## S1 File: Supplementary Figures and Tables

Minimal genetic differentiation of the malaria vector Nyssorhynchus darlingi associated with forest cover level in Amazonian Brazil

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Fig A. Selection of optimal number of clusters for STRUCTURE analysis using the Evanno method. (A) Posterior probability of the data (L(K)) for $K=1$ to 10 , (B) $L^{\prime}(K)$ for $K=1$ to 9 , and (C) L" K ) and (D) delta $K$ for $K=2$ to 9 .





Fig B. Results of fastSTRUCTURE analysis of 5561 SNP dataset. (A) Includes Ny. darlingi collected from all four municipalities (CRU: Cruzeiro do Sul area, LAB: Lábrea, PRE: Presidente Figueiredo, SAO: São Gabriel da Cachoeira) and five forest cover levels (1-5), depicting K=2-6 inferred clusters; (B) CRU only, $K=1-5$; (C) LAB only, $K=1-5$; (D) PRE only, $K=1-5$; (E) SAO only, $K=1-5$.



Fig C. Principal components analysis of 5561 SNP dataset, with shapes and colors indicating the locality and forest cover level of Ny. darlingi collection sites.


Fig D. Selection of optimal number of clusters for Discriminant Analysis of Principal Components (DAPC). (A) Bayesian Information Criterion (BIC) and (B) Akaike Information Criterion (AIC) for 1 to 30 clusters using K-means clustering in preparation for DAPC.


Fig E. Isolation by distance plot depicting Prevosti's absolute genetic distance vs. geographic distance in km for pairs of 16 collection sites. Each dot represents a pairwise comparison between two collection sites.


Fig F. Selection of candidate SNPs. (A) Plot of negative log of adjusted LFMM p-values for 5561 SNPs, highlighting SNPs with $p<0.05$. (B) Plot of log of bayenv2 Bayes factors and absolute value of Spearman's $\rho$ for 5561 SNPs, highlighting SNPs in the top $5 \%$ of Bayes factors and the top $10 \%$ of Spearman's $\rho$.


Fig G. Frequency of major allele for each candidate SNP within each collection site, plotted by the forest cover percentage at each site.


Table A. GO terms significantly enriched (Fisher's $p<0.01$ ) within 100 kb of candidate SNPs in Ny. darlingi genome.

| GO Sub-ontology | GO ID | Description | Number in <br> annotated <br> genome | Number within <br> candidate SNPs | Expected | Fisher's <br> p-value |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Biological Process (BP) | GO:0035278 | miRNA mediated inhibition <br> of translation | 3 | 2 | 0.04 | 0.0005 |
| Biological Process (BP) | GO:0018149 | peptide cross-linking | 3 | 2 | 0.04 | 0.0005 |
| Cellular Component (CC) | GO:0005576 | extracellular region | 178 | 8 | 1.82 | 0.0004 |
| Molecular Function (MF) | GO:0003810 | protein-glutamine gamma- <br> glutamyltransferase activity | 3 | 2 | 0.04 | 0.0006 |
| Molecular Function (MF) | GO:0052689 | carboxylic ester hydrolase <br> activity | 51 | 4 | 0.75 | 0.007 |
| Molecular Function (MF) | GO:0035091 | phosphatidylinositol binding | 27 | 3 | 0.4 | 0.007 |

