

Supporting Information

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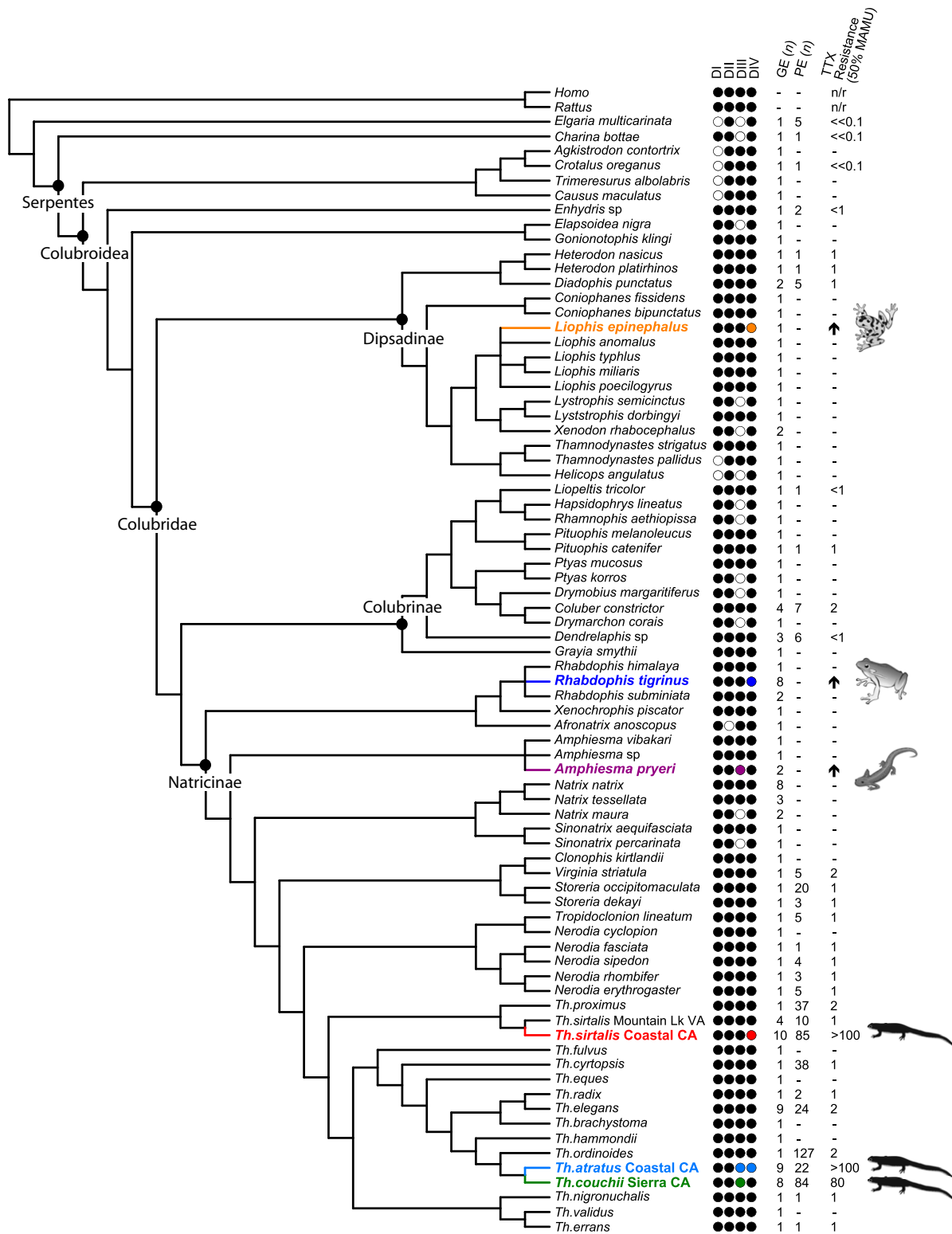


Fig. S1. Phylogenetic distribution of snake lineages that prey on tetrodotoxin (TTX)-bearing amphibians (colored branches and taxa) and also possess derived amino acid replacements at sites critical to TTX ligation in the pore-forming loops (P-loops) of the skeletal muscle sodium channel (Na_v1.4). The New World Legend continued on following page

natricines *Thamnophis sirtalis* (red), *Thamnophis atratus* (light blue), and *Thamnophis couchii* (green) prey on *Taricha* newts (1–4); the Old World natricines *Rhabdophis tigrinus* (bright blue) preys on the tree frog *Polypedates leucomystax* (5) and *Amphiesma pryeri* (purple) preys on the newt *Cynops ensicauda* (6, 7); the neotropical dipsadine *Liophis epinephelus* (orange) consumes several *Atelopus* toads (8, 9). Phylogeny of colubroid snakes and relatives is based on relationships presented in refs. 10–18. Snakes that prey on TTX-laden frogs or salamanders show derived variation in the P-loops domains DIII and DIV (colored circles); P-loops in other domains (and other taxa) lack adaptive variation (black circles). In a few cases we were unable to obtain P-loop sequences (white circles). Numbers of individuals sequenced (GE) and assayed for TTX resistance (PE) alongside measures of TTX resistance [50% mass-adjusted mouse units (MAMU)]. Direct measures of whole-animal resistance for this study were augmented with some of our previous data (19–21). We inferred elevated levels of TTX resistance (†) for *A. pryeri*, *R. tigrinus*, and *L. epinephelus* based on measures of TTX recorded in their respective prey: 60–7,000 mouse units (MU) of TTX for *C. ensicauda* (22–25); 30–920 MU for *Polypedates* spp. (26); 10–100 MU for *Atelopus* spp. (25, 27, 28).

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Table S1. Sodium channel mutational constructs (from the literature) functionally expressed ex vivo and measured for TTX resistance and/or Na⁺ conductance or Na⁺ selectivity

TTX ≥ WT	Conductance ≥ WT	Selectivity ≥ WT	Sodium channel replacement	Citations and notes
1	0		Y401C	1
1	1		W736C	2 (see figure 1D; conductance the same)
1	0		D1532A	3 (see figure 3)
1	0		F385N	3 (see figure 2)
1	0		D400A	4 [TTX]; 5 [conductance]
1	0		E755A	4 [TTX]; 5 [conductance]
1	1		K1237A	4 [TTX]; 5 [conductance]
0	0		I757C	6 [TTX]; 7 [conductance]
0	0		M1240C	6 [TTX]; 7 [conductance]
0	0		D1241C	6 [TTX]; 7 [conductance]
1	0		W402C	6 [TTX]; 7 [conductance]
1	0		E403C	6 [TTX]; 7 [conductance]
1	0		E758C	6 [TTX]; 7 [conductance]
1	0		K1237C	6 [TTX]; 7 [conductance]
1	0		W1239C	6 [TTX]; 7 [conductance]
1	0		A1529C	6 [TTX]; 7 [conductance]
1	0		D1532C	6 [TTX]; 7 [conductance]
1	1		G1530C	6 [TTX]; 7 [conductance]
1	1		W1531C	6 [TTX]; 7 [conductance]
0	0		R379Q	8
0	0		D1426Q	8
0	0		D1426K	8
1	0		Q383E	8
1	0		Q383K	8
1	0		D384E	8
1	0		D384N	8
1	0		W386Y	8
1	0		E387Q	8
1	0		E387S	8
1	0		E387Y	8
1	0		N388R	8
1	0		Q391K	8
1	0		W943Y	8
1	0		E945K	8
1	0		K1422E	8
1	0		M1425Q	8
1	0		M1425K	8
1	0		D1426N	8
1	0		A1714E	8
1	0		D1717Q	8
1	0		D1717K	3 (see figure 3), 8
1	0		D1717N*	3 (see figure 3), 8; *here the D1568N replacement of <i>Th. atratus</i> and <i>Th. sirtalis</i>
1	0		E942Q	8, 9
1	0		E945Q	8, 9
1	0		R395C	10
1	0		R750C	10
1		0	Y401C	1 [TTX]; 11–13 [selectivity]
1		0	Y401A	12
1		0	Y401D	12
1		0	Y401S	13
1		1	W736C	2 (see figure 5C); selectivity mostly the same, but more sensitive to NH ₄ ⁺
1		1	E942Q	9; selectivity same but only Li ⁺ tested
1		1	E945Q	9; selectivity same but only Li ⁺ tested
1		1	D949N	9; selectivity same but only Li ⁺ tested
1		0	D400A	4 [TTX]; 5, 14 [selectivity]
1		0	E755A	4 [TTX]; 5, 14 [selectivity]
1		0	K1237A	4 [TTX]; 5, 14 [selectivity]
0		0	W756C	6 [TTX]; 7 (see figure 6B) [selectivity]
1		0	W1239C	6 [TTX]; 7 (see figure 6B), 15 [selectivity]

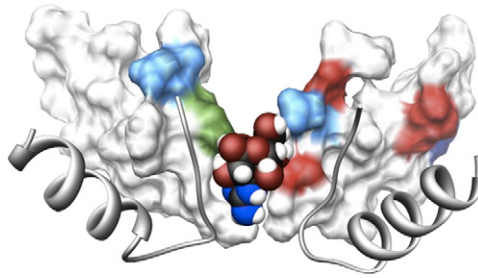
Table S1. Cont.

TTX ≥ WT	Conductance ≥ WT	Selectivity ≥ WT	Sodium channel replacement	Citations and notes
0		1	M1240C	6 [TTX]; 7 (see figure 6B), 15 [selectivity]; selectivity mostly the same
1		0	K1237C	6 [TTX]; 7 (see figure 6B), 15, 46 [selectivity]
1		0	A1529C	6 [TTX]; 7 (see figure 6B), 15, 46 [selectivity]
1		0	W1531C	6 [TTX]; 7 (see figure 6B), 15, 46 [selectivity]
1		0	D1532C	6 [TTX]; 7 (see figure 6B), 15, 46 [selectivity]
0		1	I757C	6 [TTX]; 7 (see figure 6B), 16 [selectivity]; selectivity mostly the same
1		0	W402C	6 [TTX]; 7 (see figure 6B), 16 [selectivity]
1		0	G1530C	6 [TTX]; 7 (see figure 6B), 16 [selectivity]
1		0	E403C	6 [TTX]; 11 (see figure 4D), 16 [selectivity]
0		1	D1241C	6 [TTX]; 16 (see figure 4D), 16 [selectivity]; selectivity mostly the same
1		0	E758C	6 [TTX]; 16 (see figure 4D), 16 (see figure 3)[selectivity]; similar but significantly worse for Li ⁺
1		0	R395C	10
1		0	R750C	10
1		1	F745C	10; selectivity mostly the same
1		0	I1532V*	17; *here the I1561V replacement of <i>Th. sirtalis</i>
1		0	IIDG->LVNV*	17; *here the four DIV replacements in Willow Creek <i>Th. sirtalis</i> (1.4 ^{LVNL} allele of ref. 18)

Replacement notation follows that of the original study. For the χ^2 analyses we tallied a replacement as positive (1) if it produced an effect as well or better than the wild type on TTX resistance (predictor variable) and either Na⁺ permeability or Na⁺ selectivity (response variables) and as negative (0) if it produced a statistically worse effect than the wild type.

*Only two studies (3, 17) have examined some of the naturally occurring mutations in snakes for Na⁺ conductance or Na⁺ selectivity, but these studies show that TTX-resistant mutations (or alleles) compromise sodium channel function.

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Movie S1. 3D model of the Na_v1.4 outer pore showing TTX docked in the pore and adaptive replacements in TTX-resistant snakes. The movie begins with a top-down view of the four pore loops (DI–DIV) and TTX occluding the outer pore; then the outer pore tilts forward to show angled side views of the outer pore. Note that both TTX and Na⁺ enter the pore from the extracellular side of the protein (top). The structural model of the outer pore follows ref. 1.

[Movie S1](#)

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