Manuscript: 58675\_R3

October 27th, 2018

Dear Dr. Bajaj:

On behalf of all the other authors and I please see the following comments and changes that we have made to the manuscript. We have attempted to address all your queries and make the changes you requested.

General Comments

**For editorial comments and suggestions please see below.**

*Editor: Morphometric assessment data is missing. Please provide some results with respect to the technique you have described.*

Authors: The authors are of the opinion that simple demonstrations in the figures of what taking basic morphometric measurements is sufficient for anyone emulating this technique requires from this paper. The figures themselves illustrate this fact.

*Editor: Please comment is this done with or without anesthesia?*

Authors: Techniques that require anesthesia have been so indicated in the manuscript and the authors feel mentioning where anesthesia is not required is redundant. We have mentioned anaesthesia only those method in which it is warranted. If not stated it is not required.

*Editor: This is the result for the anurans. However open question: how do you ensure that the age is same for both males and females? This point needs to be brought out in the protocol. there needs to be a greater number of animals to come to this conclusion. Please provide in tabulated form how many animals were measured etc.*

Authors: The author disagrees strongly with the editor’s comment in this instance. Distinguishing sexes by the appearance of thumb pads on males is a sexually dimorphic characteristic that is well established in the amphibian world not a discovery in this particular species. Hundreds of males and females have been identified by this method over the course of our captive colonies history however; we do not consider the tabulation of exact numbers of animals necessary since we are not establishing a new concept here. Clearly, the two animals in the figures do not represent the only specimens from which we have determined this to be a characteristic for this species.

*Editor: The figure 3 is ok in this case. However, the description of the results needs to be brought out clearly maybe one or two liner in the representative result section. Will you have some videos clips of the amphibians showing different behavior to be included in the JoVE video if needed?*

Authors: Yes, we have video of the frogs displaying reproductive behaviours during oviposition. Further comments regarding the results for this technique have been incorporated.

*Editor: Do you check the depth of anesthesia by the lack of righting reflexes?*

Authors: This question is dealt with in line 1.5.1.2

*Editor: Regarding surgery section: Please provide some data to support the technique.*

Authors: Data has been incorporated into the results section

*Editor: Please provide us representative results in the context of the techniques you have described. e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. Editor: Please rewrite the representative result to reflect the techniques you have described. All the section should have a representative result associated with it. This is important.*

Authors: The authors consider that the results and discussions for these techniques have been appropriately addressed and clearly demonstrate the context, method and outcomes of what is outlined in the protocol.

*Editor: As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:*

*a) Critical steps within the protocol*

*b) Any modifications and troubleshooting of the technique*

*c) Any limitations of the technique*

*d) The significance with respect to existing methods*

*e) Any future applications of the technique*

Authors: We appreciate the time and effort that the editor and reviewers have dedicated to the development of this manuscript. At this time we consider that we have met the requirements outlined above.

Further general comments

*Editor: Please change the title to reflect the highlighted portion of the protocol.*

Authors: Title has been changed

*Editor: Once done please ensure that the protocol is no more than 10 pages long and the highlighted section is no more than 2.75 pages including heading and spacings.*

Authors: The protocol itself is 8 pages long not including introduction, citations, discussion and results. It is unclear if the editor is referring to the protocol sections or the entire document. If this refers to the entire document then the authors will have to decide whether to go ahead with this publication.

*Editor: Any modifications and troubleshooting of the technique*

Authors: Modifications or troubleshooting is contextual and given the restriction on page limit and the fact that, modifications and troubleshooting may not have been required we feel confident that, with the help of reviewers and editors, we have addressed the protocol and the execution of the techniques clear and concise. This sentiment if true of the following editorial comments as well: (Editor: The significance with respect to existing methods and any future applications of the technique).

*Editor: Please do not make table panels as done now.*

Authors: Excruciatingly unhelpful comment.