TBE NEWS

IS THE EUROPEAN SUBTYPE OF TICK-BORNE ENCEPHALITIS VIRUS (TBEV-EU) A DISTINCT SPECIES? DELIMITATION ANALYSIS PROVIDES NEW GENOMIC EVIDENCE

Background

There have been long heated discussions in the literature about the taxonomic status of several subtypes of tick-borne encephalitis virus (TBEV). In particular, it is about the European subtype of TBEV (TBEV-EU). In terms of cladistics, this is due to the fact that the common TBEV clade includes the louping-ill virus (LIV) taxon resulting in paraphyletic relationships between these two closely related species (Fig. 1):

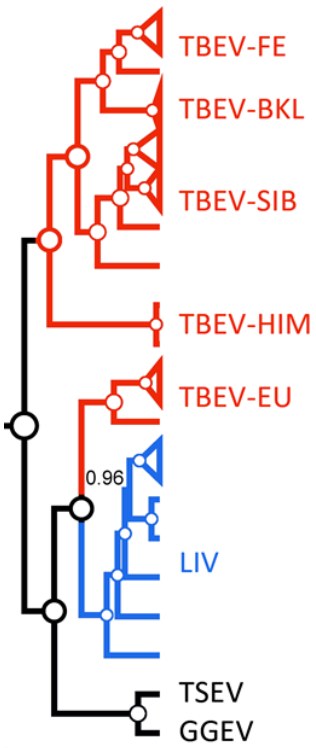

Figure 1: The paraphyly of a TBEV taxon (red color) due to LIV inclusion. White circles depict posterior probability of 1.02. TSEV – Turkish sheep encephalitis virus; GGEV – Greek goat encephalitis virus (both of them are unclassified LIV-like viruses).

In the interim, the species definition claimed by ICTV is "A species is a monophyletic group of viruses whose properties can be distinguished from those of other species by multiple criteria". To follow this objective principle, TBEV and LIV paraphyletic issue should be resolved. This could be done in two ways: whether by the separation of TBEV-E from the other TBEV subtypes (Far-Eastern [-FE], Siberian [-SIB], Baikalian [BKL], and Himalayan [HIM]) or by merging TBEV+LIV into the single species taxon. The last solution was proposed by Grard et al[6] where authors combine TBEV and LIV taxa into the single species based on amino acid (aa) p-distances (0.09) of complete polyprotein aa sequences (4 for TBEV and 4 for LIV). Charrel et al[2] came to the same conclusions on the TBEV+LIV integration based, however, on the analysis of E gene sequence (4 for TBEV and 9 for LIV). In both cases, the number of sequences analyzed was small regarding the currently available data. Moreover, the so-called "cut-off rule" (as in the case of Grard et al[6]) has one major flaw – the absence of biological rationale underlay the threshold proposed. Despite pairwise distance thresholds being able to work well in practice,[8] evolutionary methods are needed to validate their use. Also, an underlying evolutionary model makes it possible to compare alternative evolutionary hypotheses statistically.[3] Thus, the merger of TBEV+LIV applying only evolutionary distances can be questioned.

Meanwhile, a large amount of data on the eye-catching differences between virus particularities of TBEV-EU and other TBEV subtypes have been accumulated at the moment that enables consideration of TBEV-EU as an independent

taxonomic unit.

Thereby, to holistically scrutinize the paraphyletic problem of the TBEV+LIV group, we, at first, employed three available delimitation methods (GMYC, ABGD, PTP) using complete polyprotein aa sequences (n = 278) of all tick-borne flaviviruses (TBFV, 12 species), and finally, we analyzed available literature for other viral species criteria.

Delimitation Analysis

All three delimitation methods demonstrated that TBEV+LIV is not a single species unit. TBEV-E was separated into distinct taxon as well. Some methods such as PTP were tended to fraction TBEV subtypes within the clade more frequently, but none of them merged TBEV+E with other TBEV subtypes or with LIV (Fig. 2):

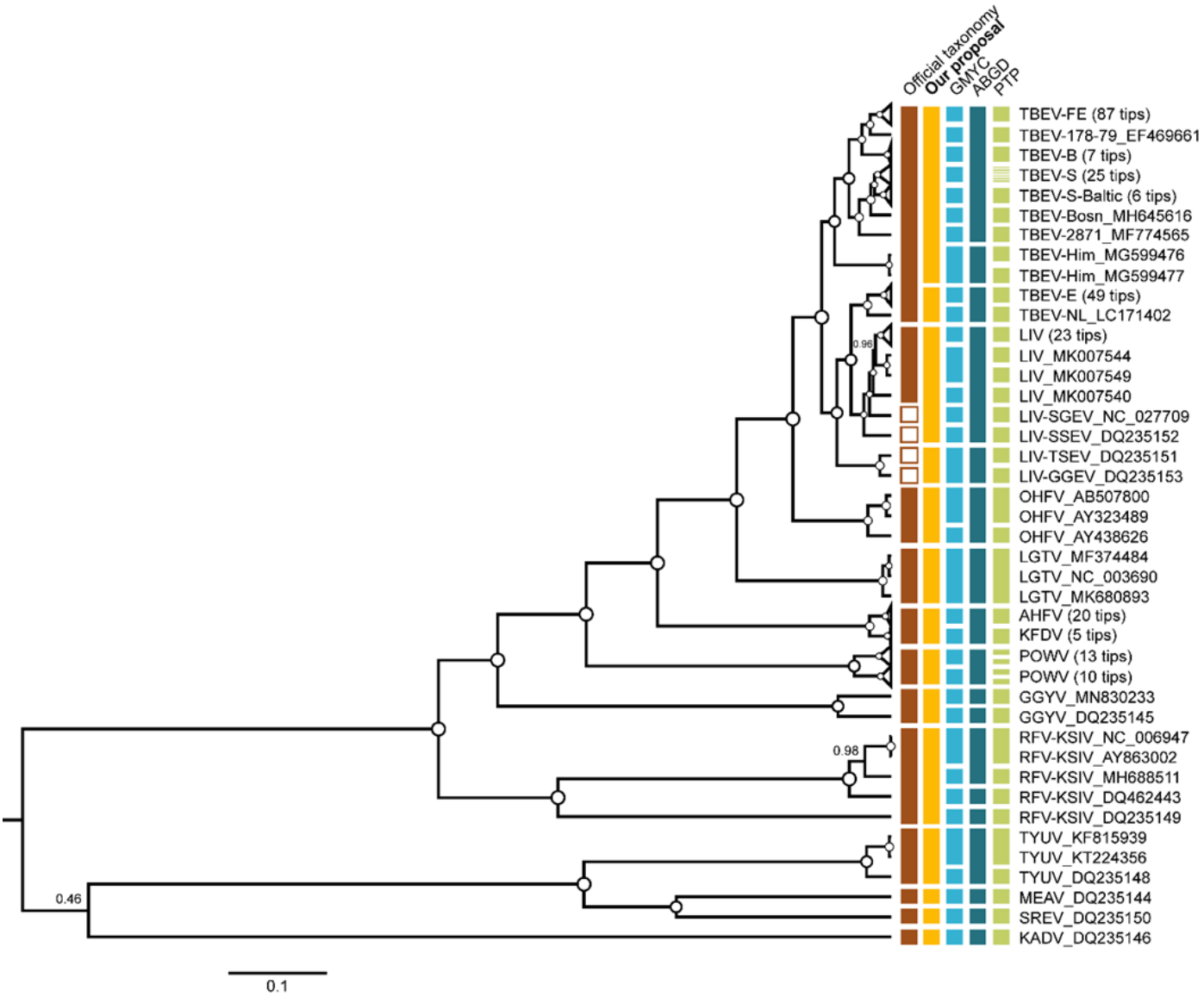

Figure 2: The phylogenetic tree of the TBFVs. The tree was reconstructed in BEAST using complete amino acid sequences (n = 278) of the polyprotein (3414 aa). For clarity, some of the wide clades were collapsed. Vertical bars to the right of tree tips indicate official classification (brown), our taxonomy proposal (orange), and delimitation results. Internal nodes with pp = 1 are marked as white circles, otherwise support values are shown by numbers ranging from 0 to 1.

Comparison of the Other Virus Particularities

We reviewed the literature for the other virus species criteria: natural and experimental host range, cell and tissue tropism, pathogenicity, vector specificity, and antigenicity. We have put all data on TBEV and LIV particularities observed into a table:

Virus	Crossing BBB and a form of a disease			Lethal cases in humans	Main vector	Host	Primary geographic location	Delimitation results	aa evolutionary distances
	Human	Sheep	Rodents						
LIV	**ME (b)**	**E**	**–**	1 case	*I. ricin.*	Sheeps, r.grouses*	British islands	distinct	differ
-EU	**ME (b)**	**M**	**E**	1–2%	*I. ricin.*	Small rodents	Europe	distinct	differ
-SIB	**E (m)**	**?**	**E**	6–8%	*I. pers.*	Small rodents	Central Russia	distinct	do not differ
-FE	**E (m)**	**E**	**E**	~20%	*I. pers.*	Small rodents	Far-East	distinct	do not differ

Table 1: Disease forms: E – encephalitis, M – meningitis, ME – meningoencephalitis; (m – monophasic, b – biphasic). *Rodents produce very low levels of viraemia as a result of LIV infection and do not support viraemic transmission as well.

As we can see, in some particularities, TBEV-EU is different from other TBEV subtypes and LIV, and sometimes it was closer whether to LIV or other TBEV members. We highlight two striking differences: reservoir host and pathogenicity in sheep. Unlike all TBEV subtypes, LIV is primarily found in red grouses and sheep inducing encephalitis and high mortality rate in both (78% in red grouses,[4] 5–60% in sheep,[7] not small rodents. Although rodents such as field voles (*Microtus agrestis*), bank voles (*M. glareolus*) and wood mice (*Apodemus sylvaticus*) raised an antibody response to infection, they could not produce a substantial viremia and did not support non-viremic transmission between co-feeding ticks.[5] This leads to the fact that LIV has patchy spatial distribution with different combinations of reservoir hosts occurring. This is exactly the opposite of the TBEV transmission patterns and natural foci structure formed by primarily small rodents. Concerning pathogenicity, in experiments with sheep, Votiakov et al[9] demonstrated that TBEV-EU did not cross a blood-brain barrier (BBB) without lethal cases contrasting TBEV-FE.

Our Proposal

We believe that the differences described above are sufficient to delineate TBEV-E and LIV (+ SSEV and SGEV) from the joint TBEV clade into two distinct species. For the rest of the TBEV subtypes (TBEV-FE, -SIB, -BKL, HIM), we proposed to classify as a single species. TSEV and GGEV can be combined into a single species taxon. After the first round of the revision, we are preparing new species names in a binomial format different from those proposed in a preprint.[1]

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