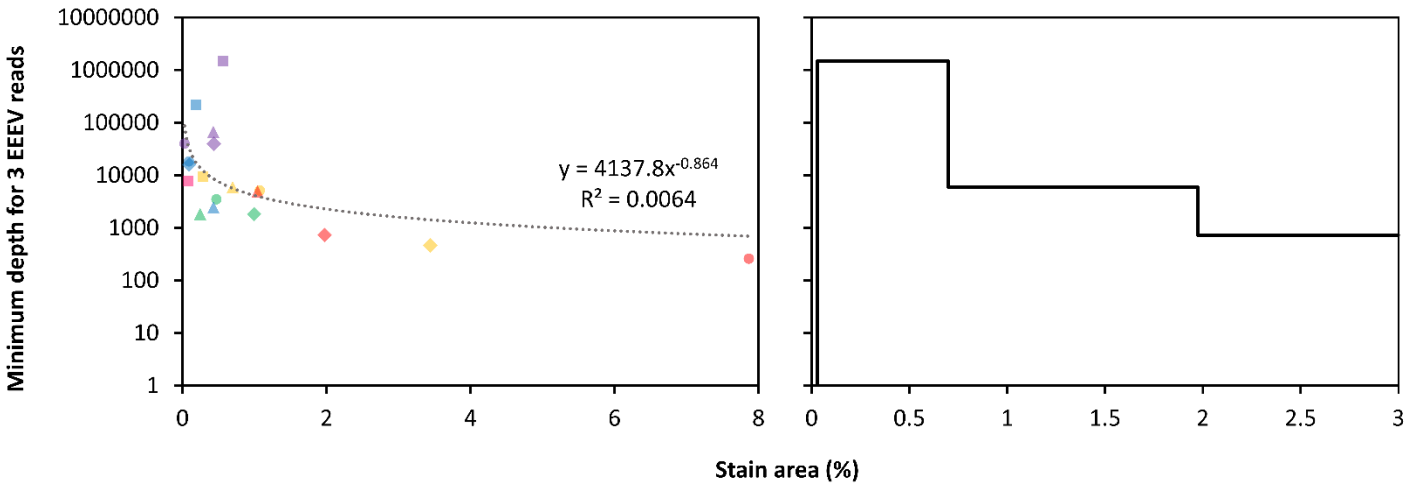

Fig. S1. *In situ hybridization* (ISH) stain quantification required to detect minimum Eastern equine encephalitis virus (EEEV) reads in samples. EEEV RNA was stained in slides made from brain tissue of EEEV-infected patients, and then stain was quantified and normalized to percent tissue area stained; tissue from the same blocks was then sequenced, and number of EEEV-mapping reads quantified and normalized to number of reads mapped/million. The minimum number of total reads required to reach 3 EEEV reads was calculated for each sample (shaped by patient, colored by brain region) and compared to percent tissue area stained (**A**). Stain thresholds were then calculated to establish minimum required reads to reach 3 EEEV reads for samples within a range of stain quantification.

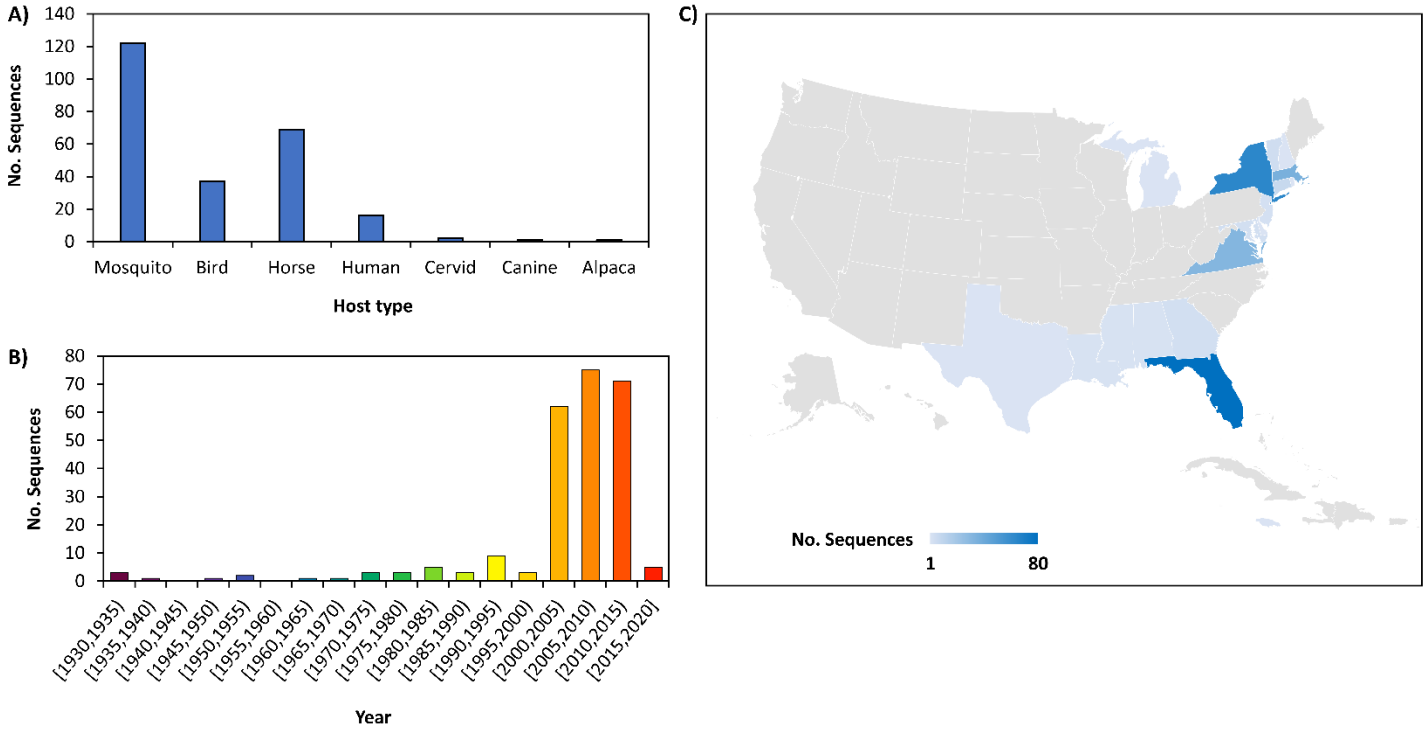

Fig. S2 Temporal, geographic, and host data for complete EEEV genomes. Complete EEEV genomes were downloaded from NCBI virus (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/) along with metadata, and then filtered for: complete records of year, host, and location; limited number of “N”s; and identity to other sequences in list. After filtering, metadata was compiled, sequence distribution host **(A)**, by year **(B)**, and location **(C)** were calculated to determine sequence diversity in subsequent analyses.