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METHODS AND RESOURCES ARTICLE

Comparative analysis of microsatellite and SNP markers for parentage testing in the golden snub-nosed monkey (*Rhinopithecus roxellana*)

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Abstract

Microsatellite markers are popular for assigning parentage, but single-nucleotide polymorphisms (SNPs) have only been applied in this area recently. To evaluate these two markers which have been previously studied in golden snub-nosed monkeys, we genotyped 12 individuals using 37 microsatellite loci and 37 SNP markers. The data showed that 32 of 37 microsatellite loci were polymorphic, and most microsatellite loci were high informative (mean PIC = 0.599). Meanwhile, 24 of 37 SNP markers were polymorphic and most were low informative (mean PIC = 0.244). For microsatellites, the combined exclusion probability with one-parent-unknown/known (CE-1P/CE-2P) nearly reached 1, while for the SNP markers, CE-2P only reached 0.9582. Under the condition of one parent known/unknown, the CE-2P and CE-1P could meet the international human parental standard (0.9973) by using five or nine microsatellite loci respectively. For SNP markers, we doubled the loci (n = 48) and simulated parentage testing, and the data showed that the CE-2P was 0.998 while the CE-1P was still low. This result indicated that the SNP loci which we used here had low polymorphism and that more loci need to be developed in the future. In addition, we corrected one case of failed identification by excluding siblings and reducing the range of candidate paternities.

Keywords *Rhinopithecus roxellana* · microsatellite · SNP · parentage testing

Introduction

The golden snub-nosed monkey is an endemic endangered species in China with a current wild population of ~22,000 individuals. According to data from the *2017 International Studbook of Golden Monkey* (Chinese Association of Zoological Gardens), at the end of 2017, there were 488 individuals in captivity in 46 institutes around the world. Genetic management is essential for the conservation of both the ex-situ and in-situ populations of endangered wildlife. Although much research has been done on the population structure and population dynamics of this species, parentage testing has not developed well.

In captivity, revising the pedigree by parentage testing can help to reduce problems caused by inbreeding such as low viability, low fertility, and high mortality. Microsatellite markers have been used in the animal husbandry industry, initially for horses, since the 1990s because of their high rate of polymorphism and low required marker numbers. Since then, they have been successfully used in many threatened animals such as *Ailuropoda melanoleuca* (Zhang et al. 2003), *Panthera tigris amoyensis* (Zhang et al. 2006), and *Grus japonensis* (Zhang et al. 2015). Single Nucleotide Polymorphisms (SNPs), a third-generation genetic marker, has the advantages of good genetic stability, rapid detection, high multi-density, and wide distribution, and has been applied to the animal kingdom, and especially the animal husbandry industry, for parentage testing (Heaton et al. 2002; Werner et al. 2004).

Microsatellites have historically been preferred in many evolution studies of golden snub-nosed monkeys, while

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SNPs have only been applied to this area recently. Microsatellites of primates such as *Homo sapiens*, *Cebus paella* and *Macaca mulatta* have been used in studies of the golden snub-nosed monkeys before specific markers for this species were published (Pan et al. 2005; Ren 2007; He 2013). Twelve years ago, the first 11 microsatellite loci of the golden snub-nosed monkey were isolated (Hao et al. 2007). Ren 2007)conducted a parentage testing for five captive golden snub-nosed monkeys in Xi'an, and Zhou et al. 2015) used 11 loci to perform parentage testing on Shennongjia golden monkeys. However, none of the CE-1P and CE-2P reached the international parental standard. In 2015, 37 SNP loci were developed from the golden snub-nosed monkey genome (Du 2015). High-resolution dissolution curve (HRM) technology has been employed on seven individuals for SNP typing and individual identification without parentage testing.

To compare the difference in accuracy of parentage testing between microsatellites and SNP markers on golden snub-nosed monkeys, we utilized the above-mentioned markers for parentage testing of 12 individuals. This research can be used to help in field surveys and genetic management of captive populations.

Materials and methods

Experimental and materials

Blood samples from Fuzhou Zoo (n = 5) and Shanghai Zoo (n = 6), a tissue sample from the Gene Resource Library of Chengdu Research Base of Giant Panda Breeding (n = 1), and fresh feces samples from Chengdu Zoo (n = 17) were used to parentage test golden snub-nosed monkeys using the 37 published microsatellite loci and the 37 SNP loci. The kinship of the above 12 individuals from which the blood and tissue samples were collected was clearly recorded (Table 1). All sample collection and utility protocols in this study were approved by the Chengdu Research Base of Giant Panda Breeding. The experimental procedures were fully in compliance with the current laws on animal welfare and research in China.

DNA extraction and quality determination

DNA was extracted using the QIAamp DNA Blood Midi Kit (Qiagen, Germany) for blood samples, the DNeasy Blood and Tissue Kit (Qiagen, Germany) for tissue samples, and the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) for feces samples, according to the manufacturer's protocol. The DNA concentration in the samples was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) at an optical density (OD value) of 260 nm; an OD260:OD280 ratio ranging between 1.8 and 2.0 was considered acceptable.

Microsatellite loci isolation PCR amplification

A total of 37 loci were synthesized for PCR amplification with reference to relevant literature (Pan et al. 2005; Haoet al. 2007; Ren 2007; He 2013). The reaction mixture contained 10–20 ng DNA, 1 µL 10 × Taq buffer, 0.2 µL dNTP Mix, 0.1µL Taq DNA polymerase, 0.2 µL each primer, 0.8 µL $MgCl_2$, and adjusted with RNase-free water to a final volume of 10 µL. The amplification reaction was performed by denaturing at 95 °C for 10 min; followed by 35 cycles of denaturing at 95 °C for 30 s, annealing temperature for 45 s, and extending at 72 °C for 45 s and a final extension at 72 °C for 10 min. The PCR products were detected by 2% agarose gel electrophoresis, and 37 loci were successfully amplified (Table 2). The PCR product was sent to Shanghai Sangon Bioengineering Co., Ltd. (Shanghai, China) for genotyping. The same operations were performed on the stool samples.

Table 1 Sample information of 12 golden monkey

Sample name	Pedigree number	Sex	Age	Source	Type
GMFZ01	480	F	12	Fuzhou zoo	Blood
GMFZ02	483	M	12	Fuzhou zoo	Blood
GMFZ03	577	M	8	Fuzhou zoo	Blood
GMFZ04	834	M	1.5	Fuzhou zoo	Blood
GMFZ05	799	F	7	Fuzhou zoo	Blood
GM6	UNK	UNK	UNK	Chengdu zoo	Cardiac muscle
GMSH07	223	M	19	Shanghai zoo	Blood
GMSH08	384	F	13	Shanghai zoo	Blood
GMSH09	790	M	4	Shanghai zoo	Blood
GMSH10	183	M	23	Shanghai zoo	Blood
GMSH11	356	F	14	Shanghai zoo	Blood
GMSH12	835	M	3	Shanghai zoo	Blood

Table 2 Microsatellite loci information used in the research

Loci	Primer sequence (5′–3′)	Repeat motif	Size range (bp)	Tm (℃)	References
GM209	F:ATCTGAATGATGTGTGGATGT R:TAGAGTAGCATTGCCT	Di	146–158	53	Hao et al. (2007)
GM219	F:GTGTATTGTGGGGCTATC R:GTGGGCTCTGACCTAGGAATC	Di	183–187	53	Hao et al. (2007)
GM108	F:CAGCGTAAGCCAGTTGCC R:GGAAAAGTCTGAAACCCACGA	Di	123–137	53	Hao et al. (2007)
GM228	F:ACCAGCCTCCAAAATTATGTG R:GAGGGGTGACTGAGTCAAA	Di	165–175	52	Hao et al. (2007)
GM213	F:GCCCTAGCAGAACATGACACT R:AGCCCATGCGTATTGAGT	Di	182–198	55	Hao et al. (2007)
GM105	F:CGGATCATTGTTGCTC R:AGATGGGAAGGTGTGTCTACA	Di	161–175	55	Hao et al. (2007)
GM227	F:CAGAAGCCACCGAAATG R:AATTCTCTCCCAAGGAATATG	Di	148–174	52	Hao et al. (2007)
GM214	F:GGGCAACAGAGCGAGACTG R:TGCAAAGATGTGAACGGAAAT	Di	134–154	53	Hao et al. (2007)
GM206	F:GGTGCTACCAGATCATTGTT R:CAGATGGGAAGGTGTGTCTAC	Di	163–183	54	Hao et al. (2007)
GM109	F:GGTGGAGGAGGGCCTAAC R:CTGATGTCCATAGGCGACCAT	Di	138–164	54	Hao et al. (2007)
GM220	F:CCCTTCTCTGTGACCTTGT R:TGAGTCCTCAGACACCAATCA	Di	165–179	52	Hao et al. (2007)
D20s206	F:TCCATTATTCCCCTCAAACA R:GGTTTGCCATTCAGTTGAGA	Tetra	163–164	52	He (2013)
D5s1457	F:TAGGTTCTGGGCATGTCTGT R:TGCTTGGCACACTTCAGG	Tetra	97–127	57	He (2013)
D7s2204	F:TCATGACAAAACAGAAATTAAGTG R:AGTAAATGGAATTGCTTGTTACC	Tetra	217–269	54	He (2013)
D14s306	F:AAAGCTACATCCAAATTAGGTAGG R:TGACAAAGAAACTAAAATGTCCC	Tetra	190–210	52	He (2013)
D1s533	F:CATCCCCCCCAAAAAATATA R:TTGCTAATCAAATAACAATGGG	Tetra	193–225	52	Pan et al. (2005)
D3s1768	F:GGTTGCTGCCAAAGATTAGA R:CACTGTGATTTGCTGTTGGA	Tetra	186–206	48	Pan et al. (2005)
D6s474	F:TGTACAAAAGCCTATTTAGTCAGG R:TCATGTGAGCCAATTCCTCT	Tetra	151–167	58	Pan et al. (2005)
D6s493	F:TCATGACAAAACAGAAATTAAGTG R:AGTAAATGGAATTGCTTGTTACC	Tetra	236–237	54	Pan et al. (2005)
D7s1830	F:GTACATGATGGGCTGTCCTC R:GATACATACTGCCAATAAATCACA	Tetra	200–228	58	Pan et al. (2005)
D10s1432	F:CAGTGGACACTAAACACAATCC R:TAGATTATCTAAATGGTGGATTTCC	Tetra	157–185	54	Pan et al. (2005)
D11s1366	F:GCTACAATGATAGGGAAATAATAGA R:GGTGGGATCCTTTGCTATTT	Tetra	241–242	54	Pan et al. (2005)
D11s2002	F:CATGGCCCTTCTTTTCATAG R:AATGAGGTCTTACTTTGTTGC	Tetra	224–252	58	Pan et al. (2005)
D15s644	F:CCTTCATTGGCAGACTCACT R:GCAGACACCAAGATGATAACG	Tetra	203–204	48	Pan et al. (2005)
D17s1290	F:GCCAACAGAGCAAGACTGTC R:GGAAACAGTTAAATGGCCAA	Tetra	170–210	60	Pan et al. (2005)

Table 2 (continued)

Loci	Primer sequence (5′–3′)	Repeat motif	Size range (bp)	Tm (℃)	References
D18s851	F:CTGTCCTCTAGGCTCATTTAGC R:TTATGAAGCAGTGATGCCAA	Tetra	253–254	50	Pan et al. (2005)
D11s925	F:AGAACCAAGGTCGTAAGTCCTG R:TTAGACCATTATGGGGGCAA	Di	172–199	55	Ren (2007)
D13s159	F:AGGCTGTGACTTTTAGGCCA R:CCAGGCCACTTTTGATCTGT	Di	168–203	55	Ren (2007)
D16s420	F:ACAAGGGTTATGGGAGGTATGAG R:GGCAATATAGTGAGAATACCAGTAG	Di	248	54	Ren (2007)
D2s141	F:ACTAATTACTACCCNCACTCCC R:TTTTCCAAACAGATACAGTGAACTT	Di	152–178	56	Ren (2007)
D6s271	F:AACAATTGGGAAATGGCTTA R:TAGGTTGTGGTGGGTGTTAC	Di	166–208	55	Ren (2007)
D6s287	F:GCCTACAAGGATTTCCAAAT R:AATGAGTGATAGCCTACAATGC	Di	286	50	Ren (2007)
D7s503	F:ACTTGGAGTAATGGGAGCAG R:GTCCCTGAAAACCTTTAATCAG	Di	148–180	55	Ren (2007)
D16S539	F:GATCCCAAGCTCTTCCTCTT R:ACGTTTGTGTGTGCATCTGT	Tetra	148–172	55	Ren (2007)
D17S791	F:GTTTTCTCCAGTTATTCCCC R:GCTCGTCCTTTGGAAGAGTT	Di	165–199	55	Ren (2007)
D4S431	F:AGGCATACTAGGCCGTATT R:TTCCCATCAGCGTCTTC	Di	246–270	55	Ren (2007)
D7s1826	F:CATCCATCTATCTCTGTAATCTCTC R:TATTTAACACACCTGTCTCAATCC	Tetra	142–162	54	Ren (2007)

SNP loci selection

Referring to the study of Du 2015) 37 SNP loci were selected (Table 3). The primers were synthesized by Shanghai Sangon Bioengineering Co., Ltd. (Shanghai, China).

HRM reaction and genotyping

HRM genotyping was performed on Roche LightCycler 96. The reaction mixture contained 10 ng DNA, 10 μL HRM Master Mix (Roche, Germany), 0.4 μL of each primer, 1.6 μL Mgcl and adjusted with RNase-free water to a final volume of 20 μL. A negative control, which contained sterile water in place of template DNA, was added during each run of the PCR to check for contamination. The amplification was achieved by the following protocol: an initial denaturation step at 95 °C for 10 min, 45 cycles of denaturation at 95 °C for 10 s, annealing for 20 s, and extension at 72 °C for 15 s. For HRM genotyping, the melting program included three steps: denaturation at 95 °C for 1 min, renaturation at 40 °C for 1 min, and a subsequent melting cycle with a continuous fluorescent reading from 65 to 90 °C at a rate of 25 acquisitions per degree celsius. Melting curve analysis was performed using the LightCycler 96 Gene Scanning Software (Roche, Germany) with default settings.

PCR product sequencing and SNPs genotyping verifing

PCR amplification using the above synthesized primers (Applied Biosystems GeneAmp PCR System 9700,USA). The reaction mixture contained 5–30 ng DNA, 2.5 μL 10× Taq buffer, 0.5 μL dNTP Mix, 0.25 μL Taq DNA polymerase, 0.5 μL each primer, 2 μL $MgCl_2$,and adjusted with RNase-free water to a final volume of 25 μL. The amplification reaction was performed by denaturing at 95 °C for 10 min; followed by 35 cycles of denaturing at 95 °C for 30 s, annealing temperature for 30 s, and extending at 72 °C for 45 s and a final extension at 72 °C for 20 min. The amplified product was sent to Shanghai Sangon Bioengineering Co., Ltd. (Shanghai, China) for cloning and sequencing, and the sequencing results were compared with the results of HRM genotyping to determine the SNP genotype.

Table 3 SNP loci information used in the research

Loci	Choromosome	SNP	Primer sequence (5′–3′)	Fragment size (bp)	Tm (℃)
002	2	C/T	F:AGAAAGACATAGGAGACCTC	43	60
			R:GAAGCCTGGCTCTGATGTTC		
003	4	G/A	F:GGTTCTAACTTACTGGGTTT	43	60
			R:TGCTATCAGCAATGTTCTCA		
004	9	T/C	F:GGAATGAAAGAACTTTGTC	48	60
			R:GTCATTTATAGACTACAGAGTCA		
008	5	T/G	F:TGTCTGCATCACATGGAGT	39	60
			R:ATTGCTTTACCACATGCTC		
014	6	A/G	F:GAGAAACCTGTAGTGTTTGG	42	60
			R:GGTCCCTTTGATCTCATTC		
015	19	T/C	F:AGCCAGATGGAAGGGAAGC	43	60
			R:CGGTGACCAGGATGAAGC		
017	9	C/A	F:CTTTCAGAAAGGCAGCAG	40	60
			R:ACAGCTCTGTCTCCCTGG		
020	1	T/C	F:CATTAGTACAGTAGTAACAGCTA	48	58
			R:CATTGCTGTGAGCATTAGT		
022	4	A/G	F:CTCCCAGGGGCTGAGCCT	39	58
			R:CCTTCCTGACCCTATCCA		
029	4	A/G	F:TGTCTGCTCCATGCTGTG	43	58
			R:CAATCAGGATCTTGCCAT		
039	2	C/T	F:AGGGGGCTCCTTGTCCAT	43	60
			R:GGTTTCCAGTGACTGATTAT		
043	4	G/A	F:TCTTCTGCCCTTTTGTCA	45	60
			R:GCAACTTTGGCATCTTTT		
044	16	G/T	F:ACTAGGATAAGACGGAAGC	45	60
			R:ATCTTGCATTTTGCCTCC		
046	1	C/T	F:GGAATAGGGGTGCCAGGACAA	84	60
			R:TGGAAATGAACTCTGACAAGG		
051	5	G/A	F:GTTGTAGGGGCTAGGTATACTG	45	60
			R:AATAGAAGATGGCGAGCCTG		
053	7	C/T	F:CAGAAATGTTGACAGAAA	43	58
			R:TCATCTGGTATTTCACTT		
061	13	C/T	F:AGGTTCCATTATCACACACT	44	60
			R:ACCTAGAGGCAGGTTTTG		
063	3	C/T	F:CAGCCTCACCTAATGTCC	44	60
			R:GAGACTCATCTAGTTTCAA		
064	3	T/C	F:GTACACCTGGTAGGACTCTGA	48	60
			R:TGTGAAGATAGAGGAAATCTG		
066	4	C/A	F:TCCTTGATGCTGACTCTT	37	60
			R:TTATTCATACAGCATGTT		
068	1	C/T	F:TCCTCTGAGACAGGTGGGG	40	60
			R:CTACACCTGCATCTCCATC		
070	12	T/C	F:TTCTTAGAGGAAGGTTTAGT	44	60
			R:CCATGCATTCTGATTTGAGG		
074	13	T/C	F:TTTGCTTCCAGCCAGCC	39	60
			R:GTGGAGAGCCCTAACCG		
079	13	A/G	F:GGGATGGTAACAAGCGAAAT	45	60
			R:TTCCATTGTTTAAGGAACCG		
087	4	G/A	F:CAGCCTAAGCCCTGGAAT	43	60
			R:TGCAAAACAGTTTGATGC		

Table 3 (continued)

Loci	Choromosome	SNP	Primer sequence (5′–3′)	Fragment size (bp)	Tm (℃)
088	4	T/C	F:AAGCACAGAAAATAGCCA	38	60
			R:GTGCAAAGCATCTCCCTC		
094	4	T/C	F:CTAGGGTTCTAGGATGTC	45	60
			R:ACAGGAACTGAGACTCAACT		
095	4	A/G	F:CCCAACCACCAGAAGAGG	39	60
			R:CTCCTCACAGAATCTGCA		
098	4	C/T	F:TTGGGGCAAGTTTCTGGG	44	60
			R:CTGCCTACATCTAAATGACA		
103	4	A/G	F:TAGACTCCGGGTGTAGCC	39	60
			R:CACTCAATTCCTGAGCCA		
111	6	T/C	F:GAACATTGTTCTGGATTTCA	45	60
			R:TATCATGTCCCTGGGCTTGT		
112	4	G/A	F:GATAACTGGTTCTGTTCTCC	43	60
			R:GTCGCAGCACAGTCAAGATG		
113	11	T/G	F:GCAAGGGAGCATGGAAAC	57	60
			R:TCCAAGACCGTCCGAAAT		
114	11	C/T	F:CTGCCCTCCAAGGTAAATCAC	43	60
			R:GTATAGTCAAATCTTTCATCC		
115	11	T/C	F:TCCTGGCTTCATTGTATTTTAC	45	58
			R:AACATAAGTGCATTTTCCAC		
116	4	T/C	F:TGAGGATGCACCAAGAAGC	58	60
			R:ACATTGGAGTTAAGGGAGAAA		
124	2	A/G	F:GCAGGTTTGCACTCTTACT	47	60
			R:AAGCAACGAGTCATGTAAG		

Statistical analysis

To test the effectiveness of the two methods, the parentage testing was performed by Cervus 3.0 software, which uses a maximum likelihood-based approach to infer parentage (Kalinowski et al. 2006). The heterozygosity and polymorphism information content of each allele from each sample was calculated at the same time. The sibling relationship was calculated using ML-Relate software (Kalinowski et al. 2007).

Results and discussion

Polymorphism analysis was carried out in 29 golden snub-nosed monkey samples (including fecal samples) with 37 microsatellite markers. The results showed that the genotyping figures of D17S791 and D4S431 were disordered, D16S539 was not monomorphic, and GM213 and D6s287 were both homozygous. A total of 197 alleles were detected in the remaining 32 loci. The observed heterozygosity was 0.045–0.857, the expected heterozygosity was 0.078–0.909, the polymorphic information content was 0.074–0.883, and the average polymorphic information content was 0.599 (Table 4). The amplification rate of blood samples and tissue samples was 100%, and no zero allele was observed. The PCR effect of stool samples was relatively poor, and four loci (D11s925, D2s141, D6s287, D16S539) failed to amplify in this test.

The 12 blood/tissue samples were subjected to HRM typing using 37 SNPs, and 29 loci were successfully genotyped, three were monomorphic (008, 068, 124), and two were homozygous (014, 043). The observed heterozygosity was 0.083–0.583, the expected heterozygosity was 0.083–0.518, the polymorphic information content was 0.077–0.373, and the average polymorphic information content was 0.244 with the remaining 24 loci (Table 5). To test the effect of this technology on stool samples, we amplified 046 with fecal DNA above. The dissolution curve failed to genotype by disorder, and the results of some samples failed to repeat. In this study, the CE-1P was 0.9582, while the CE-2P was 0.7715 for the 24 SNPs (Fig. 1).

In the parentage testing for three offspring processed by 24 SNPs, there was one instance in which one's biological father was mistakenly referred to as his sibling in the process of parentage identification using the above 24 SNP loci. ML-Related software was used for further analysis. The software estimated the kinship between individuals using

Table 4 Thirty-two microsatellite loci information of 29 samples

Loci	Allelic numbers	HObs	HExp	PIC	NE-1P	NE-2P	NE-PP
GM206	13	0.500	0.909	0.883	0.356	0.216	0.073
D13s159	13	0.500	0.883	0.849	0.424	0.268	0.104
D20s206	8	0.609	0.850	0.810	0.508	0.336	0.162
GM105	8	0.625	0.833	0.791	0.538	0.364	0.185
D5s1457	7	0.458	0.833	0.790	0.545	0.369	0.192
D17s1290	9	0.478	0.824	0.778	0.560	0.384	0.205
D7s1830	6	0.474	0.824	0.773	0.571	0.393	0.213
D1s533	8	0.615	0.811	0.768	0.570	0.393	0.208
D7s503	10	0.857	0.801	0.766	0.565	0.385	0.189
D14s306	7	0.640	0.800	0.754	0.589	0.411	0.223
D6s474	7	0.625	0.791	0.745	0.604	0.423	0.233
GM108	8	0.545	0.789	0.739	0.606	0.428	0.237
D7s2204	6	0.565	0.790	0.736	0.625	0.447	0.267
D6s493	6	0.565	0.768	0.711	0.651	0.474	0.290
D10s1432	6	0.826	0.767	0.711	0.650	0.473	0.288
D11s2002	5	0.522	0.770	0.710	0.657	0.481	0.302
GM109	5	0.346	0.707	0.639	0.731	0.563	0.389
D11s925	4	0.667	0.703	0.619	0.748	0.582	0.406
GM220	5	0.250	0.639	0.575	0.786	0.621	0.444
D15s644	9	0.400	0.602	0.569	0.786	0.600	0.387
D18s851	3	0.348	0.656	0.569	0.794	0.648	0.498
D7s1826	4	0.375	0.645	0.561	0.794	0.647	0.490
GM214	6	0.519	0.642	0.559	0.784	0.639	0.472
D16s420	5	0.174	0.557	0.481	0.845	0.707	0.553
D6s271	6	0.160	0.495	0.460	0.869	0.705	0.525
GM227	5	0.440	0.534	0.452	0.858	0.734	0.591
D3s1768	3	0.269	0.461	0.366	0.898	0.808	0.703
GM228	4	0.045	0.289	0.267	0.959	0.850	0.737
GM209	4	0.150	0.276	0.256	0.963	0.856	0.744
D2s141	2	0.167	0.290	0.239	0.961	0.880	0.803
D11s1366	3	0.091	0.172	0.160	0.986	0.916	0.847
GM219	2	0.080	0.078	0.074	0.997	0.963	0.930

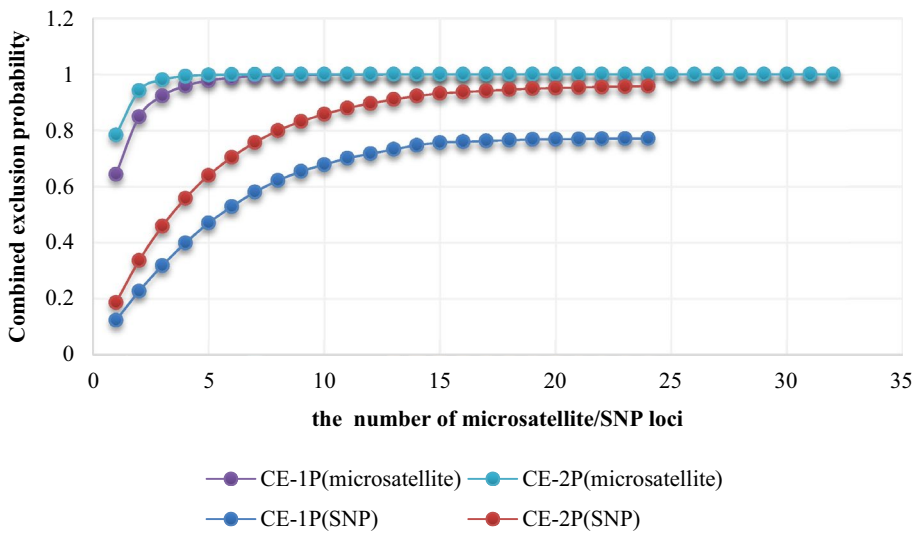

Fig. 1 The combined exclusion probability of the different number of microsatellite/SNP loci with one-parent-unknown/known (CE-1P/CE-2P)

Table 5 Twenty-four SNP loci information of 12 golden monkey

Loci	Allelic numbers	HObs	HExp	PIC	NE-1P	NE-2P	NE-PP
002	2	0.083	0.083	0.077	0.997	0.962	0.928
003	2	0.583	0.431	0.328	0.915	0.836	0.747
015	2	0.167	0.159	0.141	0.988	0.929	0.874
017	2	0.083	0.083	0.077	0.997	0.962	0.928
020	2	0.583	0.489	0.359	0.890	0.821	0.729
022	2	0.583	0.489	0.359	0.890	0.821	0.729
029	2	0.500	0.464	0.346	0.901	0.827	0.737
039	2	0.583	0.518	0.373	0.877	0.813	0.720
046	2	0.250	0.344	0.275	0.946	0.862	0.779
051	2	0.083	0.083	0.077	0.997	0.962	0.928
061	2	0.083	0.083	0.077	0.997	0.962	0.928
064	2	0.333	0.290	0.239	0.961	0.880	0.803
079	2	0.500	0.391	0.305	0.930	0.848	0.761
087	2	0.167	0.159	0.141	0.988	0.929	0.874
088	2	0.167	0.159	0.141	0.988	0.929	0.874
094	2	0.333	0.507	0.368	0.882	0.816	0.723
095	2	0.333	0.507	0.368	0.882	0.816	0.723
098	2	0.333	0.507	0.368	0.882	0.816	0.723
103	2	0.500	0.391	0.305	0.930	0.848	0.761
111	2	0.083	0.083	0.077	0.997	0.962	0.928
112	2	0.167	0.159	0.141	0.988	0.929	0.874
113	2	0.500	0.507	0.368	0.882	0.816	0.723
114	2	0.417	0.344	0.275	0.946	0.862	0.779
115	2	0.417	0.344	0.275	0.946	0.862	0.779

the maximum likelihood method. According to the software results, the SNP marker operation was able to obtain an identification result consistent with the records after the sibling individual was excluded from the suspected sire list.

The criterion for the combined exclusion probability of non-single-parent was PE > 0.9973 in the international human parentage test. In this study, a minimum of five and up to nine microsatellite loci could meet the requirements of the standard if the dam was known or unknown. According to this study, CE-1P of nine loci reached 0.9977 (top 9 in Table 4), and CE-2P of five loci reached 0.9974 (top 5 in Table 4) (Fig. 1). However, the number of samples used in this study was low. When large-scale studies are carried out in the national captive population or wild population of the golden snub-nosed monkey, the combination may be changed.

The results of this study showed that the SNP loci did not meet the identification requirements. In order to understand the demands for SNP loci in the parentage testing of golden snub-nosed monkey, we doubled the 24 loci and calculated the 48 SNP loci using Cervus software. It was found that the combined exclusion probability of the no-parent reached 0.998 when the mother was known. However, the combined exclusion probability of the no-parent was 0.948 when the mother was unknown, which was still far from meeting the requirements. Therefore, in the follow-up study, more loci need to be developed for the SNP analysis.

A microsatellite locus can have multiple alleles, while a single SNP locus usually has only two alleles. Therefore, the polymorphism of a single microsatellite marker was higher than that of a SNP marker. The polymorphism of markers was the most important factor affecting the accuracy of parentage testing. The exclusion rate of loci was related to locus polymorphism: the lower the polymorphism, the smaller the exclusion rate (Zhang 2017). In order to meet the requirements for identification, it was necessary to increase the number of SNP loci (Liu et al. 2017; Zhang 2017). The study indicated that the number of SNP loci required was also related to the species population size of the estimated model. When the population was larger, the number of SNP loci required to meet the accuracy requirements of the identification was higher (Turakulov and Easteal 2003; Yu et al. 2015).

The results showed that the presence of siblings might lead to parentage identification errors in the absence of loci polymorphic information. Even though the parentage testing has been applied to several specific species, a large number of animals still do not have molecular markers or

only a small number of molecular markers that have been developed. In this case, using ML-Related software to calculate the sibling/half sibling relationship and narrowing the suspected parentage range could be considered as an aid to improve accuracy, though it did not help improve the combined exclusion probability of the no-single-parent of the molecular markers.

Compared with the other sampling methods, fecal samples had the advantages of high availability as well as being non-invasive (Shan et al. 2018). However, fecal DNA is mainly obtained from intestinal exfoliated cells, so there were some problems such as low quantity, low quality, serious degradation, and low purity. Although the microsatellite amplification effect of the stool sample in this experiment was inferior to that of the blood sample, a large amount of data was still obtained. In the future, depth screening and optimization of experimental conditions could further improve the success rate, however, the SNP typing of fecal samples carried out by the HRM method could not accurately classify the dissolution curve in this study. This may be related to the loci we used, and does not necessarily represent the amplification ability of all SNP loci in feces. The results also showed the reading error of the HRM dissolution curve was greater than that of the gene sequencing for the fecal samples.

In this study, the accuracy of the two popular molecular markers was discussed in the parentage testing of golden snub-nosed monkey, and the results provided some reference significance for future research. Throughout the history of human parentage testing technology, as well as the laboratory's research experience in the field of parentage testing of endangered animals, such as giant pandas and red pandas, the establishment of a set of molecular markers has been a long-term process that requires patient and continuous development and screening. We expect more microsatellite markers and SNP markers to be developed and form a mature identification system in the future to provide greater resources for the conservation of golden snub-nosed monkey.

In conclusion, a small number of microsatellite loci (five to nine) with high polymorphism could be used to complete individual identification and parentage testing in this study. It was important to use microsatellite sites for individual identification and parentage testing because of the difficulty of sampling and the lack of samples of endangered species. However, there were some problems such as genetic variation and invalid alleles in microsatellite loci. There were some problems such as large samples and more loci were needed for identification, although SNP was more sensitive than microsatellite, and it has become a more favorable alternative marker in the world. In the future, researchers could choose a more suitable method for individual identification and parentage testing according to their own experimental conditions.

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Compliance with ethical standards

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

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