Dear Editor,

Enclosed please find our substantially revised manuscript “RNA-TVcurve: A Web Server for RNA Secondary Structure Comparison Based on a Multi-scale Similarity of Its Triple Vector Curve Representation”. In this revised manuscript, we have carefully addressed all the concerns by the two reviewers. We greatly appreciate the Referee’s comments on the previous draft of the paper and we hope that you and your reviewers find the revised version acceptable for publication in BMC Bioinformatics. The following is our point-by-point response to each of the criticisms by the two reviewers. I would like to take this opportunity to thank you for handling the review of our paper. The track of the revised manuscript is marked as red color.

*Our responses to the review comments are in blue and italic.*

Sincerely yours,

Ying Li, Ph.D.

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**Concern of Editor**: Reviewer reports: This manuscript was reviewed by two experts. Although Reviewer 2 recommend acceptance, Reviewer 1 gave critical comments. Therefore, I recommend the authors to revise the manuscript with taking all comments from Reviewer 1 into account (and comments from Reviewer 2 if possible). Please note that it is necessary to improve the web server and to compare with other methods.

***Response****: Thanks for providing us the chance to revise this manuscript. We have substantially revised the previous manuscript and made significant efforts in responding to the review comments/concerns. The web server of RNA-TVcurve has been substantially improved according to the comments of the two reviewers including adding novel functional module. In addition, a demonstration of the usages for each functional module has been polished and shown on the server. More details can be found below.*

**Reviewer #1**

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**Summary**: The manuscript by Li et al. presents the webserver RNA-TVcurve, the web frontend for a tool already presented in reference [29] doi:10.1186/1471-2105-13-280. RNA-TVcurve enables a combined representation of an RNA sequence in concert with its (mfe) structure as a numerical vector, named TV-curve, which can be easily plotted in 2D. Based on TV-curves, a similarity measure was introduced in [29], which can be used to compare RNAs e.g. to compute point mutation profiles or to derive phylogenetic trees. After giving a brief overview of RNA similarity related (alignment-free and alignment-based) tools, the present manuscript provides a rough overview on how to compute a similarity notion based on TV-curves. This is followed by a discussion of the webserver in- and output interfaces for three modes of operation. The results section studies an evolutionary tree derived by RNA-TVcurve in comparison to the trees derived by RNA similarities based on RNAdistance and RNApdist for a 5S rRNA dataset. Furthermore, a correlation coefficient study is performed for samples from four Rfam families.

**General comments**：

Overall, I find this manuscript to be a follow up of the original manuscript [29] without much news concerning method or results. So it basically presents the webserver while showing a flaws concerning related work and benchmarking (see detailed comments). The methods part describes RNA comparison based on TV-curves only. Thus, the two "main application modi" of the webserver "Mutation" and "Multiple" are not described at all. Only a collection of formulas without description etc. is provided at almost non-readable resolution within figure 1. Furthermore, I think an evaluation which only compares to RNAdistance and RNApdist not sufficient to benchmark RNA-TVcurve. It is more the simplest and straight-forward way, since both tools are provided by the Vienna RNA package. The claim by the authors that "no other web servers based on alignment-free methods for RNA structure comparison are available in the public domain" cannot be accepted, since they do not use webservers for the alignment-based methods RNAdistance and RNApdist neither. A short google search provided for instance doi:10.1093/nar/gku283 with available source code. The latter article also provides comparison to other tools like LocARNA, RNAStrAT, RNAforester, ...Another recent webserver in the field: doi:10.1093/nar/gkv489. I tested the webserver with the provided examples as well as a few manual inputs. Beside of being not available on 5th Sept, the webserver was working as expected. Still, there are a couple of improvements needed in order to make it a useful tool within external research. So far, it basically enables the (re)production of figures presented in [29] and the current manuscript. For the actual data, which is of interest for downstream analyses, one has to relate to the provided ZIP file. This significantly slows down and provides bad user experience. A major drawback of the webserver is its sequence-only input restriction. That way, only the automatically predicted mfe-structure of a sequence can be taken into account for comparison and downstream analyses. This restriction is only necessary for the mutation mode but obsolete for TV-curve computation and evolutionary tree generation. Here, expert knowledge about the functional structure of each RNA would be of much help. Finally, only limited reproducibility is enabled, since neither the used tool versions nor the applied scripts (and versions) are available. Also, in contrast to the manuscript statement "All the related results and source code can be freely downloaded at ...", no source code of RNA-TVcurve was found. This makes RNA-TVcurve only applicable via the webserver, which is no option for large scale analyses typical for current bioinformatics research. Given this, I cannot recommend to accept the current manuscript and webserver in its current form for publication. From my point of view, both changes in the manuscript as well as webserver are needed.

***Response****: Thanks for your comments. The web server has been substantially improved and a novel functional module RNA Pairwise has been added to perform the comparison between two RNA sequences or two sets of RNA. The input of our web server become more flexible, which include sequence or optional structure. The more comprehensive introduction on the existing RNA secondary structure alignment algorithms. The time complexity of our proposed method is pointed out. The methods of RNA 3D structure alignment are briefly introduced in the revised manuscript . The functional module of TVCurve allow to visualize multiple RNAs. We added the computational descriptions and flowcharts for the two main functional modules “Mutation” and “RNA Multiple” in the Method section. The study in [1] (doi:10.1093/nar/gku283)，include two points: 1) a new RNA secondary string representation based on strings of characters context-aware structural encoding represented by a string of characters. Each character in BEAR encodes for a specific secondary structure element (loop, stem, bulge and internal loop) with specific length. 2)Alignment of the new BEAR encodes to obtain the RNA structure alignment. The complete source codes are not provided. Just the execution file for BEAR encodes from the RNA secondary structure is available. All the related results and source code can be freely downloaded at the web server. In the revised manuscript we have improved the web server and carried out a systematic test on different types of samples for each functional module. In the following, the detailed response to each concern is provided.*

**Major comments** - manuscript (line annotated)

**Concern 1**: -45 : "A representative" -> "A most generic representative" with Sankoff you have picked one of the most expensive algorithms there are, which is by no means the standard concerning efficiency in the field! So better replace or rephrase this whole sentence.

***Response****: We have modified the sentence as suggested, by deleting “representative”.*

**Concern 2**: -48 : RNAdistance and RNApdist are both NO Sankoff-style algorithms (compare DOI:10.1186/1471-2105-5-140), so this sentence is wrong as it states this. Sankoff-like tools are PMcomp, LocARNA, CARNA, ..

***Response****: Thanks for this comment. We carefully read the paper [2] that you* [*recommend*](javascript:void(0);)[*to*](javascript:void(0);)[*us*](javascript:void(0);) *and corrected the wrong statements about RNAdistance and RNApdist. In addition, we have added more Sankoff-like and NO Sankoff-style algorithms in the revised manuscript.*

**Concern 3**: -54 : You state that alignment-based tools have extensive time requirement but in line 214 you say that RNA-TVcurve is only about 3-times faster than RNApdist (and say nothing about RNAdistance, which is much fast compared to RNApdist). Thus it seems, your tools is only in a linear factor faster than the O(n^3) time complexity algorithm RNApdist... Please comment on this!!!

***Response****: Thanks for this comment. The main computation cost of RNA-TVcurve is the wavelet decomposition, which can be conducted in terms of Mallat algorithm with time complexity O(n), where n is the length of RNA sequence. Therefore, the time complexity of RNA-TVcurve is O(n) and dramatically reduce the computational time complexity of RNApdist and RNAdistance, i.e., O(n3).*

**Concern 4**:-68 : Please recheck literature for relevant articles since I already found two recent manuscripts with a short google search.. (see general comments above) There are also methods for the distance/alignment computation for 3D RNA data, eg. DOI:10.1186/s12859-015-0696-8

***Response****: Thanks for this comment. We recheck the relevant papers including RNA secondary structure alignment and 3D structure alignment and added them in Introduction section in the revised manuscript.*

**Concern 5**:-78 : "no other web servers ... are available" : as stated already above, this is no valid argument. You did a local installation of the Vienna package to use RNAdistance and RNApdist, so why not locally installing other tools as referenced above? Furthermore, comparison to other alignment-based tools are easy to conduct, since there are e.g. webservers like Web-Beagle, LocARNA, MARNA, RNAforester, ... which provide similarity notions for the produced alignments that can be easily used for comparison.

***Response****: Thanks for this comment. The statement of “No other web servers for alignment-free RNA structural comparison are available” is originally used to express the significance of our alignment-free web server for RNA structure comparison. We are unable to find the source codes or* [*executable*](javascript:void(0);)[*file*](javascript:void(0);) *of other methods of RNA alignment free structure comparison. RNAdistance and RNApdist are two widely and easily used tools for RNA secondary structure comparison, which have been packed in the famous Vienna package. We think they may be the better choice to compare with our method. The time complexity of RNAdistance and RNApdist are O(n3). The time complexity of our RNA-TVcurve is O(n).*

**Concern 6**: -80 : "RNA-TVcurve algorithm is more efficient ..." : you never show this! Either remove of provide data to support this claim. The only notion is the "3 times faster" discussed above.

***Response****: We have removed this statement. The time complexity comparison between our method and other tools has been added.*

**Concern 7**: -81 : "results are shown ... intuitive table .." : actually I never found any table in the webserver output. It only provides figures which with floating layout. Please comment.

***Response****: We have changed it as figures. All the tables of results can be downloaded in zip file. The figures are just shown on the web interface.*

**Concern 8**: -81 : "source code can be freely downloaded" : I don’t find any option to download RNA-TVcurve nor any related script... Please comment.

***Response****: We provided* ***all the source*** *code on the “About” interface of our web server.*

**Concern 9**: -101 : "help users to intuitively and efficiently identify the difference between RNAs, especially for the long RNAs." : actually I cannot agree at all. There is no intuitive mapping of the ACGU+prime alphabet to the respective numeric encodings and thus there is no intuitive interpretation possible. Especially for long RNAs it is almost impossible to relate or study individual positions etc. Please rephrase the sentence!

***Response:*** *We rewrote the sentences to emphasized that RNA-TVcurve can provide users another angle to display and analyze the difference between RNAs, especially for the long RNAs.*

**Concern 10**: -84 : As stated above, the two modi "mutation" and "multiple" are entirely without method descriptions. So either add according descriptions within the methods section or provide a supplementary material.

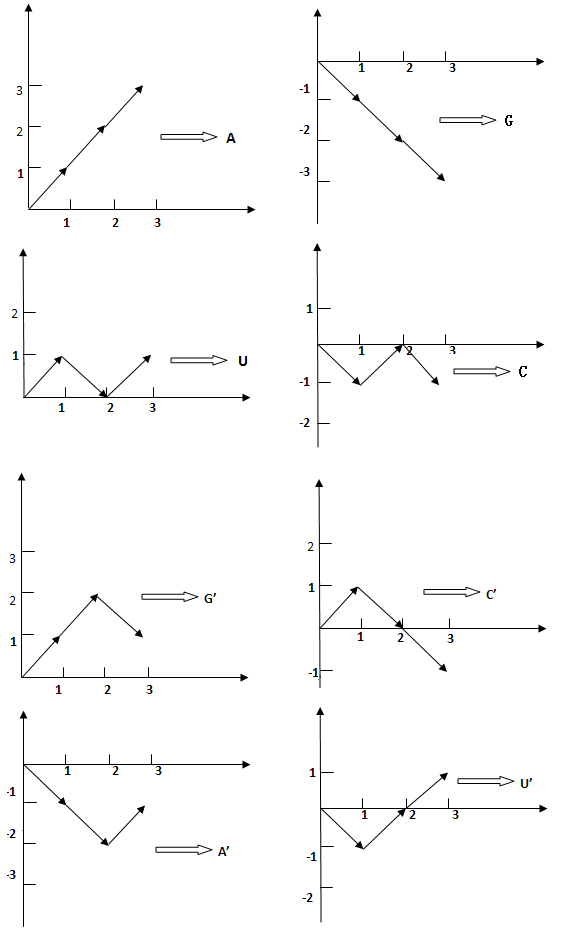
***Response****: Thanks for this comment. We added the two parts of the descriptions of computational processes for the two main functional module “Mutation” and “RNA Multiple” in the Method section.*

**Concern 11**: -95 : your numerical nucleotide representation can be compressed to the y-change   A => (1,1,1), U => (1,-1,1), ...since you always increase "x" by one. This way, the TV-curve would be the cumulative sum up to each position of the concatenation of the mapped triples. The x-coordinates are just the indices within the triplet concatenation. You actually never properly defined the plotted TV-curve data, neither here nor in [29],

as the cumulative sum over your coordinate change vectors you are mapping to.

***Response****: The unpaired A, T, C, G and paired A', U', G' and C' are represented by triple vectors respectively as follows:*

*The plots of TV-Curve Representation four unpaired nucleotides (A, T, C and G) and four paired nucleotides (A', U', G' and C') of TV-Curve are showed in the following figure:*



From the above figure, it is easy to understand how to plot the RNA TV curve. This figure is provided in the subplot of the Figure 1 in the revised manuscript.

**Concern 12**: -Fig.1 : the resolution of the image is too low. Formulas are hardly or not readable. Best provide a vector graphics or a figure with higher resolution. The triple representation is strongly distorted compared to [29]. Providing the formulas without further explanation is without use and only raises more questions than answers. Best remove all formulas from the figure since its only use is to sketch the workflow without details. If you want to keep the formulas, you will have to provide details for them within the manuscript.

***Response****: Thanks for this comment. We have changed the image quality in Figure 1 and delete the formulas from the workflow figure to make it more readable.*

**Concern 13**:-Fig.3,4,5 : There is no reason to give a step-by-step intro given your webserver is well documented. It would be sufficient to reduce the figure to sh[Schematic](javascript:void(0);) [diagram](javascript:void(0);) of functional Module ow the output page, i.e. the (c) part.

***Response****: Thanks for this comment. Due to the dramatic improvement of our web server, we renew to make the Figure 1 to introduce the web server of RNA-TVcurve including four functional modules. The original Fig 3,4 and 5 have also been changed into the new figures of* [*schematic*](javascript:void(0);)[*diagram*](javascript:void(0);) *of functional Module TVCurve, RNA mutation and RNA Multiple as Supplementary figure 1,2 and 3. In addition, schematic diagram of the novel functional module RNA Pairwise is provide as Supplementary 4.*

**Concern 14**: The chosen example can be named in the caption and the most important files contained in the ZIP can be discussed in the according manuscript section.

***Response****: The most important files are packed in the ZIP file for download and they are introduced in the revised manuscript for each functional module.*

**Concern 15**:-Fig.5 : The shown circle plots are not part of the current webserver output. Update!

***Response****: In the original version, the circle* *phylogenetic tree is provided to settle the problem that the labels of leaves on the tree are hard to read when the number of RNAs is large. In the improved web server, we changed the tree at horizontal direction in order to more clearly to display the lables of the leaves on the tree when the number of multiple RNAs larger than 20. Therefore, the circle phylogenetic tree is removed. The svg format file of the phylogenetic tree and the distance matrix file are provided for users to download, which help users to draw different format of phylogenetic tree for future analysis.*

**Concern 16**:-185 : To enable reproducibility, provide the used sequence sets within the supplementary material.

***Response****: Thank you for this comment. All the examples in the manuscript are provided as test examples listed on the web server for each functional functional module. Users can directly test the performance of each functional module of our web server through selection the different example sets listed on the web server.*

**Concern 17**:--182 : Since you derived the data set(s) for known organisms, you should provide the "true" phylogenetic tree (or at least an approximate variant) in order to enable a visual comparison of the three tree predictions.

***Response****: The evolutionary relationships are not fully clear. We just provide the known relationship among them in the table and compare the capability of different methods to capture such known evolution relationships.*

Concern 18:-183 : You use the sequences from [26] but don’t compare against the trees from [26]. Please comment! You can easily run the Neighbor Joining algorithm used in [26] on your distance matrices.

*Response: The reference [26] in the original submitted manuscript has been changed as reference [45] in the revised manuscript. The source code and execute file are not available. The three different sets used in that alignment-free including not only the sequences but also the existing structures are not provided. So we obtain the sets of RNA sequences alone by different databases search. The secondary structure for the two sets: Set I 5S RNAs RNase P and Set II RNase MRP used in our manuscript are predicted using minimum free energy algorithm RNAfold. The original inputs for these two methods are not the same sets. That is the main reason we did not directly compare with tree constructed in the [45].*

**Concern 19**: -Fig.6,7 : describe boxes/lines since figure+caption should be self-explaining

*Response: Thanks for this comment. We added the description to explain the boxes and lines in the figures in the figure legends.*

**Concern 20**: -207 : Hubert statistics is no standard textbook knowledge, so you have to provide the source reference. Furthermore, the according formula from [29] shows that you are basically doing a Pearson correlation coefficient, i.e. the results are equal to Pearson after vectorizing your matrices. So why confuse people with Hubert when you are basically doing standard Pearson? Best rewrite the paragraph.

***Response****: We have rewritten the statement of the Hubert statistics as Pearson correlation to avoid the confusion.*

**Concern 21**: -210 : "large amount" : How many are there? Provide some numbers.

***Response****: The number of the RNA sequences is 19111, which has been added in the revised manuscript.*

**Concern 22**:-217 : see comment for line 207 and adapt accordingly since you are just doing statistics on Pearson correlation coefficients.

***Response****: Thanks for this comment. We have corrected Hubert statistics as Pearson correlation.*

**Concern 23**:-226 : "fill a void in this field" is a bit of an overstatement in my opinion. Best remove or rephrase.

***Response****: We have removed this sentence in the manuscript.*

**Concern 24**:-231 : You should be aware and add a statement that RNApdist is considering the whole structure space an RNA sequence can adopt. Thus, it is even beyond suboptimal structures. Note further (for your coming research) that most structures close to mfe are very similar to the mfe-structure and thus won’t make your comparison any better unless you use reasonable filters.

***Response****: Thanks for this comment. It is very critical for our future study. We have added this statement in the revised manuscript and discussed the integration of the suboptimal structures in the conclusion section.*

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**Major comments** - webserver

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###### general ######

**Concern 23**:- No mode for pairwise comparison available !!! Within the manuscript, you make it the central point to enable (pairwise) similarity comparison of RNAs, but your whole server does not allow this! The only workaround would be to run the tree prediction to extract the distance table, which is not working if only 2 sequences are of interest. Furthermore, such a service would require sequence+structure input to be of use, which is not possible for any of the modi.

***Response****: Thanks for this comment. We added the novel functional module to perform the pairwise comparison. For this module, not only comparison of one pair of RNAs is conducted. But also a pair of RNA set can be compared one by one to help users batch computation. The distance scores are provided to further extend the application of our web server. In addition, the input of each module is corrected to allow RNA sequences and structures, or RNA sequences and part of structures, or RNA sequences alone.*

**Concern 24**:- The output pages lack a lot of information concerning what is shown and how to interpret. The webserver output has to be understandable WITHOUT reading the paper.

***Response****: We improved the web server to make it more interpretable. The outputs of the web server are more understandable. Along with the download zip files, the corresponding readme files are provided.*

**Concern 25**:-- It is not clear what Version of the Vienna RNA package you are using.

***Response****: We have provided the version of the Vienna RNA package used in our web server on the “About” page at our web server.*

**Concern 26**:-- The used RNAfold parameterization is not available, but the chosen energy parameters have a strong influence on the mfe-structure prediction. Thus, even assuming you are just using default parameters, the reproducibility is not given, since the defaults are varying between different Vienna package versions.

***Response****: Thanks for this comment. The energy parameters indeed have a strong influence on the structure prediction based on the minimum free energy. We have provided the version of Vienna package, with the default parameters of the energy parameters for RNAfold.*

The RNAfold

**Concern 27**:-- The contact page shows only email addresses but no names etc.

***Response****: We have added the names for each contact person on the contact page.*

**Concern 28**:-- Source code / program not available for local installation (claimed in paper)

***Response****: We have added the names for each contact person on the “About” page at our web server.*

**Concern 29**:-- Licence page without content.

***Response****: We have added the related content in the Licence page.*

**Concern 30**:-- The output page's layout strongly depending on monitor width due to floating image positioning. Since the images are zoomed on click (which is a "silent" feature not mentioned anywhere), you might provide only low scale figures in a fixed layout.

***Response****: Thanks for this comment. We have showcased the SVG format image on thee output page to improve the quality of the display.*

**Concern 31**:-- Language mistakes e.g. ".. check out it later.", please double check.

***Response****: We have corrected the language mistake.*

**Concern 32**:-- Stability of availability? (not available on 05.09.2016)

***Response****: We have improved the web server to increase the stability of the web serve.*

**Concern 33**:-- So far, the server is mainly useful to (re)produce the paper figures. For any information interesting for downstream analyses (like similarity values, ...) the user has to download the ZIP file, extract, and find the according data. This reduces, in my opinion, the user experience and thus might lead to reduced interest in your server. - no axis information in plots

***Response****: Thanks for this comment. For the improved web server, we try to provide more information of our results to be shown and downloaded, aiming to facilitate any downstream analysis. For RNA Pairwise Functional Module, the distance scores are directly shown on the output page. For RNA Multiple functional module, the distance matrix used to compute the phylogenetic tree is provide users to download for further analysis.*

**Concern 34**:-- no info about enlargment of images on click

***Response****: We provided the SVG format image file to make users easily enlarge the images.*

**Concern 35**:-- The output page should provide information on what is to be found in the ZIP file or according information (e.g. README file) should be added to each ZIP. It is of no use that the file content is explained in the manuscript figures.

***Response****: Thanks for this comment. This comment is very important to increase the practicability of our web server. We added the README file for each download ZIP file to explain relative details.*

###### TV-curve mode ######

**Concern 36**:-- Why do you restrict the input to one sequence? You can easily provide a tabularized overview of TV-curves etc. for more than one sequence in the output page. In its current restriction, the user has to submit individual jobs if interested in a bunch of sequences.

***Response****: Thanks for the comments. For RNA TV-curve functional module, the one sequence limitation is*

*cancelled. The input of this functional module can be the set of multiple RNA sequences and structures or*

*RNA sequences and part of structures, or RNA sequences alone.*

**Concern 37**:- It would be most reasonable and useful to allow structure input along with each sequence, to enable the representation of expert knowledge. Using the mfe only is extremely restrictive, since it is not the functional structure for most RNAs.

***Response****: Thanks for the comments. We have corrected all the functional module to allow that the input can be RNA sequence and structure or RNA sequence alone. If the input is just the RNA sequence, the secondary structure with the minimum free energy will be predicted by the tool RNAfold packed in the Vienna package.*

###### mutation mode ######

**Concern 38**:-- Again a bad user feeling: to get the mutated sequence a full ZIP download is needed. Just show the final sequences in result page. The mutation information below the figures is important but for downstream analyses the full sequence is of interest.

***Response****: Currently, not only the image is provided but also the mutated sequence and structure are given on the web page.*

**Concern 39**:-- Output: there is no information on what is shown and how to interprete the plots.

***Response****: For RNA mutation functional module, the information on the plots can be obtained from the image below, which directly provided the mutation type and mutation site with the maximum structure distance between the wild-type RNA and this RNA mutant.*

###### phylo-tree with comparison ######

**Concern 40**:- sequence names not readable in plot

***Response****: The problem has been fixed.*

**Concern 41**:-- How is the coloring derived? from FASTA-name-prefix?! This is neither documented nor generic!

***Response****: The coloring image of the phylogenetic tree is just for the example of the noncoding RNA family classification used in the original submitted manuscript, which is used to help user to more easily compare the performance among the different methods. This is documented not generic. In order to making confusion, we cancelled the coloring.*

**Concern 42**:-- ERROR mesage "Info: All invalid protein(s) are removed. ..." -> C&P error...

***Response****: This error has been corrected.*

**Concern 43**:-- no info ab out minimal sequence length

***Response****: We provide the information about the minimal sequence length.*

**Concern 44**:-- too short sequences are removed instead of error message only, this is annoying

***Response****: We have made the suggested changes.*

**Concern 45**-- TVcurve.txt (in ZIP root dir) empty : multiple\_0e9370338f312eb5618df0222851eb01ed67973c

***Response****: We corrected this error.*

**Concern 46**:-- MultipleRNAEngerge.txt (Fig5) missing

***Response****: We allow the input including the known structures. Therefore, we did not provided the minimum free energy file in the downloaded zip file.*

###### phylo-tree without comparison ######

**Concern 47** - BUG: produces mfe-plots only, eg. job multiple2\_0e9370338f312eb5618df0222851eb01 ed67973c. - Why is this not the "normal" phylo-tree mode? If you are confident in your method, it is sufficient to produce YOUR tree. To offer the trees for RNApdist and RNAdistance is an optional feature and shouldn’t be the default unless the server is for paper figure generation only.

***Response****: Thanks for the comments. We have corrected this bug of the code. For RNA Multiple and RNA Mutation module, the option “Compare with RNAdistance and RNApdist” are provided. The results based on RNA-TVcurve are computed.*

**Concern 48**: Detailed comments - manuscript (line annotated)

-25 : remove "those"

-26 : remove "in this study" since the method was proposed in [29]

-33 : what is the use/meaning of "in-house" here? remove if not needed

-41 : "usually less conserved at sequence level" : this is a quite general statement and I am not sure if true. Better restrict to something like " .. in structured regions" or similar since there are a lot of well conserved sequence motifs in RNAs too.

-55 : "graphical" -> "numerical" : the plot is only a visualization of the data

-57 : "various of" -> remove "of"

-59 : add "the" in "of the representative"

-63 : remove "graphical"

-64 : "Specifically" -> "For instance"

-85 : "methodology in" -> "methodology for pairwise RNA comparison in"

-99 : "shown in Figure 1.3" -> the diagrams are just blury and distorted at the provided image resolution.

-100 : "good" : remove since this is a very subjective view...

-115 : "TV curve" -> "TV-curve"

-117 : "Its accessible" -> "The usage"

-122 : "are two single" -> "are single"

-136 : "The functionality" -> "The output"

-137 : remove "then" + "are showed"

-155 : "lager" -> "larger"

-157 : "circle tree" are missing in the output and (given Fig. 5) are not useful anyway since you crop most of the sequence names... So best remove their annoucement here or add to server output.

-158 : "sequences four" -> "sequences of four"

-164 : space missing after "(3)"

-166,169,172 : "Module" -> "module"

-167,170,173 : space before ", (C)"

-168,171,174 : "results" -> "result"

-173 : ", ," -> remove one comma

-180 : "types RNA" -> "types of RNA"

-182,193 : "RNA Nase" -> "RNase"

-189,196 : latin organism names in italic

-191 : remove "he"

-195 : "RNA RNase" -> remove first RNA

-205 : "In the study of type" -> "Next"

-206 : "types RNA" -> "types of RNA"

-275 : "Hochsmann" -> "Hoechsmann"

-277 : "Schuste" -> "Schuster"

-325 : rewrite capital letters

***Response****: Thanks for all the detailed comments. We have carefully modified the manuscript according to your comments.*

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**Reviewer #2**

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Li et al. present a webserver RNA-TVcurve that can be used to compare RNA secondary structures, rank point mutations, and generate phylogenetic tree based on structural distance. The core algorithm implemented in the server is based on wavelet decomposition of the TVcurve. **This website may potentially be useful for RNA structural analysis.** The manuscript is also well-written. I have the following concerns of the manuscript.

**Concern 1**: line 48, 49: RNAdistance and RNAforester are not variants of Sankoff's algorithm; they are tree-alignment algorithms.

***Response****: Thanks for all the comments. We have rewritten this paragraph to introduce the alignment based RNA structure comparison algorithm according to two categories including the variants of Sankoff's algorithm and non-variants of Sankoff's algorithm. The biased statement has been corrected.*

**Concern 2**: The authors discussed time complexity issue of RNA secondary structure alignment, and they should cite recent works that aim at reducing the complexity of RNA secondary structure alignment. For example: "Zhong and Zhang, Efficient alignment of RNA secondary structures using sparse dynamic programming, BMC Bioinformatics 2013". They should also clearly present the time complexity of the programs they mentioned, specifically the one for RNA-TVcurve algorithm (citation 29 as mentioned in line 85).

***Response****: Thanks for the comment. We have added the introduction of the algorithm ERA and provided the time complexity of RNA-TVcurve in the revised manuscript. The time complexity of our method is linear.*

**Concern 3**: The method implemented for analyzing RNA mutation is confusing. Does it test all possible mutations at all sites? If yes, the time complexity would be 4^L, where L is the length of the input sequence. If it is the case, the authors should think of a better algorithm to speed it up. Otherwise please be more specific on the method description in the manuscript.

***Response****: Thanks for the comment. The functional module of RNA Mutation just focuses on the single-point mutation detection, which has been emphasized in the revised manuscript. As your mentioned, if we consider all possible mutations at all sites, it would be super time-consuming. In the future, we need do more study on this direction.*

**Concern 4**: For phylogenetic tree generation, the authors should use more accurate RNA alignment methods to construct the "ground-truth" tree for comparison other than using RNApdist and RNAdistance as they are fast but with low alignment quality. LocARNA or ERA should be considered as they implement Sankoff's algorithm and are known to generate high quality alignment.

***Response****: Thanks for this comment. For phylogenetic tree construction, the real ground-true information is used to evaluate the accuracy of the tree constructed by RNA TV-curve method. RNA distance and RNApdist are the alignment-based tools used to compare with our method. The main reason to choose these two tools mainly lies in they are the widely and easily used RNA structure comparison tools packed in the famous Vienna package. Our proposed method is based on alignment-free and the time complexity is linear.*

**REFERENCES**

1. Mattei, E., et al., A novel approach to represent and compare RNA secondary structures. Nucleic Acids Res, 2014. 42(10): p. 6146-57.

2. Gardner, P.P. and R. Giegerich, A comprehensive comparison of comparative RNA structure prediction approaches. BMC Bioinformatics, 2004. 5: p. 140.