13th April, 2019

Reference: Genome-based development of 15 microsatellite markers in fluorescent

multiplexes for parentage testing in tigers (#35446)

Subject: Comments

The proposed paper is an attempt to develop multiplex panel called TPI-plex for determining the pedigree and sex of the captive tigers. It has a great relevance for ex-situ conservation of tigers for effective conservation planning when the resources are limited for managing captive populations.

Major comments are as follows:

- 1. In the title, authors should clearly mention its application is mainly for the for captive populations.
- 2. Under abstract, the authors should be very specific to generic statements like line no. 26 "reference population" whether it is wild or captive; line no. 24 provide exact observed number of alleles instead of saying more.

3. Introduction:

- a. all generic statements should be supported by the references like at line no. 47 to 49.
- b. Authors have failed to appreciate use of "Stud Book" for captive management and literature clearly says that such information should be supported by the genetic analysis.
- c. Authors have not cited the reference of already STR available for tigers Sharma et al. (2008); and Williamson et al. (2002).
- d. Authors have failed to mention how their suggested panel TPI-plex would be beneficial than what so far used. Statement at line 74 is true.
- e. Most of the text from 100 to 110 are of findings and should go to "Results"
- 4. Material and methods: Well described and provided reference number for "Ethical Approval"

5. Results and discussion:

- a. Covered all relevant issues needed for validation of newly suggested panel of STR.
- b. Line no. 317 "how authenticate information was corrected as authors have already stated at the line no. 114 "indeterminate parentage"

c. Though authors have provided profiles of each locus and it would have been better to provide the peak ratio for correct calling purposes as proposed by Matsumoto et al. 2004. Novel algorithm for automated genotyping of the microsatellite. Nucleic Acids Research. Vol. 32:6069-6077.

Most of the figures and Tables can go as supplementary materials except Table 3; Table 5; Table 7.

Since suggested TPI-plex would be of immense useful for ex-situ conservation planning across different zoos of the world, the paper may be accepted for publication after revision.

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