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Parentage and Genetic Diversity Studies on Pangolin (*Manis javanica*) using Novel Microsatellite Markers

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Authors' Contribution
HL and JC designed research. HL analysed data and wrote the manuscript. BY developed software necessary to perform and record experiments. LL, XZ and HJ provided expertise and advice on computational analysis. XJ supported all the samples. All authors edited the manuscript.

Key words
***Manis javanica*, Microsatellites, Wildlife, Paternity testing, Genetic diversity**

ABSTRACT

The Malayan pangolin (*Manis javanica*) is an endangered mammal species exhibiting scales characteristics. The conservation and management of this species could benefit from a better understanding of its genetic diversity and structure. In this study, 24 novel SSRs were isolated from full-length transcriptome and they were used for assessing of parent-offspring relationship for *M. javanica*. All SSR markers were highly polymorphic with a mean of 6 alleles per locus, ranged from 2 to 10 alleles. The average polymorphism information content (PIC) was 0.62. The observed (H_O) and expected average heterozygosity (H_E) value was 0.56 and 0.67 tested in 39 individual samples. For parentage testing, the allele frequency date-based indicated that the combined exclusion probability values were over 99.99% when 9 SSRs were used. This study demonstrated that the microsatellite-based approach could be effectively utilized for parentage analysis in pangolins and that it has a significant application in selective breeding in endangered and valuable mammalian species.

INTRODUCTION

Pangolins are mammals exhibiting atypical morphological characteristics and belong to *Manis*, order Pholidota (Gaudin *et al*., 2006). These mammals have overlapped horny scales on the major parts of the body and a buccal system adapted to a myrmecophagy that makes them unique among the Old World mammalian fauna (Luo *et al*., 2007). A total of eight extant species of pangolins have been recognized and are widely distributed in Africa and Asia due to the exploitation of natural resources, as well as medicinal demand for pangolin scales and meat (Gaubert and Antunes, 2005). In addition, the illegal trade of pangolin is becoming increasingly serious, and all pangolin species are listed in the Conservation on International Trade in Endangered Species of Wild Fauna and Flora (CITES I) (Kumar *et al*., 2016; Li *et al*., 2017).

Although in situ protection is an important component of endangered species protection, in order to expand the existing population and increase genetic diversity, artificial breeding and ex situ conservation have become important measures to protect endangered animals (Wu *et al*., 2002). Under the conditions of artificial breeding, endangered animals can be guaranteed more comprehensive and adequate food and nutrition and can receive good health protection while avoiding harm of predators (Wu *et al*., 2002; Yusoff *et al*., 2016). Pedigree information can provide relevant data for breeders to make pertinent decisions, since it allows for maintenance of high levels of variability in the progeny, increase of the heterosis effect, and reduction of the levels of inbreeding (Spanoghe *et al*., 2015; Tarn *et al*., 1992). Therefore, it is particularly important to establish a stable population of pangolins that is kept artificially in order to avoid inbreeding depression and genetic drift, increase the effective population size and to select and optimize pairing for the purpose of long-term gene exchange between the individuals with the smallest relationship. In this way, not only can the domestic and foreign social needs of scientific research and public education be met, but also certain conditions for the release of captive breeding individuals to supplement wild populations can be created.

Since the 1980s, parentage analysis of animals has been widely expended not only from the development

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of methods for assessing the parentage of individual progeny but also from the increase in the diversity and availability of molecular markers (Luís *et al*., 2002; Zhang *et al*., 2016). SSRs (Simple Sequences Repeats) have become the markers of choice in both animal and plant species because of their discriminatory power, codominant transmission, and reproducible properties (Ellegren, 2004; Zietkiewicz *et al*., 1994). To date, microsatellite-based parentage identification technology is the most widely used and reliable method for the identification of genetic relationships among endangered species, such as Amur tigers (Zhang *et al*., 2003a), the giant panda (Zhang *et al*., 2003b), long-snouted seahorse (*Hippocampus guttulatus*) (Pardo *et al*., 2007), *Shinisaurus crocodilurus* (Luo, 2014), chimpanzees (Zhang *et al*., 2000), *Alligator mississippiensis* (Davis *et al*., 2001), *Dermochelys coriacea* (Crim *et al*., 2002), *Acipenser sturio* (Roques et al., 2016) and *Falco cherrug* (Hou *et al*., 2018). Moreover, microsatellite analysis only requires a small amount of DNA when sampling endangered and rare species by using nondestructive methods, which can be effectively analyzed and facilitated in field investigation.

In China, the Guangdong province wildlife rescue center has rescued some pangolins from the illegal trad of wild animals. These individuals were raised in Dongguan Institute of Qingfengyuan Animal Medicine; however, the source and relationship between members has not been thoroughly elucidated to date. To restore this rescued population, we combined the nondestructive extraction DNA method and the microsatellite sequences in a known transcriptome of *M. javanica* (Ma *et al*., 2019) to carry out new primers and polymorphic gene loci and to establish a highly sensitive paternity identification method for one breeding population of *M. javanica*.

MATERIALS AND METHODS

One natural death *M. javanica* and 39 saliva samples (23 males and 16 females) of breeding pangolins were provided by the Dongguan Institute of Qingfengyuan Animal Medicine in 2018. Samples were stored in RNAiso reagent (Takara, Otsu, Japan) and treated with DNase I (Takara). The total RNA (Ma *et al*., 2019) for transcriptome sequencing and locating microsatellite loci. Saliva samples were stored in 95% ethanol and kept at 4℃. From the pedigree record, we know that the parents of "BB" (♀) were "A26" (♀) and "A25" (♂) in this sample population.

The genomic DNA was isolated using a HiPure Tissue DNA mini kit (Magen Inc.). Randomly selected 30 microsatellite primers for PCR amplification to screen primers that preliminarily possessed polymorphic loci. The polymerase chain reactions (PCR) were conducted in a 10 μL reaction volume containing 5 μL 2 × EasyTaq PCR SuperMix (Trangen Inc.), 1 μL FAM-labeled M13 forward primer, 0.4 μL unlabeled M13-tailed forward primer and 1 μL reverse primer, as well as 50-100 ng of genomic DNA template in 1 μL (Boutin-Ganache et al., 2001). PCR was performed with the following protocols: one cycle of denaturation at 95 ℃ for 10 min; 35 cycles of 95℃ for 15 s with an annealing temperature 52℃ for 30 s, extension at 72℃ for 30 s, and then a final extension at 72℃ for 30 min. PCR products were analyzed with an ABI 3730XL Genetic Analyzer (Applied Biosystems). Fragment sizes were determined by comparison with the internal standard using GeneScan 500-ROX (Applied Biosystems) and GeneMarker version 1.5 (SoftGenetics, State College, Pennsylvania).

The genetic diversity parameters of SSR loci, including the number of alleles (*Na*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), alleles range and polymorphism information content (PIC), were calculated using the software CERVUS 3.0 (Kalinowski *et al*., 2007). Deviation from Hardy-Weinberg equilibrium (*HWE)* and linkage disequilibrium (LD) were performed using the software POPGENE V1.31 (Yeh *et al*., 2000) and FSTAT V2.9.4 (http://www.unil.ch/izea/softwares/fstat). For paternity testing, the exclusion probabilities of each locus based on the genotype of no known parent and on the genotype of one known parent were named NE-1P, NE-2P and NE-PP, respectively. In addition, the combined exclusion probability over the 24 loci were also calculated.

To estimate the feasibility and confidence of parentage analysis, a simulation program was designed with the software CERVUS 3.0 using the following parameters: parent pair (sex known), 70 candidate parents, 200 offspring, 30% of the candidate parents were sampled, and proportion of mistyped loci was 1%. In the program, the most-likely parents of each individual were assigned a statistic delta score and LOD score, and the true parents were evaluated depending on the scores of the candidate parents. In addition, a double-blind test was performed to evaluate the capacity of these microsatellite markers.

RESULTS

Identification and genetic diversity of microsatellite markers

According to the full-length transcriptome of *M. javanica*, a total 18,693 SSR sites were detected, including 12,120 mononucleotide repeat sequences, 3,202 dinucleotide repeat sequences, 1,594 tri-nucleotide repeat sequences, 259 tetra-nucleotide repeat sequences, 52 penta-nucleotide repeat sequences and 23 hexa-nucleotide repeat sequences. According to the obtained SSR sites, 56,079 pairs of primers were designed by the software Primer Primer 5.0.

Table I. Characterization of 24 microsatellites loci and conditions of PCR in Malayan pangolin.

Primers	Accession no.	Repeat type	FPr1(5'-3') / RPr1(5'-3')	Temperature (℃)	PSize (bp)	SSR locus
M13MJ-F2	MK121608	(TCCA)9	TTTCATACCGGGAAGTCCAC	59.8	169	PB676
			ATGGTCCTAACACCACGGAG	59.8		
M13MJ-F3	MK121609	(TTTA)8	CACCTGCATGTACCCCTTTT	59.9	226	PB210
			CCCCCTCAAAATACCACCTT	60.0		
M13MJ-F4	MK121610	(ATTC)8	GAGAGAAAGGGGAAAATCGG	60.0	237	PB220
			TGATAGGATGTGAGGAGGGG	59.9		
M13MJ-F5	MK121611	(AATT)8	TGGGGTCTGCTGTTTTCATT	60.5	194	PB235
			CTCCCTCTGTAGGTTGCCCT	60.6		
M13MJ-F7	MK121612	(TCAT)13	AGAAGTGATTTGCACCCCTG	60.1	228	PB272
			CAGTGGCCAGAATGGAGATT	60.1		
M13MJ-F9	MK121614	(ATGA)8	CCCTATGAGGTGGGCACTAA	59.9	276	PB387
			AACTCCATCAAAGGTGTGGC	60.0		
M13MJ-F10	MK121615	(GAAT)8	CCCAGATCCAAAATGAATGG	60.1	209	PB442
			TGCTGATGTTCACTCTTGCC	60.0		
M13MJ-F11	MK121616	(GATA)12	ATCCACCTAGGAACCTCAGC	58.2	249	PB464
			GACTCTTCGGGATTTCACACA	60.1		
M13MJ-F12	MK121617	(GATA)12	ATCCACCTAGGAACCTCAGC	58.2	242	PB4641
			GACTCTTCGGGATTTCACACA	60.1		
M13MJ-F17	MK121619	(ATAG)12	GTAATGGGGTATGTGGTGGG	59.8	213	PB537
			TCCCTGTTCAAACGGAATTT	59.4		
M13MJ-F20	MK121620	(AGAT)16	CAGTGCTCATCACATAGCAGG	59.5	177	PB652
			CATGCCTAGTGTTTCACGTTG	59.3		
M13MJ-F24	MK121621	(TTTTG)7	TTCAGCCAGGGTCTCTCAGT	60.0	188	PB714
			TGGGGTTTTTCCTCAATCTG	59.9		
M13MJ-F25	MK121622	(AAACA)9	CCAGAGAAAGGTAGGAGCCA	59.4	128	PB383
			TCCAGAAAACAGACCCAAGG	60.1		
M13MJ-F40	MK896876	(AG)10	GCAGCAGAACTCATCCAACA	60.0	191	PB221
			TACAGGGAATGGTTTCAGCC	60.0		
M13MJ-F41	MK896877	(AT)7	GCTCTGAGAATTGCCCATGT	60.2	194	PB251
			CACTGACGAGCAAAGCCATA	60.0		
M13MJ-F43	MK896879	(AT)8	TTGTCCTGGGGATCTTAGAAAA	59.9	186	PB681
			AATGGGCATTGTCTCCAAAA	60.3		
M13MJ-F44	MK896880	(AC)12	GGTTGGGGAGGGTGTAACTT	60.0	269	PB821
			TACTCCTTGGAGGCGTGACT	59.9		
M13MJ-F47	MK896881	(TC)10	AAATCTGTGGCCATCTCTCC	59.1	251	PB169
			GCCTTCCAGGTTGTTCAGAG	59.8		
M13MJ-F48	MK896882	(AC)9	AGAGCTGGGGAGGATGATTC	60.6	267	PB171
			ACCCTGCAGGAGTCTGAATG	60.3		
M13MJ-F49	MK896883	(TA)7	TCCCATCTAGAGAGCCATGC	60.3	160	PB1982
			ACCCAGGGTGTGTACCCATA	60.0		
M13MJ-F50	MK896884	(TG)10	GGGCTATTAATTCTGCCAGC	58.8	264	PB1983
			GTGCATGTTCACACACACATC	58.4		
M13MJ-F53	MK896885	(TG)8	ATGTCCTCATCAGGCAAACC	60.0	231	PB236
			CCTAGACTTACCTGCTTGAGCC	60.0		
M13MJ-F54	MK896886	(AT)6	GAGGGCCTTTAGGAAACTGC	60.2	197	PB2365
			GCTTCTAACAATTCCAGAGCTAAA	58.3		
M13MJ-F55	MK896887	(AT)6	GAGGGCCTTTAGGAAACTGC	60.2	198	PB2366
			GCTTCTAACAATTCCAGAGCTAAA	58.4		

Of the 30 primer pairs tested, 24 (80 %) produced a clear product following amplification of samples for *M. javanica*. Thirty-nine Malayan pangolins showed a relatively high level of genetic variability (Supplementary Table I), and all 24 loci were polymorphic (Table I), of which 10 loci contained at least 6 alleles. The number of alleles (*Na*) per locus varied from 2 to 10, the observed heterozygosity (*Ho)* in Malayan pangolin ranged from 0.167 to 0.795, the expected heterozygosity (*He*) ranged from 0.216 to 0.853, and the polymorphism information content (PIC) ranged from 0.202 to 0.853. The means of *Na*, *Ho*, *He* and PIC were 5.5, 0.558, 0.673 and 0.617, respectively. The Hardy-Weinberg test results indicated that 22 markers followed Hardy-Weinberg equilibrium. No significant pair-wise linkage disequilibrium was found between loci after Bonferroni correction ($P < 0.000181$) (Table II, Supplementary Table II).

Table II. Genetic diversity of 24 microsatellite markers in 35 *Manis javanica* individuals.

Locus	k	Na	Ho	He	PIC	NE-1P	NE-2P	NE-PP	HW
PB676	6	39	0.641	0.701	0.638	0.723	0.557	0.377	NS
PB210	6	38	0.632	0.746	0.692	0.672	0.497	0.314	NS
PB220	5	39	0.436	0.657	0.605	0.759	0.586	0.399	NS
PB235	7	39	0.385	0.635	0.584	0.772	0.601	0.411	NS
PB272	5	33	0.727	0.763	0.711	0.652	0.474	0.291	NS
PB387	5	38	0.474	0.511	0.477	0.859	0.692	0.509	NS
PB442	8	39	0.795	0.8	0.762	0.579	0.4	0.213	NS
PB464	6	37	0.568	0.796	0.754	0.598	0.418	0.235	NS
PB4641	6	37	0.459	0.749	0.704	0.658	0.477	0.285	NS
PB537	10	38	0.632	0.847	0.816	0.492	0.323	0.148	NS
PB652	6	35	0.6	0.802	0.759	0.59	0.412	0.23	NS
PB714	5	36	0.583	0.727	0.673	0.696	0.52	0.336	NS
PB383	5	36	0.722	0.737	0.678	0.691	0.518	0.339	NS
PB221	5	32	0.406	0.573	0.495	0.832	0.696	0.54	NS
PB251	4	37	0.757	0.697	0.629	0.741	0.578	0.409	NS
PB681	10	36	0.5	0.853	0.822	0.483	0.316	0.144	ND
PB821	4	32	0.5	0.708	0.637	0.735	0.572	0.405	NS
PB169	3	30	0.167	0.216	0.202	0.977	0.891	0.804	ND
PB171	2	31	0.419	0.508	0.375	0.875	0.813	0.719	NS
PB1982	4	33	0.727	0.597	0.533	0.815	0.66	0.491	NS
PB1983	3	32	0.75	0.538	0.419	0.86	0.775	0.661	NS
PB236	6	29	0.655	0.736	0.689	0.673	0.491	0.295	NS
PB2365	5	30	0.333	0.568	0.498	0.837	0.693	0.534	NS
PB2366	5	29	0.517	0.7	0.635	0.732	0.564	0.386	NS

Na the number of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, PIC polymorphic information content, ** or * Indicated significant deviation from HW after Bonferroni correction ($p<0.005$ or $p<0.01$).

Parentage analysis

The exclusion probability of individual locus ranged from 0.144 to 0.804 (Table II) when both parents information were not known (NE-PP), and its combined exclusion probability values were higher than 99% and 99.99% when 4 and 9 SSRs were used separately (Fig. 1); The exclusion probability of individual locus ranged from 0.316 to 0.891 (Table II) when one parent information was known (NE-2P), and its combined exclusion probability values were higher than 99% and 99.99% when 3 and 5 SSRs were used separately (Fig. 1); The exclusion probability of individual locus ranged from 0.483 to 0.977 (Table II) when information for both parents were known (NE-1P), and its combined exclusion probability values was higher than 99.99% when 3 SSRs were used (Fig. 1). This test confirms that the parents of "BB" (♀) were "A26" (♀) and "A25" (♂), which was consistent with the pedigree record from Qingfengyuan medicine institute. In addition, the results also supported that "C9" and "72" were parent-offspring relationships (Supplementary Table III).

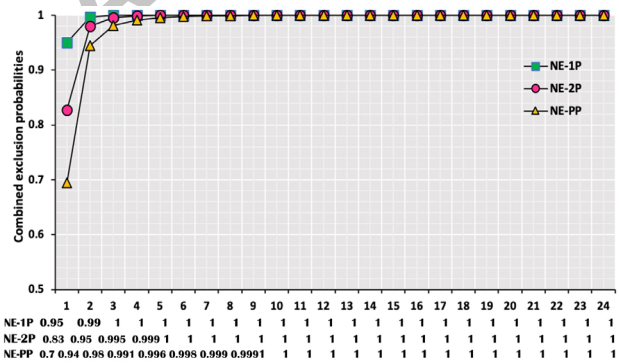

Fig. 1. Combined exclusion probabilities of 24 polymorphic loci analyzed for parent known (E-1P) and one parent known (E-2P).

DISCUSSION

In this study, we firstly used 24 newly isolated SSR loci to identify the paternity of a breeding population of *M. javanica.* Although Luo *et al*. (2007) had screened 32 polymorphic microsatellite markers for *M. javanica* in 2007, the usefulness of these loci for paternity testing was not further validated due to the rarity of species and fewer samples.

The level of population genetic diversity directly reflects the individual's ability to adapt to the environment. The greater the abundance in variation of genetic diversity within a species is, the greater the adaptability of the species to environmental changes is (Michaux *et al*., 2005). In our study, the genetic polymorphism of the artificially raised *M. javanica* population was relatively high, 20 loci of 24 SSRs

showed high polymorphism (PIC >0.5), and the average observed heterozygosity (*Ho)* and expected heterozygosity (*He*) in Malayan pangolin were 0.557 and 0.673, respectively. This finding indicated that the population of artificially raised pangolin can avoid inbreeding under correct guidance, which is conductive to the recovery of the pangolin population (Botstein *et al*., 1980).

Compared with *He* of other rare and protected animals, such as the giant panda (*He* = 0.637) (Zhang *et al*., 2003b), American alligator (*He* = 0.76) (Davis *et al*., 2001), Chinese crocodile (*He* = 0.54) (Luo, 2014), Rhesus macaques (*He* = 0.62) (Morin *et al*., 1997) and Lynx (*He* = 0.53) (Carmichael *et al*., 2000), the genetic diversity of *M. javanica* was not significantly higher than that of other species. The low genetic diversity in *M. javanica* observed in this preliminary microsatellite survey suggests an urgent need for population management and conservation of this species, since the loss of genetic diversity in endangered species is often associated with inbreeding and a reduction in reproductive fitness (Reed and Frankham, 2001).

In our study, the combined exclusion probability for NE-1P and NE-2P were higher than 99.99% when 4 SSRs were used. Moreover, when 9 SSRs were used, the values of three type parents information was higher than 99.99%. In previous reports on paternity testing, usually 6 SSRs markers may make the exclusion probability of pedigree confirmation more than 99% (Zajc, 1999). 10 and 18 polymorphic SSR loci can be used to accurately identify the relationship between individuals of Amur tiger (Zhang *et al*., 2003a) and *Acipenser sturio* (Roques et al., 2016) separately. These results indicated that the selected SSRs had a very high power and were suitable for parentage analysis. The more loci that we selected, the higher the accuracy was that could be reached in parentage analysis.

In conclusion, the parentage analysis investigated in this study, which was based on kinship testing and inferential methods, was demonstrated to be particularly hopeful regarding the development of pedigree information validation or parental assignment programs. As a genetic marker, microsatellites can objectively reflect the genetic diversity of pangolin and can be used in a paternity test. Therefore, the combination of microsatellite markers and traditional ecological research for carrying out genetic diversity analysis and the paternity test of pangolin will promote the protection, as well as rational development and utilization, of rare species resources and scientific resource management.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20190816080848

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

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

Boutin-Ganache, I., Raposo, M., Raymond, M. and Deschepper, C.F., 2011. M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques,* **31**: 24-26.

Carmichael, L.E., Clark, W. and Strobeck, C., 2000. Development and characterization of microsatellite loci from lynx (*Lynx canadensis*), and their use in other felids. *Mol. Ecol*., **9**: 2197-2199. https://doi.org/10.1046/j.1365-294X.2000.105323.x

Crim, J.L., Spotila, L.D., Spotila, J.R., Reina, M.R., Williams, C.J. and Palanino, F.V., 2002. The leatherback turtle, *Dermochelys coriacea*, exhibits both polyandry and polygyny. *Mol. Ecol.,* **11**: 2097–2106. https://doi.org/10.1046/j.1365-294X.2002.01591.x

Davis, L.M., Glenn, T.C., Elsey, R.M., Dessauers, H.C. and Sawyer, R.H., 2001. Multiple paternity and mating patterns in the American alligator, *Alligator mississippiensis*. *Mol. Ecol*., **10**: 1011–1024. https://doi.org/10.1046/j.1365-294X.2001.01241.x

Ellegren, H., 2004. Microsatellites: Simple sequences with complex evolution. *Nat. Rev. Genet*., **5**:435–445. https://doi.org/10.1038/nrg1348

Gaubert, P. and Antunes, A., 2005. Assessing the taxonomic status of the Palawan pangolin *Manis culionensis* (Pholidota) using discrete morphological characters. *J. Mammal.,* **86**: 1068–1074. https://doi.org/10.1644/1545-1542(2005)86[1068:ATTSOT]2.0.CO;2

Gaudin, T.J., Emry, R.J. and Pogue, B., 2006. A new

genus and species of pangolin (Mammalia, Pholidota) from the late Eocene of Inner Mongolia, China. *J. Vertebr. Paleontol.,* **26**: 146–159. https://doi.org/10.1671/0272-4634(2006)26[146:ANGASO]2.0.CO;2

Hou, X., Xu, P., Lin, Z., D'Urban-Jackson, J., Dixon, A., Bold, B., Xu, J. and Zhan, X., 2018. An integrated tool for microsatellite isolation and validation from the reference genome and their application in the study of breeding turnover in an endangered avian population. *Integr. Zool*., **13**: 553–568. https://doi.org/10.1111/1749-4877.12305

Kalinowski, S.T., Taper, M.L. and Marshall, T.C., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol*., **16**: 1099-1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kumar, V.P., Rajpoot, A., Mukesh, Shukla, M., Kumar, D. and Goyal, S.P., 2016. Illegal trade of Indian Pangolin (*Manis crassicaudata*): Genetic study from scales based on mitochondrial genes. *Egypt J. Forensic Sci*., **6**: 524–533. https://doi.org/10.1016/j.ejfs.2016.06.008

Li, L., Wei, L., Ma, J. and Chen, J., 2017. Development and characterization of 21 SNP markers in *Manis javanica* based on high-throughput sequencing. *Conserv. Genet. Resour.,* **3**: 1–3. https://doi.org/10.1007/s12686-017-0822-1

Luís, C., Cothran, E.G. and Oom, M.M., 2002. Microsatellites in Portuguese autochthonous horse breeds : usefulness for parentage testing. *Genet. Mol. Biol*., **25**: 131–134. https://doi.org/10.1590/S1415-47572002000200003

Luo, D., 2014. *Parentage and genetic diversity studies in Luokeng Chinese crocodile (*Shinisaurus crocodilurus*) using microsatellite DNA marker*. PhD thesis, Guangxi Normal University, Guilin city, China.

Luo, S.J., Cai, Q.X., David, V.A., Zhang, L., Martelli, P.L., Norman, T.L., Ferrand, N.C., Gauber, S.C., Ramos, P., O'Brien, W.E., Antunes, S.J., Johnosn, A. and Warren, E., 2007. Isolation and characterization of microsatellite markers in pangolins (Mammalia, Pholidota, *Manis* spp.): Primer note. *Mol. Ecol. Notes,* **7**:269–272. https://doi.org/10.1111/j.1471-8286.2006.01577.x

Ma, J.E., Jiang, H.Y., Li, L.M., Zhang, X.J., Li, H.M., Li, G.Y., Mo, D.Y. and Chen, J.P., 2019. SMRT sequencing of the full-length transcriptome of the Sunda pangolin (*Manis javanica*). *Gene,* **692**:208–216. https://doi.org/10.1016/j.gene.2019.01.008

Michaux, J., Hardy, O., Justy, F., Fournier, P., Kanz, A., Cabria, M., Davison, A., Rosoux, R. and Libois, R., 2005. Conservation genetics and population history of the threatened European mink *Mustela lutreola*, with an emphasis on the west European population. *Mol. Ecol*., **14**: 2373-2388. https://doi.org/10.1111/j.1365-294X.2005.02597.x

Morin, P., Kanthaswamy, S. and Smith, D.G., 1997. Simple sequence repeat (SSR) polymorphisms for colony management and population genetics in rhesus macaques (*Macaca mulatta*). *Am. J. Primatol*., **42**: 199-213. https://doi.org/10.1002/(SICI)1098-2345(1997)42:3<199::AID-AJP3>3.0.CO;2-S

Pardo, B.G., López, A., Martínez, P. and Bouza, C., 2007. Novel microsatellite loci in the threatened European long-snouted seahorse (*Hippocampus guttulatus*) for genetic diversity and parentage analysis. *Conserv. Genet.,* **8**: 1243–1245. https://doi.org/10.1007/s10592-006-9241-7

Reed, D.H. and Frankham, R., 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution*, **55**: 1095-1103. https://doi.org/10.1111/j.0014-3820.2001.tb00629.x

Roques, S., Berrebi, P., Rochard, E. and Acolas, M., 2016. Parentage assignment in the critically endangered European sturgeon (*Acipenser sturio*) based on a novel microsatellite multiplex assay: a valuable resource for restocking, monitoring and conservation programs. *Conserv. Gene. Resour.,* **8**: 313-322.

Spanoghe, M., Marique, T., Rivière, J., Lanterbecq, D. and Gadenne, M., 2015. Investigation and development of potato parentage analysis methods using multiplexed SSR fingerprinting. *Potato Res*., **58**: 43–65. https://doi.org/10.1007/s11540-014-9271-3

Tarn, T.R., Ta,i G.C.C., De long, H., Murphy, A.M. and Seabrook, E.A., 1992. Breeding potatoes for long-day, temperate climates. *Pl. Breed Rev*., **9**: 217–332. https://doi.org/10.1002/9780470650363.ch8

Wu, S., Ma, G.Z., Tang, M., Chen, M. and Liu, N., 2002. The status and conservation strategy of pangolin resource in China. *J. Nat. Resour*., **17**: 174–180.

Yeh, F., Yang, R., Boyle, T. and Boyle, T., 2000. *POPGENE32: Microsoft Windows-based freeware for population genetic analysis.* University of Alberta, Edmonton. pp. 32.

Yusoff, A.M., Tan, T.K., Hari ,R., Koepfli, K., Wee, W.Y., Antunes, A., Sitam, F.T., Rovie-Ryan, J.J., Karuppannan, K.V., Wong, G.J., Lipovich, L., Warren, W.C., O'Brien, S.J. and Choo,

S.W., 2016. De novo sequencing, assembly and anlysis of eight different transcriptomes from the Malayan pangolin. *Sci. Rep*., **6**: 28199. https://doi.org/10.1038/srep28199

Zajc, I., 1999. Utility of canine microsatellite in revealing the relationship of pure bred dogs. *J. Hered*., **90**: 104-107. https://doi.org/10.1093/jhered/90.1.104

Zhang, J., Ma, W., Wang, W., Gui, J. and Mei, J., 2016. Parentage determination of yellow catfish (*Pelteobagrus fulvidraco*) based on microsatellite DNA markers. *Aquac. Int.,* **24**: 567–576. https://doi.org/10.1007/s10499-015-9947-y

Zhang, Y., Lawrance, S.K., Ryder, O.A., Zhang, Y. and Isaza, R., 2000. Identification of monozygotic chimpanzees by microsatellite analysis. *Am. J. Primatol*., **52**: 101–106. https://doi.org/10.1002/1098-2345(200010)52:2<101::AID-AJP4>3.0.CO;2-6

Zhang, Y., Li, D., Rao, L., Xiao, Q. and Liu, D., 2003a. Identification of polymorphic microsatellite DNA loci and paternity testing of Amur tigers. *Acta Zool. Sin*., **49**: 118–123.

Zhang, Z., Shen, F., Sun, S., David, V., Zhang, A., O'Brien, A. and Stephen, J., 2003b. Paternity assignment of giant panda by microsatellite genotyping. *Hereditas(Beijing)*, **25**: 504–510.

Zietkiewicz, E., Rafalski, J.A., Consulting, R. and Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-Anchored polymerase chain reaction amplification. *Genomics,* **20**: 176–183. https://doi.org/10.1006/geno.1994.1151

Pakistan J. Zool., pp 1-7, 2021. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20190816080848

Supplementary Material

Parentage and Genetic Diversity Studies in Pangolin (*Manis javanica*) using Novel Microsatellite Markers

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Supplementary Table I. Genotype result with 24 SSRs from 39 individuals of *M. javanica.*

Gene ID	PB676a	PB676b	PB210a	PB210b	PB220a	PB220b	PB235a	PB235b	PB272a	PB272b	PB387a	PB387b	PB442a	PB442b	PB464a	PB464b
A35	195	195	252	252	255	263	201	201	236	240	290	290	236	248	254	258
2	179	191	232	244	255	255	201	213	220	240	278	290	232	236	254	258
A31	187	187	248	252	255	255	197	209	236	236	290	294	244	244	254	254
A34	191	195	240	248	255	255	201	201	220	240			228	248	266	266
129	187	191	244	256	255	255	201	213	240	240	290	294	224	252	262	266
18	179	179	248	248	255	255	213	213			288	292	228	248	266	266
A8	195	195			255	255	201	201	220	220	290	290	248	248		
172	191	195	244	244	251	251	201	201	220	232	278	290	228	228	250	250
1	191	195	252	252	255	255	201	201	240	240	290	290	228	244	254	254
15	195	195	244	248	267	267	213	213	220	240	290	290	236	252	254	266
89	195	195	248	248	267	267	201	201	220	240	290	290	224	228	254	254
421	191	195	244	248	255	263	201	201	232	244	290	290	228	248	262	262
B11	191	199	244	252	259	259	201	201	232	240	292	292	224	224	250	250
29	191	191	244	244	255	259	201	201			290	294	224	244	250	262
A3	191	195	240	248	255	259	201	201	232	244	290	290	228	236	250	250
12	187	191	244	244	255	263	201	201	220	240	278	294	236	248		
E2	191	191	252	252	255	255	213	221	220	220	290	294	228	228	262	262
E11	191	191	240	248	255	259	201	213	220	244	290	290	228	236	250	250
14	191	191	240	252	255	255	201	205	236	236	290	290	228	236	262	262
5	187	187	248	252	267	267	209	209	240	244	278	290	228	240	266	266
B3	187	191	252	252	255	259	217	217	220	244	290	290	228	236	254	262
E3	191	195	244	248	267	267	201	213	220	232	288	290	228	236	250	266

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0030-9923/2021/0001-0001 $ 9.00/0

E4	179	195	244	256	255	255	213	213	240	244	290	290	228	228	258	262
C9	195	199	244	244	255	259	213	213	232	240	290	290	224	252	254	262
C46	191	195	244	248	259	259	201	221	220	232	290	294	228	252	262	274
246	191	195	244	248	255	259	201	221	220	232	278	290	228	252	262	274
72	195	195	244	248	255	255	213	213			290	294	236	252	254	266
A28	187	191	244	244	255	259	209	217	240	244	290	294	236	244	258	266
A21	195	195	244	244	259	259	201	201	220	240	278	290	236	236	254	258
244	191	195	244	256	255	259	209	221			290	290	228	244	262	262
A14	187	195	248	252	255	255	201	201			278	290	236	248	262	262
97	195	195	248	248	251	263	201	201	240	240	290	290	228	236	262	266
47	191	195	248	256	259	267	201	201	232	236	290	290	244	248	262	262
51	187	195	248	252	263	263	201	213	240	240	278	290	228	248	258	262
240	191	195	244	244	255	259	197	197			290	290	228	236	250	254
BB	171	191	244	252	255	255	201	213	220	244	290	290	228	236	258	262
A25	171	191	232	244	255	267	201	201	240	244	290	290	236	244	258	266
A26	179	191	244	252	255	267	201	213	220	240	288	290	228	228	258	262
135	191	195	240	248	255	259	201	213	220	220	288	288	232	236	262	266

Supplementary Table II. P-value for genotypic disequilibrium.

Locus A		Locus B	P-value
HM01	X	HM02	0.01207
HM01	X	HM03	0.80717
HM01	X	HM04	0.12728
HM01	X	HM05	0.09
HM01	X	HM06	0.12069
HM01	X	HM07	0.22413
HM01	X	HM08	0.1688
HM01	X	HM09	0.20185
HM01	X	HM10	0.70663
HM01	X	HM11	0.43225
HM01	X	HM12	0.74431
HM01	X	HM13	0.23199
HM01	X	HM14	0.55844
HM01	X	HM15	0.19116
HM01	X	HM16	0.32348
HM01	X	HM17	0.12562
HM01	X	HM18	0.8538
HM01	X	HM19	0.79793
HM01	X	HM20	0.14746
HM01	X	HM21	0.6496
HM01	X	HM22	0.14949
HM01	X	HM23	0.05601
HM01	X	HM24	0.55351
HM02	X	HM03	0.44851
HM02	X	HM04	0.54967
HM02	X	HM05	0.59406
HM02	X	HM06	0.64214
HM02	X	HM07	0.80786
HM02	X	HM08	0.52225
HM02	X	HM09	0.17906
HM02	X	HM10	0.30036
HM02	X	HM11	0.01348
HM02	X	HM12	0.12029
HM02	X	HM13	0.73699
HM02	X	HM14	0.72127
HM02	X	HM15	0.22141
HM02	X	HM16	0.8137
HM02	X	HM17	0.51283
HM02	X	HM18	0.65833
HM02	X	HM19	0.87717
HM02	X	HM20	0.86232
HM02	X	HM21	0.28094
HM02	X	HM22	0.63431
HM02	X	HM23	0.41761
HM02	X	HM24	0.19612
HM03	X	HM04	0.98899
HM03	X	HM05	0.84326
HM03	X	HM06	0.5304
HM03	X	HM07	0.40402
HM03	X	HM08	0.47341
HM03	X	HM09	0.43627
HM03	X	HM10	0.88069
HM03	X	HM11	0.52543
HM03	X	HM12	0.69808
HM03	X	HM13	0.67149
HM03	X	HM14	0.35123
HM03	X	HM15	0.26812
HM03	X	HM16	0.19264
HM03	X	HM17	0.10424
HM03	X	HM18	0.41562
HM03	X	HM19	0.47757
HM03	X	HM20	0.17435
HM03	X	HM21	0.46319
HM03	X	HM22	0.32051
HM03	X	HM23	0.95304
HM03	X	HM24	0.06971
HM04	X	HM05	0.2313
HM04	X	HM06	0.67924
HM04	X	HM07	0.27283
HM04	X	HM08	0.02304
HM04	X	HM09	0.66029
HM04	X	HM10	0.77391
HM04	X	HM11	0.39413
HM04	X	HM12	0.91141
HM04	X	HM13	0.23819
HM04	X	HM14	0.9133
HM04	X	HM15	0.98851
HM04	X	HM16	0.18409
HM04	X	HM17	0.48322
HM04	X	HM18	0.49797
HM04	X	HM19	0.00967
HM04	X	HM20	0.80768
HM04	X	HM21	0.42293
HM04	X	HM22	0.27645
HM04	X	HM23	0.88815
HM04	X	HM24	0.33087
HM05	X	HM06	0.84909
HM05	X	HM07	0.1908
HM05	X	HM08	0.23627
HM05	X	HM09	0.76446

HM05	X	HM10	0.30721	HM07	X	HM22	0.73391
HM05	X	HM11	0.7379	HM07	X	HM23	0.83105
HM05	X	HM12	0.20562	HM07	X	HM24	0.3787
HM05	X	HM13	0.39214	HM08	X	HM09	0.0088
HM05	X	HM14	0.77529	HM08	X	HM10	0.57359
HM05	X	HM15	0.88714	HM08	X	HM11	0.92514
HM05	X	HM16	0.67725	HM08	X	HM12	0.25656
HM05	X	HM17	0.67493	HM08	X	HM13	0.86083
HM05	X	HM18	0.51486	HM08	X	HM14	0.00435
HM05	X	HM19	0.03964	HM08	X	HM15	0.13449
HM05	X	HM20	0.14616	HM08	X	HM16	0.88565
HM05	X	HM21	0.44163	HM08	X	HM17	0.75699
HM05	X	HM22	0.20464	HM08	X	HM18	0.33554
HM05	X	HM23	0.83283	HM08	X	HM19	0.04145
HM05	X	HM24	0.67	HM08	X	HM20	0.39431
HM06	X	HM07	0.31536	HM08	X	HM21	0.15236
HM06	X	HM08	0.64859	HM08	X	HM22	0.91844
HM06	X	HM09	0.11804	HM08	X	HM23	0.33326
HM06	X	HM10	0.14899	HM08	X	HM24	0.28203
HM06	X	HM11	0.95355	HM09	X	HM10	0.98453
HM06	X	HM12	0.34728	HM09	X	HM11	0.3342
HM06	X	HM13	0.51873	HM09	X	HM12	0.17054
HM06	X	HM14	0.02431	HM09	X	HM13	0.99757
HM06	X	HM15	0.01685	HM09	X	HM14	0.07736
HM06	X	HM16	0.18083	HM09	X	HM15	0.0042
HM06	X	HM17	0.29649	HM09	X	HM16	0.19196
HM06	X	HM18	0.00906	HM09	X	HM17	0.87134
HM06	X	HM19	0.215	HM09	X	HM18	0.74174
HM06	X	HM20	0.69681	HM09	X	HM19	0.15391
HM06	X	HM21	0.41431	HM09	X	HM20	0.85486
HM06	X	HM22	0.86022	HM09	X	HM21	0.12583
HM06	X	HM23	0.31196	HM09	X	HM22	0.23373
HM06	X	HM24	0.07359	HM09	X	HM23	0.25779
HM07	X	HM08	0.02232	HM09	X	HM24	0.63395
HM07	X	HM09	0.19072	HM10	X	HM11	0.2012
HM07	X	HM10	0.31826	HM10	X	HM12	0.20583
HM07	X	HM11	0.1808	HM10	X	HM13	0.86486
HM07	X	HM12	0.16942	HM10	X	HM14	0.39022
HM07	X	HM13	0.66558	HM10	X	HM15	0.41025
HM07	X	HM14	0.09159	HM10	X	HM16	0.80757
HM07	X	HM15	0.47902	HM10	X	HM17	0.55322
HM07	X	HM16	0.17351	HM10	X	HM18	0.40225
HM07	X	HM17	0.25236	HM10	X	HM19	0.1579
HM07	X	HM18	0.96525	HM10	X	HM20	0.99572
HM07	X	HM19	0.07236	HM10	X	HM21	0.93739
HM07	X	HM20	0.94804	HM10	X	HM22	0.25663
HM07	X	HM21	0.75507	HM10	X	HM23	0.18518

HM10	X	HM24	0.85123	HM15	X	HM16	0.30529
HM11	X	HM12	0.16348	HM15	X	HM17	0.91123
HM11	X	HM13	0.02638	HM15	X	HM18	0.42772
HM11	X	HM14	0.98098	HM15	X	HM19	0.82736
HM11	X	HM15	0.22355	HM15	X	HM20	0.96355
HM11	X	HM16	1	HM15	X	HM21	0.45319
HM11	X	HM17	0.64598	HM15	X	HM22	0.99797
HM11	X	HM18	0.82471	HM15	X	HM23	0.40757
HM11	X	HM19	0.19725	HM15	X	HM24	0.77764
HM11	X	HM20	0.5313	HM16	X	HM17	0.24188
HM11	X	HM21	0.45482	HM16	X	HM18	0.69736
HM11	X	HM22	0.87246	HM16	X	HM19	0.97464
HM11	X	HM23	0.01207	HM16	X	HM20	0.99913
HM11	X	HM24	0.23873	HM16	X	HM21	0.95435
HM12	X	HM13	0.03583	HM16	X	HM22	0.26442
HM12	X	HM14	0.58598	HM16	X	HM23	0.83036
HM12	X	HM15	0.49145	HM16	X	HM24	0.70964
HM12	X	HM16	0.81696	HM17	X	HM18	0.10428
HM12	X	HM17	0.80058	HM17	X	HM19	0.77359
HM12	X	HM18	0.46543	HM17	X	HM20	0.2237
HM12	X	HM19	0.4021	HM17	X	HM21	0.85283
HM12	X	HM20	0.48268	HM17	X	HM22	0.5554
HM12	X	HM21	0.09446	HM17	X	HM23	0.8962
HM12	X	HM22	0.89529	HM17	X	HM24	0.76493
HM12	X	HM23	0.12964	HM18	X	HM19	0.02185
HM12	X	HM24	0.87428	HM18	X	HM20	0.33659
HM13	X	HM14	0.94906	HM18	X	HM21	0.53341
HM13	X	HM15	0.73699	HM18	X	HM22	0.98087
HM13	X	HM16	0.35957	HM18	X	HM23	0.1646
HM13	X	HM17	0.31105	HM18	X	HM24	0.06536
HM13	X	HM18	0.93344	HM19	X	HM20	0.36083
HM13	X	HM19	0.52486	HM19	X	HM21	1
HM13	X	HM20	0.88764	HM19	X	HM22	0.98406
HM13	X	HM21	0.63138	HM19	X	HM23	0.43272
HM13	X	HM22	0.92975	HM19	X	HM24	0.97583
HM13	X	HM23	0.85812	HM20	X	HM21	0.51
HM13	X	HM24	0.55558	HM20	X	HM22	0.07101
HM14	X	HM15	0.73775	HM20	X	HM23	0.62627
HM14	X	HM16	0.65721	HM20	X	HM24	0.42623
HM14	X	HM17	0.0987	HM21	X	HM22	0.60572
HM14	X	HM18	0.76949	HM21	X	HM23	0.13953
HM14	X	HM19	0.34203	HM21	X	HM24	0.93004
HM14	X	HM20	0.64971	HM22	X	HM23	0.44822
HM14	X	HM21	0.30076	HM22	X	HM24	0.24156
HM14	X	HM22	0.42583	HM23	X	HM24	0.11514
HM14	X	HM23	0.54156				
HM14	X	HM24	0.45344				

P-value for genotypic disequilibrium based on 27600 permutations. Adjusted P-value for 5% nominal level is: 0.000181; Adjusted P-value for 1% nominal level is: 0.000036.

Supplementary Table III. Paternity test results of Malay pangolins.

Off-spring ID	Loci typed	Candidate mother ID	Loci typed	Pair loci compared	Pair LOD score	Pair top LOD	Pair confi-dence	Can-didate father ID	Loci typed	Pair loci com-pared	Pair LOD score	Pair top LOD	Pair confi-dence	Trio loci com-pared	Trio LOD score	Trio top LOD	Trio confi-dence
A35	15																
A25	22	BB	22	20	7.46E+00	7.46E+00	*	15	15	14	-1.02E+01	0.00E+00		21	-7.01E+00	-7.01E+00	-
A34	22	E3	15	14	-4.79E+00	-4.79E+00	-	135	13	12	-7.32E+00	-7.32E+00	-	14	-1.48E+01	-1.48E+01	-
129	21	A8	20	17	-2.14E+01	0.00E+00		12	21	18	-4.95E+00	-4.95E+00	-	19	-2.65E+01	-2.65E+01	-
18	12	E3	15	12	-6.19E+00	-6.19E+00	-	15	15	12	-1.15E+01	-1.15E+01	-	12	-1.19E+01	-1.19E+01	-
172	23	E3	15	14	-1.26E+01	-1.26E+01	-	A3	23	23	-7.71E+00	-7.71E+00	-	23	-1.42E+01	-1.42E+01	-
1	24	2	24	24	-1.39E+01	-1.39E+01	-	15	15	15	-2.04E+01	0.00E+00		24	-2.05E+01	-2.05E+01	-
15	15	A8	20	11	-9.32E+00	-9.32E+00	-	18	12	12	-1.15E+01	0.00E+00		15	-1.22E+01	-1.22E+01	-
89	23	C46	24	23	-1.40E+01	-1.40E+01	-	C9	14	13	-1.66E+01	0.00E+00		23	-2.68E+01	-2.68E+01	-
B11	22	E11	24	22	-1.90E+01	-1.90E+01	-	C9	14	14	-1.46E+01	-1.46E+01	-	22	-3.24E+01	-3.24E+01	-
29	22	E3	15	13	-9.46E+00	0.00E+00		12	21	19	-1.38E+01	0.00E+00		22	-1.49E+01	-1.49E+01	-
A3	23	E11	24	23	-1.05E+01	-1.05E+01	-	172	23	23	-7.71E+00	-7.71E+00	-	23	-1.53E+01	-1.53E+01	-
12	21	2	24	21	-8.89E+00	0.00E+00		129	21	18	-4.95E+00	-4.95E+00	-	21	-1.24E+01	-1.24E+01	-
5	24	A31	23	23	-1.88E+01	-1.88E+01	-	51	23	23	-1.47E+01	-1.47E+01	-	24	-3.11E+01	-3.11E+01	-
B3	20	BB	22	18	-6.16E+00	-6.16E+00	-	A14	21	17	-7.33E+00	-7.33E+00	-	20	-1.90E+01	-1.90E+01	-
E4	24	BB	22	22	-1.60E+00	-1.60E+00	-	129	21	21	-1.38E+01	0.00E+00		23	-8.01E+00	-8.01E+00	-
C9	14	C46	24	14	-7.88E+00	0.00E+00		72	23	13	2.15E+00	2.15E+00	+	14	-9.19E+00	-9.19E+00	-
72	23	A28	24	23	-5.76E+00	-5.76E+00	-	C9	14	13	2.15E+00	2.15E+00	+	23	-6.16E+00	-6.16E+00	-
A21	23	E3	15	15	-1.89E+01	0.00E+00		A14	21	20	-1.44E+01	0.00E+00		22	-1.89E+01	-1.89E+01	-
244	23	C46	24	23	-7.30E+00	-7.30E+00	-	135	13	12	-1.69E+01	0.00E+00		23	-2.32E+01	-2.32E+01	-
A14	21	BB	22	19	-5.99E+00	-5.99E+00	-	135	13	11	-5.38E-01	-5.38E-01	-	20	-1.25E+01	-1.25E+01	-
51	23	97	23	22	-1.17E+01	-1.17E+01	-	A35	15	15	-9.74E+00	-9.74E+00	-	22	-2.39E+01	-2.39E+01	-
135	13	A26	22	13	-9.62E+00	0.00E+00		A34	22	12	-7.32E+00	0.00E+00		13	-1.25E+01	-1.25E+01	-
421	24	E3	15	15	-1.25E+01	0.00E+00		A35	15	15	-1.32E+01	0.00E+00		15	-1.95E+01	-1.95E+01	-
A8	20	421	24	20	-9.22E+00	-9.22E+00	-	A35	15	11	-9.70E+00	0.00E+00		20	-1.26E+01	-1.26E+01	-
A26	22	BB	22	20	4.97E+00	4.97E+00	*	E4	24	22	-4.13E+00	-4.13E+00	-	22	-1.51E+01	-1.51E+01	-
2	24	E3	15	15	-4.49E+00	-4.49E+00	-	18	12	12	-1.22E+01	0.00E+00		15	-1.27E+01	-1.27E+01	-
A31	23	14	23	22	-2.55E+01	0.00E+00		135	13	13	-2.90E+01	0.00E+00		23	-3.41E+01	-3.41E+01	-
E2	24	BB	22	22	-9.71E-01	-9.71E-01	-	135	13	13	-1.15E+01	-1.15E+01	-	23	-1.58E+01	-1.58E+01	-
E11	24	E3	15	15	-5.86E+00	-5.86E+00	-	29	22	22	-8.61E+00	-8.61E+00	-	24	-1.23E+01	-1.23E+01	-
14	23	47	24	23	-1.49E+01	0.00E+00		244	23	22	-1.48E+01	0.00E+00		23	-2.15E+01	-2.15E+01	-
E3	15	2	24	15	-4.49E+00	-4.49E+00	-	A34	22	14	-4.79E+00	0.00E+00		15	-5.40E+00	-5.40E+00	-
C46	24	246	24	24	-3.51E+00	-3.51E+00	-	135	13	13	-1.48E+01	0.00E+00		24	-9.24E+00	-9.24E+00	-
246	24	C46	24	24	-3.51E+00	-3.51E+00	-	135	13	13	-1.10E+01	0.00E+00		24	-9.34E+00	-9.34E+00	-
A28	24	E3	15	15	-1.56E+01	0.00E+00		12	21	21	-7.31E+00	0.00E+00		24	-1.99E+01	-1.99E+01	-
97	23	E3	15	15	-1.31E+01	-1.31E+01	-	51	23	22	-1.17E+01	0.00E+00		22	-2.09E+01	-2.09E+01	-
47	24	14	23	23	-1.49E+01	0.00E+00		135	13	13	-1.19E+01	-1.19E+01	-	24	-2.34E+01	-2.34E+01	-
240	22	A28	24	22	-1.96E+01	0.00E+00		244	23	22	-1.42E+01	-1.42E+01	-	22	-2.24E+01	-2.24E+01	-
BB	22	A26	22	20	4.97E+00	4.97E+00	*	A25	22	20	7.46E+00	7.46E+00	*	22	1.58E+01	1.58E+01	*