A concise point-by-point response to the editorial points

Response: Thanks. We have modified our manuscript according to your opinions. We are grateful to you for constructive comments on how to improve our work. We have taken into consideration every comment and finished the point-by-point responses.

Commented [AA1]: I do not understand – why expected? Do you mean they served as positive test for paternity assessment?

Response: Thanks for your question. We don't mean that they served as positive test for paternity assessment. We wanted to say that their relationship was unclear and should be identified.

As we described in the manuscript, we confirmed that T12-T20 were parents and they might mate and produced offspring (T01-T11). T01, T02, T06, T07, T08 and T09 six tigers were born from the same womb, and T03, T04 and T05 three tigers were from the same womb. However, their biological parents were unclear.

We are sorry for the misunderstanding and we have revised this sentence. Please see line 99.

Commented [AA2]: Reference needed.

Response: Thanks for your suggestion. We have cited related references at this place.

Commented [AA3]: In contradiction with lines 263, 264

Response: Thanks for your suggestion. We speculated that you pointed out this sentence is in contradiction with lines 408, 409. We are sorry for the mistakes, in fact, we wanted to say a same thing in these two places. We have corrected and unified the descriptions in the revised manuscript, please see lines 156-158 and 247-248, respectively.

To be more explicit, we have changed the original 220-221 lines to "The repetition numbers of the motifs of unsequenced alleles were deduced in reference to the repeat sequence structures of both the sequenced alleles and the reference genome, and the observed sizes of both sequenced and unsequenced alleles."

For example, at locus DA2S1059, allele A was sequenced and its repeat sequence structures is $(AGAT)_{10}$. Its repeat sequence structure in the reference genome is $(AGAT)_{8}$. Using these information, we could determine that the motif of this locus is a tetra-nucleotide (AGAT), and its numerical nomenclature of allele A is 10. If the observed sizes of allele A and allele B are 354 and 358, respectively. We will deduce that numerical nomenclature of allele B should be 11.

Commented [AA4]: Proper genotyping/ allele assignment would require allelic ladders. It seems they were not made/used. Am I right?

Response: Thanks for your question. We made allelic ladders in this study. The allelic ladders of each locus were shown in the following table (Table R1).

Table R1. The allelic ladders of each locus

Locus	Allelic ladders
AMEL	Y, X
DA3S1123	11.2, 12.2, 13.2, 14.2, 15.2
DA2S1059	8, 9, 10, 11, 12
DB1S1259	18.1, 19.2, 20.1, 21.1, 22.2, 23.2, 25.3, 26.3
DD3S86	13, 14, 15, 16, 17, 18, 19,
DE1S613	11, 12, 13.2, 14.3, 15.3, 17, 18, 19.1
DF1S579	9.1, 11.1, 12.1, 13.1, 14.1, 15, 17, 18
DA2S1575	7.1, 8.1, 9.1, 10, 11, 12, 13, 14
DF2S497	10, 11, 12, 13, 14
DA3S1145	15, 16, 17, 18, 19
DD2S793	9, 10, 11, 12, 13
DD4S705	17.3, 18.2, 19.3, 20.3, 22, 22.3, 24.3
DB1S542	11, 12, 13, 14, 15, 16
DA1S1290	7, 10, 11, 12, 13, 14, 15
DA1S1470	9, 10, 11, 12, 13, 14, 15
DC1S1364	12.2, 13.2, 14.2, 15.2, 16.2, 17.2, 18.2

Please add this this information to the ms as Suppl. Mat. Using the format of, for example, https://strbase.nist.gov/str_D1S1656.htm, demonstrating sequences found for each STR.

Commented [AA5]: I do not understand - how could some alleles not be observed?

Response: Thanks for your question. We are sorry that we mistakenly wrote "alleles" instead of "genotypes." It should be "…and even some genotypes were not observed". The observation of genotypes with low frequencies usually requires a larger sample size.

We have performed a Hardy Weinberg exact test by Genepop software (version 4.7) and this description has been deleted in the revised manuscript.

Commented [AA6]: Debatable - you could have performed an exact test as provided, for instance by Arlequin or Genepop software.

Response: Thanks for your valuable suggestion. Following your suggestion, we have performed a Hardy Weinberg exact test by Genepop software (version 4.7). The Markov chain method was employed to estimate the probability of deviation from HWE with the default parameters (dememorization number =1000, number of batches = 100, and number of iterations per batch = 1000). We have updated the results of Hardy-Weinberg equilibrium exact test in Table 3.

There are three differences compared to the previous results. DA3S1123 and DD3S86 show departures from HWE and DA1S1290 conforms to HWE.

Commented [AA7]: Reference(s) needed for the formulas used

Response: Thanks for your suggestion. The related references have been added in the

revised manuscript.

Commented [AA8]: I do not understand - do you mean that a formal model of total codominance was applied?

Response: Thanks for your question. We meant that the undetected and null alleles were removed in these formulas and just the way they are. This sentence is a bit redundant and we have deleted it in the revised manuscript.

Commented [AA9]: This formula is not correct nor the description; please check the used reference

Response: Thanks for your suggestion. Kloosterman et al. 1993 used this formula (Equation 4) to calculate the discrimination power (DP). The technical specification for judicial authentication named "Specification of the calculation methodology of forensic parameters for autosomal STR" was released by the Ministry of Justice of the People's Republic of China. It stipulated this formula to calculate DP based on the genotype frequency, describing k is the number of genotypes of a genetic marker, and p_i is the frequency of the i^{th} genotype. YiPing et al 2016 used this formula in their book *Science of Medico-legal Physical Evidence* (*Fa Yi Wu Zheng Xue*, in Chinese). So we think this formula is correct.

Yes, but nomenclature is misleading, as pi is used as an allele frequency above and not a genotype frequency. Why not using gi, for instance?.

Commented [AA10]: Nonsense -please omit; STRs are not intrinsically codominant- it depends on the typing methodology

Response: Thanks for your suggestion. We have deleted this sentence in the revised manuscript.

Commented [AA11]: Requires better explanation; I cannot see how fragment length in bp is translated into repeat numbers. Please provide also a table where amplicon length is provided along with numerical nomenclature for each allele. Table S5 does not seem to apply correctly the nomenclature rules.

Response: Thanks for your suggestions. We have added a column in Table S5 to explain the numerical nomenclature for each allele. The information of the amplicon length were also provided in Table S5.

The numerical nomenclature of alleles with complex repeats were designated based on the method of Hellmann et al. 2006. The amplified fragments of both DB1S1259 and DD4S705 loci consist of a complex hypervariable region mainly based on a tetrameric motif of (AAAG) repeat and a pentameric motif of (AAAAG) repeat. The nomenclature was based on assuming a general tetrameric repeat structure. The naming of sequenced allele of DB1S1259 locus (allele 20.1, Table S5) depends on 16 (AAAG) [16 repeats] + (AAAAG) [1.1 repeats] + 3 (AAAG) [3 repeats] = 16 + 1.1 +3 = 20.1. In the same way, the naming of sequenced allele of DD4S705 locus (allele

18.2, Table S5) depends on 16 (AAAG) [16 repeats] + 2 (AAAAG) [2.2 repeats] = 16 + 2.2 = 18.2. The numerical nomenclature of alleles with simple repeats were designated based on the nomenclature rules in Mayr et al. 1995 and Olaisen et al. 1998. We think the nomenclature rules were applied correctly in Table S5.

I am not sure if the rules of nomenclature are correctly applied, but as you now provide a supplementary table where this explanation is included, I think there is not a major problem; anyway, please check at the light of examples of https://strbase.nist.gov/index.htm

Commented [AA12]: See previous comment

Response: Thanks. We have answered this question in AA3. We have corrected and unified the two descriptions in the revised manuscript. Please see lines 156-158 and 247-248, respectively.

Commented [AA13]: Too many loci show significant deviation; need explanation. A genotype/phenotype distribution table would be required.

Response: Thanks for your suggestions. Based on references, we thought the deviation from HWE in allele frequencies at these microsatellite loci might be caused by natural selection, inbreeding, human disturbance, population degradation and small population size. We have added the relevant contents in the revised manuscript, please see lines 253-256. We also have added a genotype distribution table following your suggestion, please see Table S7.

In order to solve this issue, is is necessary to compare observed and expected genotype distributions per locus. You have provided the observed one in S Table7, but I cannot find the expected values. Please add.

Commented [AA14]: No ladder was made/employed.

Response: Thanks for your suggestions. We have answered the question about laddars in AA4. We made allelic ladders in this study.

Please revise carefully; some typos remain, for instance: inparentage…