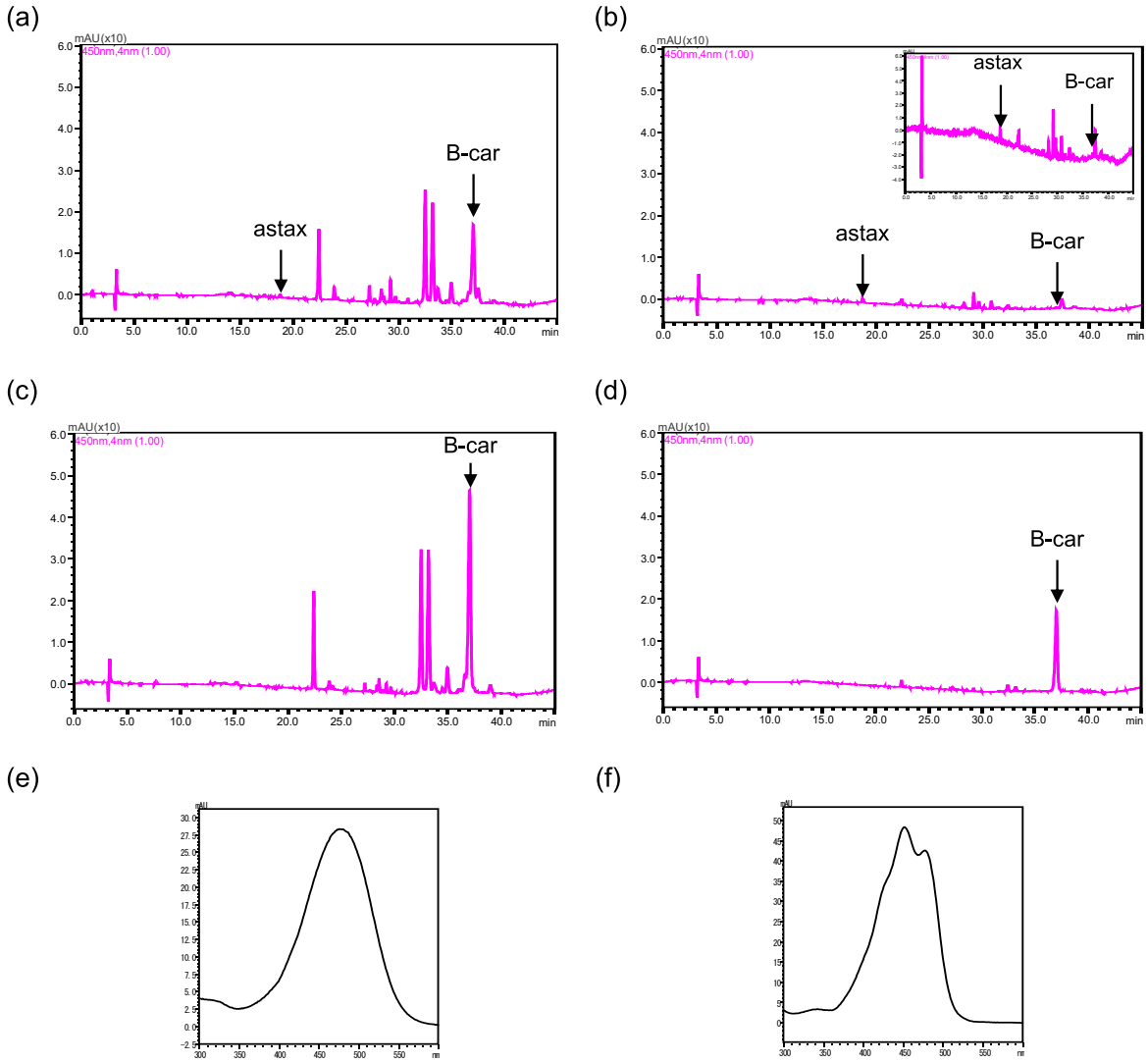
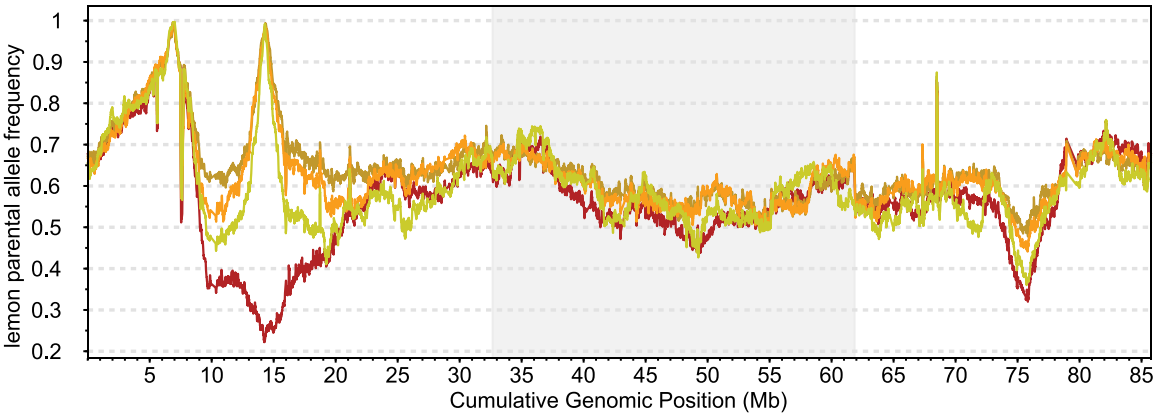
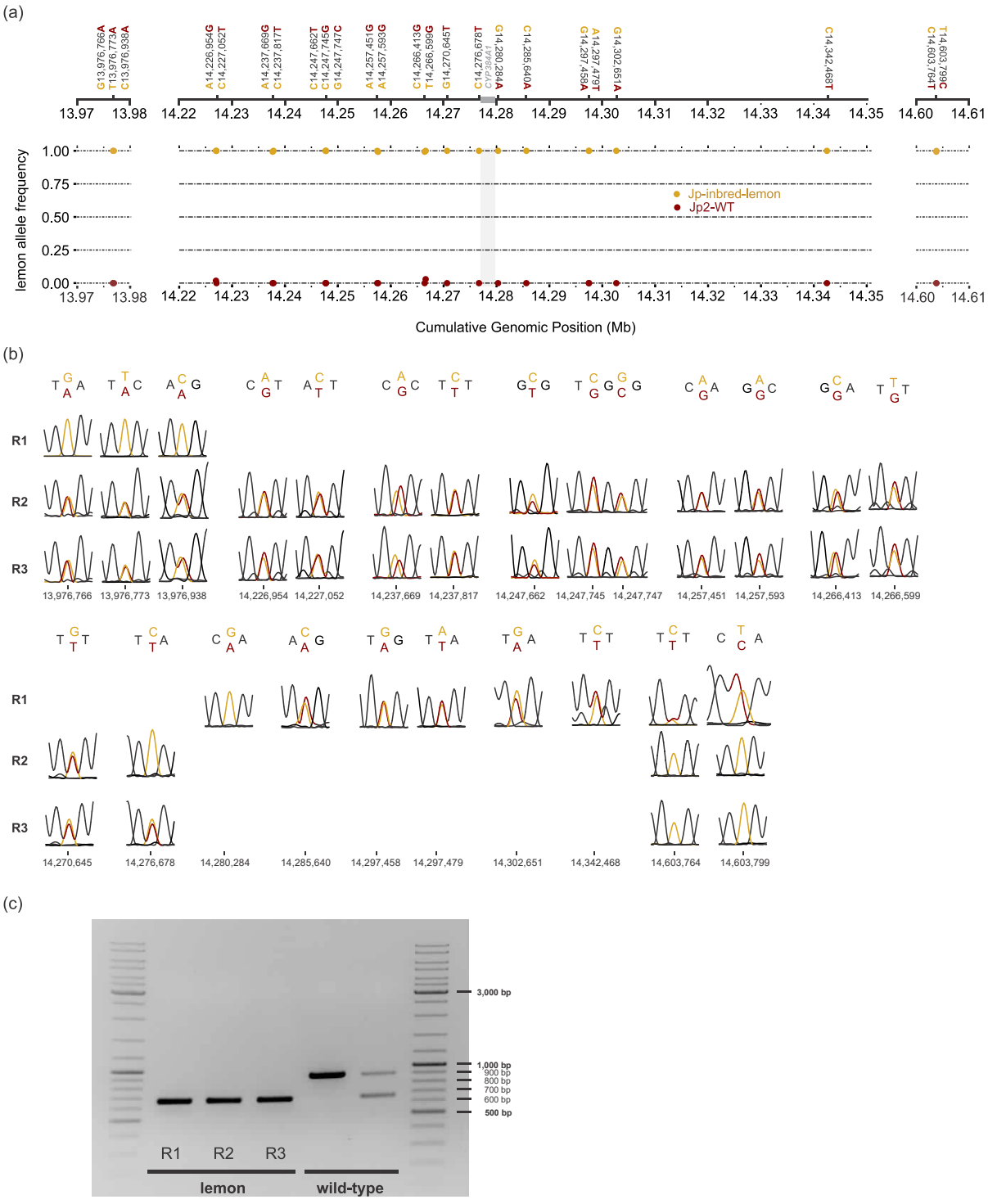


Supplementary Figure 1. HPLC analysis of the carotenoid profiles of lemon and wild-type *T. kanzawai* mites. The top two rows are the retention times of the HPLC system, (a) wild-type feeding *T. kanzawai,* (b) wild-type diapausing *T. kanzawai,* (c) lemon feeding *T. kanzawai*, and (d) lemon diapausing *T. kanzawai.* The 'astax' and 'B-car' labels refer to astaxanthin and β-carotene, respectively. The bottom row shows the absorption spectrum of (e) astaxanthin, and (f) β-carotene.

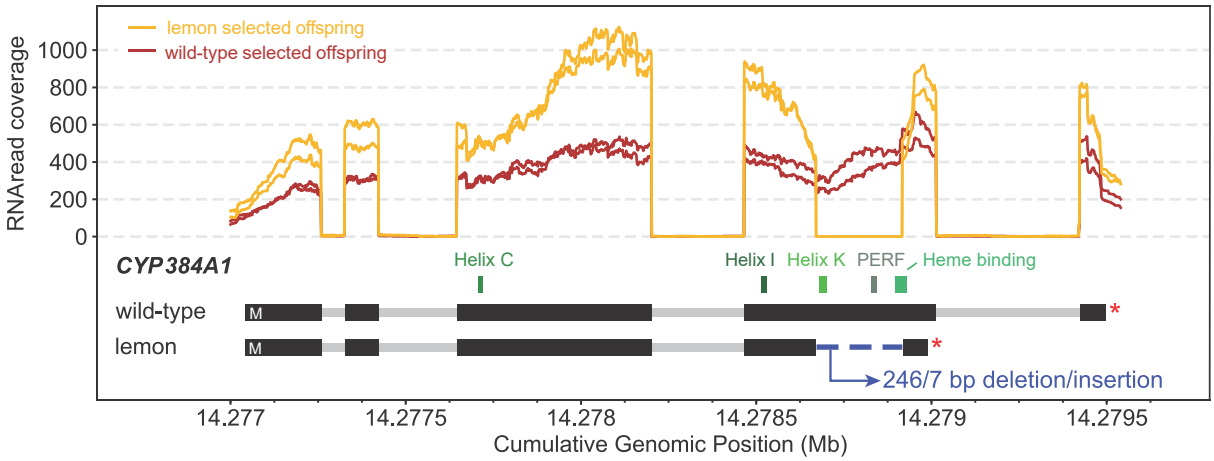


Supplementary Figure 2. Sliding window allele frequency profiles for populations used for bulk segregant analyses. Frequencies of the parental Jp-inbred-lemon alleles of the three lemon selected pools (orange and yellow lines) and one wild-type offspring pool (red line) are plotted in a sliding window analysis as indicated (window size and offset parameters are as for figure 4a). The three *T. urticae* reference chromosomes are shown in alternating white and grey and are ordered by decreasing length. A region of fixation for all samples was observed on chromosome 1 proximal to the lemon locus, presumably as a result of the purging of a deleterious allele, but did not impact mapping of the lemon phenotype (this figure and figure 4a, and as confirmed by fine-mapping, see main text and supplementary figure 3).

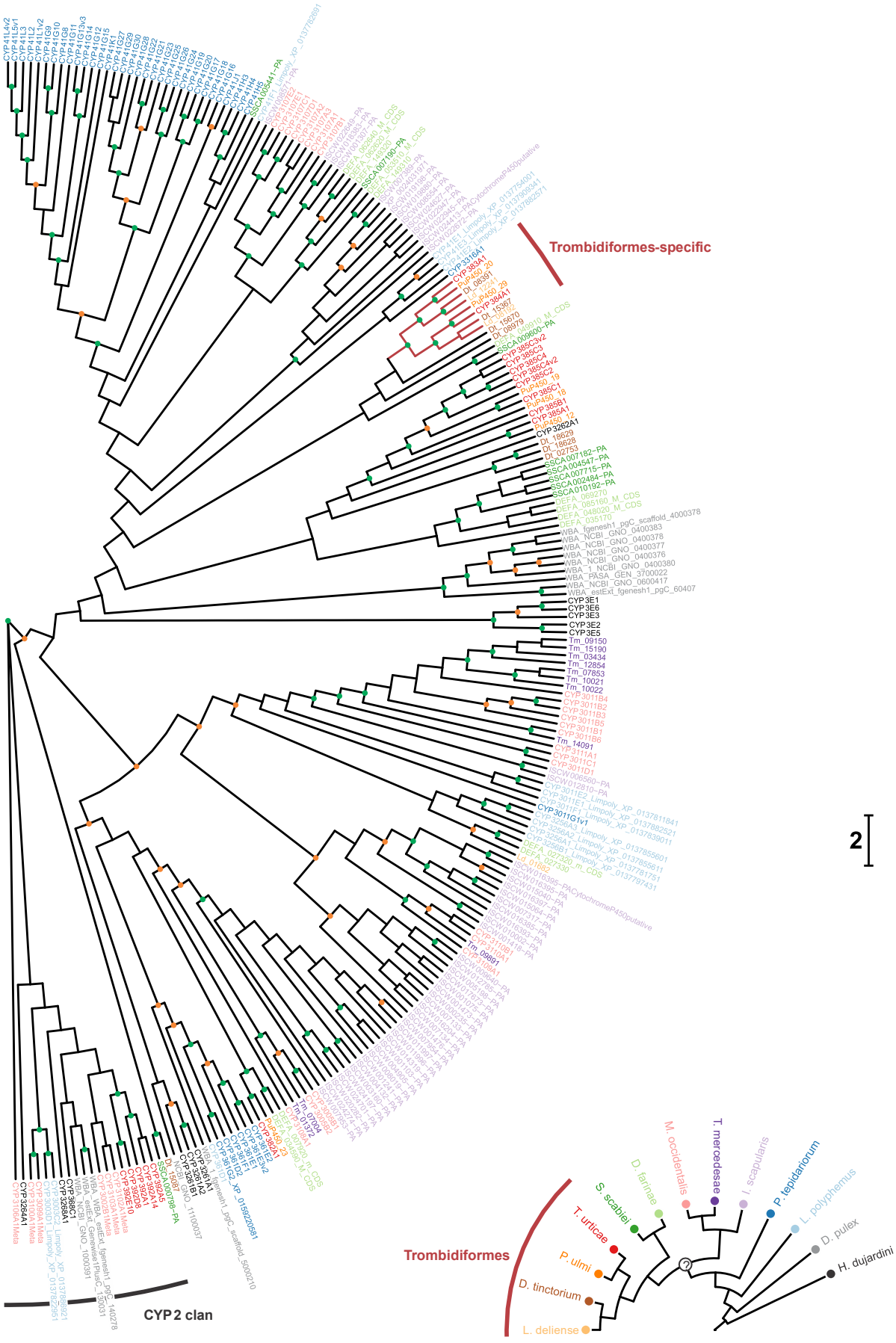


Supplementary Figure 3. Three recombinant *T. kanzawai* females reveal a narrow minimal candidate region of 8.96 kb for the lemon phenotype. (a) SNP-based markers distinguishing the parents used for mapping the lemon phenotype are ordered according to their genomic positions on the first chromosome. Nucleotides are color coded, with orange and red originating from the Jp-inbred-lemon and Jp2-WT parent, respectively. Shown at bottom is the genotypic information at these sites as assessed from Illumina read alignments of the parents that were used for marker selection. The genomic location of *CYP384A1* is indicated by grey shading. (b) Trace data are plotted for three diploid lemon female mites (R1, R2, and R3) that showed the closest recombination breakpoints to *CYP384A1*. Genotypes at indicated markers were assessed by detecting heterozygosity/homozygosity at the variable positions.

Single females were iteratively genotyped starting from the most distant markers. Females R2 and R3 were heterozygous 5' of *CYP384A1* until positions 14,270,645 and 14,276,678, respectively. Female R1 exhibited homozygosity for the lemon haplotype at the 3' end of *CYP384A1* until position 14,285,640, after which heterozygosity was observed at all distal markers. Trace data are color coded with orange and red to indicate peaks corresponding to the variants from Jp-inbred-lemon and Jp2-WT, respectively; black indicates non-variable positions. See supplementary table 4 for an overview of the total number of informative recombinants within the ~630 kb region. (c) The three recombinant females were homozygous for the lemon deletion variant in the fourth exon of *CYP384A1.* An agarose gel is shown with the PCR-amplified *CYP384A1* fragments of R1, R2, and R3, along with homozygous and heterozygous phenotypically wild-type females as controls. Primers used for amplification flanked the deletion variant. As shown in supplementary table 4, all tested lemon females of the segregating population were homozygous for the deletion within the fourth exon of *CYP384A1.*



Supplementary Figure 4. RNA-Seq read coverage of lemon and wild-type selected offspring within the *CYP384A1* coding sequence confirms the deletion in the fourth exon. Shown is the gene model for *CYP384A1* (exons are in black, introns are in gray; "M" and asterisk, start and stop codons, respectively). The five structural motifs of cytochrome P450s are indicated by green rectangles as labelled. As revealed by the predicted gene models in wild-type and lemon mites (bottom), the gene model in lemon harbors a 246/7 bp deletion/insertion that results in a frameshift and a premature stop that removes three of the five critical P450 motifs. The location of the lemon specific structural variant is indicated by the dashed blue line.


Trombidiformes-specific
CYP 2 clan
2
Trombidiformes
S. scabiei
T. urticae
P. ulmi
D. tinctorium
L. deliense
D. farinae
M. occidentalis
T. mercedesae
I. scapularis
P. tepidariorum
L. polyphemus
D. pulex
H. dujardini

Supplementary Figure 5. Maximum-likelihood phylogenetic analysis suggests a 1:1:1:1 orthology of CYP384A1 for mites of the Trombidiformes order. The branches that lead to these Trombidiformes-specific cytochrome P450s are depicted in dark red. Gene IDs are colored according to species identity. The phylogenetic reconstruction was rooted using CYP2 clan members, and the phylogenetic position of the 13 animal species is outlined in the species tree in the lower right. A monophyletic origin for mites (Chelicerata: Acari) remains debated. Nodes with bootstrap support values between 75 and 90 are indicated by orange circles, whereas nodes that are supported by a bootstrap value greater than or equal to 90 are depicted by green circles. The scale bar represents 2 amino acid substitutions per site.