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From: Pradeep Uchil <pdu@mcbl.iisc.ernet.in>

The following points will be elaborated in the discussion.

1)The realibility of the tree topology pointed out by the fact that all the three methods used i.e., likelihood, parsimony, distance gave the same tree topology. The high bootstrap support values also convincingly puts forth above point.
2)The early divergence of P20778 with respect to the other isolates used.
3)The date of divergence for the above node which clearly indicates that the virus has been around earlier than 1958 (the time when JEV cases were first reported in India). This will be attributed to sylvan cycle (virus life cyle restricted to the wild animals) which at some time point was disturbed by the humans to make the human beings now an accidental host.
4)The other North Indian isolates are of different origin since they club with the Japanese strain as against P20778 which going by its close branching order with the chinese isolate L48961 might have its origin in China.
5)The usage of nonstructural proteins like NS4a, NS2b, NS5 and more importantly NS3 as a good phylogenetic marker.

#Apart from discussion part, we still need to mention about the trees we will be showing, in the text. We were planning to show only two trees
1)tree based on the NS3 gene
2)tree based on the full length amino acid sequence. ✓

#In the results section the 'Dating the divergence node of P20778' is still incomplete. In this section there will be a table which shows the divergence dates of P20778 based on different genes. We have done the analysis for three genes : envelope, NS3 & NS5.

There are certain areas where we would want your expert coments:
1) This problem we had already discussed once. But that was long back. Hence I am mentioning it all over again. This is regarding Table. 2 and Table 2.1 . Table 2 will be finally shown. Here we need to get an error value for Ps/Pn. Ps and Pn are average values for the proportion of syn or nonsyn substituions obtained from MEGA. They don't provide a method by which standard error values can be obtained for the ratio. Firstly, is it correct to take ratio of Ps and Pn? If yes how does one get an error value for the ratio ? yes given
2)Table 2 represents an analysis with all the JEV sequences taken into consideration. Table 2.1 is with GP78 sequence deleted from the analysis. GP78 seems to skew the results a lot. If you look at the % variable sites (aa + nt) for capsid gene most of the nucleotide changes seem to change the amino acid sequence too. This seems absurd since capsid remains bound to the RNA and wrt to envelope is not subject to positive selection to an extent as denoted by the numbers denoted. When GP78 seuence is deleted from the analysis (table 2.1) a more realistic picture is obtained with envelope gene being the one with highest number of variable sites. Table 2.1 also shows that NS3 is one of the most conserved gene with highest Ps/Pn ratio and least % variable amino acids. must clear
This discrepancy is also observed in the bootstrap values that are obtained when GP78 sequence is included. Tree 1 and Tree 2 are obtained using maximum likelihood analysis . Eventhough the tree topology is consistent the bootstrap support values are low when GP78 sequence is included. Can you please give your comments on these points?
If you need any more information I will be be happy to give it to you as soon as possible.
Awaiting for your reply
Pradeep.D.Uchil

Can you compute total # of changes (non syn changes) in each w.r.t. consensus seq. & show something amiss with GP78?