

Assessing the Importance of Eco-evolutionary Variables in Driving Venom Evolution Across Diverse Animal Lineages

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Abstract. Animal venoms are novel phenotypic traits that critically enhance fitness and ecological adaptation. Although venom composition and function are extensively documented, our understanding of the ecological and evolutionary drivers shaping these systems relies heavily on snake-focused studies, which may not generalize across broader venomous taxa. Here, we assembled a macroevolutionary dataset spanning 125 diverse species to evaluate how ecological and morphological variables influence five key venom traits, including delivery mode, function, complexity, specialized organs, and biological function. Using generalized, linear models and Random forest variable importance analyses, we show that diet type plays a surprisingly limited role across most models. Instead, body size consistently emerges as the more important predictor for venom delivery, complexity, and biological function, supporting a mechanical constraints hypothesis where larger body size provides more capacity for larger venom glands and more complex toxins. Additionally, prey handling strategy dominates predictions of venom function and specialized organs. Notably, habitat type ranks among the top three predictors for all five venom traits, demonstrating the pervasive influence of physical and ecological environments on venom evolution. While diet type shows detectable statistical effects in linear models, its importance may be largely overshadowed by body size and handling strategy. To conclude, venom evolution is a multifactorial process driven by ecological contexts and morphological constraints. These findings reframe the traditional diet-driven hypothesis and advocate for a generalized, multi-variable framework in future comparative venom studies.

Keywords: Venom evolution, venomous taxa, phenotypic adaptation, ecological drivers, macroevolution

1. Introduction

In nature, many animals exhibit novel phenotypic traits that enhance their fitness and ecological adaptation [1, 2]. For example, the elaborate and colorful tail feathers of male peacocks are a novel phenotypic trait that enhances their fitness benefits by attracting mates through sexual selection. However, in some desert-dwelling beetles, such as *Stenocara gracilipes*, novel phenotypic traits occur in the form of raised bumps on their elytra, which allow them to harvest water from fog in their arid environment [3]. Among these innovations, venom and venomous characteristics represent one of the most striking evolutionary innovations. Venom can be broadly defined as toxic secretions

that are delivered directly into the internal environment of a living recipient (e.g., through stinging, biting, etc.), where they disrupt normal physiological functioning against the recipient's interests and to the benefit of the venomous organism [4]. Venom systems have evolved independently across nearly all major animal taxa, including invertebrates, such as arthropods, and vertebrates, such as reptiles, amphibians, and mammals [5]. The global distribution of these venomous animal groups spanning all biogeographic realms is visualized in Figure 1. More importantly, this repeated convergence has resulted in diversity in venom composition, modes of action, delivery mechanisms, and associated organs or cells [6, 7]. Venom varies among neurotoxins, hemotoxins, cytotoxins, and cardiotoxins, with the ability to act on one specific pathway (e.g., neural pathway) using different biochemical components, which is to say, different biochemical components can disrupt the same biological process (e.g., nerve signaling) by attacking different targets or steps, each contributing in its own way. For instance, cone snail venoms contain different protein components, including alpha and beta conotoxin (CTX), enabling them to cut off neural signal transmission of their prey. Venoms also vary substantially in their biological functions and ecological roles. In animals, venoms are commonly used for predation, defense against predators, and in some cases, are involved in reproduction or intraspecific competition. Such functional diversity demonstrates that venom evolution is closely linked to ecological contexts and organismal fitness. For example, the predatory cone snail *Conus geographus* uses a fast-acting insulin-like venom to induce hypoglycemic shock in passing fish, effectively immobilizing prey much larger than itself [8]. In contrast, the platypus *Ornithorhynchus anatinus* produces venom only during the breeding season, and males use it primarily in intrasexual combat to gain dominance over rivals [9]. These collectively illustrate that venom evolution is closely tied to ecological context and organismal fitness, raising the question of which specific selective forces drive its diversification.

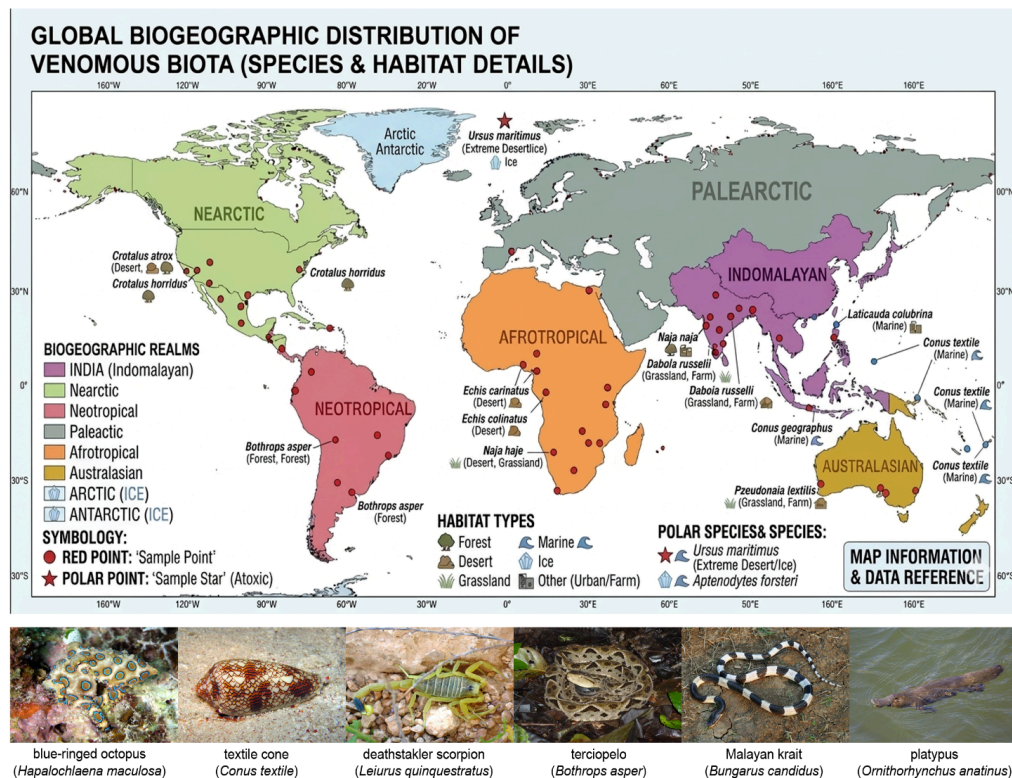


Figure 1. Global biogeographic distribution of venomous species

Studies have demonstrated the critical roles of ecological factors in shaping the evolution and diversification of venom, including diet, habitat, life history, and species interactions such as predator–prey dynamics and courtship behaviors [5, 7, 10]. Among these factors, diet has been widely regarded as a major selective force, as venom is often directly involved in food acquisition or deterrence. For example, a rattlesnake uses its venom to quickly immobilize rodents, allowing it to swallow them safely. Numerous case studies across species, particularly in snakes, support the hypothesis that dietary specialization drives venom evolution [6, 11, 12]. For example, the sea snake *Aipysurus eydouxii*, which feeds exclusively on fish eggs, exhibits relaxed selection on venom genes, resulting in a simplified venom composition and frequent null or deleterious mutations [12]. Diet also influences venom-related traits in other species. In some frogs, such as dendrobatid (poison dart frogs), individuals do not synthesize their own toxins but instead sequester alkaloids from arthropod prey - known as diet-acquired chemical defense [13]. These studies highlight the strong link between feeding ecology and venom function, whether the venom is self-synthesized (e.g., cardiotoxic steroids in some amphibians or snakes) or diet-acquired (e.g., alkaloids in dendrobatids). However, most existing evidence is taxon-specific, and it remains unclear whether diet-driven venom evolution represents a general pattern across diverse venomous lineages. Furthermore, the extent to which other ecological factors, such as habitat structure, predation risk, or social behaviour, interact with diet to shape venom diversity has not been systematically explored. Despite substantial progress in venom composition and function in individual and restricted taxa, comparative studies that address the ecological drivers of venom diversity across multiple lineages and geographic regions remain limited. In particular, the roles of diet, biogeography, and species interactions in shaping venom evolution are poorly integrated within an eco-evolutionary unified framework. To address these gaps, this study investigates what ecological and morphological variables affect venom function and diversification across a broad range of venomous species. Using a standardized data set collected across venomous taxa (Figure 1), we aim to unravel (1) whether and how a set of ecological variables (e.g., biogeographic zone, habitat, activity pattern) and morphological traits (i.e., body size) influence venom characteristics and functions; and (2) whether such effects vary across venom characteristics and functions, i.e., venom delivery mode, venom function and complexity, specialized venomous organs, and putative biological functions; and (3) to what extent diet type contributes to venom function and diversity relative to other eco-morphological predictors, and which factors consistently rank as the more important determinants across different venomous traits.

2. Materials and methods

2.1. Data compilation and standardization

To investigate how ecological and morphological variables drive venom evolution and diversity across venomous taxa, we constructed a standardized comparative dataset. This dataset integrated ecological, morphological, trophic, reproductive, and venom-related traits for 125 venomous species across 11 major taxonomic groups: Actinopterygii (n = 2), Amphibia (n = 28), Arachnida (n = 14), Aves (n = 10), Cephalopoda (n = 3), 1 from Chilopoda (n = 1), Cubozoa (n = 1), Gastropoda (n = 7), Insecta (n = 14), Mammalia (n = 24), and Reptilia (n = 21). All trait definitions, categorization, and criteria are summarized in Table 1. Variables were defined a priori to ensure conceptual consistency and avoid post hoc categorization bias. Only adult ecological information was recorded to minimize confounding effects of ontogenetic dietary or habitat shifts. When multiple sources were available, concordant information across independent references was prioritized. Categorical variables were

coded as binary or multi-level factors, while continuous variables, such as body size, were standardized to consistent measurement units. Missing information was explicitly coded as "no data" rather than inferred, ensuring transparency and reproducibility of trait assignment.

2.2. Data sources

Ecological and morphological data were primarily compiled from peer-reviewed literature and publicly available natural history resources, including Wikipedia, which provided accessible species-level summaries of habitat, activity patterns, diet, and reproductive modes. These data were cross-validated against specialized databases where available venom-related data were collected from multiple sources. Venom delivery mode and specialized venom organs were coded based on anatomical descriptions from the primary literature. Venom composition and complexity data were extracted from: (i) UniProtKB/Swiss-Prot Tox-Prot program [14], a manually curated database of venom proteins from all venomous taxa; (ii) taxon-specific databases including ArachnoServer [15], ConoServer [16], and iVenomDB [17]; and (iii) the VenomZone web portal, which provides curated information on venoms from six major venomous taxa (snakes, scorpions, spiders, cone snails, sea anemones, and insects) [18]. Venom function categories (e.g., prey subjugation, defense) were assigned based on ecological studies and behavioral observations following the framework of Fry et al. [19].

2.3. Ecological and morphological trait definitions

Nine ecological and morphological traits were selected to capture three hierarchical levels of effects on venom function and evolution. Biogeographic variables included: (i) biogeographical zone (Biogeo_zone), referred to as the primary zoogeographic realm (i.e., Nearctic, Indomalayan, Afrotropical, Neotropical, Australasian, Palearctic, Indo-Pacific, Global) following standard biogeographic classifications; and (ii) distribution width (Distri_width), categorized as regional (restricted range) or widespread (broad distribution) [20, 21]. Habitat and trophic ecology variables included: (i) habitat type (Habitat_type), classified as forest, grassland, desert, freshwater, marine (including coastal), cave, urban, farmland, or various (≥ 3 types); (ii) patterns of daily activity (Activity_pattern), classified as nocturnal, diurnal, or crepuscular based on primary activity periods [22]; (iii) primary types of diet (Diet_type), classified into major prey and diet categories (reptiles, mammals, fish, birds, amphibians, invertebrate_arthropods, invertebrate_others, plant, or various); In animals, diet specialization pattern is generally classified as specialist (species primarily uses a narrow range of resources) or generalist (species primarily uses a wide range of resources) [23, 24]. Although specialization and generalization in diet patterns exist along a continuum both within and between species, each species' primary prey resource and trait was considered as its diet pattern; and (iv) prey handling pattern (Prey_hand), classified as venom injection (using specialized structures to inject venom for prey immobilization) or non-venom (relying solely on physical means) [25, 26]. To assess the impact of morphological and reproductive traits, we also collected (i) average adult body size (Body_size), measured as snout-vent length (SVL) in meters; (ii) the presence or absence of aposematism (Aposematism), binary coding for presence (1) or absence (0) of conspicuous warning coloration signaling toxicity [27, 28]; and (iii) reproductive type (Reproductive_type), classified as oviparous, viviparous, or other/specialized [29, 30].

2.4. Venom trait definitions

To assess venom function and diversity, five main venom traits were collected, including venom delivery mode, venom function and diversity, and specialized venom organs and putative biological function of venoms. Venom delivery mode (Venom_delivery) was defined as the mechanical method of introducing venom into a target, classified as active injection (possession of specialized delivery organs with muscular action to actively force venom into the target) or passive injection/transfer (venom entry via contact, passive flow, or target's own movement against spines/structures) [6]. In venomous species, specialized venom organs refer to dedicated anatomical structures for both venom synthesis and delivery, such as glands connected to fangs, stings, or spines [5, 7]. The presence or absence of such specialized venom organs was coded as Yes or No (toxins secreted from generalized glands or lacking dedicated injection structures). Primary venom function (Venom_function) was defined as the principal biological role of venom, classified into six categories: prey subjugation, defense, intraspecific competition, digestion, reproduction, and parasitic (e.g., facilitating parasite transmission) [5, 10, 31-34]. This functional classification prioritizes ecological context over biochemical detail, enabling cross-taxon comparisons of venom adaptive significance. Major biochemical compound classes of venom toxins are categorized based on dominant toxin superfamilies, e.g., phospholipase A2, three-finger toxins, serine proteases, or others [18, 35]. Following Zancolli et al. [35], venom complexity (Venom_complexity) was defined as a categorical proxy for biochemical diversity based on explicitly identified major toxin families in the venom. The venom complexity was categorized into three levels: Low (1-2 toxin families), Medium (3-4 toxin families), or High (≥ 5 toxin families). This standardization allows biochemical diversity to be quantified without requiring full proteomic datasets for every species, providing a robust and comparable metric of functional diversity across large phylogenetic scales. Putative biological function (Bio_function) was defined as the primary pathophysiological effect of venom at the organismal level, inferred from major components, classified as neurotoxin, cytotoxin, hemotoxin, myotoxin, cardiotoxin, or uncategorized/other [5, 6, 11, 33, 36]. Toxicity measures are often derived from medically oriented research, potentially biasing representation toward human-relevant effects. However, functional toxin categories (e.g., neurotoxicity, cytotoxicity) are physiologically conserved across vertebrates, supporting cross-context inference.

Table 1. Definitions and categorization of variables

Feature category	Variable name	Definition	Categorization	Rational
Biogeography	Biogeo_zone	The primary zoogeographic realm in which the species is natively and consistently found.	Neartctic, Indomalayan, Afrotropical, Neotropical, Australasian, Palearctic, Indo-Pacific, Global.	Tests historical biogeography effects
	Distri_width	The geographical range extent of the species.	Regional (restricted range), Widespread (broad distribution).	Links to niche breadth and evolutionary lability

Table 1. (continued)

Habitat & Tropical Ecology	Habitat_type	The primary physical environment(s) where the species lives and forages.	Forest, Grassland, Desert, Freshwater, Marine (including coastal), Cave, Urban, Farmland, Various (applies if species regularly uses ≥ 3 of the above types).	Captures physical and biotic environment
	Activity_pattern	The primary period of daily activity.	Nocturnal (active at night), Diurnal (active during daylight), Crepuscular (active at dawn/dusk).	Relates to predator-prey encounters
	Diet_type	The major taxonomic categories of consumed prey/food.	Reptiles, Mammals, Fish, Birds, Amphibian, Invertebrate_arthropods, Invertebrate_others, Plant, Various (≥ 3 of the above categories).	Refines dietary specialization
	Pre_handle	The primary mechanism used to subdue and process prey during foraging.	Venom_injection: Uses specialized anatomical structures (e.g., fangs, stings) to inject venom for prey immobilization, regardless of hunting strategy (active or ambush). Non-venom: Relies solely on physical means (e.g., constriction, swallowing alive) for prey subjugation; any toxins are used solely for defense.	Captures the ecological context of venom use.
Morphological & Reproductive traits	Body_size	Snout-vent length (SVL), measured in meters (m).	Continuous variable.	A key constraint on prey size and energetics
	Aposematism	Presence of conspicuous, contrasting color patterns that signal toxicity or unpalatability.	0 (absent), 1 (present).	Signals a defensive venom function
	Reproductive_type	The primary mode of offspring development and birth.	Oviparous: Reproduction involves laying eggs, regardless of egg environment (terrestrial, aquatic) or subsequent development. Viviparous: Offspring develop internally and are born live. Other/Specialized: Represents unique systems not fitting the above (e.g., monotremes, eusocial reproduction).	May correlate with life-history traits

Table 1. (continued)

Venom characteristics	Venom_delivery	The mechanical method of introducing venom into a target.	Active injection: Possesses specialized delivery organs (e.g., hollow fangs, stings) and uses muscular action to actively force venom into the target. Passive injection/transfer: Venom enters the target via contact, passive flow (e.g., grooved teeth), or the target's own movement against spines/structures.	Describes the mechanical integration of the venom system.
	Venom_function	The principal biological role of the venom.	Prey subjugation, Defense, Intraspecific competition, Digestion, Reproduction, Parasitic (e.g., facilitating parasite transmission).	Core functional classification
	Venom_complexity	A categorical proxy for the biochemical diversity of major toxin families in the venom.	Low: 1-2 distinctly identified major toxin families. Medium: 3-4 distinctly identified major toxin families. High: ≥ 5 distinctly identified major toxin families. No data: Information unavailable. Note: Only explicitly named, distinct families (e.g., Three-finger toxins, SVMPs, Phospholipase A2) are counted. Vague descriptors (e.g., "peptides") or "no data" are not counted toward complexity	Biochemical diversity metric
	Specialized_venom_organisms	Presence of anatomical structures dedicated specifically to both venom synthesis and delivery.	Yes: Has dedicated glands connected to specialized delivery structures (e.g., fangs, stings, spines). No: Toxins are secreted from generalized glands (e.g., skin, saliva) or lacks dedicated injection structures.	Indicates morphological adaptation for venom use.
	Bio_function	The primary pathophysiological effect of the venom at the organismal level, inferred from its major components.	Neurotoxin, Cytotoxin, Hemotoxin, Myotoxin, Cardiotoxin, Uncategorized/Other.	Links chemistry to biological effect

2.5. Statistical analyses

All statistical analyses were conducted in R (version 4.2.2; R Core Team, 2022), loaded with packages, e.g., car, DHARMA, emmeans, lme4, MASS. Model fits were evaluated using the stimulateResiduals function (package "DHARMA") to assess residual distributions and overall model adequacy. Likelihood ratio tests for the fixed effects were performed using the Anova function in the 'car' package. The post hoc comparisons for interaction terms were performed using the 'emmeans' package.

2.5.1. Data preparation and exploratory analysis

Prior to analysis, the compiled dataset was inspected for consistency and missing values. Continuous variables such as body size (SVL) were transformed into comparable units, e.g., log-transformation to meet normality distribution. Categorical variables were converted into factors following the categorization described in Table 1. To minimize biases caused by missing values, all subsequent analyses were performed using complete-case subsets specific to each model, ensuring that only species with relevant data for the variables under consideration were included. An initial exploratory phase was conducted to visualize overall variable distributions and detect potential correlations among the nine ecological and morphological and five venom variables, providing a preliminary overview of trait variation across taxa for the subsequent comparative analyses.

2.5.2. Generalized linear models

To test whether ecological and morphological traits predict venom characteristics independently, we conducted a series of comparative generalized linear models (GLMs). First, pairwise associations between categorical variables were evaluated using a chi-square test of independence. For the primary GLM analyses, ecological and morphological variables, or their interactions, were included as explanatory variables. In this first analysis step, we constructed a separate model for each of the five venom characteristic response variables (e.g., `venom_delivery`, `venom_function`, `venom_complexity`, `specialized_organs`, and `bio_function`). The model family and link function were determined by each response variable. For these binary or categorical response variables, we employed GLMs with binomial or Poisson error distributions. These models included the nine ecological and morphological variables as fixed effects. This explanatory analysis provided a preliminary overview of how each ecological and morphological variable independently affects venom characteristics.

2.5.3. Random forest analyses

To quantify the predictive power and variable importance of ecological-morphological traits for each specific venom characteristic, we conducted Random Forest (RF) classification and regression models using the `randomForest` package in R. For each of the five venom traits (`venom_delivery`, `venom_function`, `venom_complexity`, `specialized_venom_organs`, and `putative_biological_function`), an RF model was built with all nine ecological-morphological variables as predictors (`biogeo_zone`, `distri_width`, `habitat_type`, `diet_type`, `activity_pattern`, `log_body_size1`, `aposematism`, `prey_handle`, `reproductive_type`). Model parameters included 500–1000 trees (`ntree`), with importance metrics computed. Variable importance was assessed using the mean decrease in accuracy for classification models (binary factors) or the mean decrease in node impurity (Gini index) for categorical factors with more than two levels. This non-parametric approach is robust to multicollinearity and captures non-linear relationships and complex interactions among predictors. For specific binary venom traits where model separation was encountered in preliminary logistic regressions, we employed Firth's bias-reduced logistic regression using the `logistf` package to obtain reliable parameter estimates and p-values, but the primary inference for variable importance was derived from the random forest models.

3. Results

3.1. Effects of ecological and morphological variables on venom traits

The exploratory analyses based on (G)LMs models showed the differential effects of ecological and morphological variables on venom function and diversity (Table 2). Specifically, diet type had a significant effect on venom delivery, specialized organs and venom complexity, but not on venom and ecological function. However, we found significant effects of body size and aposematism on venom and biological functions. Both prey handling and reproductive type showed a significant effect on biological function, whereas the venom function was only significantly affected by prey handling type. For other variables regarding biogeography and habitats, there were no significant impacts on venom function and diversity (Table 2).

3.2. Variable importance in random forest models

The random forest models (RF models) revealed consistent yet nuanced patterns across five venom characteristic variables (Figure 2). Body size emerged as the most important predictor for venom delivery, venom complexity, and biological function, while prey handling was the dominant variable for venom function and specialized organs. Habitat type consistently ranked within the top three predictors across all five models, underscoring its pervasive influence on venom-related traits. Biogeographic zone and diet type also contributed, particularly for venom delivery, complexity, and specialized organs, respectively (Figure 2). Overall, body size and ecological context (i.e., habitat, biogeography, diet) appeared to be the primary determinants shaping venom diversity, whereas handling-related behaviors (i.e., prey handling type) specifically influenced venom function and the evolution of specialized delivery organs.

Table 2. Statistical results regarding the significance of ecological and morphological variables in influencing venom function and diversity

Ecological & Morphological variables	Venom delivery	Venom function	Venom complexity	Specialized venom organs	Biological function
	P	P	P	P	P
Biogeo_zone	1.00	1.00	1.00	1.00	1.00
Distri_width	1.00	1.00	1.00	1.00	1.00
Habitat_type	1.00	1.00	1.00	1.00	1.00
Activity_pattern	1.00	1.00	1.00	1.00	1.00
Log_body_size1	1.00	0.02	1.00	1.00	0.02
Aposematism	1.00	0.02	1.00	1.00	0.01
Diet_type	< 0.001	0.82	0.04	0.005	1.00
Prey_handle	1.00	< 0.001	1.00	1.00	< 0.001
Reproductive_type	1.00	0.92	1.00	1.00	< 0.001



Figure 2. The random forest variable importance plots

4. Discussion

4.1. Body size as a primary driver of venom evolution

Our combined exploratory analyses and random forest analyses revealed that ecological and morphological factors differentially influenced venom traits examined, with diet type playing a surprisingly limited role in shaping venom function and diversity. These results challenge the prevailing narrative that diet is the dominant selective force driving venom evolution [11, 33]. Instead, body size and ecological context, specifically habitat and biogeography, emerged as the primary determinants of venom diversity, while prey handling strategy predominantly influenced venom function. Both analytical approaches converged on body size as the most important predictor for venom delivery, venom complexity, and biological function. This pattern likely supports a constraints hypothesis that larger body size may allow for larger venom glands and correlates with the capacity to produce a more complex toxin arsenal. Species with larger body size and robust fang structures are more capable of active venom injection, whereas species with smaller body size often rely on passive delivery or non-venomous strategies [5]. Furthermore, the significant effect of body size on biological function suggests that predator-prey size dynamics, rather than prey taxonomy, drive the evolution of venom physiology and function. Larger predators that target larger, more mobile prey tend to evolve fast-acting neurotoxins, whereas smaller or ambush predators may rely on hemotoxins that require slower incapacitation [11].

4.2. The role of prey handling strategy in venom function

The distinction between venom function and biological function is critical. Prey handling type was the dominant predictor of venom function (e.g., predation vs defense) and specialized organs, whereas it did not significantly affect biological function. This indicates that while the ecological

role of venom is dictated by how an animal interacts with prey, the physiological mode of action is not. Unsurprisingly, species that inject venom to subdue prey almost invariably use it for predation, while those with non-venomous handling rely on venom solely for defense (e.g., poison dart frogs, slow lorises). This pattern is also supported by the strong effect of aposematism on venom function in our RF models. Importantly, reproductive type also influenced biological function, suggesting that life-history strategies impose energetic constraints that shape venom biochemistry and physiological function.

4.3. Reassessing the diet-driven hypothesis

Contrary to previous assumptions [11], diet type played a surprisingly limited role in shaping venom function and diversity in most models across species. While exploratory analyses based on (G)LM models showed a significant effect of diet type on venom delivery, presence of specialized organs, and venom complexity, it was rarely among the top predictors in the RF models (ranking third only for venom complexity and specialized organs). This discrepancy suggests that while diet types exert a detectable statistical effect, their importance is overshadowed by body size and prey handling strategy, indicating that how venom is delivered is shaped less by what is eaten than by the physical context and the predator's own size. Notably, the weak support based on our findings for the classic diet-driven hypothesis that dietary generalists evolve more complex venoms [11] suggests that our metric (the counts of toxin families) may not capture finer-grained biochemical variation and functions. The findings that diet type did not emerge as a significant predictor also indicate that the physiological effect of venom (neurotoxic vs. hemotoxic vs. cytotoxic) is determined more by predator size and ecological niche than by the specific prey taxon. Our results suggest that while diet undoubtedly influences venom evolution in some specialized lineages (e.g., sea snakes feeding on fish eggs) [12], it is not the universal, overriding driver across all venomous taxa.

4.4. Habitat and biogeographic influences on venom diversity

Our robust results showed the consistent ranking of habitat type among the top three predictors across all RF models. This demonstrates that the physical and ecological environment imposes fundamental constraints on venom evolution. Forest dwellers frequently use active injection to subdue agile prey in complex three-dimensional environments, whereas desert or aquatic taxa may depend more on passive transfer. Importantly, freshwater and marine species frequently produce neurotoxic venoms (e.g., cone snails, sea snakes), while terrestrial forest species may show a mix of hemotoxic and cytotoxic activities. Biogeographic zone also contributed significantly to venom delivery and complexity, suggesting historical faunal assembly and prey community structure influence toxin diversity over macroevolutionary timescales.

5. Conclusion and implications

Our results demonstrate that venom function and evolution are a multifactorial process where ecological context (habitat, prey handling strategy) and morphological constraints (i.e., body size) consistently override dietary impacts as the more dominant drivers. The fact that diet type had no significant effect on venom function or ecological function based on (G)LMs, and ranked poorly in RFs, suggests that caution is needed when generalizing snake-focused findings to the broader diversity of venomous animals. Our findings do not refute the diet-driven hypothesis but reframe it: diet probably acts through its correlates (prey size, escape behavior, habitat) rather than through prey

taxonomy per se. Future comparative studies should therefore adopt a more nuanced framework that considers morphological constraints and ecological context alongside dietary variables. We acknowledge several methodological limitations. First, venom data availability varies substantially across taxa, with medically important groups (e.g., snakes) overrepresented relative to less-studied venomous lineages. Second, dietary and ecological data were drawn primarily from natural history literature rather than systematic field studies, introducing potential observer bias. We addressed this by prioritizing concordant information across independent references and explicitly coding missing values to prevent artificial inflation of associations. Importantly, because our primary hypothesis concerns relative differences among species in venom traits associated with dietary variation, systematic reporting biases are unlikely to produce consistent directional artifacts aligned with dietary categories. The categorical standardization approach, while abstracting away fine-scale molecular variation, enables detection of broad evolutionary patterns across phylogenetically diverse lineages.

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