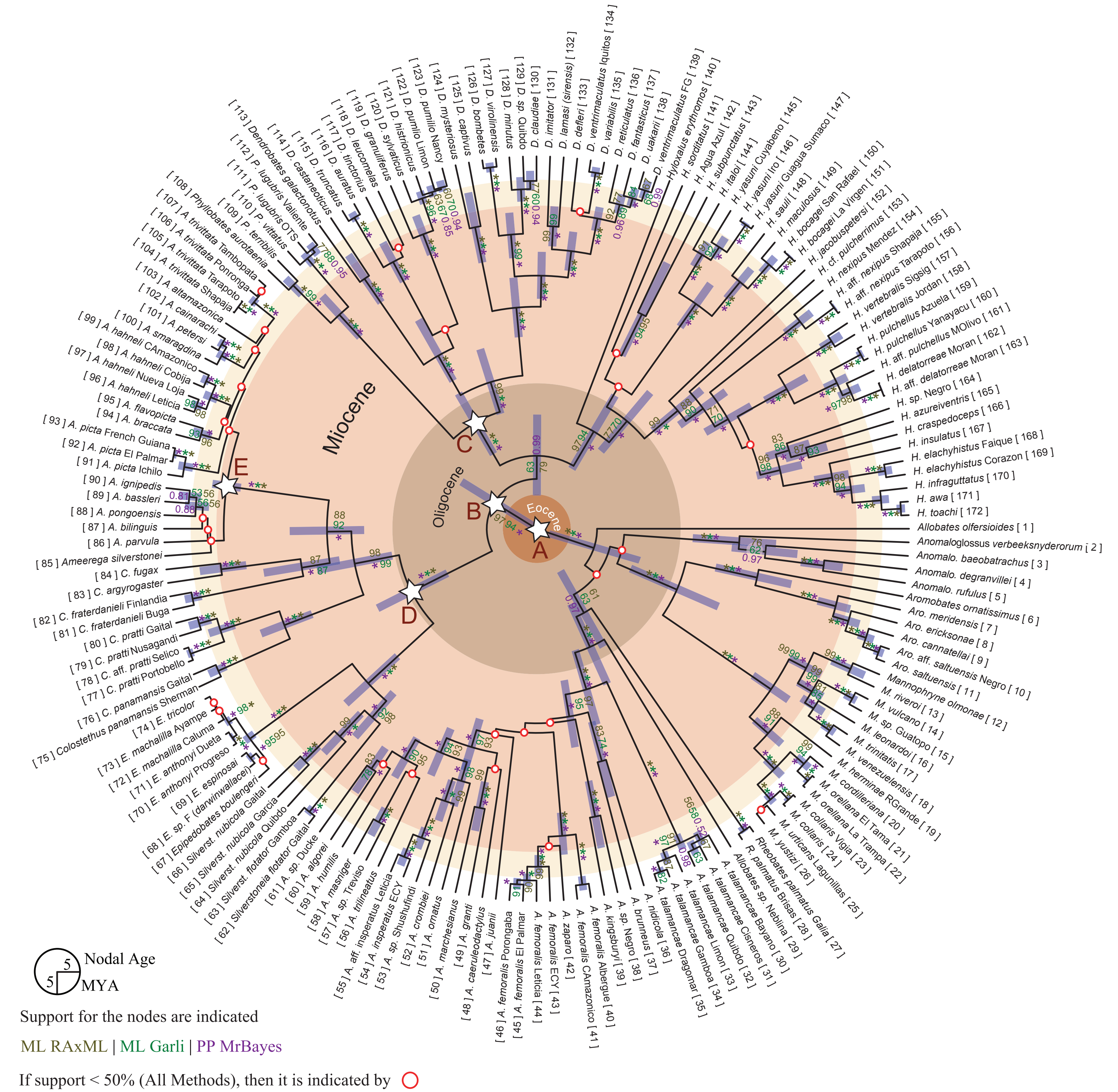
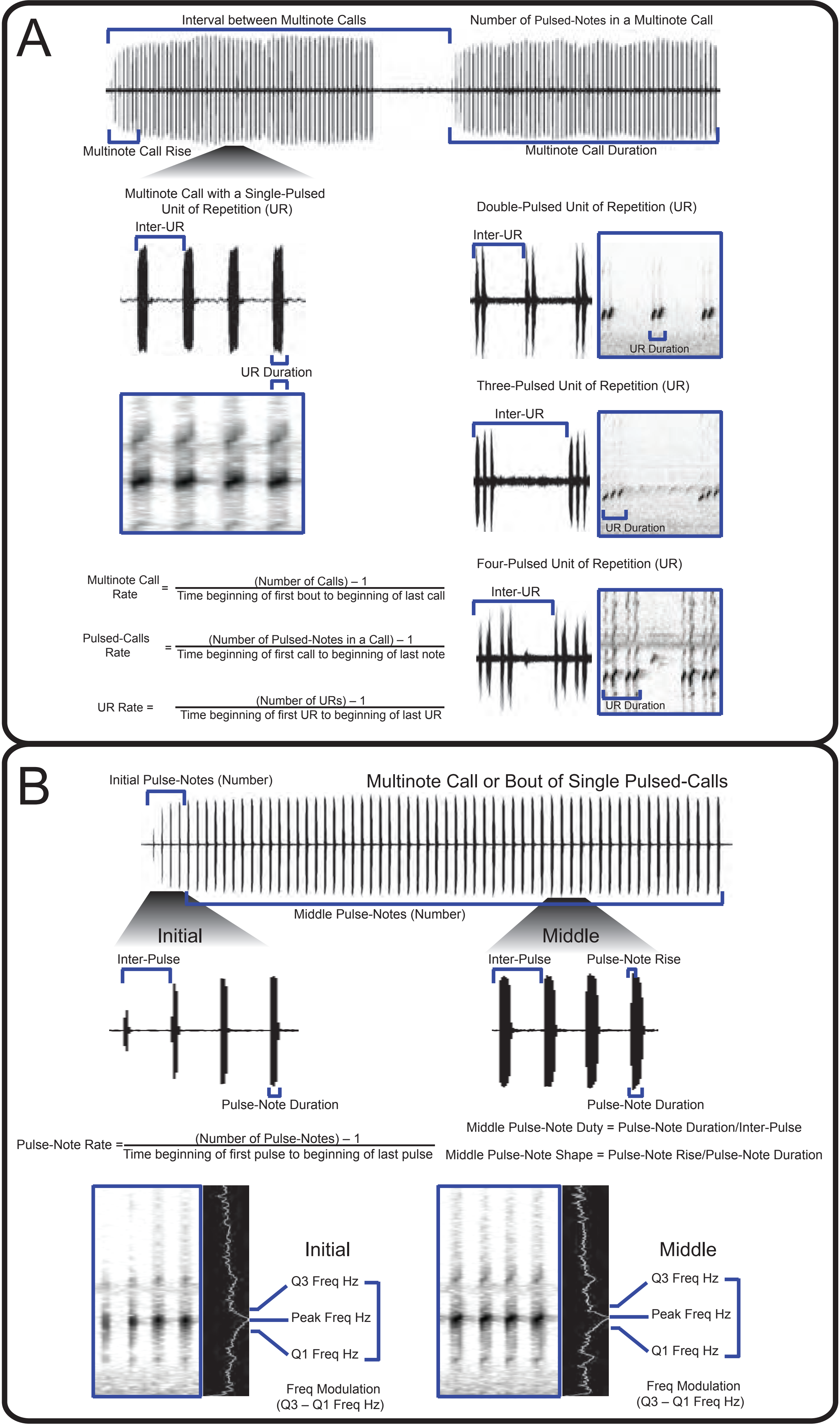
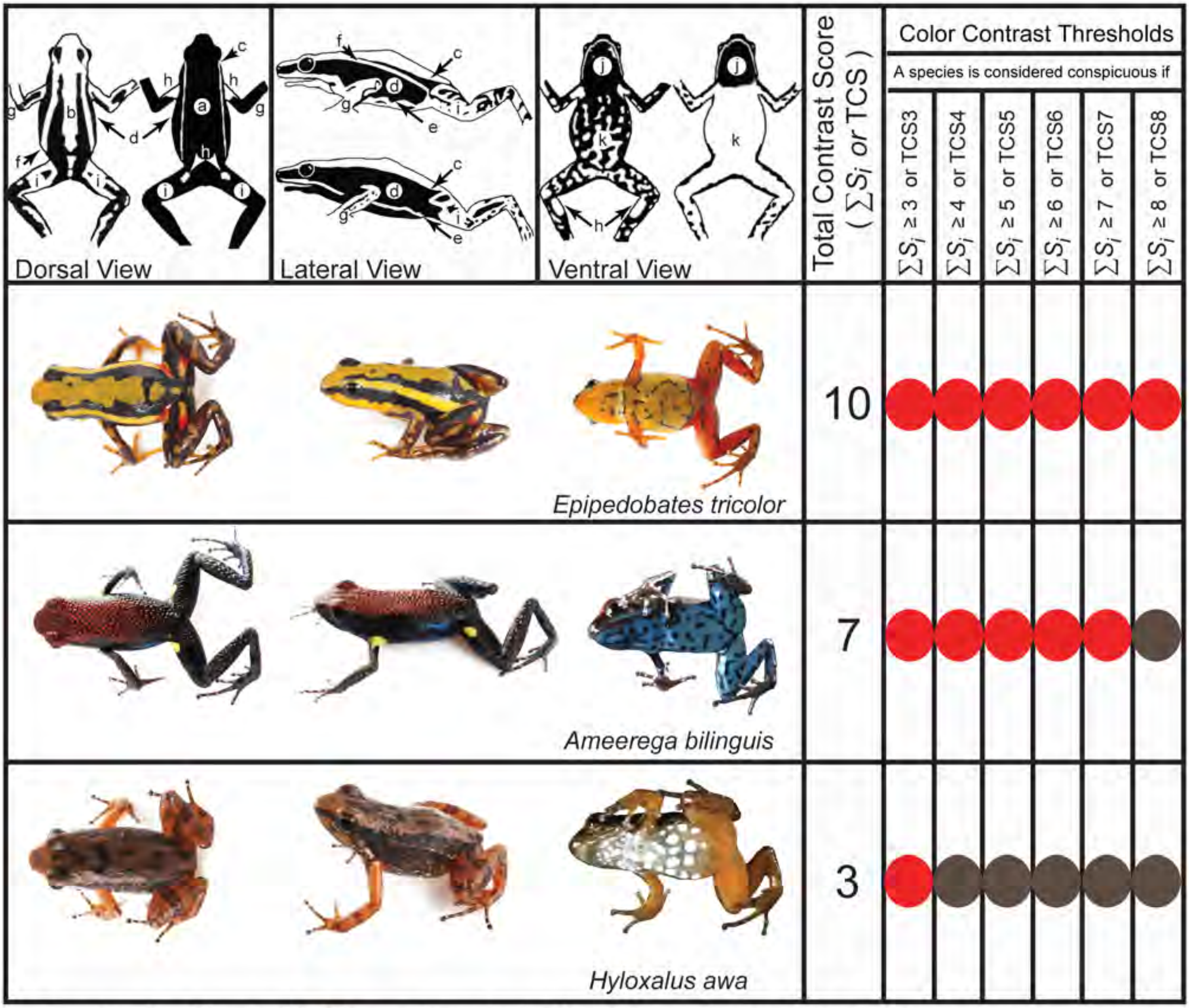


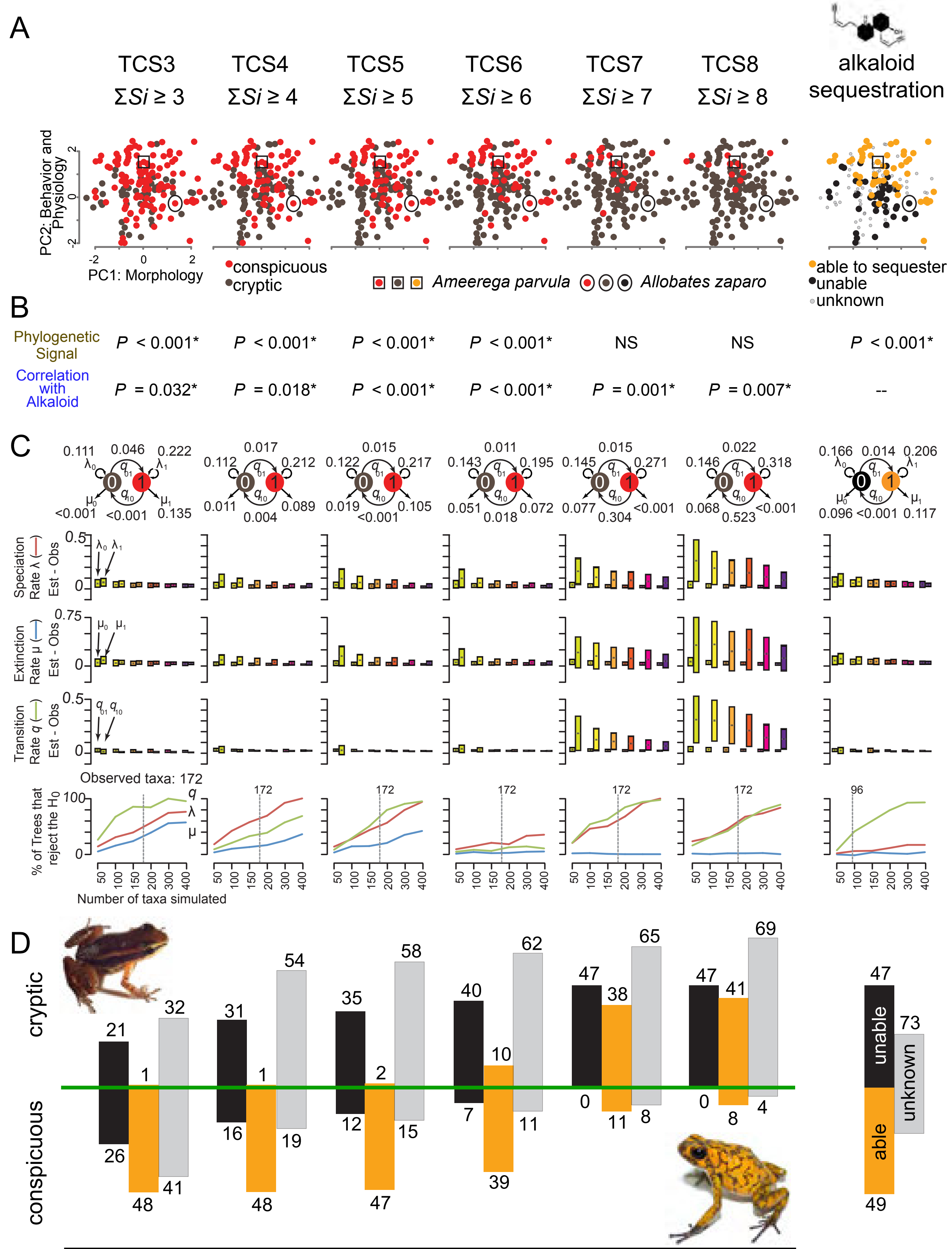
Supporting Figure S1. Chronogram, nodal support, and nodal age uncertainty of the Dendrobatidae (poison frog) Tree of Life. The chronogram shows nodal support under each different estimation method and node age uncertainty (blue bars). The species name and the phylogenetic identifier (Phy ID) number within square brackets are used in Tables S1– S3 and Dataset S1. Nodal support is given by non-parametric bootstrap proportions (ML-RAxML and ML-Garli) and Bayesian posterior probabilities (PP). The node-age calibration points are shown as open stars. These include (node A) crown node of Dendrobatidae (Mean = 38.1 ± 4.2 millions of years-MYA); (node B) the crown node of Dendrobatinae + Hyloxinae + Colostethinae (Mean = 31.6 ± 5.6 MYA); (node C) the crown node of Dendrobatinae (Mean = 24.1 ± 3.1 MYA); (node D) the crown node of Colostethinae (Mean = 27.1 ± 3.2 MYA); and (E) the crown node of *Ameerega* (Mean = 8.7 ± 1.9 MYA).



Supporting Figure S2. Definitions of call variables for (A) multinote calls and units of repetition (URs); and (B) single-note pulses (smallest acoustic units).



Supporting Figure S3. Binary characterization of conspicuousness of three exemplar poison frog species using color contrast thresholds. We characterized eleven frog segments: dorsal background (a), dorsal stripe (b), dorsolateral stripe (c), lateral background (d), ventrolateral stripe (e), oblique lateral stripe (f), arm dorsal (g), flash mark (h), thigh dorsal (i), throat (j), and abdomen (k). Frog diagrams were modified from s previous characterization (Silverstone 1976). Total contrast score (TCS or ΣSi) was determined after adding the conspicuousness or cryptic states from all frog segments (Table S1 – S2). Poison frogs might have multiple natural predators (e.g., snakes, crabs, and birds) with diverse visual sensitivities under different light conditions. For this reason, each species was considered conspicuous (a red dot) or cryptic (brown dot) based on its ΣSi in relationship (i.e, more or equal than, ≥) to an increasing series an integer threshold values from 3, 4, …, 8. In the case of *Epipedobates tricolor*, its ΣSi was 10 and this species is considered conspicuous under all color contrast thresholds (i.e., value of 1-conspicuous under binary variables ΣSi ≥ 3, ΣSi ≥ 4, …, ΣSi ≥ 8). For *Ameerega bilinguis*, its ΣSi was 7 and this species is considered conspicuous under the thresholds 3 – 7 (i.e., value of 1-conspicuous under binary variables ΣSi ≥ 3, ΣSi ≥ 4, …, ΣSi ≥ 7 and 0-cryptic under the variable ΣSi ≥ 8). For *Hyloxalus awa*, its ΣSi was only 3 and this species is considered conspicuous only under the threshold 3 (i.e., value of 1-conspicuous under binary variable ΣSi ≥ 3 and 0-cryptic under variables ΣSi ≥ 4, ΣSi ≥ 5, …, ΣSi ≥ 8.



0.719	0.823	0.854	0.823	0.604	0.573	Accuracy
0.649	0.750	0.797	0.848	1.000	1.000	Precision
0.980	0.980	0.959	0.796	0.224	0.163	Sensitivity

Supporting Figure S4. Diversity analyses of the aposematic syndrome in poison frogs. (A) Distribution of conspicuousness and alkaloid sequestration. To visualize the relationship between call and aposematism, we depicted each species based on its conspicuousness, alkaloid sequestration ability, and plotted them in call space (PC1: morphology, versus PC2: behavior/physiology). Note the location of *Allobates zaparo* (visual Batesian mimic) and its aposeme model *Ameerega parvula*. (B) Probability values for presence of phylogenetic signal (Pagel's lambda test) and correlations between each conspicuousness variable with alkaloid sequestration ability. Significant correlations ($P < 0.05$) indicate the aposmatic phenotype. No phylogenetic signal was detected in the variables TCS7 and TCS8 variables. All other conspicuousness variables are significantly correlated with alkaloid sequestration. (C) Diversification analysis results under each characterization of conspicuousness: λ = speciation, μ = extinction, and q = transition between character states. Alternative states are indicated by subscripts: 0 (cryptic coloration or unable or lack of alkaloid sequestration) and 1 (conspicuousness or alkaloid sequestration). The results of power analyses are indicated by bar charts. These bars indicate mean and ± one standard deviation of the differences between the values of the parameters estimated (200 simulated data) and observed (observed data). Line plots indicate the percentage of simulated trees that rejected the constrained model as a function of the number of terminals under different constraints (i.e., $\lambda0 \neq \lambda1$, $\mu0 \neq \mu1$, and $q10 \neq q01$). (D) Distribution of taxa based on their aposematic phenotypic value. The variable TCS5 or $\Sigma Si \geq 5$ is the best qualitative (binary) measurement for classifying taxa as aposematic (i.e., conspicuous when they also are able to sequester alkaloids) based on joint criterion for accuracy, precision, and sensitivity indices when they are closer to 1.00 (see Supporting Text for details).