaques by inferring identity-by-descent segments	yes
Guerier	2012	Cons Genet	Parentage analysis in a managed free ranging population of southern white rhinoceros - genetic diversity pedigrees and management	yes
Huck	2014	Proc Soc R B	Correlates of genetic monogamy in socially monogamous mammals - insights from Azaras owl monkeys	yes
Josi	2021	Evolution	Age- and sex-dependent variation in relatedness corresponds to reproductive skew territory inheritance and workload in cooperatively breeding cichlids	yes
Langergraber	2007	PNAS	The limited impact of kinship on cooperation in wild chimpanzees	yes
Muralidhar	2014	Mol Ecol	Kin-bias breeding site selection and female fitness in a cannibalistic neotropical frog	yes
Noordwijk	2012	Behav Ecol Socio	Female philopatry and its social benefits among Bornean orangutans	yes
Pacheco	2024	Heredity	Relatedness-based mate choice and female philopatry - inbreeding trends of wolf packs in a human-dominated landscape	yes
Palomares	2017	Sci Rep	Territoriality ensures paternity in a solitary carnivore mammal	yes
Sanderson	2015	Mol Ecol	Banded mongoose avoid inbreeding when mating with members of the same natal group	no
Smith	2003	Proc R Soc Lond B	Wild female baboons bias their social behaviour towards paternal half sisters	no
Snyder-Mackler	2016	Genetics	Efficient genome-wide sequencing and low-coverage pedigree analysis from noninvasively collected samples	yes
Vigilant	2015	Behav Ecol Socio	Reproductive competition and inbreeding avoidance in a primate species with habitual female dispersal	yes
Wikberg	2014	Anim Behav	The effect of male parallel dispersal on the kin composition of groups in white-faced capuchins	yes
Chakrabarti	2020	Sci Rep	The role of kinship and demography in shaping cooperation amongst male lions	yes
Diaz-Aguirre	2018	Behav Ecol Socio	Kinship influences social bonds among male southern Australian bottlenose dolphins (*Tursiops cf. australis*)	yes

Tab S2: Final list of publications identified in the literature review. Sample size refers to the number of samples originating from populations without managed breeding. Sample size was not always explicitly reported in the source articles and therefore represents our best estimate; it should be interpreted as an approximate indication of study scale rather than an exact value. The number of genetic markers corresponds to the number of markers used for relatedness estimation. When multiple marker sets of different sizes were used within a study, we calculated the mean number of markers and rounded it up. If studies did not explicitly specify the type of genetic relatedness inferred, we assumed that general relatedness was intended, encompassing all possible degrees.

Freudiger et al_TableS2

Table S3: Overview of datasets and estimators used. For STR and imputed SNP, we tested the performance of all estimators on the largest dataset. For the reduced subsets, we used only the best-performing estimator.

marker type	marker number	estimator	median	25% quantile	75% quantile	range
STR	37 *(range: 27-41)*	quellergt	-0.1262	-0.1918	-0.0570	-0.602–0.339
	37 *(range: 27-41)*	lynchli	-0.1067	-0.1809	-0.0316	-0.539–0.328
	37 *(range: 27-41)*	ritland	-0.1259	-0.1694	-0.0798	-0.417–0.304
	37 *(range: 27-41)*	lynchrd	-0.1244	-0.1715	-0.0794	-0.445–0.216
	37 *(range: 27-41)*	wang	-0.1059	-0.1802	-0.0341	-0.491–0.295
	37 *(range: 27-41)*	dyadml	-0.0447	-0.0717	-0.0174	-0.361–0.390
	37 *(range: 27-41)*	trioml	-0.0479	-0.0753	-0.0255	-0.380–0.379
	19 *(range: 12-20)*	dyadml	-0.0379	-0.0683	0.0137	-0.342–0.503
	9 *(range: 6-10)*	dyadml	-0.0283	-0.0643	0.0823	-0.428–0.875
SNP (imputed)	6,940,242 *(7× WGS)*	PI-HAT	0.0027	-0.0082	0.0128	-0.109–0.076
	6,940,242 *(7× WGS)*	r_{ab}	-0.0615	-0.0871	-0.0447	-0.237–0.031
	6,940,242 *(7× WGS)*	Two-out-of-three IBD	-0.0612	-0.0868	-0.0444	-0.237–0.034
	98,833 *(100% exome)*	PI-HAT	0.0065	-0.0107	0.0229	-0.118–0.111
	9,844 *(10% exome)*	PI-HAT	0.0101	-0.0181	0.0365	-0.156–0.152
	999 *(1% exome)*	PI-HAT	0.0308	-0.0392	0.0952	-0.312–0.328
SNP (unimputed)	2,655,057 *(1× WGS)*	PMR	-0.0465	-0.0605	-0.0314	-0.106–0.116
	63,271 *(0.1× WGS)*	PMR	-0.0424	-0.0605	-0.0248	-0.131–0.121
	16,735 *(0.05× WGS)*	PMR	-0.0491	-0.0713	-0.0265	-0.162–0.124
	699 *(0.01× WGS)*	PMR	-0.0769	-0.1608	0.0076	-0.601–0.421

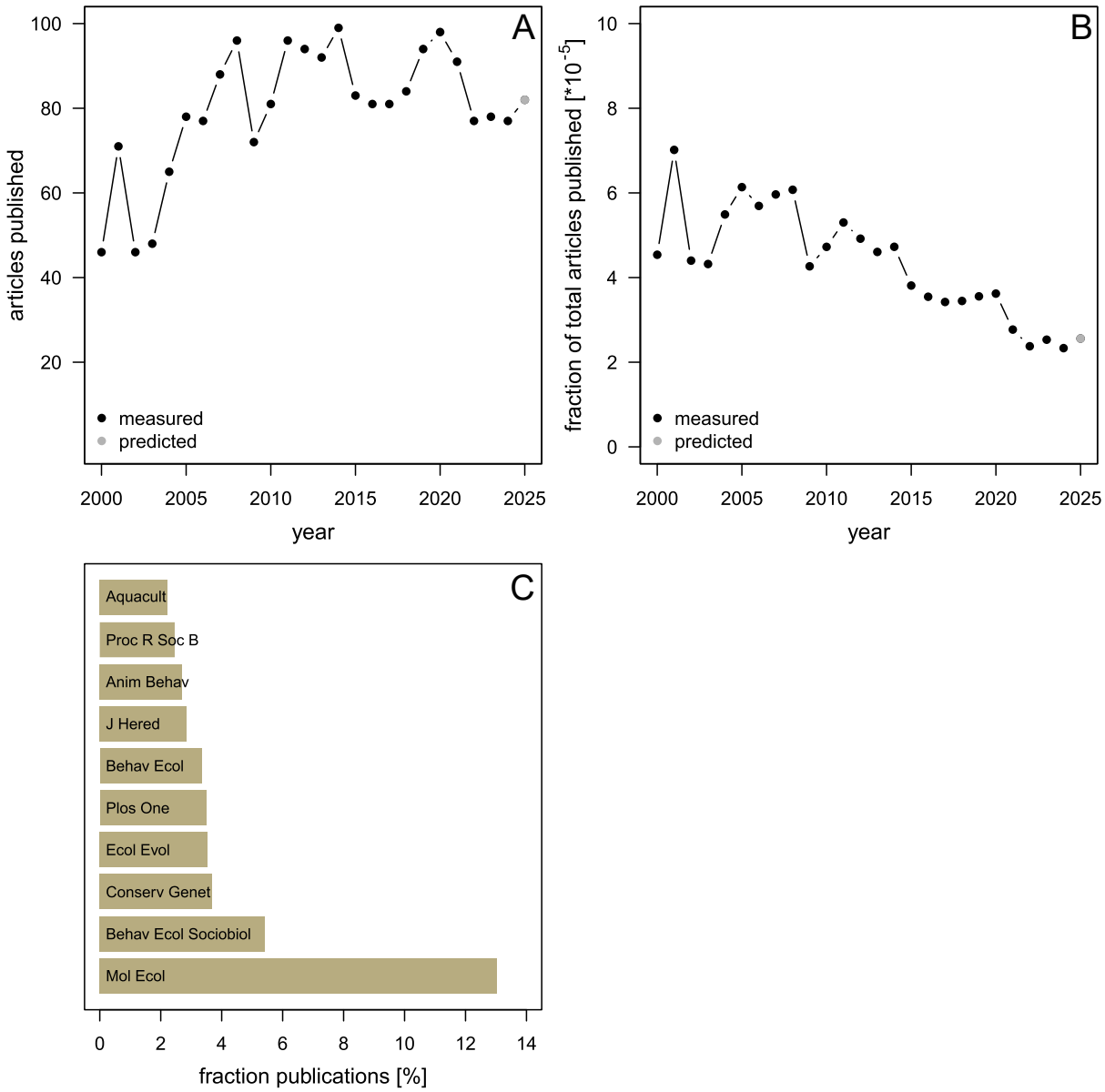


Figure S1: Number of publications across time and journals. (A) The number of articles published per year that we included in the systematic literature review. For 2025, we interpolated based on the first 6 months in this review. **(B)** The number of articles published per year normalised by the total number of articles found on Web of Science for each year (found via keyword search "the OR a"). The number of articles published in 2025 is predicted based on data of the first 6 months. **(C)** The ten journals in which the most articles have been published. In total, articles were published in 271 different journals.

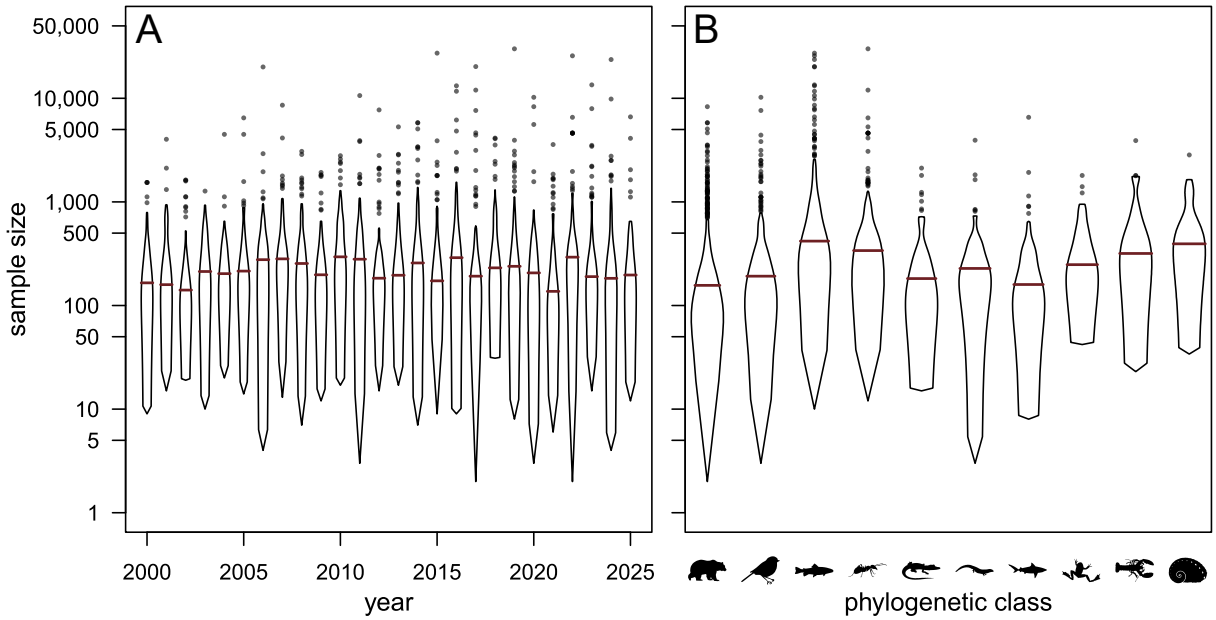


Figure S2: Sample size across time and phylogenetic class. (A) Sample size used across years, shown on a log-scale. **(B)** Sample size per study species across the ten most common phylogenetic classes. The violin extent covers the interquartile range and whiskers (calculated as 1.5 × IQR), following boxplot conventions. Red horizontal lines indicate the median value. Individual points indicate outliers that fall beyond the whisker boundaries.

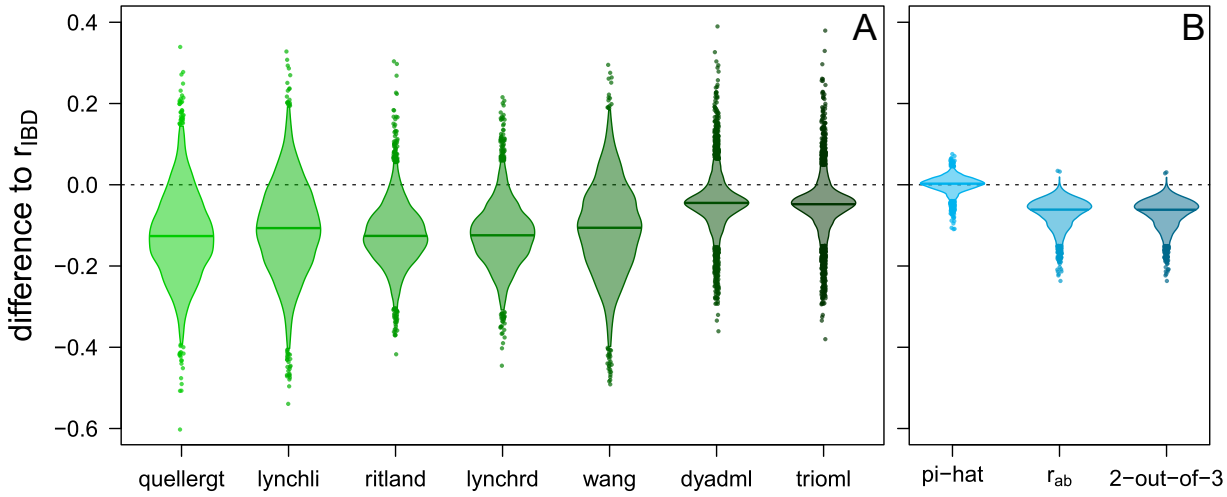
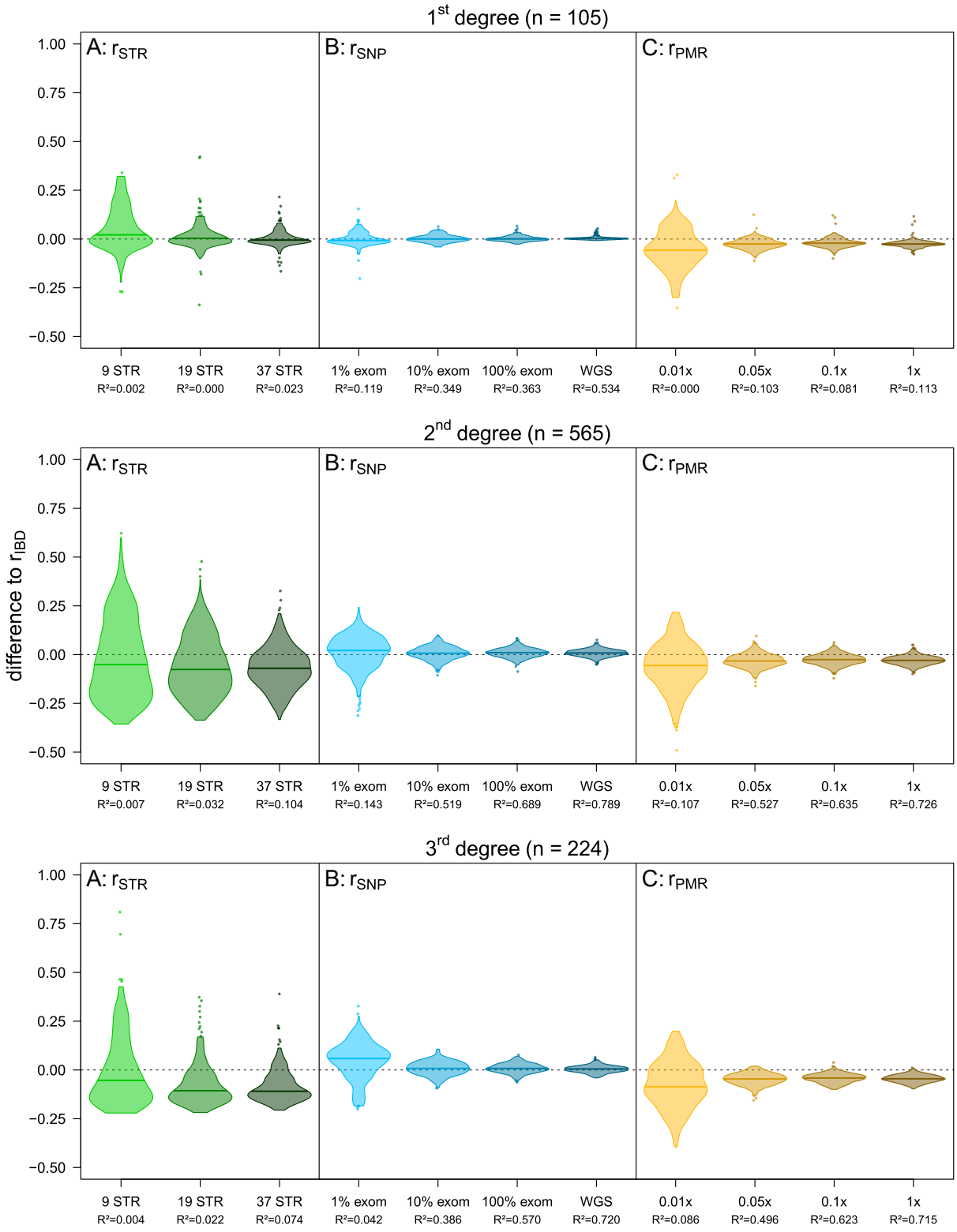


Figure S3: Performance of different estimators. The precision and accuracy of different STR-based **(A)** and SNP-based **(B)** relatedness estimators. The plot shows the deviation of r_{STR} and r_{SNP} from r_{IBD} for each dyad in the data set ($n_{STR/SNP}$ = 4,753, n_{PMR} = 4,371), using all available markers per dataset (n_{STR} = 37, n_{SNP} = 6,940,242). The violins depict the distribution of data within the 50% interquartile ranges (IQR). All data outside of the IQR are shown as dots. The coloured lines within the violins show the median. The dashed line indicates perfect agreement among r_{IBD}, r_{STR}, and r_{SNP}. The median deviances between the different estimators and r_{IBD} are: quellergt: -0.124, lynchli: -0.106, ritland: -0.123, lynchrd: -0.123, wang: -0.107, dyadml: -0.043, trioml: -0.051, PI-HAT: 0.003, r_{ab}: -0.061, and 2-out-of-3: -0.061.


1st degree (n = 105)
2nd degree (n = 565)
3rd degree (n = 224)
A: rSTR
B: rSNP
C: rPMR
difference to rIBD
1.00
0.75
0.50
0.25
0.00
−0.25
−0.50
9 STR
19 STR
37 STR
1% exom
10% exom
100% exom
WGS
0.01x
0.05x
0.1x
1x
R²=0.002
R²=0.000
R²=0.023
R²=0.119
R²=0.349
R²=0.363
R²=0.534
R²=0.000
R²=0.103
R²=0.081
R²=0.113
R²=0.007
R²=0.032
R²=0.104
R²=0.143
R²=0.519
R²=0.689
R²=0.789
R²=0.107
R²=0.527
R²=0.635
R²=0.726
R²=0.004
R²=0.022
R²=0.074
R²=0.042
R²=0.386
R²=0.570
R²=0.720
R²=0.086
R²=0.496
R²=0.623
R²=0.715

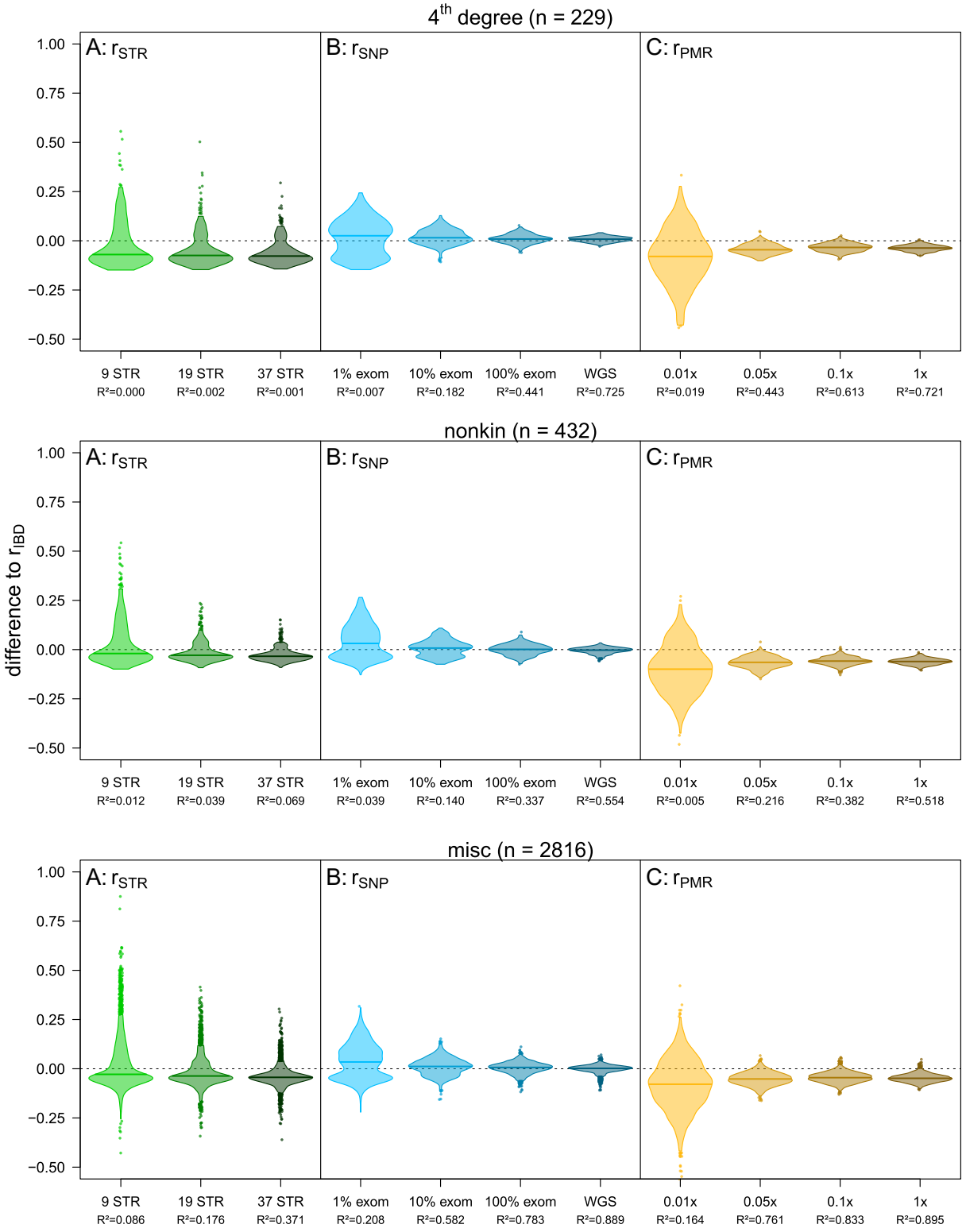


Figure S4: The performance of differently sized marker sets across degrees of relatedness. The precision and accuracy of STR-based, SNP-based, and PMR-based relatedness using datasets with various marker numbers. The plot shows the deviation between r_{STR} and r_{SNP}, respectively, from r_{IBD} for each dyad of each degree of relatedness. Nonkin are dyads without a link in the pedigree, and miscellaneous ('misc') are dyads which were more distant relatives or followed more complex relatedness patterns. The violins depict the distribution of data within the 50% interquartile ranges (IQR). All data outside of the IQR are shown as dots. The coloured lines within the violins show the median. The dashed line shows the perfect agreement between r_{IBD} and r_{STR}, r_{SNP}, and r_{PMR}, respectively.