

Supplementary Material File 2 – Supplementary Figures

Molecular evolution of α -latrotoxin, the exceptionally potent vertebrate neurotoxin in black widow spider venom.

Authors: Jessica E. Garb ^{1*} and Cheryl Y. Hayashi ²

Addresses:

1. Department of Biological Sciences, University of Massachusetts Lowell, Lowell, MA 01854, USA
2. Biology Department, University of California Riverside, Riverside, CA, 92521, USA

* Corresponding Author

Email: Jessica_Garb@uml.edu



Figure S1. Alignment of *Latrodectus tredecimguttatus* and *Steatoda grossa* alpha-latrotoxin (ALTX) venom proteins inferred from nucleotides. Amino acids color coded by physicochemical property, dots indicate identical residues, dashes indicate gaps, vertical arrows indicate predicted furin cleavage sites based on (K/R)_{X-3}(K/R)_{0-1R} consensus. *L. tredecimguttatus* ALTX annotated with signal peptide, post-translationally cleaved Cterminus, and ANK (ankyrin repeats) from Swiss-Prot entry P23631. *S. grossa* ANK repeats predicted with Rep V1.1 (www.embl.de/~andrade/papers/rep/search.html). Wing, body, and head domains annotated from Orlova et al. (2000). Asterisks indicate cysteine, that when mutated in *L. tredecimguttatus* sequence, obliterates toxin action (Ichtchenko et al. 1998).

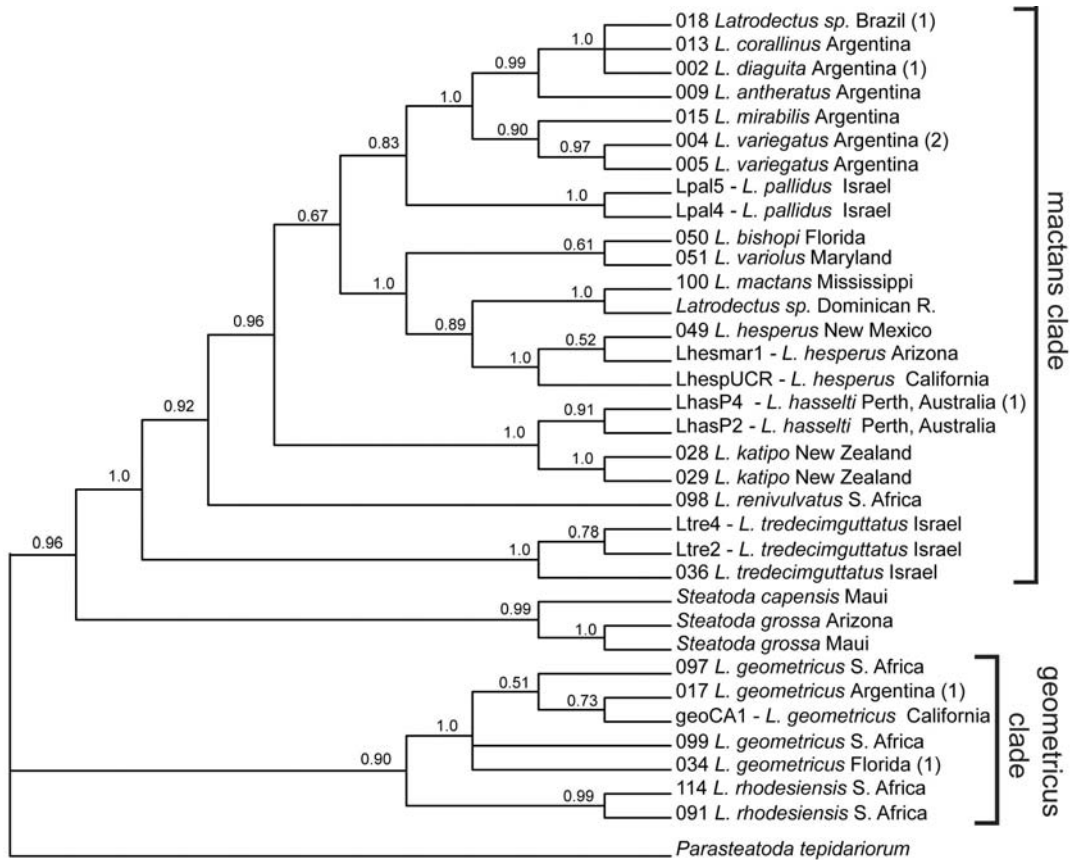


Figure S2. Partitioned Bayesian consensus tree of 659 bp alignment of mt COI data, with clade posterior probability values above nodes. Numbers in parentheses indicate additional identical sequences not included in analysis. Corresponding maximum likelihood and parsimony trees are in Figure 3 and Figure S3, respectively.

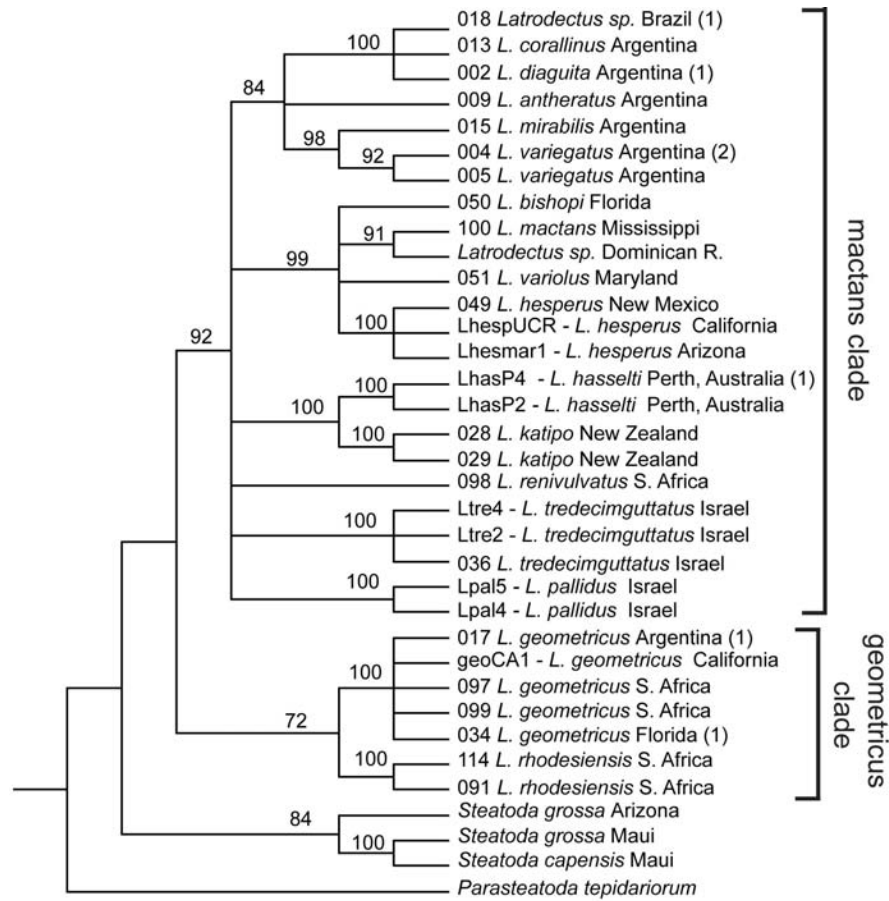


Figure S3. Parsimony consensus tree of 659 bp alignment of mt COI data, with bootstrap support values from 1000 replicates above nodes. Numbers in parentheses indicate additional identical sequences not included in analysis. Corresponding maximum likelihood and Bayesian are in Figure 3 and Figure S2, respectively.

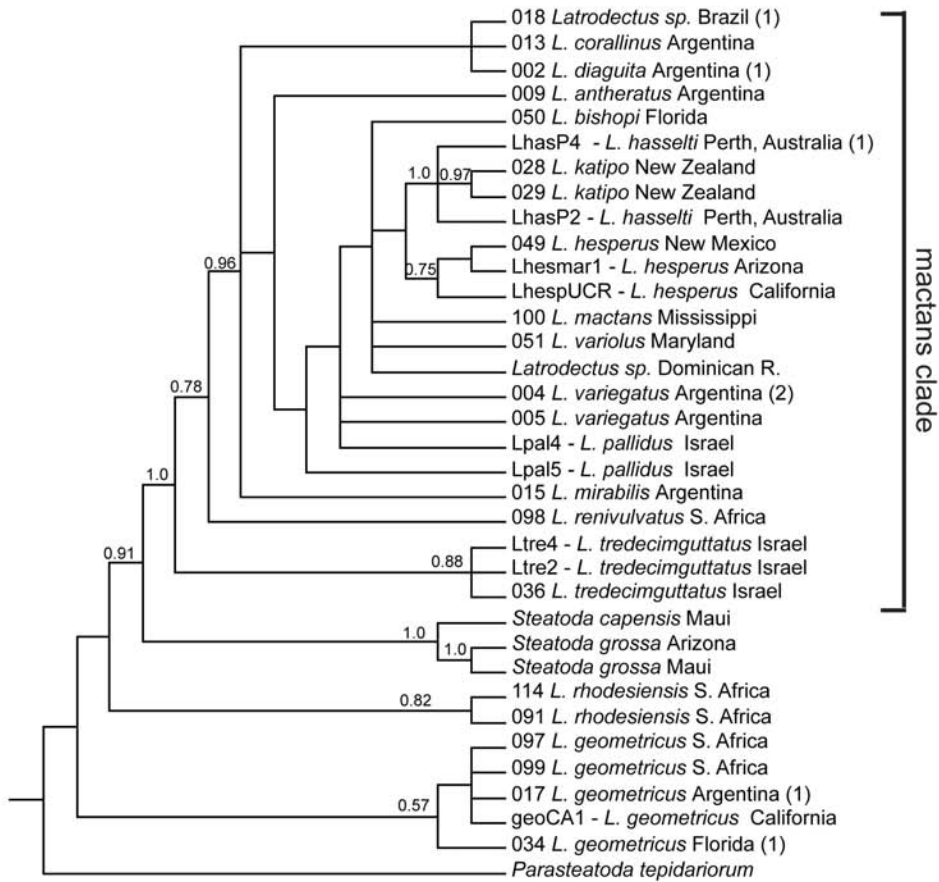


Figure S4. Strict consensus of 12 maximum likelihood trees (-ln L=1127.98572) derived from mt COI data excluding third codon positions (440 characters), clade posterior probability support values for clades in Bayesian consensus tree resulting from analysis of this data shown above nodes, nodes without numbers above nodes were collapsed in Bayesian consensus tree. Numbers in parentheses indicate additional identical sequences not included in analysis. Corresponding parsimony tree is in Figure S5.

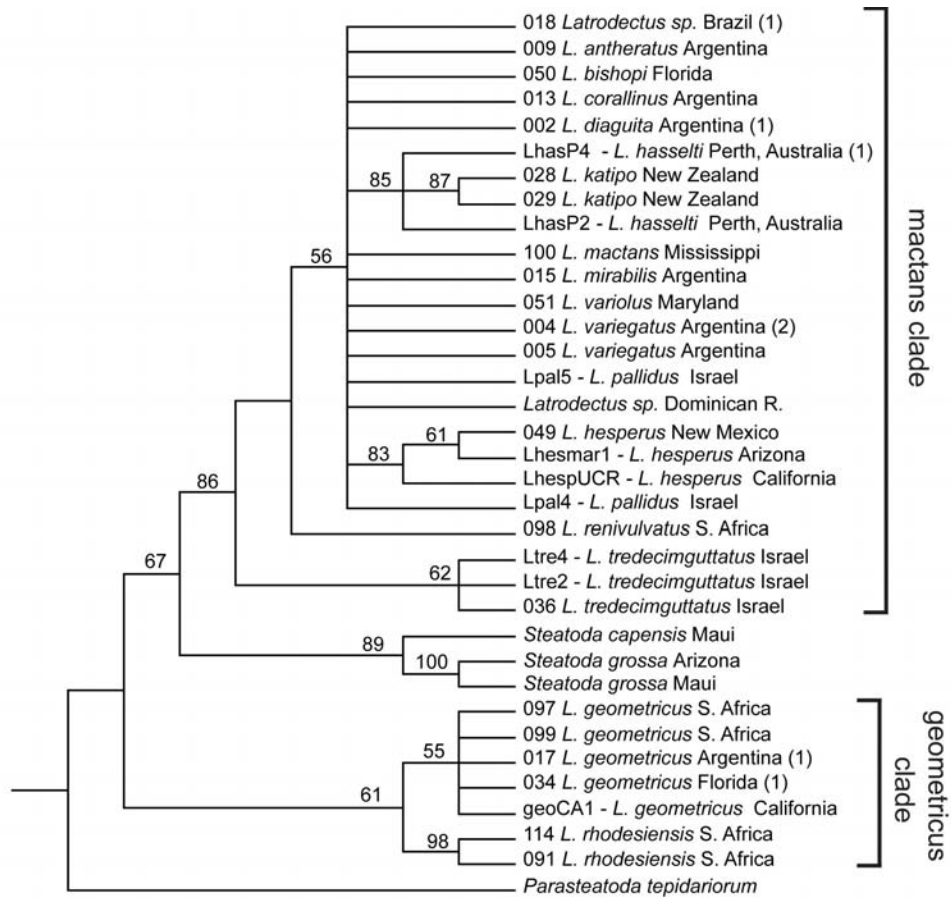


Figure S5. Parsimony consensus tree of mt COI data excluding third codon positions, with bootstrap support values from 1000 replicates above nodes. Numbers in parentheses indicate additional identical sequences not included in analysis. Corresponding maximum likelihood and Bayesian results in Figure S4.

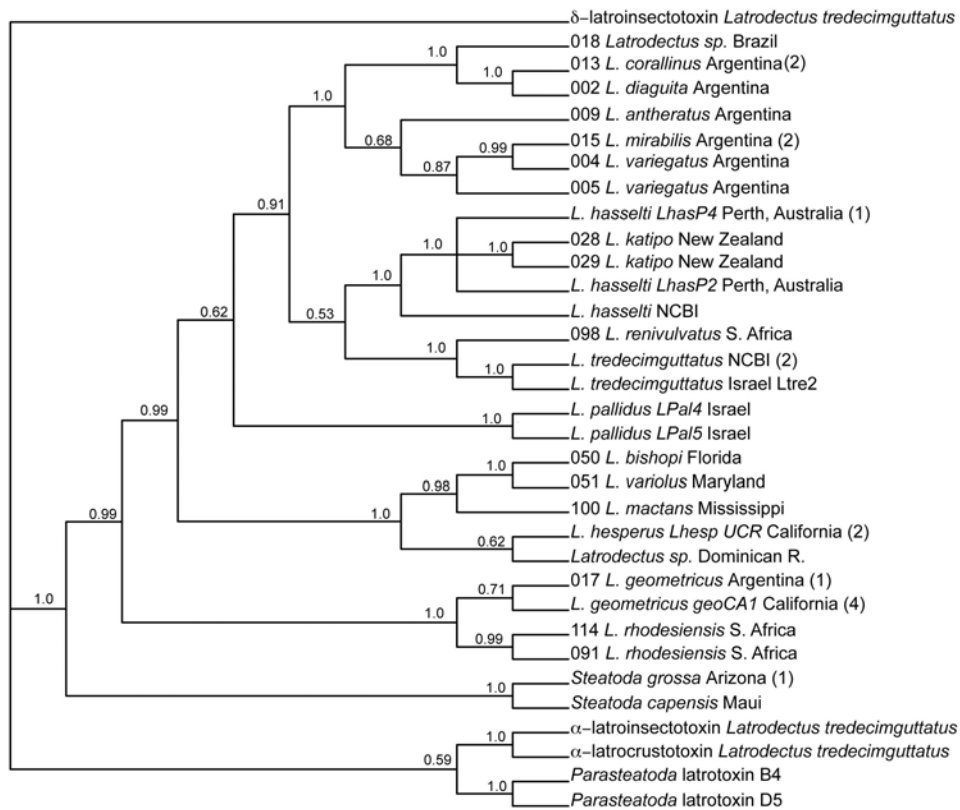


Figure S6. Partitioned Bayesian consensus tree of 642 bp α -latrotoxin sequence alignment from this study, with published latrotoxins. Values at node are clade posterior probabilities. Numbers in parentheses after terminal indicate number of additional identical sequences collected.

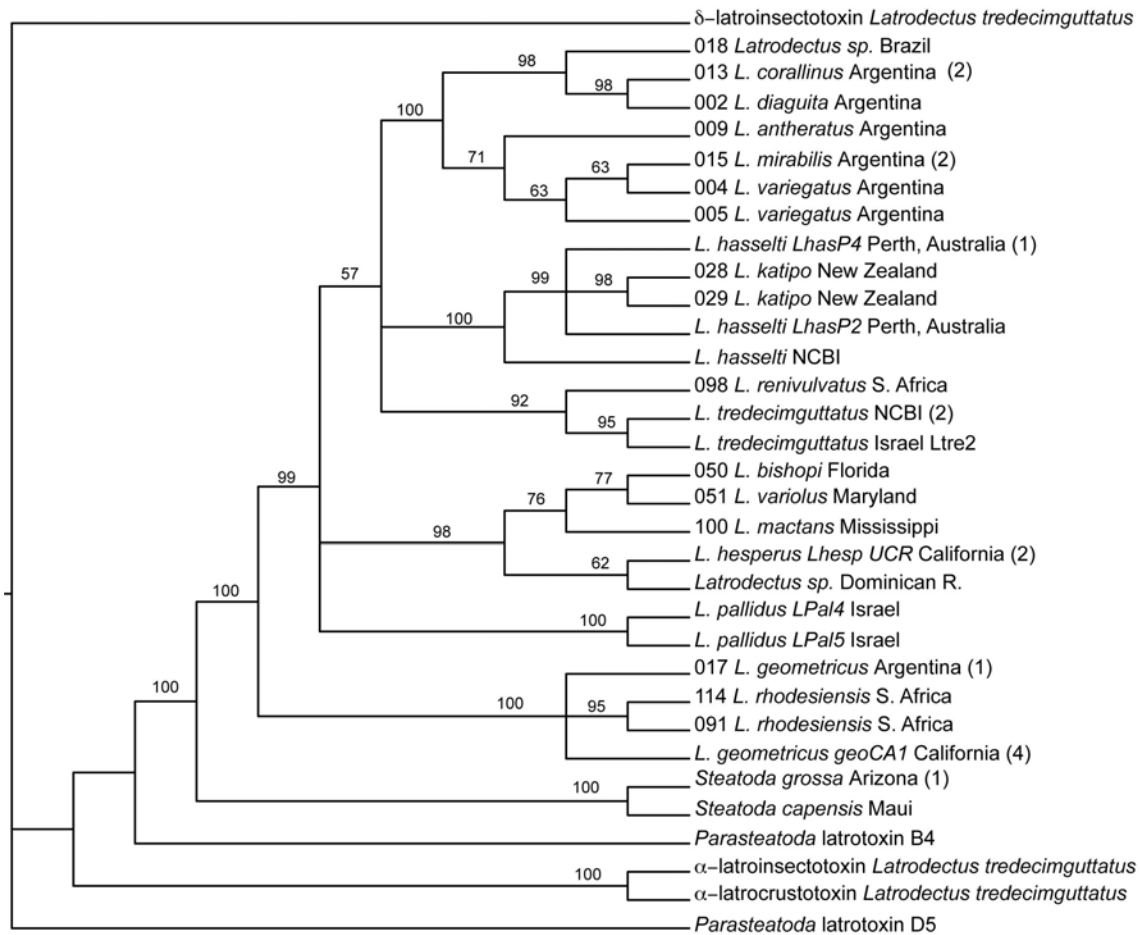


Figure S7. Consensus of six most parsimonious trees from 642 bp α -latrotoxin sequence alignment from this study, with published latrotoxins. Numbers at node are bootstrap values from 1000 replicates. Numbers in parentheses after terminal indicate number of additional identical sequences collected.

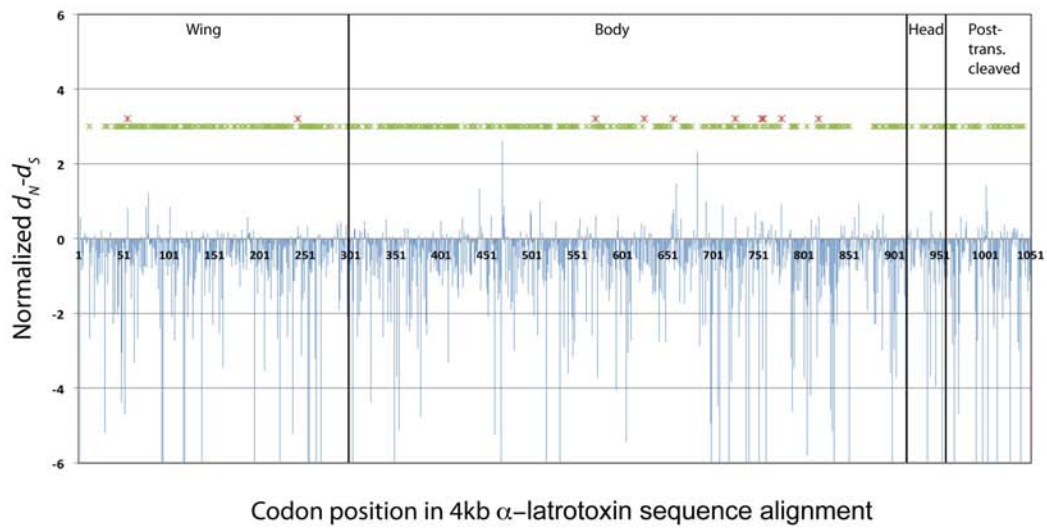


Figure S8. Plot of normalized d_N-d_S (blue) computed in FEL module of HyPhy for each codon position in 4kb α -latrotoxin alignment along the four domains (wing, body, head, and post-translationally cleaved); Green asterisks indicate 370 codons found to be negatively selected at a significance level of 0.1; red asterisks show 10 codons found by FEL analysis to be positively selected at 0.1 p-value. Codons 1-300 correspond to wing domain, 301-916 correspond to the body domain, codons 917-953 correspond to the head domain, and codons 954-1051 correspond to the C-terminal post-translationally (post-trans.) cleaved region.

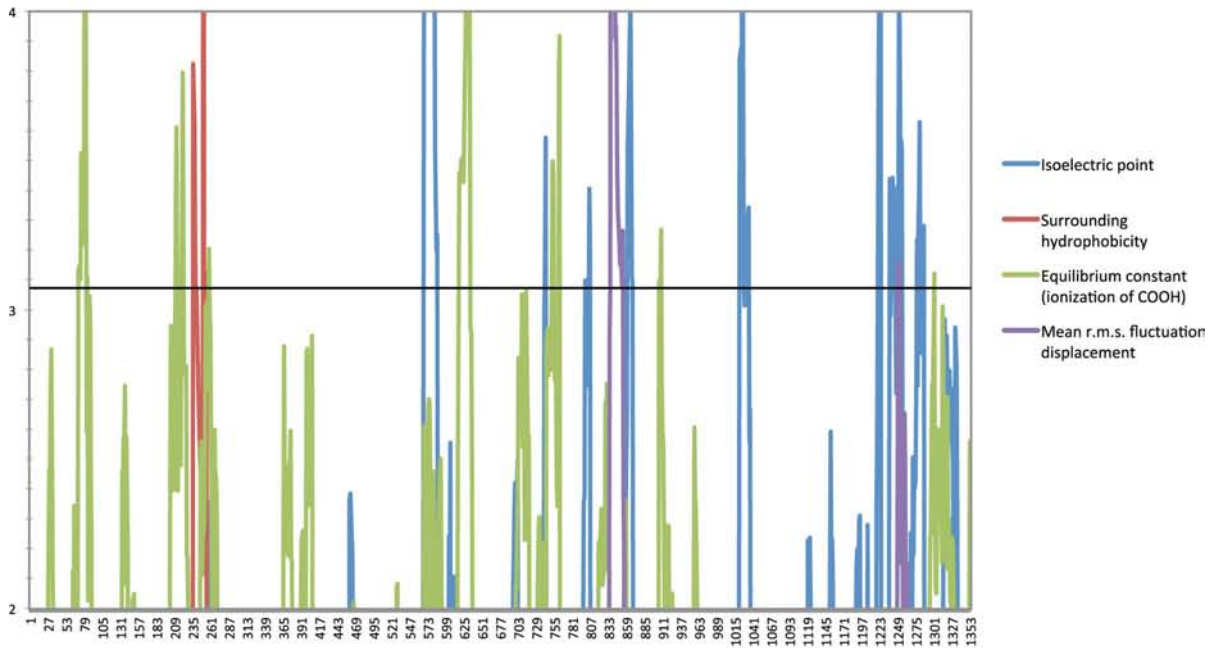


Figure S9. Sliding window analysis of positive selection for alpha-latrotoxin alignment conducted in TreeSAAP. Shown are magnitude category 8 changes (most extreme) for three properties (Isoelectric point, Equilibrium constant, and Mean r.m.s. fluctuation displacement) with positive Z-scores (>3.09 ; indicated by horizontal line) over large windows (≥ 20 20bp windows), Z-scores >3.09 indicate positive selection. Also shows magnitude 8 category changes for surrounding hydrophobicity.