



**Figure S5** Putative stem-loop alignments of the R1-like (red) and R2-like (blue) sequences from the cholinergic loci of representative species from three different animal phyla (these are in addition to the species presented in Figure 6). The fractional pairing of the stem is given below each stem; the length of the putative loop is shown above each stem. Splice sites at the 5'-end of the first VAcHT coding exon are indicated with a thin arrow, and the VAcHT initiation codon is indicated with a thick arrow. \*The *T. rubripes* genome reflects a whole genome duplication, and therefore has 2 copies of the CGL. Official *T. rubripes* gene names for the CGLs are still unassigned or provisional; for the present investigation, we refer to the CGL on Chromosome 1 as CGL a and the locus on Chromosome 4 as CGL b.