Author's response to reviews

Title: Comparative analysis of oral treponemes associated with periodontal health and disease

Authors:

Meng You (fiona_scu@yahoo.com.cn)
Sisu Mo (mosisu@126.com)
W Keung Leung (ewkleung@hkucc.hku.hk)
Rory M. Watt (rmwatt@hku.hk)

Version: 3 Date: 27 March 2013

Author's response to reviews: see over
Dear Dr. Harris,

My co-authors and I wish to submit our revised manuscript entitled ‘Comparative analysis of oral treponemes associated with periodontal health and disease’ (MS: 1789142786880073) to be considered for publication in *BMC Infectious Diseases*.

We thank the three reviewers for their insightful and informative comments. All of the reviewers consider our study to be of interest and importance within this research field, and appear to be fairly satisfied with the manuscript pending some minor amendments and clarifications. After careful consideration of each of the issues raised, we have revised our manuscript accordingly. A detailed point-by-point response is included below.

We thank you in advance for considering our revised manuscript, and we look forward to hearing from you in the near future.

Yours sincerely,

Rory Watt
**Editor’s Comments:**
The sentence: “Subjects consented to their individual data being published.” has been added to the methods section (page 7)

**Point-by-point response to the reviewers’ comments**

**Reviewer 1 (Dr. J. Christopher Fenno):**

No specific changes were requested.

**Reviewer 2 (Dr. Jacques Izard):**

**Major issues**

None

**Minor issues**

1) **Title.** Should read “oral treponemes associated”.
   
   **Response:** Agree – changed

2) **Abstract, line 4.** Too many “and”
   
   **Response:** one “and” has been removed

3) **Abstract, line 5.** “Oral treponemes (genus *Treponema*)” is the correct terminology
   
   **Response:** Agree – changed

4) **Page 4, line 3.** Too many “and”
   
   **Response:** one “and” has been removed

5) **Reference 15.** Please include the chapter of interest.
   
   **Response:** “chapter 1, pp9-18” added to reference

6) **References.** Please be sure that the italics are present where they should be.
   
   **Response:** This has been done

7) **Page 5, 2nd paragraph.** *Treponema medium* is missing from that list.
   
   **Response:** we thank the reviewer for spotting this significant oversight. This has been inserted, along with the corresponding reference.

8) **Page 5, 2nd paragraph, 2nd line.** Please remove “(Borrelia)”. This is past antiquated.
   
   **Response:** Agree. The word “Borrelia” has been removed

9) **Page 5, 2nd paragraph.** *Treponema pallidum* can be an infectious agent in the oral cavity. This should be mentioned.
   
   **Response:** Agree. A sentence and corresponding reference has been added to the bottom of page 5

10) **Page 7, line 13.** It should be mentioned for “each subject” as it leaves the reader unclear is all plaque of all subjects were pooled or not.

   **Response:** Agree. The phrase “from each subject” has been added to clarify this point.


   **Response:** Agree. The details for this reference have been changed

12) **Page 19, 1st paragraph last line.** The authors should mention that this reinforce previous observation both microscopy (Rosebury, Microorganisms indigenous to man, 1962) and by next generation sequencing (Genome Biol. 2012 Jun 14;13(6):R42. doi: 10.1186/gb-2012-13-6-r42.) and reference 51.
Response: We have modified the text, and have included references to next generation sequencing [including the suggested one from Genome Biol. (2012)]. As the 1962 book reference refers to microscopy-based studies, and does not refer to genetic analyses, we do not think it is entirely appropriate within this specific context.

13) Page 19, last line. Reference 14 indicates 49 phylotypes (98.5% cut off)
Response: Agree. We have corrected this error.

14) Page 20, last line. As-yet-to-be-cultivated

Reviewer 3: (Dr. Floyd Dewhirst)
1) The limitations of the work are not really addressed. The authors could add discussion of the advantages and disadvantages of their approach compared to next gen sequencing.
Response: We have added several sentences to the discussion section, which clearly outlines the limitations of our study.

Discretionary Revisions:
1) The authors have done an excellent job of placing their 99% similarity OTUs in the context of previous published work and HOMD similarity for novel OTU’s. So that all your OTUs can be related to the HOMD taxonomic scheme, I strongly urge you to combine supplementary Tables 1 & 2 so that there are columns for % similarity and Hit_HOMD_clone_name for all 110 of your OTUs.
Response: Agree. We have changed the format of Supplementary tables 1 and 2, so that all 110 OTUs can now be directly related to the HOMD taxonomic scheme. Correspondingly, we have added a sentence to the results section to indicate this.

2) For your readers who are interested in seeing the neighbor joining tree with branch lengths (rather than just topological ordering) I encourage you to include a tree (even as a supplementary Figure).
Response: Agree. We have included a new Supplementary Figure (additional file 4), which shows a Neighbour-Joining (NJ) tree with branch lengths proportional to genetic distances (i.e. a phylogram) analogous to the ultrametric NJ tree (cladogram) included in figure 4.

Minor Essential Revisions:
None noted.

Other minor changes we have made to the manuscript during our revisions
During our revisions, we thoroughly checked and re-analyzed all the treponeme OTU sequence data (included in the newly-revised additional files 1 and 2), and consequently re-evaluated the phylogenetic relationships. We realized that the NJ tree shown in the original figure 4 was not calculated using the precise methods reported in the materials and methods section. This has been corrected in the revised version. It should be noted that the ultrametric NJ tree shown in Panel A of the revised figure 4 is consistent with the NJ phylogram shown in the new Additional Figure 4. It may be noted that here are a few very minor differences in the tree topology, but this has no notable bearing on any results or conclusions.

Upon closer inspection of the NJ trees, we observed that phylogroup 5 contain two distinct clades. We have added a couple of sentences to the discussion section to speculate that the taxonomic classification of three OTUs (7P63, 7P6, 7P35) within the oral treponeme phylogroup framework may be revisited in the future; in light of additional 16S rRNA gene sequence data that may become available.