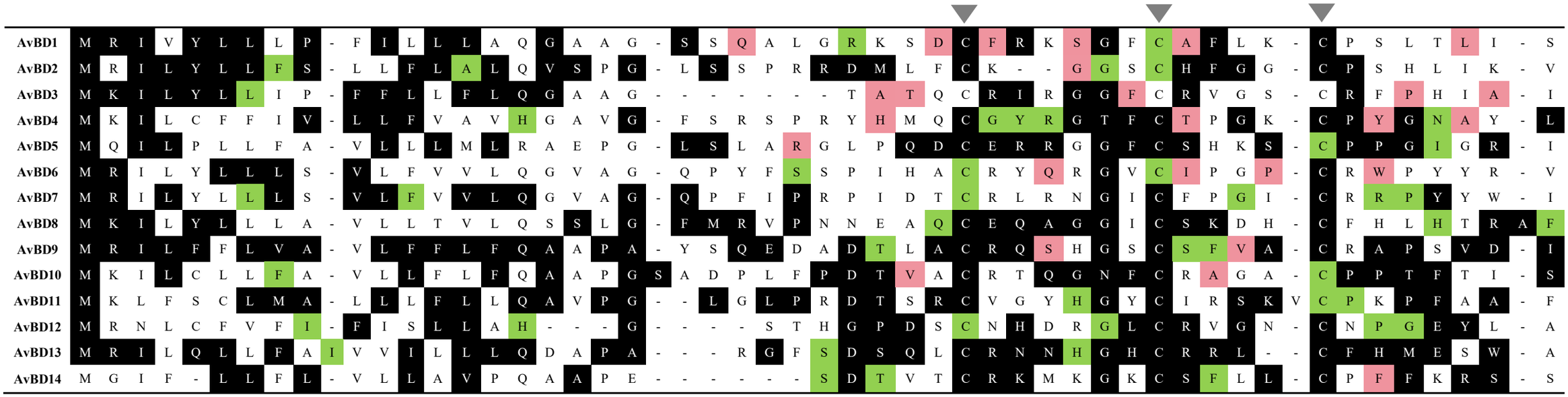

Selection acting on individual amino acid residues across species was evaluated using four methods from HyPhy: Fixed Effects Likelihood (FEL), Mixed Effects Model of Evolution (MEME), Fast Unconstrained Bayesian Approximation (FUBAR), and Single-Likelihood Ancestor Counting (SLAC), along with three tests (M1a vs. M2a, M7 vs. M8, and M8 vs. M8a) in the PAML software package. Residues exhibiting significant evidence of positive selection, supported by all four HyPhy tests or all three PAML models, are shaded in red. Negative selection on residues was assessed using FEL, FUBAR, and SLAC; residues showing significant evidence of negative selection in all three methods are shaded in blue. Residues shaded in black are fixed at the codon level, meaning that every individual in all species shares the same triplet codon. The six conserved cysteine (C) residues characteristic of β-defensin genes are marked with gray triangles.