

Isolation and characterization of 113 polymorphic microsatellite loci for the Tibetan frog (*Nanorana parkeri*) using next generation sequencing

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Abstract We developed and characterized 113 polymorphic microsatellites in the Tibetan frog (*Nanorana parkeri*) using 454 GS-FLX next generation sequencing technology. These loci were tested in 46 individuals from two *N. parkeri* populations from the Tibetan plateau. The average number of alleles per locus was 8.09 (range = 2–20). The average observed heterozygosities (H_O) per locus in the two populations were 0.58 (range = 0.04–1) and 0.29 (range = 0–0.87), respectively. These microsatellites will be useful for population and conservation genetics studies of *N. parkeri* and other closely related species.

Keywords Microsatellite · *Nanorana parkeri* · Tibetan plateau

Nanorana parkeri is a frog species endemic to the Tibetan plateau and occurs across a relatively narrow latitudinal (28–31°N) and longitudinal (84–97°E) region (Ma et al. 2009). It occupies an extensive altitudinal range from 2850 to 5100 m above sea level (m.a.s.l.), thereby being the amphibian species with the highest altitudinal distribution in the world and the only species of ranid frog found above 4500 m.a.s.l. (Hu et al. 1987; Zhang et al. 2012). However, the information regarding population genetic structure, mating system and dispersal patterns remain unknown due to the lack of molecular genetic markers. We describe here the development of a total of 113 polymorphic microsatellites in *N. parkeri* using 454 GS-FLX next generation sequencing technology. The markers will be useful for population genetics and genomic investigations of this species.

Two adult individuals of *N. parkeri* were sampled from Maizhokunggar County (29.7539°N, 91.6682°E; 3800 m.a.s.l.), Tibet, China. The genomic DNA was extracted from behind legs using the standard phenol–chloroform extraction protocol (Sambrook and Russell 2001). The two individuals were sequenced using 454 (Roche) GS-FLX genome sequencer and each was sequenced on 1/8 of a Pico Titer plate. We obtained 150008 reads amounting to a total of ca. 53 Mbp (average read length = 359 bp). Sequences containing di-, tri-, tetra-, penta- and hexanucleotide repeat motifs were identified using the Tandem Repeats Finder program (Benson 1999) with the default conditions. Redundant sequences were removed by aligning the sequences using the program DNASTar version 7.1 (DNASTAR Inc, Madison, WI, USA). Out of 1588 reads with repeat motifs, 361 sequences (22 %) with six or more repeats and sufficient flanking regions were retained for primer design. Primer pairs were designed by the program Primer3 version 1.1.1 (Rozen and Skaletsky 2000). The

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Table 1 Details for 113 polymorphic microsatellite loci isolated from *Nanorana parkeri*

Locus	GenBank accession no.	Repeat motif	Primer sequences	Fluorescence labels	T_a (°C)	Size range (bp)	N_A	H_O	H_E
Npar001	KC818479	(TG) ₁₅	F: TGTTTCCCACCTTTGATGT R: GTCGGCAGCCACTATTTTC	FAM	55	351–375	8	0.383	0.421
Npar002	KC818480	(ATA) ₈	F: TGGCGCTATATAAATCCTG R: GCATTGCCTATTACAACACT	FAM	55	134–146	3	0.064	0.063
Npar003	KC818481	(TC) ₁₂	F: CCGCTTTTATTTACCAG R: CAATGAGGATGGAGAACAG	TAMRA	55	189–279	12	0.319	0.696
Npar004	KC818482	(GATA) ₇	F: ACTGGCAGGTTTACACTTAC R: GACGACAGAGCAGCACC	HEX	55	235–255	6	0.426	0.509
Npar005	KC818483	(ATAC) ₆	F: GCTTACTGCATACAGGGAGAG R: CTGGCTCAGGCTAAACAGAT	TAMRA	55	202–214	3	0.149	0.142
Npar006	KC818484	(TCTA) ₂₉ (TCTG) ₇	F: TACACCTTTATTTTGAAAAGAG R: ATATTACCACAGAGGGCTAC	FAM	55	330–382	14	0.897	0.897
Npar007	KC818485	(ATAG) ₁₁ (ATGG) ₄	F: ATGATTGATTTTGTTCACAC R: TGACGCTTGCTAGGAGTTC	HEX	55	298–366	13	0.723	0.856
Npar009	KC818487	(ATAG) ₉	F: TCTGCTGTCTGTCAGGACT R: ATGTTCCAGATACCAGTGCC	FAM	55	342–390	10	0.723	0.787
Npar010	KC818488	(CATA) ₁₂	F: TCTCAAGTTCCTTATGCC R: TAATCATTACCAGCCCTAAT	FAM	55	186–230	12	0.766	0.828
Npar011	KC818489	(GATA) ₁₇ (GATG)	F: GTTTCCTCGCTCAGGTTGT R: CAATGATTTGGAGGGTGTC	HEX	50	241–309	15	0.804	0.859
Npar012	KC818490	(TAT) ₉ (TA)	F: CCATTCTTGATGGTGGTAC R: AAACCCTATTGTTCTGAAAC	TAMRA	50	239–254	2	0.085	0.082
Npar013	KC818491	(TAT) ₁₀ (GAT) (TAA)	F: AAGCCATTTCTATACGACAT R: ACCGAGCCTAAACACCTT	FAM	55	338–362	5	0.213	0.519
Npar014	KC818492	(TTA) ₈	F: AATGGGTACGTTTGACTGG R: CAGGTCTACGTTGTGACAGT	FAM	55	191–203	4	0.213	0.301
Npar015	KC818493	(AGAT) ₁₃	F: GAGCACAGTTAGGTTTCATAG R: CACTAATATGATACCAGTGC	FAM	55	255–295	11	0.702	0.787
Npar016	KC818494	(ATCT) ₉ (ATTT) ₂	F: CTGACCTACCACCGGCTAT R: CACCGTGTACATTCTTTACC	TAMRA	55	237–253	2	0.064	0.063
Npar017	KC818495	(TATC) ₁₄	F: CTGTATCCCAGGCACCAT R: ACCAAGGACTACGAGAACC	FAM	50	313–353	11	0.851	0.860
Npar018	KC818496	(TTA) ₁₂ (TT) ₃	F: AGGGGCGTGTATGATAGG R: GCAGGGATTCACAGAGG	FAM	55	192–198	2	0.128	0.121
Npar019	KC818497	(TATC) ₁₄	F: CATTAGATTTAATTTGGGTC R: CATTAGATTTAATTTGGGTC	HEX	55	262–294	9	0.702	0.822
Npar020	KC818498	(TAA) ₁₀ (TA)	F: GAAATACATTGGGACCTGTG R: GTGAACCGAGCTTGAGGG	TAMRA	55	245–254	4	0.192	0.609
Npar021	KC818499	(TATC) ₁₅	F: ATGTGATGCCGCACTTTCT R: TCTGATGGGAGCGTCTGTT	FAM	50	332–364	8	0.723	0.799
Npar022	KC818500	(TAGA) ₈	F: TTCGCAAAGACAAGATGGT R: CATTGTGAGTGCCCTGAT	FAM	55	196–264	15	0.766	0.846
Npar025	KC818502	(AGAT) ₁₇	F: TAATTTGCATCTAAGCACAGT R: GGGAACTTTATGGGTCAAC	FAM	55	293–337	11	0.723	0.808
Npar026	KC818503	(TATG) ₁₁	F: AATCGCAAGCGTCCAGAC R: CGGTGACTCCGTACAGGTC	FAM	55	181–247	15	0.830	0.867
Npar027	KC818504	(GATA) ₁₂ (GATG) ₄	F: GTAGCCACAACATCCAGT R: AGAGCCCAGCTTAACTCTT	HEX	55	257–289	9	0.489	0.641
Npar028	KC818505	(TCTA) ₁₆ (TCAA)	F: TTACTTTGCTGGTCTCCCT R: ACTGTACTCTGGTGCTTTG	TAMRA	55	222–262	11	0.745	0.758

Table 1 continued

Locus	GenBank accession no.	Repeat motif	Primer sequences	Fluorescence labels	T_a (°C)	Size range (bp)	N_A	H_O	H_E
Npar029	KC818506	(ATA) ₉	F: ATGACATTGGTGGTTGGT R: ACAGACATAAAGAGGCATT	FAM	55	321–348	5	0.213	0.236
Npar030	KC818507	(AG) ₁₃	F: GCTTTGTAGAGCAGCGGTAG R: GCTCATCACAAAGCTCCCAC	FAM	55	189–195	4	0.192	0.230
Npar031	KC818508	(TA) ₂₃	F: GCTGACCGTGTCCATCCCT R: CCTTATGAGCTGCACCCAGT	HEX	55	264–284	5	0.222	0.561
Npar032	KC818509	(TG)(CA) ₂ (TGTA) ₉	F: TGGACTTATCGCACAGGTAG R: TAGATCGAGGATATGGAGATG	TAMRA	55	228–264	8	0.575	0.775
Npar033	KC818510	(CA) ₁₂	F: TAGCCTTACCAGTGTCTCC R: GCAAGTGTTTGTCTGGGTT	FAM	55	304–322	7	0.426	0.639
Npar034	KC818511	(GATA) ₁₂	F: AGCAGAAAATGAGCACTAT R: TGAAAATGGGGTCTACTCT	FAM	55	168–236	17	0.723	0.890
Npar035	KC818512	(AGAT) ₆	F: ATGGAGCTGAAAAGCAGG R: GGCAGGTACAATGTAAACG	HEX	55	252–272	5	0.170	0.200
Npar036	KC818513	(AAT) ₉	F: GCACAAACTGTTGGCACT R: TGACCCACTGTTCTCCTTG	TAMRA	55	223–235	4	0.277	0.357
Npar038	KC818515	(CATA) ₁₀	F: TGCAGCCTCATAGTTGTAT R: ACTGGAGTGATGCTTTGTT	FAM	55	170–202	8	0.702	0.760
Npar039	KC818516	(ATCT) ₆	F: CCACCTCTGTCCCTCCT R: TGCCACTGGAGTTCAATAC	HEX	55	252–296	9	0.447	0.694
Npar040	KC818517	(ATT) ₁₁	F: GTCACCAGTATTGCCTCT R: GCTACCTCCTCATGTATCA	TAMRA	55	223–235	5	0.319	0.388
Npar041	KC818518	(TGG) ₉	F: TTGTGTTGTGCCCTTACC R: GCTCCAGGGACAGACC	FAM	55	314–323	4	0.298	0.549
Npar042	KC818519	(CAT) ₈	F: ACATACTTGAGACATCCCAC R: CTGGACGAGCAAATACAAG	FAM	55	185–203	4	0.277	0.264
Npar043	KC818520	(TAGA) ₂₂ (TTGA)	F: TTTCACAGATGTCAGCCAAGA R: AGCAGGTAGGCAGGGAAGG	HEX	55	217–257	10	0.702	0.878
Npar044	KC818521	(ATT) ₈	F: ATCTAGCTGGCAGATGAAT R: TAAGACCGACCACCGAAC	TAMRA	55	222–228	3	0.319	0.390
Npar045	KC818522	(ATT) ₉	F: GCTTCATCTTTCAGTGGC R: CAGGGGCTCTGTTTACC	FAM	55	302–305	2	0.106	0.271
Npar046	KC818523	(GT) ₁₃	F: GAACCTGCTGATTCCAAG R: TGTAACCACATGCCTAATAC	FAM	55	190–198	4	0.043	0.084
Npar048	KC818525	(TTAG) ₁₁	F: AAGGTACGAGCCAGAATCAC R: GCTGCCAAATGGTCAACT	TAMRA	55	208–224	5	0.553	0.490
Npar049	KC818526	(TGTA) ₉	F: ACCTATTGGCAAGTCAC R: TTTACAGTATTACAGGCATT	FAM	55	303–315	4	0.277	0.480
Npar050	KC818527	(TAGA) ₂₁ (CAGA) ₆	F: AGGCTTGTTTCCTTCTTCTG R: TCTAGTGTGCTGGCTGTG	FAM	55	171–227	15	0.889	0.882
Npar051	KC818528	(TATC) ₉ (TAT)	F: CATTATTCATAACTGCCACC R: CTGGAGCCACTGCTTTGTT	HEX	55	247–255	3	0.319	0.553
Npar052	KC818529	(AATG) ₆	F: TCATACAACCAGAACCAGC R: AGGACTAAGAATACACCC	TAMRA	55	174–214	2	0.234	0.241
Npar053	KC818530	(CATA) ₉	F: CCGACACGCTGAGGAAG R: ATGGGAATCTGGGATGC	FAM	55	294–310	4	0.277	0.488
Npar054	KC818531	(GATA) ₁₃	F: CGTGGGCCACATAAATAAG R: TTGGAAAATCAACTCAAAAG	FAM	55	180–202	9	0.532	0.750
Npar055	KC818532	(TA) ₁₅ (TC)	F: TGGAATTTATTAATAACTGGAGC R: AGGGGTCAGGTTTGTGATGTT	HEX	52	198–268	7	0.106	0.534

Table 1 continued

Locus	GenBank accession no.	Repeat motif	Primer sequences	Fluorescence labels	T_a (°C)	Size range (bp)	N_A	H_O	H_E
Npar056	KC818533	(ATCT) ₁₈	F: GGCTACTCGGGACTGTTAT R: AGCGACAGGCATACTCTTAT	TAMRA	55	196–252	14	0.787	0.807
Npar057	KC818534	(GGT) ₁₃	F: TGACTGTCTCGGGATTTC R: CTTTTGGCAGATTACCC	FAM	55	286–306	5	0.023	0.068
Npar058	KC818535	(CTAT) ₁₆	F: GAGTGTAACCAAAACAAATC R: CAGTTGACAGGGCTAAAAGT	FAM	55	168–208	11	0.766	0.851
Npar059	KC818536	(TCTA) ₂₃	F: TTGTTATCGCTCCCAAAC R: GACAGGAGAAACAGCAGAC	HEX	55	228–272	11	0.681	0.761
Npar060	KC818537	(ACAT) ₉	F: ACGCAGTGCTTTACAATCT R: TTTCTGGTGACACTATCTG	TAMRA	55	198–210	4	0.277	0.354
Npar061	KC818538	(ATAG) ₁₂	F: GGCCTGGATGATGGACT R: ATTGAACCCTACGGACTAAG	FAM	55	295–331	10	0.745	0.797
Npar062	KC818539	(TA) ₂₆ (GA) ₄	F: CTTTGTCTTCCAAGCCACC R: AATTAGCCCTCCTTAACCT	FAM	50	173–197	6	0.383	0.584
Npar063	KC818540	(AGAT) ₇	F: AGCAGCTCTACAGTTCTG R: TAGGGATAGAGCCAATGTG	HEX	55	234–246	4	0.128	0.521
Npar064	KC818541	(ATAC) ₈	F: TACAGGGATATGGAACTAC R: CTATTCATTCTGCCTCTTGC	TAMRA	55	203–227	7	0.447	0.626
Npar066	KC818542	(GT) ₁₇	F: TTCCTGGGTTTGGTTTGT R: GCATTTTGTCTGGCTAC	FAM	55	160–210	12	0.298	0.523
Npar067	KC818543	(TATC) ₁₂	F: AAACCATAAGAGAGTTTACAAT R: TGCCAGTATGGTCAAAG	HEX	55	230–262	9	0.617	0.647
Npar068	KC818544	(ATAC) ₂₇ (AC) ₆	F: GGAAGTGATCCCCATTTCG R: GCTGACTTTCTGAAGCAAAC	TAMRA	55	177–245	11	0.702	0.773
Npar069	KC818545	(CTAT) ₁₅	F: GATTAGCCAATAGGGGAAGC R: CCATCGTAGAGCCAATCAAAC	FAM	55	246–330	17	0.681	0.883
Npar070	KC818546	(TATC) ₁₂	F: ATTTGAAGGGTAAGAGTGG R: CTCAGTGCCAGCTACTTATC	FAM	55	178–210	9	0.553	0.763
Npar071	KC818547	(TAGA) ₁₁	F: CAGATGAGAATGGTGGCAG R: ACCCCTAGAAACCAGAACTT	HEX	55	219–263	11	0.787	0.808
Npar072	KC818548	(ATCT) ₁₈ (CT) ₂	F: TGACGGGTGTACTCACTTCT R: GCATCTGTTTATCTTCTAGGAT	TAMRA	55	189–239	14	0.851	0.867
Npar073	KC818549	(TAGA) ₁₄	F: AAGAGGAAGTGATGACGG R: TGGATACACCACTGATCG	FAM	55	267–327	11	0.511	0.799
Npar074	KC818550	(TCTA) ₁₀	F: CGGTAGTGGTTTCTCAG R: CTCCAAATGCACCTACG	FAM	55	177–225	11	0.617	0.796
Npar076	KC818551	(GATA) ₈	F: CAGACAGACCGATAGACAG R: GACTAAGAAACCGCCAGAC	TAMRA	55	204–248	9	0.787	0.833
Npar077	KC818552	(TCTA) ₁₈	F: AAATCTGCGTGGGTTCGGTT R: CAGGCTGGGGCTTTACTGG	FAM	55	270–338	13	0.644	0.845
Npar078	KC818553	(TTG) ₁₀	F: TAAACAACCTCCTCCTCTT R: AGATTAGCCTGGATCAGC	FAM	55	165–177	4	0.255	0.248
Npar079	KC818554	(ATCT) ₂₀	F: TAGGAACTGGCAGTGGGAT R: TTAAAGGGAACGACAACAG	HEX	55	225–253	8	0.766	0.824
Npar080	KC818555	(TCTA) ₁₂	F: CAGATGCAGGAAGTGAAT R: TGTGACTGACGACCGAAT	TAMRA	55	264–316	10	0.766	0.780
Npar081	KC818556	(ATAG) ₁₀	F: GTGGACCGCAAATCGCAG R: CCCGCTAATTGAACAACAT	FAM	55	264–326	11	0.787	0.792

Table 1 continued

Locus	GenBank accession no.	Repeat motif	Primer sequences	Fluorescence labels	T_a (°C)	Size range (bp)	N_A	H_O	H_E
Npar082	KC818557	(CTAT) ₁₅	F: GCAAGAGCTTTAATCCTTAC R: GGTGGCTGGAATACAAAC	FAM	55	150–178	8	0.702	0.740
Npar083	KC818558	(TG) ₂₁ (AG) ₂	F: CGGAAGATGCTGAGATTAG R: TCAGAATGTTATGGCTGAT	HEX	55	229–252	6	0.426	0.411
Npar084	KC818559	(AAAT) ₇ (AC) ₂	F: CACGGGACAAATTAAGAGT R: ATACAGTTGAGAAGGGTGG	TAMRA	55	196–200	2	0.106	0.102
Npar085	KC818560	(CTAT) ₁₃	F: GGTGTTATAAACAGACTTTTCT R: CAGTCTAGCCTTAATTACAATA	FAM	55	278–322	10	0.6809	0.735
Npar086	KC818561	(GATA) ₆	F: GTAGTGAAGCCATTGATGAT R: CTGCGGTGATGTTCTTTC	FAM	55	165–193	5	0.1064	0.124
Npar087	KC818562	(TG) ₁₂	F: ATACACGGATAGATTTCAAAC R: TACCCTTAATAACGAGATGAC	HEX	52	211–241	10	0.447	0.680
Npar088	KC818563	(AC) ₁₇ (AG) ₃	F: CCGAGAAGTACACGAGG R: AGGTCCATCCAGTACAACATC	TAMRA	55	274–284	3	0.128	0.518
Npar089	KC818564	(TGTA) ₁₀	F: CTTCGTGGCTGAGTTGCTGT R: CGGGCTCAAATTGTAAAAGAGT	FAM	55	275–307	7	0.553	0.720
Npar090	KC818565	(TACA) ₈ (CA) ₂	F: GACTTTGTGAGGGGAAAC R: AACCTGTACTATAAATAACGG	FAM	55	134–166	3	0.085	0.584
Npar091	KC818566	(TA) ₁₄	F: TCAAGAACAGGCTATGAAAC R: AGCGAGATGTGAGATTGG	HEX	55	219–221	2	0.128	0.121
Npar092	KC818567	(TTG) ₁₂	F: CATTCTGGGATTATGAG R: TACCTGGAAGTAAAGCAG	TAMRA	55	265–280	6	0.362	0.576
Npar093	KC818568	(GATA) ₂₇	F: ATCCATACGGTGGGCAAAT R: GACCCAGAAACTGACACTTCA	FAM	55	246–350	18	0.553	0.813
Npar094	KC818569	(ATCT) ₁₇	F: TTTGACAGTTGGCTGACTC R: TAAAATAGAATGGCAGGAT	FAM	55	160–228	16	0.702	0.848
Npar095	KC818570	(TATC) ₁₅	F: GCAGGTAGGGGTAGTAAAT R: GTCACCACTTTGTAGACTTGT	HEX	55	210–280	18	0.745	0.901
Npar097	KC818572	(AAT) ₁₂	F: AAGATAGATTTATGGGAGTAGC R: TGGCAGCATTTATAGAGTTG	FAM	50	280–289	4	0.404	0.438
Npar098	KC818573	(TTG) ₈	F: TCCGATTGGGAAGACTGT R: GTCGGCACATAGGGACAC	FAM	55	157–187	11	0.553	0.787
Npar099	KC818574	(GATA) ₁₄	F: CAATGCAATATGAAAAGCTC R: ACTGTCCTGTCGGTTAGGC	HEX	55	197–253	15	0.8723	0.905
Npar100	KC818575	(TAT) ₈	F: TGGAGCACTGTCAAAGC R: GTATTGCGATTGTAATGTCT	TAMRA	55	259–370	7	0.128	0.659
Npar101	KC818576	(AGAT) ₁₃	F: ACCCTCTTCGTTTCGCTAT R: GAACAGTACAGGCTGCTTC	FAM	55	258–314	12	0.575	0.777
Npar102	KC818577	(TGTA) ₁₃ (TG) ₆	F: ACAGAAGAAGCCAGCAATG R: GATAGGTGGGAGCAAGGTA	FAM	55	151–171	6	0.234	0.285
Npar103	KC818578	(AAT) ₁₁	F: AAGTCACATCACAAGTTTC R: TTCTCAAGCAAAAAGGATAAG	HEX	55	199–214	5	0.362	0.416
Npar104	KC818579	(AAT) ₁₀ (GAT) ₂	F: GCCCTCTATTCCTTTTGT R: CTACTTTCCTGCTTCCAT	TAMRA	55	257–269	4	0.255	0.564
Npar105	KC818580	(AC) ₁₅ (GC)	F: AGAGCCATTTACATCCACG R: TAGCCACAATCATATTCCTT	FAM	55	279–333	12	0.064	0.820
Npar106	KC818581	(CTAT) ₉ (CCAT)	F: GATGGGAAGCAACCAAAAC R: CGACTGGACAGCGGCACT	6-FAM	55	142–158	4	0.234	0.593

Table 1 continued

Locus	GenBank accession no.	Repeat motif	Primer sequences	Fluorescence labels	T_a (°C)	Size range (bp)	N_A	H_O	H_E
Npar107	KC818582	(TG) ₁₆ (TC) ₂ (TT)	F: GAAAGTGGTGGGAGTGAG R: GAAAACCTAATAAAGACAGGC	HEX	55	193–309	20	0.596	0.933
Npar109	KC818583	(ATA) ₈	F: CACCTGTCTTTACATCCAT R: GTCGTTTATTAATTTATTGAGC	FAM	55	267–282	6	0.340	0.594
Npar110	KC818584	(CA) ₁₅	F: AAATGGTTTCCCTTGAT R: ACTATCTGAGCACAGTAT	FAM	50	147–183	8	0.106	0.610
Npar112	KC818585	(TATC) ₂₅ (TA) ₂	F: AGATTTGAGCTAGGCTGC R: AGAAGATATTGCGAGTGG	TAMRA	55	239–291	13	0.723	0.868
Npar114	KC818586	(TCT) ₁₀	F: GATTAACCTCTGCGGTACG R: GGAGATTCCCCTGTAAGAC	FAM	55	133–148	5	0.468	0.554
Npar115	KC818587	(AGAT) ₁₁	F: CTGTTAGCAAGACACCACCAT R: AAGCAAGTCAACCCATTTCCG	HEX	55	185–229	10	0.404	0.624
Npar116	KC818588	(TAA) ₁₅ (CAA) ₂	F: CCACATTCCAATACCTCCT R: CTAGAGTTTCCCACCAGTT	TAMRA	55	253–268	6	0.702	0.759
Npar117	KC818589	(CTAT) ₁₃	F: GCTATACTACCCTATCTTTG R: TTGTGTCAGTACATACCCTGT	FAM	55	267–291	7	0.702	0.755
Npar118	KC818590	(TAGA) ₈ (TAGC)	F: TGAGGATCTACAGCCAAAT R: TCAATGTAAATACTTGTGGGT	FAM	55	146–186	10	0.468	0.659
Npar119	KC818591	(ATCT) ₁₅	F: TTATGTTTGCTTACCTTTTC R: TATGCCTAGAACTTGATGC	HEX	55	186–254	12	0.553	0.716
Npar120	KC818592	(TCTA) ₁₄	F: CATAGTAAGGGCAGGAGC R: GAGCGAGGATGGGAAATAC	TAMRA	55	255–279	7	0.638	0.715
Npar121	KC818593	(TCTT) ₁₆	F: TTCATAGCACCCAGTCTTAG R: CGCTCTCAACCAATTTGC	FAM	55	270–294	6	0.468	0.651
Npar122	KC818594	(ATA) ₉ (ATT) ₂ (TA) ₄	F: GATGGATTTGGGTTGTTT R: ATGTGACCGGCCAGAAG	FAM	55	130–142	2	0.170	0.256
Npar123	KC818595	(TTAT) ₆	F: GGCATATTGGCTCTGTTGT R: TTTCTCACCTCCTGTTTG	HEX	55	199–203	2	0.319	0.431
Npar124	KC818596	(AG) ₁₄ (AC)	F: GTGACAATTTAGAAGGACGGC R: TGTTGCCCAACTGTAAGC	TAMRA	55	247–259	6	0.340	0.634

T_a the annealing temperature of the touchdown PCR thermal cycling, N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

five most common motifs were AC, TC, ATCT, AT and ATT.

Ten samples from different populations in Lhasa region (Tibet, China) were used for preliminary testing of all the primers. Non-denaturing 8 % polyacrylamide gel electrophoresis (PAGE) was used to examine polymorphism of the loci, and 124 pairs of primers were found to be potentially useful. After the initial tests, template DNA of 46 individuals from two populations (23 individuals from each) was further used to test polymorphism of the 124 loci. The two populations were Maizhokunggar and Leiwuqi (31.1572°N, 96.3571°E; 4302 m.a.s.l.) from central and southeastern Tibet, respectively. The forward primers of these loci were labeled with FAM, HEX or TAMRA fluorescent dyes (Invitrogen, Life Technologies Corporation, Shanghai). Multiple PCR was performed for 3–4 pairs of primers per 20 μ l reaction volume mixture which

contained 30 ng of genomic DNA, 0.2 μ M of each PCR primer and 10 μ l Premix Ex Taq II Version 2.0 [2 \times ; TaKaRa Biotechnology (Dalian) Co., Ltd]. A touchdown PCR protocol was used for multiplexing: 94 °C for 5 min, followed by 16 cycles at 94 °C for 30 s, 63 °C for 30 s (decreased 1 °C per cycle), 72 °C for 40 s, then 25 cycles at 94 °C for 30 s, T_a (Table 2) for 30 s, 72 °C for 40 s, then a final extension at 72 °C for 10 min, using a Mastercycler[®] Pro S thermal cycler (Eppendorf China Ltd, Beijing). PCR products were genotyped using an ABI PRISM 3730xl DNA Analyzer (Applied Biosystems, Life technologies, NY, USA) together with a size standard ET-550-R. Genotypes were scored using GeneMarker version 1.75 program (Softgenetics, State College, PA, USA). Of the 124 loci screened, six loci were discarded because of multiple peaks and five loci were monomorphic in both populations. The observed (H_O) and expected (H_E)

Table 2 Genetic variability of the 113 microsatellite loci in the two *Nanorana parkeri* populations from the Tibetan plateau

Locus	Maizhokunggar ($n = 23$)				Leiwuqi ($n = 23$)			
	N_A	H_O	H_E	P_{HWE}	N_A	H_O	H_E	P_{HWE}
Npar001	8	0.609	0.603	0.4879	3	0.130	0.127	1.0000
Npar002	3	0.087	0.086	1.0000	1	0	0	NP
Npar003	12	0.609	0.819	0.0055	1	0	0	NP
Npar004	6	0.696	0.688	0.5285	2	0.130	0.125	1.0000
Npar005	3	0.261	0.240	1.0000	1	0	0	NP
Npar006	11	0.913	0.876	0.7347	7	0.870	0.736	0.9158
Npar007	7	0.565	0.559	0.7007	8	0.870	0.844	0.7762
Npar009	9	0.696	0.813	0.1608	4	0.739	0.675	0.8432
Npar010	12	0.870	0.898	0.1452	3	0.652	0.577	0.5679
Npar011	13	0.909	0.914	0.5181	5	0.696	0.727	0.3012
Npar012	2	0.174	0.162	1.0000	1	0	0	NP
Npar013	5	0.435	0.476	0.2663	1	0	0	NP
Npar014	4	0.391	0.501	0.1797	1	0	0	NP
Npar015	10	0.739	0.841	0.1825	4	0.652	0.631	0.4001
Npar016	2	0.087	0.085	1.0000	1	0	0	NP
Npar017	11	0.957	0.898	0.9438	4	0.783	0.728	0.8059
Npar018	2	0.261	0.232	1.0000	1	0	0	NP
Npar019	9	0.826	0.862	0.3345	5	0.609	0.647	0.2068
Npar020	4	0.348	0.518	0.0257	1	0	0	NP
Npar021	8	0.870	0.800	0.7547	5	0.565	0.776	0.0092
Npar022	13	0.826	0.911	0.0502	5	0.696	0.674	0.6109
Npar025	9	0.783	0.703	0.9458	3	0.652	0.550	0.9050
Npar026	14	0.870	0.887	0.4180	6	0.783	0.751	0.7209
Npar027	9	0.565	0.745	0.1604	2	0.391	0.496	0.2708
Npar028	11	0.913	0.864	0.0670	5	0.565	0.570	0.5178
Npar029	4	0.391	0.409	0.4891	2	0.044	0.044	NP
Npar030	3	0.348	0.382	0.3534	1	0	0	NP
Npar031	5	0.429	0.768	0.0000*	1	0	0	NP
Npar032	6	0.696	0.659	0.4595	5	0.478	0.496	0.2182
Npar033	7	0.522	0.585	0.3498	2	0.348	0.394	0.4663
Npar034	12	0.870	0.864	0.2712	7	0.565	0.723	0.0771
Npar035	5	0.348	0.380	0.2321	1	0	0	NP
Npar036	4	0.435	0.514	0.2544	2	0.087	0.085	1.0000
Npar038	7	0.957	0.843	0.9213	2	0.435	0.394	0.8577
Npar039	8	0.609	0.628	0.1414	2	0.305	0.322	0.6202
Npar040	5	0.609	0.623	0.1260	1	0	0	NP
Npar041	3	0.565	0.501	0.8744	1	0	0	NP
Npar042	4	0.522	0.437	0.9079	1	0	0	NP
Npar043	8	0.870	0.814	0.7413	5	0.522	0.749	0.0108
Npar044	3	0.609	0.532	0.8277	1	0	0	NP
Npar045	2	0.217	0.415	0.0346	1	0	0	NP
Npar046	4	0.087	0.167	0.0222	1	0	0	NP
Npar048	5	0.609	0.496	1.0000	3	0.478	0.479	0.5846
Npar049	3	0.565	0.518	0.7631	2	0	0.085	0.0222
Npar050	15	1.000	0.932	1.0000	4	0.783	0.758	0.5778
Npar051	3	0.652	0.565	0.9275	1	0	0	NP
Npar052	2	0.478	0.372	1.0000	1	0	0	NP

Table 2 continued

Locus	Maizhokunggar ($n = 23$)				Leiwuqi ($n = 23$)			
	N_A	H_O	H_E	P_{HWE}	N_A	H_O	H_E	P_{HWE}
Npar053	4	0.565	0.598	0.0660	1	0	0	NP
Npar054	6	0.826	0.762	0.8571	3	0.218	0.202	1.0000
Npar055	6	0.217	0.477	0.0000*	1	0	0	NP
Npar056	14	1.000	0.930	1.0000	3	0.565	0.492	0.8623
Npar057	2	0.053	0.149	0.0811	1	0	0	NP
Npar058	11	0.870	0.903	0.3023	4	0.652	0.739	0.2370
Npar059	9	0.913	0.841	0.9240	2	0.435	0.394	0.8577
Npar060	4	0.565	0.567	0.5934	1	0	0	NP
Npar061	10	0.783	0.881	0.2078	4	0.696	0.600	0.9496
Npar062	5	0.696	0.599	0.8183	2	0.087	0.085	1.0000
Npar063	4	0.217	0.241	0.3103	1	0	0	NP
Npar064	6	0.696	0.769	0.0280	2	0.174	0.162	1.0000
Npar066	12	0.565	0.801	0.0296	1	0	0	NP
Npar067	8	0.696	0.773	0.3915	3	0.522	0.463	0.7855
Npar068	9	0.826	0.800	0.3377	4	0.609	0.661	0.4411
Npar069	12	0.652	0.892	0.0005	7	0.696	0.672	0.5803
Npar070	9	0.522	0.815	0.0000*	4	0.609	0.639	0.2652
Npar071	10	0.870	0.855	0.3140	6	0.696	0.727	0.5709
Npar072	11	0.957	0.879	0.8922	7	0.739	0.787	0.3615
Npar073	10	0.609	0.840	0.0038	3	0.391	0.382	0.7126
Npar074	11	0.870	0.892	0.5378	3	0.391	0.414	0.4517
Npar076	8	0.870	0.825	0.7631	6	0.739	0.807	0.0683
Npar077	11	0.571	0.868	0.0000*	7	0.739	0.789	0.4417
Npar078	4	0.522	0.445	0.9079	1	0	0	NP
Npar079	7	0.826	0.802	0.4805	6	0.739	0.771	0.5855
Npar080	10	0.913	0.872	0.4316	4	0.609	0.642	0.3300
Npar081	9	0.826	0.827	0.5646	6	0.783	0.749	0.5575
Npar082	8	0.870	0.800	0.7949	4	0.522	0.557	0.4174
Npar083	5	0.478	0.442	0.8542	2	0.348	0.348	0.7126
Npar084	2	0.174	0.162	1.0000	2	0.044	0.044	NP
Npar085	10	0.870	0.879	0.2203	3	0.478	0.444	0.7304
Npar086	3	0.174	0.166	1.0000	1	0	0	NP
Npar087	8	0.870	0.827	0.7429	1	0	0	NP
Npar088	2	0.261	0.232	1.0000	1	0	0	NP
Npar089	5	0.696	0.587	0.8812	2	0.391	0.415	0.5814
Npar090	3	0.130	0.330	0.0052	1	0	0	NP
Npar091	2	0.174	0.162	1.0000	2	0.087	0.085	1.0000
Npar092	6	0.739	0.762	0.2538	1	0	0	NP
Npar093	15	0.609	0.906	0.0000*	5	0.478	0.504	0.4927
Npar094	14	0.913	0.929	0.5440	7	0.478	0.619	0.0008
Npar095	17	0.826	0.938	0.0071	7	0.696	0.770	0.3628
Npar097	4	0.826	0.692	0.9731	1	0	0	NP
Npar098	9	0.565	0.787	0.0363	4	0.565	0.553	0.5556
Npar099	14	0.913	0.896	0.3849	10	0.826	0.871	0.0537
Npar100	6	0.261	0.514	0.0015	2	0	0.085	0.0222
Npar101	11	0.783	0.873	0.0778	3	0.348	0.465	0.1240
Npar102	5	0.217	0.314	0.0667	2	0.217	0.198	1.0000

Table 2 continued

Locus	Maizhokunggar ($n = 23$)				Leiwuqi ($n = 23$)			
	N_A	H_O	H_E	P_{HWE}	N_A	H_O	H_E	P_{HWE}
Npar103	5	0.522	0.577	0.2767	2	0.174	0.162	1.0000
Npar104	4	0.522	0.434	1.0000	1	0	0	NP
Npar105	7	0.044	0.652	0.0000*	5	0.087	0.589	0.0000*
Npar106	4	0.435	0.379	1.0000	1	0	0	NP
Npar107	15	0.391	0.877	0.0000*	11	0.783	0.889	0.1383
Npar109	6	0.652	0.711	0.3429	1	0	0	NP
Npar110	6	0.217	0.352	0.1025	1	0	0	NP
Npar112	12	0.913	0.911	0.3428	5	0.565	0.648	0.1297
Npar114	5	0.826	0.696	0.9402	2	0.130	0.125	1.0000
Npar115	9	0.783	0.813	0.5055	1	0	0	NP
Npar116	6	0.652	0.657	0.5957	3	0.739	0.571	0.9654
Npar117	7	0.826	0.844	0.2312	3	0.609	0.577	0.7631
Npar118	10	0.870	0.881	0.1229	2	0.087	0.085	1.0000
Npar119	11	0.783	0.850	0.0035	3	0.304	0.382	0.2507
Npar120	7	0.783	0.825	0.2341	4	0.478	0.522	0.3145
Npar121	5	0.435	0.716	0.0028	4	0.522	0.530	0.5691
Npar122	2	0.348	0.394	0.4663	1	0	0	NP
Npar123	2	0.652	0.496	0.9798	2	0	0.085	0.0222
Npar124	6	0.696	0.554	1.0000	1	0	0	NP

N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, P_{HWE} the value from tests for Hardy–Weinberg equilibrium, NP not performed

* Denote significant ($P < 0.05$) departure from Hardy–Weinberg Equilibrium after Bonferroni correction

heterozygosity, and the number of alleles (N_A) per locus were calculated using the Excel Microsatellite Toolkit (Park 2001). GENEPOP' version 007 (Rousset 2008) was used to test the significant deviations from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium (LE).

Basic information and genetic variation of the 113 loci are summarized in Table 1. N_A varied from 2 to 20 with an average value of 8.09. Genetic variability of the 113 polymorphic microsatellites in the two populations is shown in Table 2. Forty monomorphic loci were observed in the Leiwuqi, whereas none in the Maizhokunggar. H_O ranged from 0.044 to 1.000 and H_E between 0.085 and 1.000 in the Maizhokunggar. In the Leiwuqi, H_O varied from 0 to 0.870 and H_E ranged from 0 to 0.889. After the Bonferroni correction (Rice 1989), seven loci (Npar031, Npar055, Npar070, Npar077, Npar093, Npar105 and Npar107) were found to deviate significantly ($P < 0.05$) from HWE in the Maizhokunggar, and only one locus (Npar105) showed significant ($P < 0.05$) deviation from HWE in the Leiwuqi. Significant ($P < 0.05$) LD were observed between twelve pairs of loci in the Maizhokunggar and five pairs of loci in the Leiwuqi. Nevertheless, we did not find any pair of loci significantly ($P < 0.05$) deviating from LE in both populations.

The set of markers developed in this study should be useful for future population genetic studies on *N. parkeri* for a better understanding of population differentiation, breeding system and dispersal ability as well as for quantitative trait locus (QTL) mapping. These markers are also possibly valuable genetic resources for other closely related species.

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