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# DIVERGENT ROADS: DISTINCT EVOLUTIONARY TRENDS ACROSS *CROTALUS*

**RECOGNIZED THROUGH MULTI-OMIC APPROACHES**

Durban, J.1, Sanz, L.1, Trevisán-Silva, D.2, Neri-Castro, E.3, Alagón, A.3, Calvete, J.J.1,\*

1 Laboratorio de Venómica Evolutiva y Traslacional, Instituto de Biomedicina de Valencia, CSIC, Jaime Roig 11, 46010 Valencia, Spain; 2 Instituto Butantan, São Paulo, Brazil; 3 Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México

*E-mail address*: jcalvete@ibv.csic.es

**Background:** The most recent common ancestor for the rattlesnakes has been estimated at 12.7 Mya (mid-Miocene) with a center of origin in the north-central Mexican Plateau. Biogeographical data suggest dispersals northward into North America (late Miocene, 6–8 Mya), southward into Central America dating back to the late Miocene/early Pliocene (6.4−6.7 Mya), and a relatively recent (1.8−1.1 Mya) South American dispersal during the middle Pleistocene (1.1−1.0 Mya). *Crotalus* venoms are classified, according to their predominant expression of hemorrhagic PIII-SVMPs or heterodimeric neurotoxic PLA2, as type I or type II. This venom dichotomy is a shared trend throughout the Americas. However, the evolutionary routes followed by North, Central, and South American venoms are punctuated by their own characteristics. In North America, type-I and type II venoms are scatterly distributed across phylogeny. Type-I and type-II venom phenotypes in North American rattlesnakes appear to be due with recent lineage-independent losses of the genes coding for the the neurotoxic PLA2 complex subunits. In Arizona, *C. s. scutulatus* type-I/type-II venom dichotomy correlates with the transition from a haemorrhagic to a neurotoxic phenotype along a South Central to South Eastern axis. The predominance in adult South American rattlesnakes (*Crotalus durissus* sp.) of type II venoms exhibiting increasing incapacitation and lethal activities to rodents represents an adaptive paedomorphic trait driven by the gain of neurotoxicity along the South America's North-South axis. On the other hand, in the Central American rattlesnake, *C. s. simus*, the two venom phenotypes are linked by an ontogenetic transition from type II to type I venom, and this compositional shift appears to be post-transcriptionally modulated by age- dependent changes in the concentration of miRNAs.

**Methods:** Multi-omic analyses of the transcriptional and translational venom gland activities also support a role for miRNAs in the ontogenetic venom compositional changes in congeneric Mexican rattlesnakes, *C. simus* and *C. tzbacan*. The finding of dual-action miRNAs, which silence the translation of neurotoxic heterodimeric PLA2 crotoxin while simultaneously up-regulating SVMP mRNAs, potentially explains the existence of the mutually exclusive type-II and type-I venom dichotomy among rattlesnakes.

**Discussion/Conclusion:** The hypothesis that alterations of the distribution of miRNAs in response to an external cue may contribute to the mechanism generating adaptive venom variability deserves further detailed studies.

 **Key Words**: *Crotalus* venom, venom phenotype dichotomy, venom ontogeny, miRNAs