



**Figure S4** Alignment length (bp) varied significantly among sample coverage classes for all (A; Kruskal-Wallis test:  $\chi^2 = 274.87$ ,  $df = 17$ ,  $P < 2.2e-16$ ), nonsynonymous (B; Kruskal-Wallis test:  $\chi^2 = 41.71$ ,  $df = 16$ ,  $P = 0.00044$ ), and synonymous (C; Kruskal-Wallis test:  $\chi^2 = 40.09$ ,  $df = 16$ ,  $P = 0.00076$ ) sites. Alignment length for noncoding sites (D; Kruskal-Wallis test:  $\chi^2 = 27.42$ ,  $df = 17$ ,  $P = 0.05221$ ), however, did not differ significantly among coverage classes. Whiskers extend to 1.5 times the interquartile range. Note that coverage class one does not contain amplicons with coding regions that were annotated.