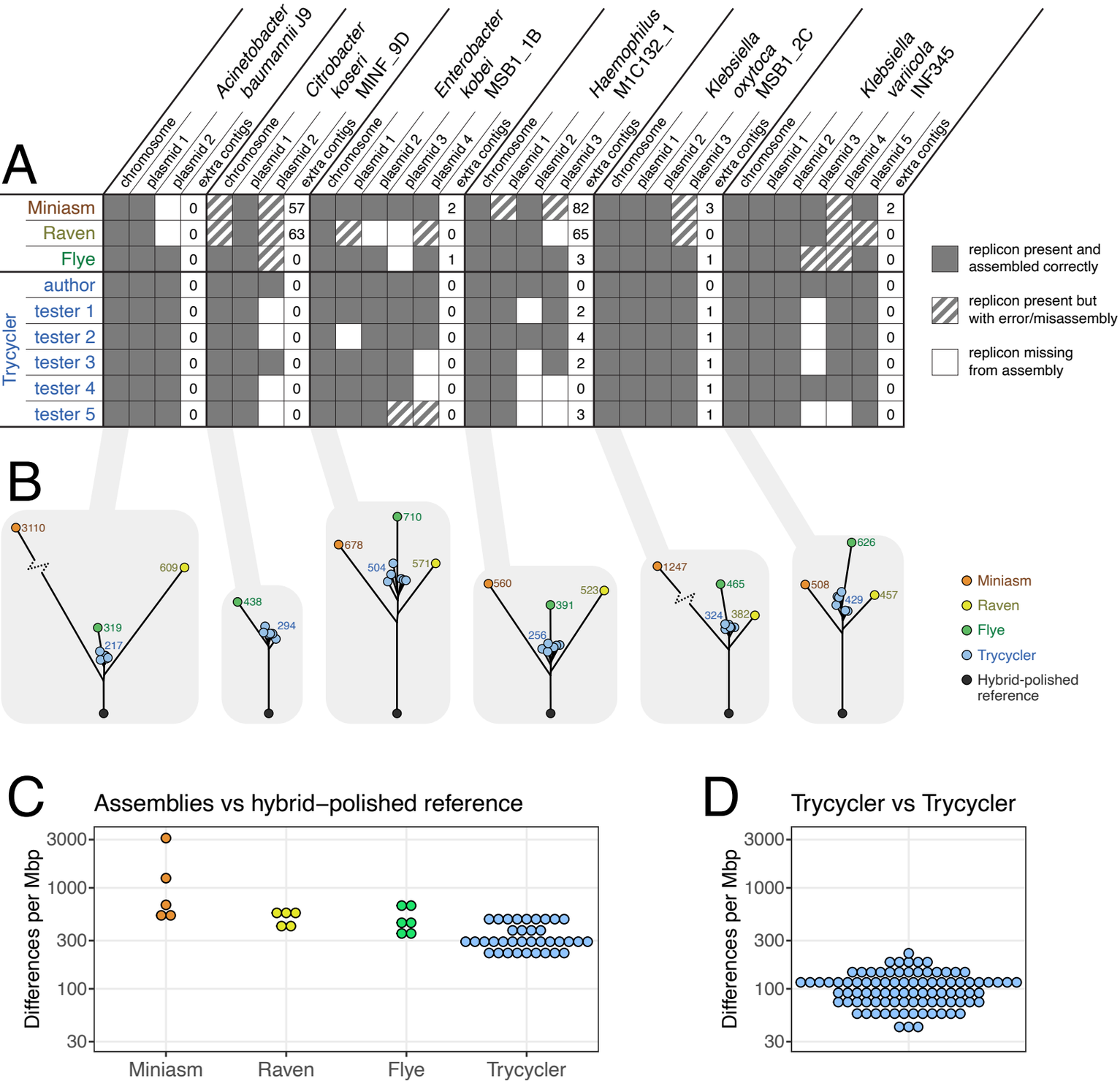

Figure 3: results for the multi-user test which aimed to assess the consistency of Trycycler assemblies when it was run by different users. Results include assemblies from three different long-read assemblers (Miniasm/Minipolish, Raven and Flye) and Trycycler assemblies from six different users (the author of Trycycler and five independent testers).

A: Presence/absence matrix for the replicons in the test genomes. Each replicon was classified as either present in the assembly, absent from the assembly or present but with an error/misassembly (see Table S3 for more detail). The number of additional contigs (e.g. spurious sequences or contaminant replicons from barcode crosstalk) is also indicated for each assembly. All Trycycler assemblies contained an accurate chromosome, and only one Trycycler assembly contained misassemblies. However, in many cases the Trycycler testers excluded a true plasmid or included an additional plasmid (e.g. from cross-barcode contamination).

B: Neighbour-joining trees of all available assemblies for each of the chromosomes. Hybrid-polished (Medaka+Pilon) versions of the author's Trycycler assemblies were included as reference sequences. The values indicate the number of single-bp differences per Mbp between each assembly and the polished reference (values for Trycycler are the mean of all six Trycycler assemblies). For each genome, the Trycycler assemblies cluster tightly and are closer to the polished reference than the assemblies from other long-read assemblers.

C: Differences between each assembled chromosome and the hybrid-polished reference. Values are single-bp differences per Mbp of sequence. Trycycler assemblies contain fewer differences, on average, compared to the single-assembler assemblies.

D: Differences between alternative Trycycler assemblies of each chromosome. Values are single-bp differences per Mbp of sequence, and there are 90 values (6 genomes × 15 unique pairwise combinations per genome).