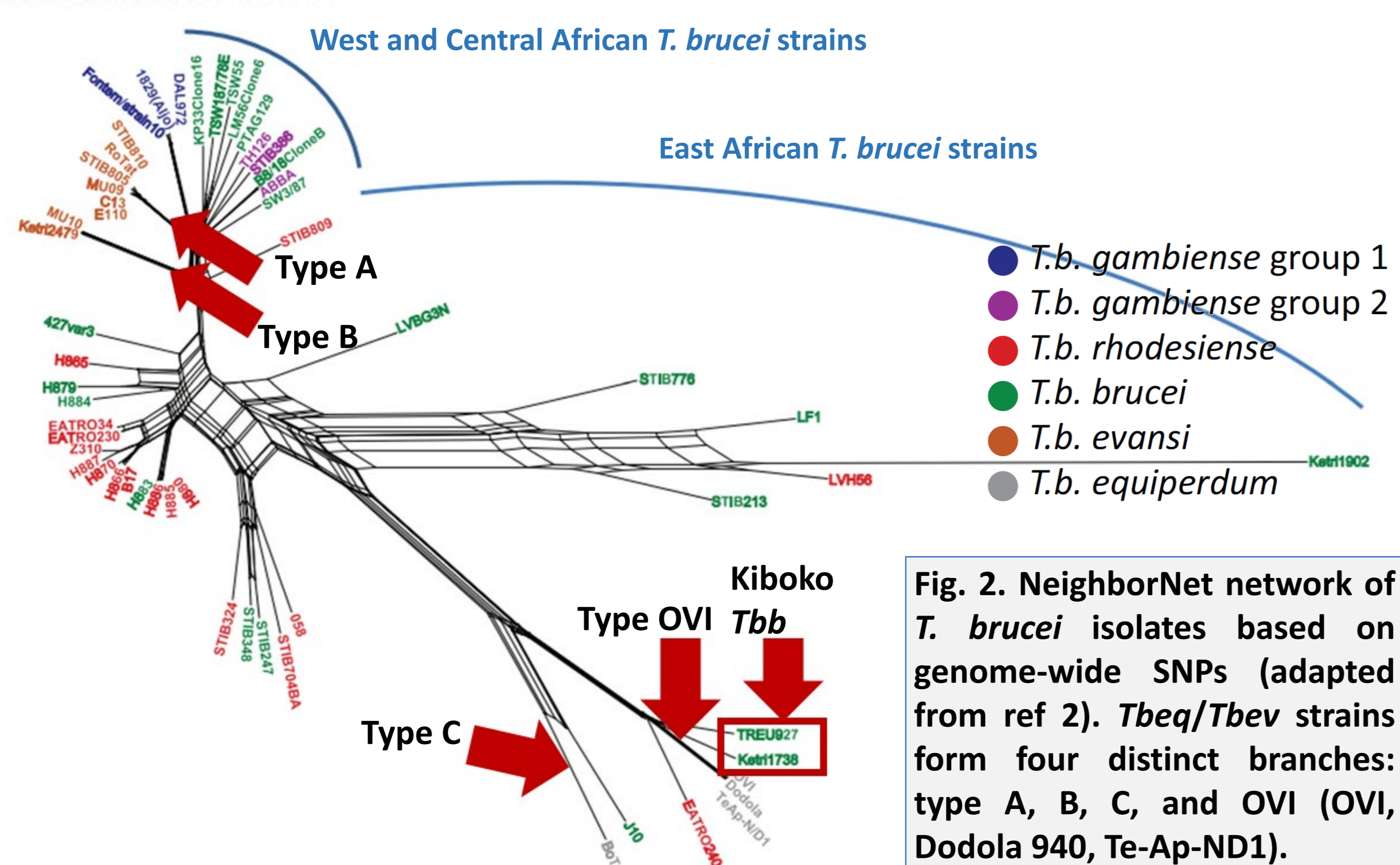
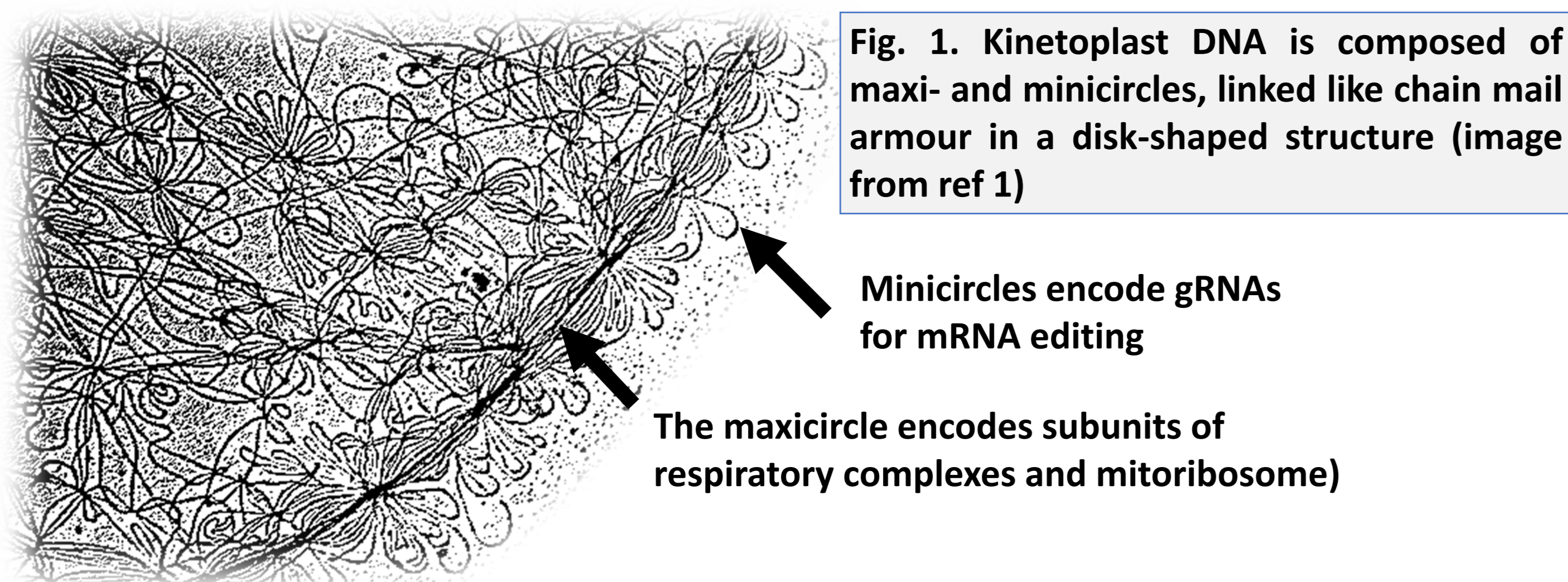


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The kinetoplast: a complex mtDNA system



Type A *Tbev* Vietnam has a complete maxicircle

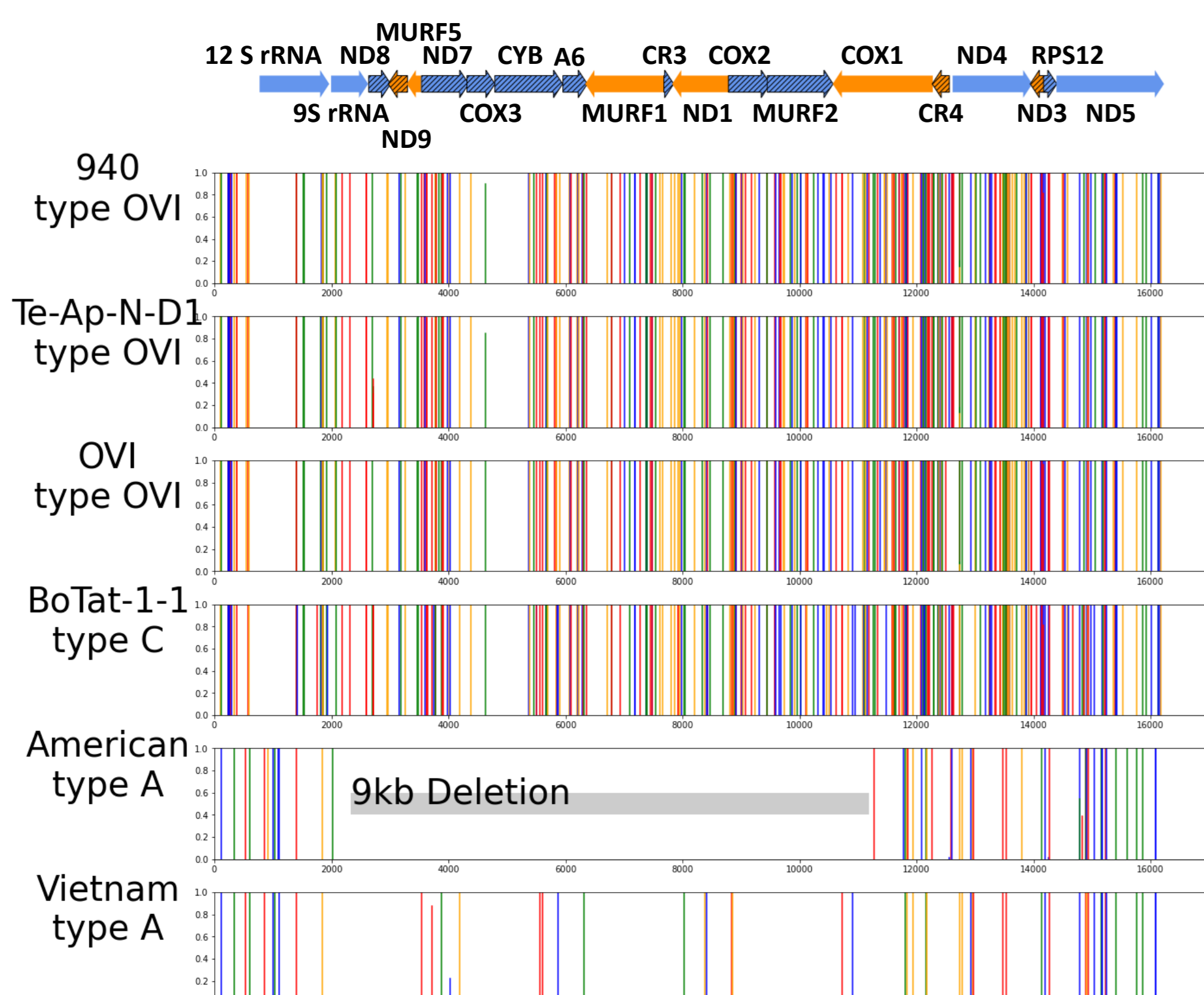


Fig. 3. Maxicircle SNP analysis, based on comparison with the *Tbb* EATRO 1125 reference. Type C and type OVI strains are related, with some group-specific SNPs (coloured lines).

Maxicircle-containing type A strains share similar SNPs and typically have a 9-kb deletion, with the notable exception of the Vietnam strain (1998, ref 6).

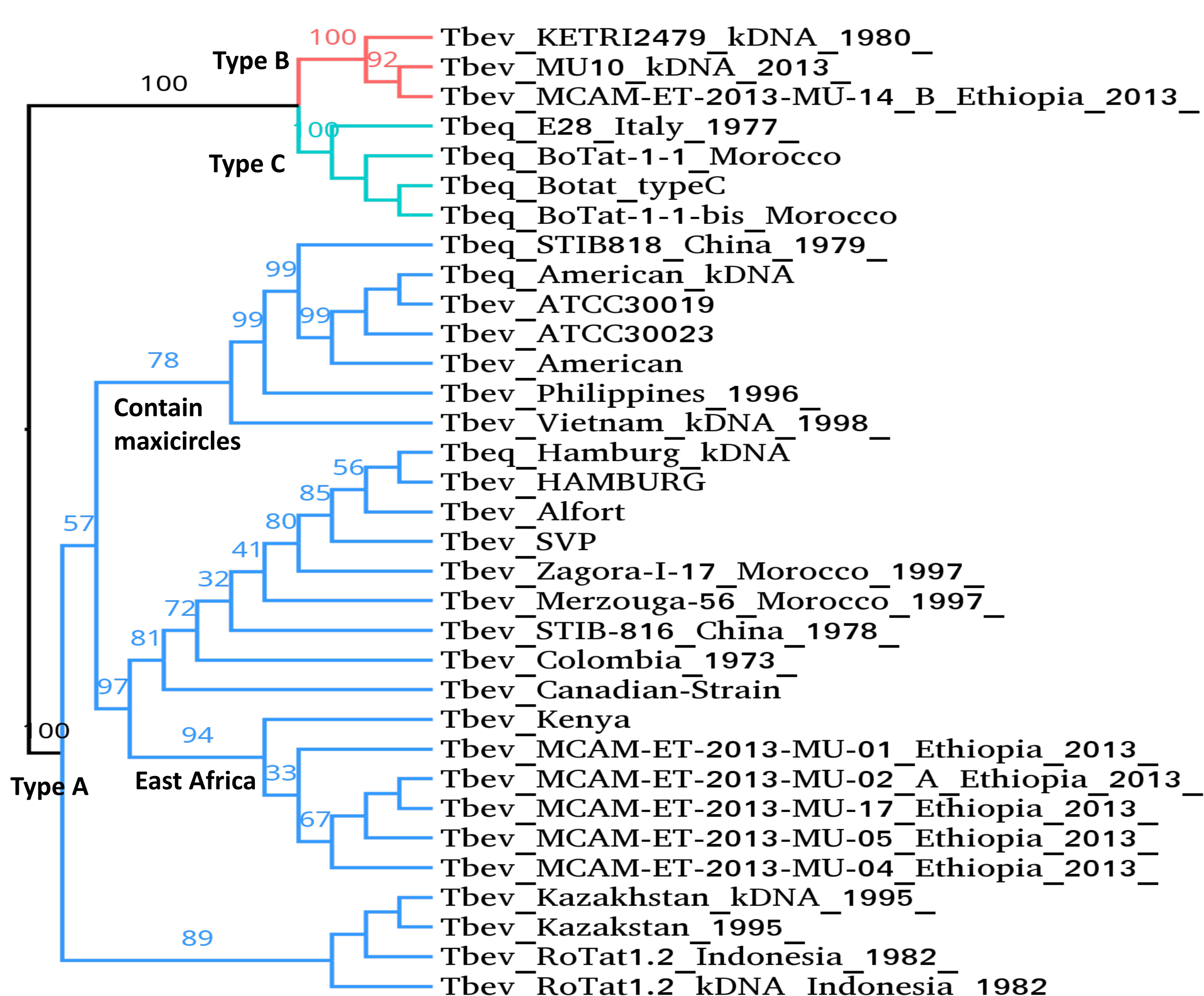


Fig. 4. *Tbev/Tbeq* phylogeny based on type A, B and C minicircles. Maxicircle-containing type A *Tbev* isolates form a monophyletic clade (1000 bootstraps). The Vietnam strain resides on the outermost branch of this clade, consistent with the subsequent occurrence of the 9-kb deletion.

Three groups of *Tbeq/Tbev* are defined by presence of a single minicircle type

Mitochondrial DNA in kinetoplasts (also called kinetoplast DNA or kDNA) is unique in structure and gene content (Fig. 1). *Trypanosoma brucei brucei* (*Tbb*) kDNA contains 20-50 identical copies of ~23-kb maxicircles and 5-10k highly heterogeneous 1-kb minicircles. Only the genes encoding a subunit of the F₁F₀-ATP synthase (A6) and mitoribosomal protein subunit 12 (RPS12) are essential in the mammalian bloodstream stage. Both genes require post-transcriptional editing directed by gRNAs encoded on minicircles.

T. b. equiperdum (*Tbeq*) and *T. b. evansi* (*Tbev*) evolved from *Tbb* on multiple occasions by switching from obligatory transmission by tsetse flies to direct transmission between mammals, either mechanically via biting flies or vampire bats (*Tbev*) or sexually (*Tbeq*; only in equids). Monophyletic subgroups of *Tbev* include strains historically designated as *Tbeq* and vice versa (ref 2,3). *Tbeq* and *Tbev* are considered kDNA independent and all strains have undergone partial (and sometimes complete) kDNA loss. Compared to ~391 minicircle classes identified in *Tbb* (ref4), minicircle complexity in *Tbeq/Tbev* is highly reduced.

Here we have used a recently developed bioinformatics pipeline (ref 5) to assemble kDNA from 56 *Tbeq/Tbev* isolates. Comparison with each other and with the pleomorphic *Tbb* reference strain EATRO 1125 reveals the following main findings:

- (1) We confirm that the minicircle genomes of three groups of *Tbeq/Tbev* consist of thousands of copies of a single minicircle class that is specific and therefore diagnostic for each group: types A, B and C, respectively.
- (2) One *Tbev* type A isolate has a complete maxicircle, a feature previously thought to be restricted to *Tbeq*.
- (3) A 4th group of isolates, here termed OVI-group, has 43-45 different minicircle classes per network, providing sufficient capacity for nearly complete editing of A6 and RPS12. This suggests that kDNA independence in these isolates evolved relatively recently in these strains.

Three groups of *Tbeq/Tbev* are defined by presence of a single minicircle type

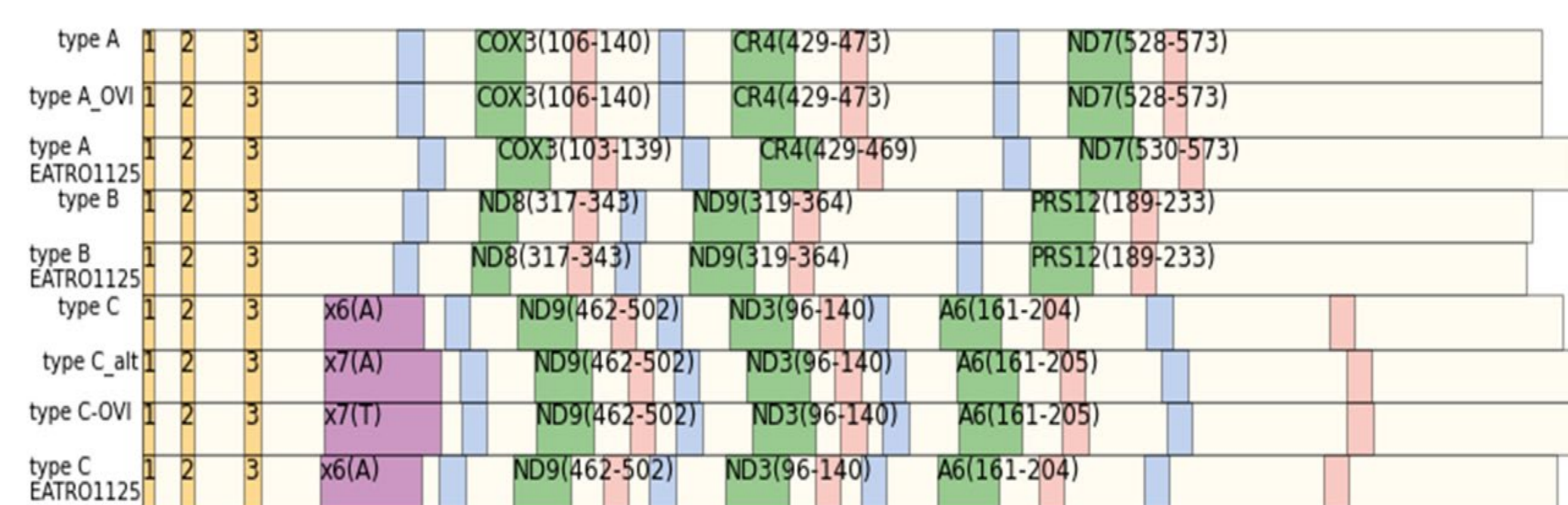


Fig. 5. kDNA assembly confirms that three groups of *Tbeq/Tbev* contain just a single type of minicircle class that is therefore diagnostic for each group: A, B, or C. Homologs of these minicircle classes can be found in *Tbeq* OVI-group strains and in *Tbb* EATRO 1125. Type C minicircles have a varied number of 12-bp repeats.

OVI-group strains have higher minicircle diversity and nearly complete – but ‘streamlined’ - gRNA coverage for A6 and RPS12 mRNAs

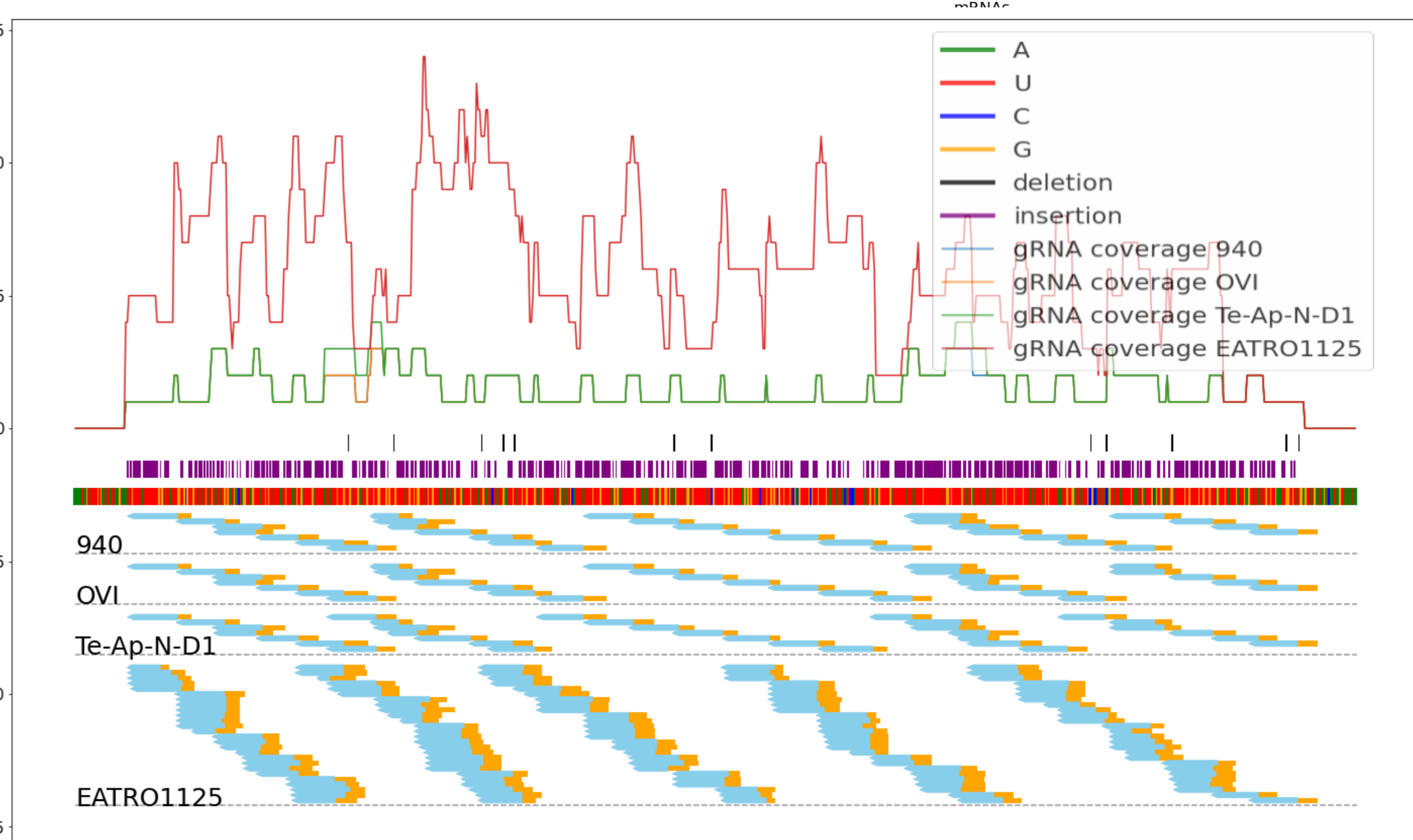
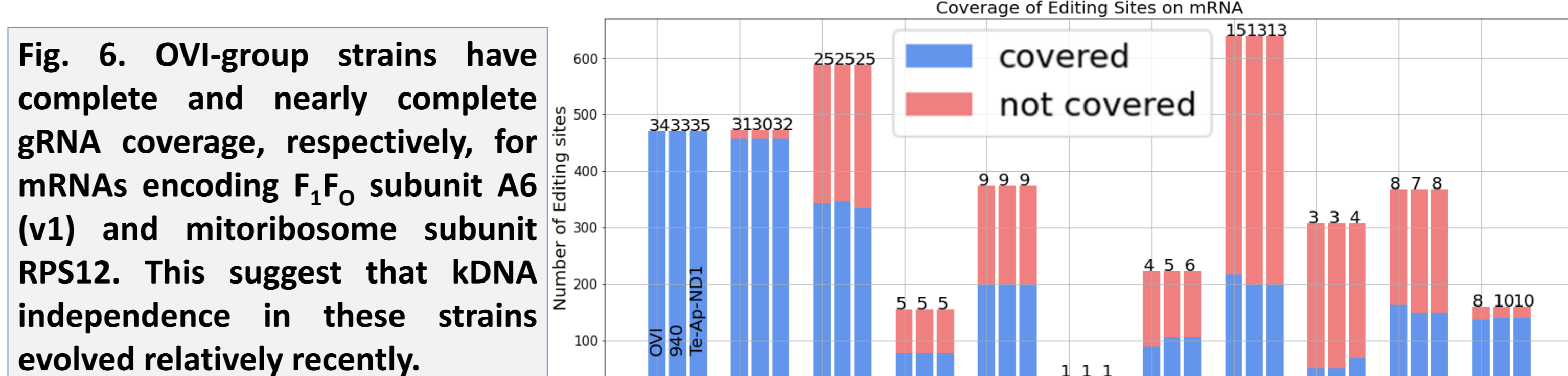


Fig. 7. Compared to the pleomorphic *Tbb* strain EATRO 1125, which shows highly redundant gRNA coverage of the mRNA A6 editing sites, OVI-group *Tbeq* feature a ‘streamlined’ editing capacity, with most editing sites being covered by a single gRNA species. A very similar scenario is observed for the RPS12 mRNA (not shown). gRNAs are shown in blue, with anchor regions in yellow.

Acknowledgements & References

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