Symbiosis
https://doi.org/10.1007/s13199-024-01031-0

A review of the venom microbiome and its utility in ecology and evolution including future directions for emerging research

Marina E. De León · Eduardo G. P. Fox · Sara Dunaj · Ronald A. Jenner · Carl N. Keiser · Jason Macrander, et al. ***[full author details at the end of the article]***

Received: 24 July 2024 / Accepted: 24 December 2024

Abstract
Microbes play vital roles in ecological systems, yet their presence and functions within venom environments of venomous organisms remain understudied. Despite the prevalent belief in the sterility of venoms, recent findings reveal diverse microbial communities within venom systems. This review aims to explore the relationships between venoms and microbes, highlighting their potential roles in evolutionary processes, ecological interactions, and therapeutic advancements. Venoms, composed of toxins utilized in hunting or defense, represent a rich source of natural products with applications in drug discovery and therapy, exemplified by FDA-approved venom toxin-derived drugs. Understanding microbial resistance mechanisms against antimicrobial peptides can illuminate coevolutionary processes and guide therapeutic development. Integrating hologenomic evolution and microbial ecology frameworks will facilitate comprehensive research on venom-microbiome interactions, and reveal the evolutionary drivers of venom diversification. Investigating and investing in these relationships promises advancements in understanding evolution, ecology, and biotechnology, with implications for human health and ecological conservation. This review synthesizes existing knowledge, identifies many gaps in literature, and investigates critical unanswered questions in the field of venom microbiology, encouraging ongoing and future collaborative research.

Keywords Bacteria · Host-microbiome · Omics · Natural Products · Symbiosis · Venom

1 Introduction

Microbes are essential factors in ecological niches, yet their presence and functions are vastly underexplored in venom systems (Gibbons and Gilbert 2015). Given the fundamental importance of microbial life in every ecosystem, venom-associated microbiomes likely influence evolutionary pathways, ecological interactions of venom, and offer potential avenues for biotechnological and therapeutic advancements. Venom is a biological agent consisting of toxins used to support behaviors such as hunting or defense. This systematic review aims to investigate the interactions between venoms and microbes, elucidating potential microbial properties of venoms and their

The iVAMP consortium iVAMP is a collaborative, open-source group of researchers around the world with a shared interest in studying the interactions between microorganisms, parasites, and venom. Our long-term aim is to investigate venom glands as potential microenvironments for microorganisms, establishing an inclusive network of scientists, promoting sample sharing, data collaboration, and engaging the general public in research projects. iVAMP seeks to expand the direction and reach of the fields of host-microbe interactions, microbiology, and venomics through collaboration and open data sharing. The consortium aspires to compile collaborative data into an open-source platform, making valuable research accessible to the public. Future efforts include cataloging species and NCBI Sequence Read Archive (SRA) IDs for select venom and venom-associated bacterial transcriptomes on the iVAMP website as the consortium continually works towards establishing a comprehensive resource for the scientific community and the general public. iVAMP emphasizes continuous inquiry, discussion, and engagement with the general public to foster interest and understanding of ongoing research projects. iVAMP provides a unique opportunity to highlight the transformative impact of non-hierarchical structures in practice, exemplified by the leadership of underrepresented groups of people authoring this review. This representation not only enriches the diversity of perspectives within our group but also serves as a powerful example of inclusivity in STEM. Additionally, it underscores our commitment to fostering career development opportunities for individuals from underrepresented regions, such as the global South. Through collaborations and funding initiatives facilitated by iVAMP, we strive to create a supportive environment where researchers from diverse backgrounds can thrive and contribute to our shared mission of advancing scientific knowledge.

Published online: 27 March 2025

contributions to evolutionary processes, ecological dynamics, as well as promising applications in biotechnology and therapeutics. Toxins from both microbes and venomous animals have proven to be a rich library of natural products for drug discovery, with examples such as botulinum toxin, discovered from *Clostridium botulinum* used in ophthalmology, neurology and dermatology, while diphtheria toxin discovered from *Corynebacterium diphtheriae* is being adapted for cancer treatment (Ting and Freiman 2004; Shafiee et al 2019). Additionally, six venom toxin-derived drugs are already FDA approved and in clinical use (Herzig et al. 2020). Venoms contain mixtures of bioactive molecules, including small molecules, salts, peptides and proteins, collectively referred to as toxins (Kaas and Craik 2015). Venom systems have evolved independently more than 100 times in an extremely diverse range of taxa spanning at least eight phyla (Schendel et al. 2019). The majority of animal venoms have evolved for predation and/or defense but can also serve a remarkable variety of functions, including intraspecific competition, conspecific communication, chemical detoxification; detection of envenomed prey, courtship and mating, offspring care, defense against microbial pathogens and ectoparasites, and interestingly, venom is also used by some animals during self- and allogrooming to detox and suppress microbial pathogens, and by other animals during mating as well as to compete for mates (Schendel et al. 2019).

Cocktails of toxic compounds are either termed venoms or poisons, depending on the mode of delivery. Venom apparati are the anatomical structures or organs involved in the production, storage and active delivery of animal venoms; for example, typically there is a main venom gland producing the venom, which then accumulates in a sac or flows via ducts to be injected into the target via delivery structures, typically fangs, stingers, or spines that create a wound (Van Marle and Piek 1986). Venom is typically produced by specialized glands that are usually directly attached to a delivery system, with but few exceptions such as the slow loris employing a two-step venom delivery system (Grow et al. 2015). In contrast, poisons are toxic compounds that can accumulate either in specialized glands, such as the parotid glands of toads, or in single cells distributed throughout the organism but are not associated with a delivery mechanism (Brodie 2009). Poison may passively enter the bloodstream through inhalation, ingestion, or through the skin via absorption (Harris and Arbuckle 2016). This is because the molecules are typically small enough to pass through the membrane barriers. However, with some exceptions (e.g., venom peptides), venom molecules tend to be too large to pass through the cellular membranes or enter through paracellular absorption and therefore require specialized sharp piercing devices to breach the external barrier and penetrate the victim's body.

In recent years, next-generation sequencing technology has advanced our knowledge of host-associated microbiomes (Gill et al. 2006; Clooney et al. 2016). Animals are inhabited by and have coevolved with complex microbial communities that are regionally specific such as the gut, reproductive tract, or oral microbiomes, and provide a suite of functions that span from immune response to reproduction. However, few studies focus specifically on venom microbiomes (Torres et al. 2017; Dunbar et al. 2019; Klein et al. 2019; Dunaj et al. 2020; Yang et al. 2021; Esmaeilishirazifard et al. 2022). The dearth of studies focusing on venom microbiomes may be due to the prior assumption that venom glands are sterile due to the antimicrobial properties of many venoms. However, it has become increasingly apparent that venoms and their associated microbiomes are more complex than previously thought (Ul-Hasan et al. 2019, Dunbar et al. 2020). Many, if not most venoms contain components such as antimicrobial peptides (AMPs) and the literature on venom antimicrobial properties is extensive. Given that microbes inhabit every ecological niche on Earth (Martiny et al. 2006), it should come as no surprise that venom systems also contain resident microbes, and that these microbes likely evolved resistance mechanisms to venom AMPs (Fig. 1) (Esmaeilishirazifard et al. 2022). Studying the resistance mechanisms employed by venom-resident microbes against AMPs will aid in the understanding of the coevolutionary dynamics between venomous organisms and their microbial communities. Additionally, understanding how these microbes withstand the antimicrobial properties of venom components may have useful implications for the development of novel therapeutics and antimicrobial agents. A framework is needed for researching venom-microbiome interactions that takes into account hologenomic evolution (which includes microbial and host genomes) and microbial ecology. This approach should guide studies aimed at understanding the evolutionary drivers of venom evolution, as well as those focused on discovering natural products and novel therapeutics.

1.1 A comprehensive summary of venom microbiology and venomics

While the study of microbiomes falls under the broader umbrella of microbiology, the specific methodologies employed to study venom microbiology and venom microbiomes are distinct. Venom microbiology, which traditionally focuses on culture techniques, may not capture the full microbial diversity present in a sample. In contrast, venom microbiomes, which are studied using molecular genetics and profiling techniques, offer a more comprehensive understanding of the microbial diversity present in a sample by encompassing a broader range of microorganisms, including those that may be difficult to culture. Both

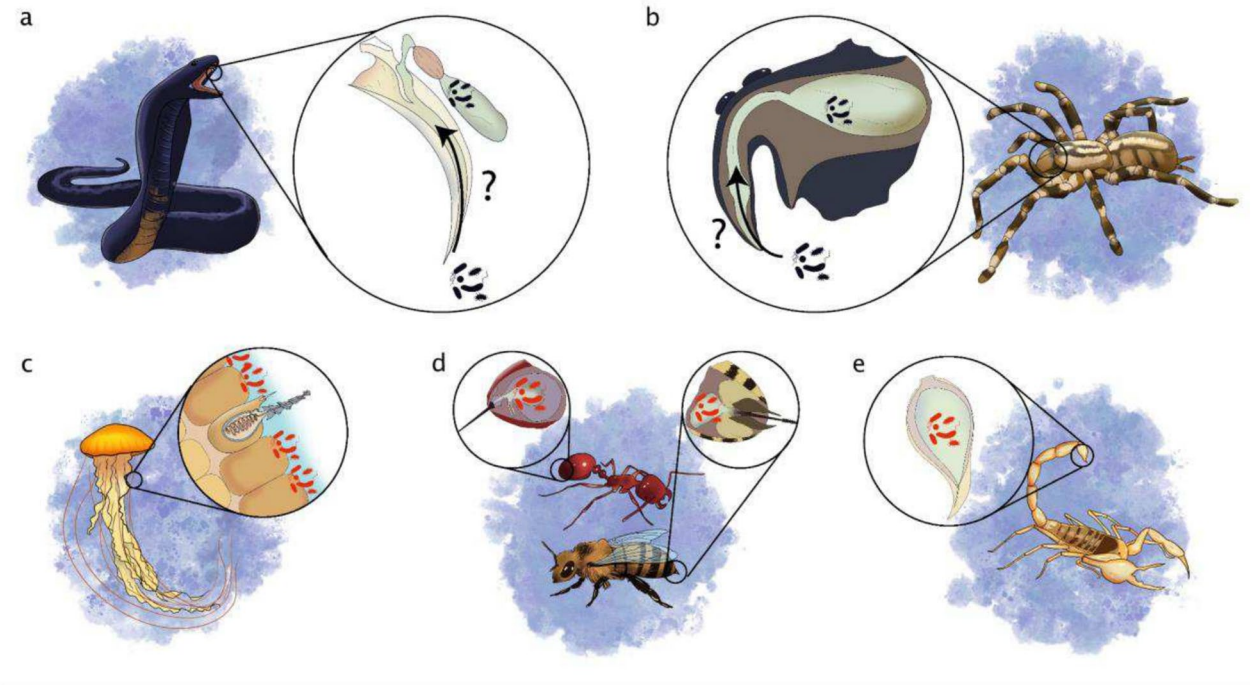

Fig. 1 Hypotheses for the bacterial colonization of venom glands. Bacteria colored red indicates their confirmed presence within the venom gland. Bacteria colored black indicate unconfirmed presence within the venom gland. Hypothetical modes of entry by bacteria are represented by arrows and "?". **a)** Snake venom gland exhibiting bacterial colonization, potentially through environmental entry via hollow fangs. **b)** Similarly, a spider venom gland showing bacterial colonization, possibly facilitated by environmental entry through hollow fangs. **c)** Jellyfish surface mucus harbors environmental bacteria, possibly facilitating toxin transmission to nematocysts upon contact with cnidocytes. **d)** Bacteria have been observed within the venom glands of hymenopterans, but colonization method is unknown. **e)** A scorpion telson containing bacteria within venom gland tissue, potentially due to vertical transmission from mother to embryo during reproduction. The figure background was sourced from BioRender, individual organisms were drawn using Procreate version 5.3.10, and the figure was assembled in the 2024 version of Adobe Illustrator

culture -dependent and -independent studies are valuable and function in synergy with one another in understanding the biology of host-microbe interactions. For example, *Streptomyces* spp. was identified in the venom duct of *Conus* (a genus of predatory sea snails) using culture-dependent methods (Peraud et al. 2009; Quezada et al. 2017), while *Stenotrophomonas* spp. was identified in venom of multiple *Conus* species using culture-independent methods (Torres et al. 2017). The findings of these studies, taken together, have significantly contributed to our understanding of symbiosis and coevolution as they pertain to venom microbes and host ecology.

Venomics is the study of animal venoms and their components through integration of genomics, transcriptomics, and proteomics, providing a broad characterisation of venoms (Calvete 2017; Wilson and Daly 2018; Walker et al. 2020). Research in this field involves identifying and characterizing venom toxins, and studying the evolutionary relationships between venomous species to understand the diversity and adaptations of venomous organisms. Despite significant progress in venomics, many venom systems have not been thoroughly studied and their venom composition and the full range of toxins they produce remain largely unknown. The limited scope of venom microbiome studies has primarily focused on a select few animal hosts. Some highlighted hosts leave a substantial void in our understanding of host-microbe coevolution, microbial diversity across taxa, and the functional roles of microbes in venoms. The vast majority of venomous animal hosts remain unexplored. This limitation restricts our understanding of the breadth and depth of microbial associations, the evolutionary dynamics shaping ecological relationships, and the potential roles of microbes in venom production, modification, and delivery.

The 'omics' revolution has propelled venom research, leading to the emergence of venomics as a recognized field. Understanding the evolutionary processes that shape venom composition and the ecological roles of specific toxins is an active area of research. Researchers such as those associated with the Initiative for Venom Associated Microbes and Parasites (iVAMP, https://ivamp-consortium.github.io/) are investigating the diversity and function of microorganisms within the venom microbiome and how they interact with the host and venom components.

Studies are being conducted to understand the roles of the venom microbiota in toxin production, venom composition, venom potency and function, as well as venom evolution (Ul-Hasan et al. 2019).

Advanced systems biology approaches such as metagenomics, metaproteomics, and metabolomics, are frequently used to study collective communities of microorganisms, encompassing bacteria, viruses, protists, unicellular eukaryotes, and fungi, residing within a specific environment or organism. These techniques enable the exploration of microbial composition, diversity, and function in various ecosystems and organisms. The increasing biomedical interest in venom toxins is driving advancements in their artificial synthesis. This interest also motivates researchers to try and overcome challenges associated with obtaining and growing venom gland secretory tissues in vitro, as well as navigate the difficulties in obtaining venom directly from venomous animals and purifying toxins while respecting biodiversity and bioprospecting laws. Sourcing the genetic material for these cultures is also covered by international laws on bioprospecting to foster accessibility and beneficial sharing as an addition to the Convention on Biological Diversity, commonly shortened to the Nagoya Protocol (Knauf et al. 2019). For instance, a method for culturing venom gland secretory cells from the aggressive 'Novateiro' ant *Pseudomyrmex triplarinus* was patented (patent no. C12N5/0601) (Hink 1985; Hink and Butz 1985). Although there have been no recent developments to this method, this technology sets the framework for not only producing toxins in vitro, but also for culturing venom-associated microbes. Similar culturing methods have been described for venom tissues from the Brazilian armed spider *Phoneutria nigriventer* (Silva et al. 2008) and the venomous snail *Conus cumingii* (Viswanathan et al. 2018). The feasibility of tissue culturing for venom production is contingent upon the specific organism and its cellular characteristics. While producing snake toxins in vitro using venom gland organoids has been successful (Vogt 2020), challenges could arise when dealing with venomous arthropods, whose cells are notoriously difficult to maintain in culture. It has been shown that bacterial culture media containing venom can be used for selection of venom-adapted strains of *Enterococcus faecalis* over wild type strains (Supplementary Table 1) (Torres et al. 2017; Dunbar et al. 2019; Klein et al. 2019; Dunaj et al. 2020; Yang et al. 2021; Esmaeilishirazifard et al. 2022). However, this is only practical where large amounts of filter-sterilized venom are available. It is currently unknown whether venom culture media would allow growing otherwise unculturable species. If traditional and novel culture methods are combined, successfully isolated symbiont strains from invertebrate venom apparatuses could be adapted into venom gland cell cultures, enabling studies about the biological features of this untapped microbiological diversity in controlled Petri dish environments. For example, organoid cultures, with their abilities to replicate complex tissue structures and functions, offer a versatile and powerful platform for advancing our understanding of microbiomics, ranging from infection studies to exploring host–microbe interactions in various physiological contexts. Organoid cultures utilize microinjection, triple co-cultures to mimic complex interactions, and monolayer methods that help to study cell signaling pathways, immune responses, or gene expression patterns during host–microbe interactions, all in order to create more complex models that simulate host–microbe dynamics (Dutta and Clevers 2017).

Unlike venomics, which integrates proteomics with metabolomics, transcriptomics and/or genomics of venom components, microbiomics often relies on metagenomics for a comprehensive analysis of microbial communities. Other key techniques in microbiomics include 16S/18S/ITS rRNA metabarcoding and sequencing, whole genome sequencing for microbial community profiling and shotgun metagenomic sequencing for a more detailed genomic analysis of gene function in microbial populations. Research in microbiomics often aims to understand the interactions between microorganisms and their hosts, elucidating the impact of microbes on host health and disease. Ongoing research in venomics and venom microbiomes focuses on uncovering novel toxins, understanding the evolutionary aspects of venom, and investigating venom proteomics and genomics. Exploring the diversity and function of venom-associated microorganisms and investigating the potential therapeutic applications arising from these discoveries is necessary for understanding venom biology. While the importance of microbiomes in secreted toxins, such as tetrodotoxin (TTX) in newts, has been substantiated (Vaelli et al. 2020), the role of the microbiomes in venoms is much less understood.

Known symbiont model systems in the broader microbiome community encompass a diverse range of organisms including corals, squids, the human gut, and aphids (O'Brien et al. 2019). These symbiont-model systems have been instrumental in uncovering the functional importance of symbiotic relationships and their impact on host health, development, and ecology. Studying the venom microbiome can also provide information on the functional significance of venomous host-microbe interactions, and reveal their effects, if any, on venom composition, toxin evolution, and potential therapeutic applications, while advancing our understanding of host-microbe dynamics in specialized ecological niches. Here

we provide an overview of venom-microbiome systems, and a set of best practices to help guide the direction of this emerging field of research, especially focusing on multidisciplinary studies.

2 Venomous host systems

There exists a prevailing bias in venom microbiome studies towards Arthropoda, particularly spiders, scorpions, and stinging insects (hymenopterans, such as ants, bees and wasps). This bias likely stems from the accessibility of these models for exploratory studies and their well-known association with microbiota, such as the bacterial endosymbionts *Wolbachia*, which are important for understanding arthropod vector-borne diseases. In this review, we broaden the scope beyond arthropods, encompassing other animal taxa that have been subject to venom microbiome studies. The following section synthesizes, in detail, the limited research conducted on organisms that have demonstrated evidence of microbial associations with their venom, bringing to light many aspects of these systems that are yet to be fully understood.

2.1 Cnidaria

Cnidarians are a diverse group of marine animals encompassing jellyfishes, hydra, sea anemones, and corals. Representing the earliest lineage of venomous animals, cnidarians deliver their venom through organelle-derived cellular structures called nematocysts (Ozbek 2011; Sierra and Gold 2024). The nematocysts are contained within specialized cells called cnidocytes, and function like microscopic harpoons for venom delivery, serving as a defining synapomorphy for the entire phylum (Daly et al. 2007; Fautin 2009; Jouiaei et al. 2015). While the term "gland" is informally used to describe these structures involved in venom production, it is more functional than anatomically precise and does not imply the same complexity as glands in other animals. Beyond nematocysts, venom expression has also been reported in different forms of gland cells (Moran et al. 2012), ultimately contributing with proteinaceous toxins to the protective surface mucus layer (SML), common to all cnidarian ectodermal layers. Mucus secreted by mucocytes provides the SML substrate for microbes to colonize, sustaining microbial communities that work synergistically within the host to develop an adaptive immune system to defend against pathogens (Fig. 1) (Bourne et al. 2016). This unique combination of an external secreted mucus containing both mutualistic/commensal microbes and venom is distinctive to cnidarians among other venomous animals (Bakshani et al. 2018; Rivera-Ortega and Thomé 2018; Savoca et al. 2022). Over 100 cnidarian-derived AMPs have been deposited to UniProt (The UniProt Consortium 2019), and of specific note is SHK from *Stichodactyla helianthus,* which has antimicrobial and ion channel-blocking properties (Castañeda et al. 1995; Kim et al. 2017). A synthetic truncated version SHK-186 is a potent and selective blocker of the potassium channel $K_V1.4$ which has passed Phase 1b clinical trial for plaque psoriasis (Castañeda et al. 1995; Tarcha et al. 2017). Cnidarian toxins have demonstrated promise in areas such as pain management and neuroscience, warranting further investigations into their bacteria-venom relationships (Osmakov et al. 2013; Liao et al. 2019).

Although cnidarians lack a multicellular, localized venom gland or point of envenomation, they were most likely the first venomous lineage to coevolve with their prokaryotic symbionts that occur and thrive within the SML (Rivera-Ortega and Thomé 2018). Within this SML there are several peptides that behave both as AMPs, as well as cytotoxic or ion-channel targeting peptides (Mariottini and Pane 2014; Mariottini and Grice 2016; Augustin et al. 2017; Logashina et al. 2017; Jayathilake and Gunathilake 2020; Kvetkina et al. 2021; Hernández-Elizárraga et al. 2022). The microbial assemblages associated with zooplankton represent another aspect of the ecological dynamics involving pelagic jellyfish and sessile cnidarians (Roberts and Suttle 2023; Savage et al. 2023). By consuming zooplankton in the water column, cnidarians can incorporate associated microbial communities into their diet (Clinton et al. 2021). Common pathogens transferred to cnidarians via zooplankton consumption are likely kept at bay through established beneficial microbial assemblages, innate immune repertoire, or a combination thereof. Because of this, innate immune genes co-expressed with beneficial bacterial peptides may result in strong selective pressures for anti-pathogen peptides to be co-expressed with toxins used to immobilize prey and avoid targeting beneficial microbial communities, exemplifying another way that selection can influence the origin of venom assemblages by moving through trophic levels. Jellyfish-associated microbes have been implicated as the cause of disease in farmed fish, where pathogens may be directly transferred via contaminated stingers (Clinton et al. 2021). These examples not only highlight the ecological significance of microbial communities in cnidarians, but also underscore the potential for coevolutionary mechanisms that may shape the venom composition across evolutionary time scales (O'Hara et al. 2021). Nematocysts do not contain bacteria, and there is currently no evidence supporting the sequestration of toxins by cnidarians from bacteria, but it is plausible to hypothesize that bacteria on the surface of cnidarians could influence venom production, or that a trade-off occurs between venom production and immune response, particularly if secretory cells are involved in both AMP release and toxin production. Exploring symbiotic

relationships between bacteria and toxin production may reveal underlying mechanisms and ecological implications in the relationships between microbial assemblages and venom evolution in pelagic jellyfish and other cnidarians.

2.2 Mollusca

2.2.1 Neogastropoda: Conidae

The Conidae family, namely cone snails, are amongst the first venomous hosts in which microbial biodiversity of venom glands was explored and include culture-dependent as well as culture-independent studies (Peraud et al. 2009; Quezada et al. 2017; Torres et al. 2017). Likewise, they are also amongst the first venomous hosts to be of interest in spearheading the field of venom biology from the discovery of the peptidic drug ziconotide (Prialt) in the early 1980s to the emergence of venom transcriptomics, expanding venom research to what is today known as venomics (Hu et al. 2012).

Cone snails serve as a strong venom-microbe model system. Conidae are incredibly diverse, with over 900 species known ("MolluscaBase Eds." 2024). The venom arsenal of each individual species is diverse, with species possessing hundreds to thousands of peptides in a venom cocktail (Lewis et al. 2012). Their venom gland mimics that of the gut in resembling a long tract with known distinct functions from one end to the other (Marshall et al. 2002; Hu et al. 2012). The muscular bulb "pushes" venom out along and through the tract for carrying out either prey capture or defense behavior (Marshall et al. 2002; Dutertre et al. 2014).

Current literature supports the hypothesis that venoms possess a core microbial community across venomous host systems (Torres et al. 2017). The reason, however, remains under discussion. Exploration of the *Californiconus californicus* venom microbial community has resulted in several hypotheses: (a) core venom microbes seek and receive refuge from their host, deduced from studies comparing and contrasting various organs of the host and its surrounding sediment and seawater environment over time (Ul-Hasan et al. 2019); (b) the host selects for microbes from the environment resulting in a niche community that contributes to venom functionality by specific mechanisms of action, such as venom microbe small molecules and metabolites influencing the post-translational modifications of host conopeptides (Torres et al. 2017); and (c) core venom microbes may produce antimicrobial compounds themselves to compete with one another and prevent pathogens from colonizing their surrounding environment, thereby serving as mutualists to the host (Ul-Hasan dissertation data).

The significance of incorporating both culture -dependent and -independent studies, as well as longitudinal and latitudinal experiments ranging from in the field versus in captivity, points towards more questions for discovery in venom microbiomes. *Streptomyces* are known symbionts of many animal hosts, and this may also be true based on culture-dependent work in Conidae (Peraud et al. 2009; Quezada et al. 2017). Culture-independent work supports these findings (Ul-Hasan dissertation data), and additionally suggests *Stenotrophomonas* may play a symbiotic role in the venom across multiple Conidae species (Torres et al. 2017). Captive experiments that place the host in various sterile environments mimicking those observed in aquaculture also implicate the role of *Streptomyces* in venom activity on a metabolomics level (Ul-Hasan dissertation data). Further, integration of not only 16S amplicon sequencing but also 18S amplicon sequences led to the identification of more than 50% Ochrophyta and approximately 30% of rare taxa when compared to surrounding seawater and other tissues in *C. californicus* (Ul-Hasan dissertation data). There is tremendous room for deeper exploration on additional amplicon identification as well as metagenomics sequencing. Additionally, pairing microbial sequencing of the venom and host tissues (as well as environmental sampling for point of reference) with simultaneous metabolomics and proteomics profiling thus far suggests a microbial community gradient along the venom tract that may be paired with host venom function.

C. californicus can be bred and maintained in captivity, contributing to exploration of questions pertaining to horizontal and vertical gene transfer in host development and venom microbes associated throughout life stages. Aspects such as these are important to consider for identifying a strong host system for holistic venom microbiome exploration such that results can be built upon over time and by different research groups. The population of *C. californicus* also makes power analyses for identification of the minimum sample size for an experiment easy to pinpoint. While it is unfortunate so few venom microbiome studies exist within the expansive ocean of microbiome research conducted to date, it also presents an opportunity for researchers across all career stages to pursue and expand this nascent field with the intention of establishing a strong foundation that enhances current holobiont theory, and enriches natural product discovery as we know it.

2.3 Arthropoda

2.3.1 Arachnida: Araneae (spiders)

All families of spiders except the Uloboridae have a pair of venom glands which connect to the fangs via two venom ducts. Spider venom is injected in very small quantities, often < 1 µl (Wigger et al. 2002), and contains bioactive compounds that act primarily to paralyze/kill prey and defend against predators, though potential roles

in pre-digestion have also been hypothesized (Kuhn-Nentwig et al. 2011). Spider fangs have specialized structures such as serrated edges, recurved tips, and microscopic channels that facilitate the efficient injection of venom into prey or predators. These unique adaptations could influence the types of microbes that colonize the venom delivery system. Spider chelicerae, include two hollow fangs which typically serve as conduits for venom delivery via a direct connection to venom glands situated in the basal chelicerae in mygalomorph spiders or prosoma in araneomorph spiders (Lüddecke et al. 2022). This fang anatomy, effectively forming an open biological syringe, has prompted speculation that the venom duct and accompanying venom gland could be colonized by environmental microbes (Fig. 1) (Esmaeilishirazifard et al. 2022). The venom gland ecological niche potentially favors microbiota capable of tolerating specific toxins/AMPs present in spider venoms, though it has been hypothesized that AMPs in venom function to defend against microbial colonization (Langenegger et al. 2019; Lüddecke et al. 2022). However, Esmaeilishirazifard et al. (2022) found bacterial taxa living inside the venom apparati, rather than simply on the outside of the fangs, and Dunaj et al. (2020) cross-validated 16S data with RNA sequencing data to verify that several microbial symbionts were active inside venom glands of theridiid spiders (Supplementary Fig. 1). Interestingly, *Latrodectus* spp black widows have fewer unique bacterial taxa in their venom glands compared to other theridiid spiders, suggesting that differences in the toxic environment may influence microbial community composition (Dunaj et al. 2020).

In addition to bioactive proteins and neurotoxins, spider venoms have been found to contain AMPs. These AMPs are mostly linear, α-helical peptides which exhibit antimicrobial properties, but they also disrupt the integrity of eukaryotic cell membranes and are therefore often referred to as "lytic peptides" (Langenegger et al. 2019). AMPs have been described in the venoms of several spider families, including Lycosidae (Yan and Adams 1998; Budnik et al. 2004), Zodariidae (Lazarev et al. 2011), Agelenidae (Benli and Yigit 2008), and Theraphosidae (Abreu et al. 2017). Some spider AMPs have been reported to show both antifungal and antiviral properties (Yan and Adams 1998; Ji et al. 2019). Antimicrobial effects of spider venoms are not universal, as the venom from *Steatoda nobilis* exhibited no inhibitory effects against laboratory strains of *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), or *Listeria monocytogenes*, nor against two bacterial species directly isolated from *S. nobilis* fangs—*Pseudomonas azotoformans* and *Staphylococcus capitis* (Dunbar et al. 2020).

Spider venom-associated microbes are of particular interest for medical arachnology among increasing efforts for development of novel venom-derived antimicrobial and antiparasitic drugs (Nixon et al. 2021). A particularly promising outcome of investigating venom-associated microbes in spiders is resolving the dubious role of bacteria in spider bite pathology. Reports of bacterial infections after alleged spider bites are common, and some of the symptoms frequently attributed to spider bites (e.g., necrotic arachnidism or loxoscelism) may actually be due to subsequent bacterial infections (Vetter et al. 2003). Secondary bacterial infections at the site of a spider bite may be due to microbes living on the fangs or inside the venom apparatus of spiders or may even be due to secondary colonization of the wound (e.g., by scratching). Only spiders from the family Sicariidae and *Hemiscorpius* scorpions are known to contain phospholipase D in their venoms, which is largely accepted as the cause of dermonecrotic lesions (Swanson and Vetter 2005; Isbister and Fan 2011; Hauke and Herzig 2017; Torabi et al. 2017). For *Loxosceles* species with a known ability to cause necrotic lesions, misdiagnosis of necrotic arachnidism is common from areas where these spiders do not even occur (Swanson and Vetter 2005). Several other spiders were also implicated in necrotic arachnidism before a range of systematic prospective studies with verified taxonomic specimen identifications clarified that their venoms were not likely to be causing necrotic lesions (Isbister and Gray 2003; Isbister and Hirst 2003; Isbister and Gray 2004; Vetter et al. 2006). Furthermore, some lesions could be treated with antibiotics, suggesting bacteria as the causative agent of the reported pathologies (Isbister 2001). Frequent misdiagnosis of spider venom as the causal origin of bite symptoms versus bacterial infection, demonstrates the urgent need to further understand venom-microbe interactions.

Monteiro et al. (2002) found that removing *Clostridium perfringens* bacteria (using the antibiotic penicillin G) from the *Loxosceles intermedia* spider venom microbial community can decrease the severity of necrotic lesions in bite wounds (Monteiro et al. 2002). Bacterial communities on the cuticle of the hobo spider *Tegenaria agrestis* have been investigated by exposing individual spiders to MRSA bacteria, but no later evidence for mechanical vectoring of bacteria was detected (Gaver-Wainwright et al. 2011). Venom from the recluse spider *Loxosceles gaucho* was reported to exhibit antimicrobial properties, while also increasing biofilm formation of *Pseudomonas aeruginosa* bacteria (de Oliveira Domingos et al. 2018). These examples raise questions about context dependency of microbe-venom interactions. In some cases venom may have antimicrobial properties and limit bacterial infection after envenomation, while in other cases venom may carry pathogenic microbes that can cause subsequent infections associated with spider bite pathologies. Future research

should focus on the interactions and co-evolution of spider venom components and microbial communities and might clarify whether bacteria or bacterial enzymes are present in spider venoms.

2.3.2 Arachnida: Scorpiones (scorpions)

Scorpions host distinct bacteria within their telsons (venom-producing organs). *Smeringurus mesaensis* and *Hadrurus arizonensis* scorpion species contain unique and phylogenetically diverse telson microbiomes (Fig. 1) (Shimwell et al. 2023). Scorpions produce venom from paired venom glands inside the telson that drain into a single duct terminating at the aculeus, these glands also contain mucous-secreting cells increasing the viscosity of the venom (Kennedy et al. 2021). Microbes in these glands may influence the composition of the venom, suggesting a potential link between the presence of bacteria in the scorpion's telson and the composition of antimicrobial peptides in its venom. Some of these peptides may be produced by the telson bacteria rather than the scorpion itself (Shimwell et al. 2023). Intracellular *Mollicutes* bacteria may be passed vertically from the mother's secretory vesicles in secretory cells of the telson to their embryos, presumably during reproduction, and there is evidence of a long-term coevolutionary relationship between scorpions and specific bacterial symbionts (García-Santibañez et al. 2022). The exact nature of this relationship is still under investigation. Detailed investigations of the roles of these microbiomes in the telson will require improvements in the telson gland extraction process to avoid possible environmental contamination, as the scorpion venom glands connect directly to the cuticle via the telson muscle, as opposed to most arthropods which have distinct compartmented tissues (Kennedy et al. 2021).

Numerous AMPs have been isolated from scorpion venoms, including a rare component of scorpion venom, hadrurin, from the Mexican scorpion *Hoffmannihadrurus* (*Hadrurus) aztecus*. Hadrurin is similar to other peptides found in the secretions of two *Glandirana* (*Rana)* frog species, specifically the N-terminal segment of gaegurin 4 and brevinin 2e (Torres-Larios et al. 2000). This partial similarity may imply some shared structural or functional features between hadrurin and these frog peptides that are potentially driven by convergent antimicrobial adaptations. The diversity of AMP sequences isolated from scorpion venoms allows for powerful structure–activity relationship studies (Torres-Larios et al. 2000). Many of these peptides are linear cationic amphipathic alpha-helices and can be synthesized and modified to improve their drug-like properties (Amorim-Carmo et al. 2022). Genomic studies using palpal muscle tissue from *Mesobuthus martensii* scorpions have identified genes for venom peptides (Cao et al. 2013). However, if some peptides in scorpion venoms are produced by bacteria within the venom glands, these peptide genes might have been transferred horizontally between different organisms sharing the same bacterial taxa associated with their glands. This hypothesis introduces a novel perspective on how the venomous components of scorpions may be influenced by microbial processes.

2.3.3 Myriapoda: Chilopoda (centipedes)

The first pair of centipede legs are modified into powerful venomous claws, known as forcipules, which serve as specialized tools for injecting venom into prey and adversaries, portraying evolutionary innovation for successful predation. Centipede venom glands and forcipules, characterized by a glandular epithelium surrounding the porous proximal part of the venom duct, suggests a regulated secretion process, with muscular control implicated in venom release and electron-dense granules formed in secretory cells preceding venom production (Dugon 2015). Centipede venom, containing diverse disulfide-constrained peptides with unique pharmacological effects on neuronal targets and anti-thrombotic properties, presents promising avenues for discovering drugs that address conditions such as ion channel dysfunction and thrombosis (Undheim et al. 2016). Based on these observations, further medically-based investigation into centipede biochemistry is warranted.

Centipede venoms are of evolutionary interest as there is substantial evidence that they acquire toxins from microbes by horizontal gene transfer (HGT). At least five toxin gene families were transferred from bacterial and fungal donors into centipede venoms at various times during their evolutionary history, including enzymes and smaller proteins (Undheim and Jenner 2021). Three of these toxin families function as virulence factors in bacterial donor taxa, which suggests that HGT can be an effective mechanism for the transfer of bacterial weapons into animal hosts.

Although the extent to which HGT has contributed to venom evolution in centipedes is exceptional, a range of microbial donors, including bacteria and fungi, have transferred venom components to other animal taxa as well, including cnidarians, arachnids, hymenopterans, and lepidopterans (Huang et al. 2021; Undheim and Jenner 2021; Walker et al. 2023). Given the repeated acquisition of venom components, profiling the microbiota of venom systems may produce insights into their role as possible reservoirs for innovations in venom evolution. To date, no microbial studies of the venom system of centipedes have been published.

2.3.4 Hexapoda: Insecta: Hymenoptera (ants, wasps and bees)

Hymenopterans are a large and diverse group of insects with over 150,000 species, comprising bees, wasps, and ants (Peters et al. 2017). These insects are infamous for their stings, which are the result of a specialization of the outer reproductive apparatus of females into a piercing projection, enabling the injection of secreted toxins into aggressors and prey. Anatomically, the venom apparatus of hymenopterans is composed of different interconnected secretory glands and tissues derived from female reproductive accessory glands, primarily represented by the Dufour's gland (aka "alkaline gland"), the venom gland (aka "acid gland") and the venom reservoir (Bridges and Owen 1984). In some wasp lineages, secretions deriving from the ovaries' common oviduct (termed "calyx fluid") may also contain toxins and venom-adjuvant factors, including symbiotic viruses and virus-like particles (Dicke et al. 2020; Salvia et al. 2023). Hence, the diverse glands and secretory tissues from which hymenopteran insect venom components are derived provide a unique opportunity for host-associated viruses and microbes.

Microbes have been reported from the venom glands of few hymenopterans, potentially affecting the function of their venom toxins (Fig. 1). A first report of a fungus infecting the venom apparatus of a wasp (Lebeck 1989) came from *Comperia merceti* (Hymenoptera: Encyrtidae) which is a gregarious parasitoid of the brown-banded cockroach *Supella longipalpa* (Blattaria: Blattellidae) ootheca. The wasp injects a yeast (suspected *Candida* sp., Deuteromycotina) into its host during oviposition and it is believed to participate in parasitism by disrupting the host's development (Lebeck 1989). This yeast infection is not limited to the wasp venom apparatus, as it has been observed in various organs throughout the wasp life stages, being most prevalent in the male's hemolymph. Inside the venom glands, the yeasts were observed in large numbers embedded within the wall of the venom reservoir, inside a capsular cell wall, and their presence in other regions of the venom apparatus could not be discarded (Lebeck 1989). The yeast is injected into the cockroach egg along with the parasitoid eggs, and was previously believed to participate in host's tissue digestion favoring the nutrition of the parasitic larvae (LeBeck 1989), however this assumption was not supported by later experimental evidence (Gibson and Hunter 2009). As it stands, the effects of the fungal yeasts within the venom of *C. merceti* parasitoids remains unclear. Similarly, occasional presence of spore-forming *Nosema* spp. yeasts in several tissues of honeybees, including within the venom reservoir have been detected (Copley and Jabaji 2012). Systemic infection by *Nosema* (also known as nosemosis) is considered a serious disease in honeybee colonies. This infection has been observed to affect the content and diversity of venom proteins, likely due to its debilitating effects on infected honeybees (Zakaria and Mohammed 2010). As yeasts detected in the venom of these hymenopterans are present in several other tissues and not all individuals of the host species are infected (i.e. meaning there is no specific mutualism), suggesting that the presence and effects of yeasts in hymenopteran venom may not have an adaptive role in their venom biology.

Ants are remarkable amongst insects for the chemical diversity within their secretions, supported by a number of secretory glands (Attygalle and Morgan 1984; Billen 1991). The venom compositions vary greatly across the different ant subfamilies, from mixtures of water-soluble acids with bioactive peptides or toxic oils to alkaloids (Touchard et al. 2016). The final composition of the injected mixture derives from different fluids from distinct parts of the venom apparatus, e.g. the Dufour's gland, typically produces apolar compounds like terpenoids or alkadienes (Mitra 2013) that are administered via the stinger. The metabolic pathways underlying the biosynthesis of some ant venom toxins has not been elucidated, as illustrated by alkaloids that require the participation of enzymes yet undescribed from animals, thus raising the possibility of microbial involvement (Blum and Hermann 1978; Pankewitz and Hilker 2008; Fox and Adams 2022). Sequestration of plant secondary metabolites has been the suspected origin for some species (Fig. 2), while different lineages of Myrmicinae ants have also been empirically or experimentally demonstrated to produce venom alkaloids belonging to structural families including pyridine nicotinoids, terpenoids, and piperidine toxins (Touchard et al. 2016; Fox and Adams 2022). Venom alkaloids seem to have evolved several times during ants' evolution, and have become prominently prevalent among species of the tribe Solenopsidini within Myrmicinae (Jones et al. 1996; Chen et al. 2019). As structurally heterogeneous molecules, there is no absolute metabolic pathway for producing alkaloids, and researchers suspect symbiotic microbes may be involved in the biosynthesis of the intermediate precursors for many insect alkaloids (Beran et al. 2019). In fact, there is experimental evidence suggestive of the participation of venom symbionts in the biosynthesis of alkaloids, as exemplified by the induced suppression of venom alkaloids by feeding ants with antibiotics at sub-lethal doses as observed in *Solenopsis* fire ants and in *Aphaenogaster* myrmicine ants (Rojas et al. 2011; Lenoir and Devers 2018).

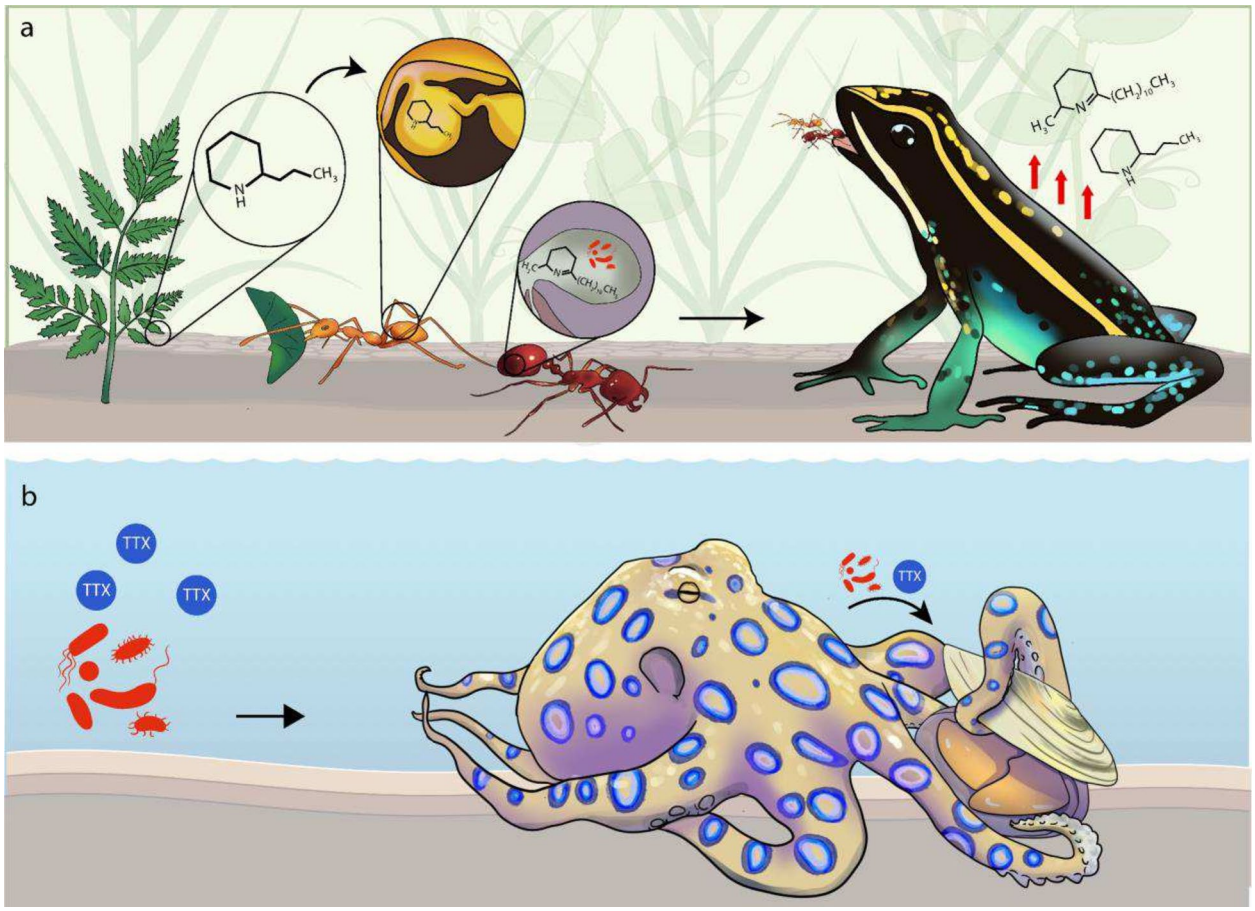

Fig. 2 Venom toxins move through trophic levels by host sequestration. **a)** Hemlock plants may produce alkaloids of the piperidines class. Hypotheses propose that some insects may ingest the leaves containing alkaloids and at least partially incorporate them to their venom composition. Animals, such as poison dart frogs, that predate these insects can further sequester the toxic alkaloids. The toxin may be digested and modified with help from gut bacteria before reaching the skin gland/tissue to be used as poison. **b)** Bacteria present in the water column secrete TTX, which blue ringed octopuses sequester and use in their venom cocktails for predating clams and other prey items. The figure background was sourced from BioRender, individual organisms were drawn using Procreate version 5.3.10, and the figure was assembled in the 2024 version of Adobe Illustrator

The only attempt to identify and locate suspected symbionts was documented in a survey for microbes within the venom apparatus of two fire ant species – the red imported fire ant, *Solenopsis invicta,* and the tropical fire ant, *Solenopsis geminata* – using high-throughput (Illumina) 16S rRNA sequencing of dissected venom reservoirs. Subsequent analysis estimated the microbial diversity associated with the dissected venom reservoirs and found that Pseudomonadota (Proteobacteria) was the most abundant phylum identified throughout samples from different field sites, followed by Mycoplasmatota (Tenericutes) and Bacillota (Firmicutes); most abundant genera were *Mesoplasma*, followed by *Exiguobacterium* and *Pseudomonas*. These results were similar when compared to observations from a distant non-alkaloid-producing ant, *Diacamma rugosum,* suggesting that the microbial composition associated with venom gland reservoirs may correlate with different venom chemistries of distant ant lineages (Yang et al. 2021). No direct imaging/detection methods (e.g. light and fluorescence microscopy) have been attempted to confirm the presence of the 16S rRNA sequenced microbiota inside the venom reservoir, thus the possibility of contamination from the proximate hind gut or abdominal tergite during dissections cannot be ruled out (e.g. microbes, being widespread, can be present in many structures surrounding the venom apparatus, including the stinger (see also Supplementary Fig. 1). Moreover, detected microbes are commonly present in the guts of insects, including fire ants (Yang et al. 2021). Therefore, the presence of microbial symbionts within venom apparatuses of ants warrants more robust investigation, such as the use of fluorescence microscopy markers to confirm the presence and location of microbes within distinct tissues.

2.3.5 Hexapoda: Insecta: Neuroptera: Myrmeleontidae (antlions)

Antlion larvae, predatory insects known for paralyzing their prey with venom, have been the subject of investigation regarding the origin and composition of their venom. Three different paralytic and insecticidal proteins have been identified in the venom system of *Myrmeleon bore*, and these were shown to be produced by bacteria (Yoshida et al. 2001). Larval venom contains *Enterobacter aerogenes*, which produces a toxic heat-shock protein, GroEL (Yoshida et al. 2001). *Bacillus cereus* was isolated from its esophagus and was found to produce the toxin sphingomyelinase C, and *Bacillus sphaericus* was isolated from its crop and found to produce the pore-forming protein sphaericolysin (Nishiwaki et al. 2004; Nishiwaki et al. 2007a, b; Walker et al. 2018). The presence of other bacterial species in the head, crop and gut of *M. bore* and other antlion species brings up questions regarding the potential role of microbial symbionts in venom production (Dunn and Stabb 2005; Liu et al. 2016). Interestingly, *Pantoea* bacteria were found in the crop of *M. bore* (Nishiwaki et al. 2007a, b). *Pantoea agglomerans* has been shown to be a gut symbiont in the grasshopper *Schistocerca gregaria* and they produce antimicrobial phenols that confer resistance to fungi (Dillon and Charnley 1995). The mechanisms by which these bacteria contribute to venom synthesis, and the extent to which venom components originate from the digestive system, remain areas requiring further investigation.

2.4 Chordata

2.4.1 Vertebrata: Reptilia: Squamata (scaled reptiles)

Squamate reptiles, comprising lizards, snakes, and amphisbaenians, represent the largest group of terrestrial vertebrates with over 10,000 described species (Vitt and Caldwell 2013; Singhal et al. 2021). The evolution of an advanced venom delivery apparatus occurred in multiple squamate lineages, including helodermatid and anguimorph lizards (Koludarov et al. 2017), and nearly 20% of all snake lineages (> 200 species) are considered to be medically significant by the World Health Organization (Hutson 2010). It has been suggested that even lineages lacking an obvious specialized delivery apparatus (e.g., varanid lizards) have highly derived secretory glands that are specialized to deliver venom toxins via mechanical stimulation during biting for either defensive or predatory justification, where the physical action of biting manually stimulates the release and delivery of venom (Fry et al. 2006; Koludarov et al. 2017). Further, several lineages previously thought to be harmless or unable to produce complex toxins have been shown to possess complex toxicological weaponry (Koludarov et al. 2017). Some lineages of snakes undergo ontogenetic shifts in their venom toxin profiles, related to dietary shifts (Cipriani et al. 2017; Schonour et al. 2020), and overall venom toxin complexity appears to be related to dietary complexity (Holding et al. 2021). Dietary complexity may also be related to oral and gut-microbiome complexity (Smith et al. 2021), but whether this is reflected in other host sites is unexplored.

Advanced snakes, which include highly venomous and medically important species such as cobras and pit vipers, are the best known and studied venomous reptiles in the context of their venom diversity and chemistry. However, aside from early studies that sought to identify antimicrobial peptides from snake venom glands, bacterial infections after snakebite, and a few veterinary studies on reptile oral microbiota, few studies have investigated the presence or function of microbes in venom-associated glands of venomous reptiles. Nearly all of these studies employed techniques designed to propagate and classify culturable bacteria (Dehghani et al. 2016; Ghosh et al. 2018), whereas through advances in modern sequencing technologies we now know this approach largely fails to detect the major part of the diversity of microbial life present in any given sample (Hug et al. 2016).

Venoms represent a key adaptation that has played a main role in the diversification of venomous animals like snakes (Calvete et al. 2007). While there have been some studies dedicated to culturing microbes from snake venoms (Goldstein et al. 1979), few have used comprehensive sequencing techniques to describe the snake microbiome in the gut (Pulford et al. 2019), mouth (Blaylock 2001), and venom (Esmaeilishirazifard et al. 2022). Most research thus far has sought to fight bacterial infections after snake bites, which might originate from venom, fangs, and saliva of the snake (Clark et al. 1993; Garg et al. 2009). Given what we now know about the near-ubiquity of vertebrate host-associated microbial life with both host external and endogenous tissues (Ley et al. 2008; Rosenberg and Zilber-Rosenberg 2018), the lack of inquiry using modern methodologies (amplicon and shotgun sequencing, FISH, proteomics, metabolomics) into the presence and function of microbes in the venom and associated glands of squamate reptiles is surprising. By and large the most interest garnered regarding microbial interactions with a venomous squamate lineage is that of oral bacteria of monitor lizards. Long thought to be the main causative agent in monitor lizard-inflicted wounds (Auffenberg 1981), oral bacteria (and possibly those from venom) were subsequently discounted when both venom glands and complex venom-like proteins were identified in multiple monitor lizard lineages (Koludarov et al. 2017). Furthermore, a single study utilizing 16S metabarcoding failed to find any sepsis-causing bacteria in captive adults or neonate monitor lizards (Goldstein et al. 2013). However, we know that captivity influences the microbiome of alligators (a

non-squamate reptile lineage) (Keenan et al. 2013), iguana microbiomes (Eliades et al. 2021), and the prevalence of sepsis-causing and pathogenic bacteria in saliva samples of wild vs captive komodo dragons (Montgomery et al. 2002). Lastly, it has been shown that both external and endogenous microbiomes of captive monitor lizards are largely shared with those from the environment (Hyde et al. 2016). This somewhat polarizing yet exciting shift in the literature focus has led to several opposing hypotheses regarding the purpose of microbes (in this case sepsis-causing bacteria) in the venom or oral flora of monitor lizards. First is the "bacteria as venom" model where bacteria are recruited to aid in prey acquisition and defense (Auffenberg 1981), whereas the opposing "passive acquisition" model posits that microbes present are purely incidental and constrained by the external environment (Fry et al. 2009). A third, "lizard epidemic" model has been proposed (Bull et al. 2010) where microbes are directly transferred between actively feeding lizards, and thus are recruited via specific life-history traits and selected by the oral and venom environment to aid in prey acquisition and defense. This latter model is elegant and considers the evolution and life history of both host and microbes, and should be investigated more broadly when dealing with species that have large social interactions, such as co-feeding or communal nesting/sleeping.

Harboring a complex and potentially pathogenic oral microbiota is not restricted to monitor lizards, with several snakes known to possess bacteria capable of causing medical complications via snakebite (Shek et al. 2009; Lam et al. 2011; Krishnankutty et al. 2018). However, these studies did not specifically investigate microbes present in venom and have largely relied on mouth swabs. Potentially pathogenic bacteria such as *Escherichia coli, Shigella spp., Providencia spp.* and *Chryseobacterium spp.* are often found in great abundance in the oral cavities of a diverse array of snake species (Shek et al. 2009; Lam et al. 2011; Lukač et al. 2017). Recent studies have found that the oral and gut microbiomes of sympatric venomous snake species reflect host ecology and venom type, and that the gut microbiomes of venomous and non-venomous snakes significantly differ in bacterial composition (Qin et al. 2019; Smith et al. 2021). Future studies on the selective pressures driving venom phenotypes should also address the possible microbial contributions to venom, as well as the ecology of microbes inhabiting the venom apparatus of venomous squamates.

Variations in snake venom glands and delivery mechanisms across families provide diverse ecological niches for microbes. Front-fanged snakes like Atractaspididae, Elapidae, and Viperidae have large glands possibly allowing for great microbial colonization, and hollow fangs for high-pressure venom injection (Mackessy 2009). Conversely, rear-fanged snakes like Colubridae, Homalopsidae, and Lamprophiidae feature smaller glands and lack hollow fangs, resulting in slower venom delivery through multiple puncture sites possibly facilitating uptake of microbes from the victim (Mackessy and Baxter 2006; Mackessy 2009). Venomous lizards, such as Helodermatidae (gila monsters and bearded lizards) and Anguimorpha genera (*Lanthanotus* and *Varanus*), possess simple venom delivery systems with glands located on the mandible, accompanied by infralabial mucus glands. Venom is delivered passively through ducts leading to teeth in the lower jaw (Fry et al. 2006; Koludarov et al. 2017). During prey subjugation or defensive bites, these lizards often employ biting, holding, and head thrashing behaviors, which may facilitate the entry of more venom into wounds (Fry et al. 2006; Koludarov et al. 2017). These variations in anatomical structures and mechanisms may influence the colonization and function of bacteria within the venom glands.

Snake venom has been explored as a source of new antimicrobial compounds and several toxin classes have been described as antibacterial (Samy et al. 2012). These antimicrobial properties have long contributed to the general assumption that venom glands and ducts are sterile. However, little effort has been made to investigate the extremophile microbes that are able to thrive in this hostile environment, or their evolutionary responses (i.e. antimicrobial compound production) to unique selection pressures present in the venom gland/duct system. The longstanding dogma that oral secretions and venom microbiomes are either derived from prey or are sterile is now being challenged as the oral and gut microbiome have been shown to be independent of prey-associated microbes (Costello et al. 2010), and non-venomous snakes often have substantially lower abundance of microbial inhabitants within their oral cavities than venomous snakes (Lam et al. 2011). Furthermore, microbes have been isolated and found to be unique to the venom-gland microenvironment of venomous snakes (Esmaeilishirazifard et al. 2022). Overall, squamate reptiles that exhibit cooperative feeding present an interesting opportunity for colonization and spread of venom, oral, or gut microbes between predators, prey, and the environment.

The vast majority of studies on microbes present in the oral cavities or venom/saliva of reptiles have focused on culture-based techniques rather than sequence-based approaches. However, there has been some overlap in the findings using either approach, and the majority of bacteria identified in the oral cavities of reptiles belong to the phyla Pseudomonadota (formerly Proteobacteria), Bacteroidota, Bacillota (formerly Firmicutes), Actinomycetota (formerly Actinobacteria), and Acidobacteriota, typically in decreasing relative abundances (Ghosh et al. 2018). Pseudomonadota, Bacteriodota, and Bacillota are the dominant phyla in the gut of wild reptiles (Colston and Jackson 2016). However, aquatic and marine reptiles often have high abundances of

Fusobacteriota (formerly Fusobacteria) and Mycoplasmatota (formerly Tenericutes) in both oral and gut samples, potentially relating to the environmental influence on the microbiome of aquatic reptile hosts (Keenan et al. 2013; Smith et al. 2021). Recently, *Patescibacteria* were identified in the oral microbiome of venomous sea snakes (*Laticauda laticaudata*), which have not been previously known to occur in reptile guts or oral microbiomes (Smith et al. 2021). Chloroflexota (formerly Chloroflexi) are known to inhabit the oral cavities of several medically significant elapid snake species, including king and Indian cobras (Krishnankutty et al. 2018). Many of the bacteria known to inhabit the oral cavities of venomous reptiles are potentially pathogenic. The most common infections associated with medically significant snakebites are attributed to *Enterococcus faecalis*, and a recent study identified two novel strains of the bacteria that were unique to the venom gland/delivery system of the venomous snake *Naja nigricollis* (Esmaeilishirazifard et al. 2022). Current investigations into reptile oral cavities and venom microbiomes have identified prevalent bacterial phyla, including potential pathogens, thus further research, utilizing advanced sequencing techniques to elucidate the diversity, functions, and bioactive properties of these venom bacteria-based chemicals, is required to understand the medical impact of the venom microbiome in envenomed patients.

2.4.2 Underexplored microbes in venom systems

The investigation of the venom microbiome represents a burgeoning field within scientific research, primarily focusing on bacterial communities, and even fewer focusing on bacterial contributions to venom composition and movement through trophic levels. Fungi and Archaea present challenges regarding their cultivation and genome studies, leading to a bias towards bacterial components in venom microbiome research. However, this oversight may withhold information as the full spectrum of venom-associated microorganisms including parasites, viruses, fungi, and archaea, remains inadequately studied. The following sections present unique aspects of venom-microbe systems as far as they are currently understood while addressing areas of each system that are in critical need of further study.

2.4.3 Bacterial synergy in trophic transfer of venom toxins

Investigating the transmission of venom toxins across trophic levels presents a novel research area for understanding how bacteria contribute to venom and poison composition in various organisms. Myrmicine ants, mites, and beetles produce toxic alkaloid-rich poisons and venoms. When ingested by anurans such as dendrobatid and mantelline frogs and bufonid toads, the alkaloids present in these poisons and venoms may become sequestered within the frog's cells (Jones et al. 1999; Valderrama-Vernaza et al. 2009). These amphibians can, thus, utilize ant, mite, and beetle prey alkaloids for chemical defense against predation, facilitated by cutaneous mucous glands and serous glands aiding in alkaloid sequestration (Darst et al. 2005). The process is likely facilitated by gut bacteria, though the mechanisms are unknown, warranting further research (Fig. 2).

Exploring the movement of venom components through trophic levels helps us understand host-microbe dynamics impacting chemical defense, offering potential biomedical data for drug development. Toxins are often initially discovered in animals and later found in bacteria, with potential for purification and synthesis for pharmaceutical use. Microsymbionts contributing to venom toxin sequestration may reveal key ecological dynamics, informing how bacteria influence animal chemical defense strategies. Batrachotoxin (BTX), a medically significant natural toxin, likely originates from plant metabolites ingested by Melyridae beetles and oribatid mites, undergoing structural modification by arthropod gut microsymbionts (Daly et al. 1994; Dumbacher et al. 2004; Saporito et al. 2007). BTX is also found in the skin and feathers of some birds such as the Pitohui, which also feed on toxic beetles (Dumbacher et al. 2004). Further investigation of the functional attributes of microbes in toxin modification or chemical synthesis in this system are needed to understand putative biochemical pathways or novel bioactive compounds applicable in medicine.

Similar to BTX, the alkaloid tetrodotoxin (TTX) is a potent sodium channel neurotoxin (Bane et al. 2014). Some caudatans (newts and salamanders), and various phylogenetically diverse marine animals secrete or inject TTX as a defense, while other animals use the toxin for predation (Chau et al. 2011). In some marine animals the source of TTX is direct dietary accumulation from environmental bacteria containing TTX (Chau et al. 2011). In contrast, the production of TTX in newts is uncertain, and the synthesis mechanisms, whether internally regulated or facilitated by bacterial symbionts, remain unclear, as bacteria within the skin microbiome of toxic newts can produce TTX (Vaelli et al. 2020). Unlike anurans, which are known to sequester toxins directly from venomous prey, caudatans are likely to sequester TTX from environmental bacteria associated with diet in the same way that marine animals do. In fact, many of the TTX-producing bacterial strains isolated from the skins of newts are from the same genera as those identified in marine animals. For many marine animals, the toxin is known to be produced by symbiotic bacteria, which may be a possible reason why so many different animals can utilize TTX (Vaelli et al. 2020). Blue-ringed octopuses (genus *Hapalochlaena*) sequester TTX from marine bacteria (Whitelaw et al. 2019). The venom glands in octopuses are connected

to the salivary papillae, near the beak or mouth of the octopus, and they assist in delivering venom to subdue prey or deter potential threats (Fig. 2). These animals have developed selective uptake mechanisms, potentially involving specialized transport proteins to concentrate and store TTX in the venom glands. The molecular mechanisms of bacterial TTX production and the mechanisms of uptake and storage are ongoing areas of research. Sequestration of TTX and/or TTX-producing bacteria requires protection against the toxin for the host as the molecular target of TTX, voltage gated sodium channels, is fundamental to life processes throughout the animal kingdom. Target site resistance via voltage gated sodium channel mutations have been considered to be standard for TTX resistance, having been detected in TTX utilizing species such as *Hapalochlaena spp*. (Geffeney et al. 2019). New research is also investigating the origin and role of soluble toxin-binding proteins in these toxin pathways. For instance, the discovery of saxiphilin, a saxitoxin-binding protein isolated from frogs and toads challenges previous ideas about how these animals resist their own toxins and suggests that sequestration mechanisms could be important for protection (Abderemane-Ali et al. 2021). Both the diversity of animals in which TTX has been found and the means of obtaining, sequestering, producing, secreting, or injecting the chemical raise questions about the mechanisms by which this toxin can travel through trophic levels from bacteria to predator to prey, and the likelihood of environmental and gut bacteria mediating the chemical's ability to traverse environments and hosts.

2.4.4 Parasites and additional micro-eukaryotes

The diverse relationships between venomous animals and eukaryotic parasites in the venom environment remain underexplored, but interactions between venoms and various parasitic organisms are known to exist. Venomous animals may use their venom defensively against parasites, and in some cases, parasites can modulate venom production, affecting the defensive behaviors of venomous organisms.

Parasites, spanning a diverse range of taxa including Arthropoda, Nematoda, Platyhelminthes, and Protozoa among others constitute ubiquitous selective pressure on host populations (Poulin and Morand 2000). Given the small points of entry into venom glands, infection by metazoan parasites in the venom environment seems unlikely, though single-celled eukaryotic parasites (protists) could potentially reside in the venom microenvironment if they are able to survive. However, it is more likely that venomous animals are trying to defend themselves against parasites, rather than coevolve with or symbiotically recruit parasites like they do with bacteria and viruses. Slow lorises generate their venom through a combination of specialized brachial glands located inside their elbows and salivary glands (Nekaris et al. 2013). The branchial glands secrete a toxic compound, which the slow loris transfers to its mouth by licking. There it is combined with toxic saliva, with the slow loris envenomating victims through its bite, using the venom to immobilize potential prey or threats (Gardiner et al. 2018). The venom from two slow loris species, *Nycticebus javanicus* and *Nycticebus coucang*, exhibits ectoparasite repellent properties, impairing and often causing the death of various arthropods (Grow et al. 2015). This suggests its potential role in repelling parasites, particularly ectoparasites like ticks. In another example, nematode parasites interfere with *Lasius* ant venom production, resulting in a reduction of defensive behaviors (Yanoviak et al. 2008). Some parasites, including certain helminths, produce molecules with venom toxin-like properties (Dzik 2006). These molecules are often involved in interactions with the host's immune system and can modulate immune responses (Dzik 2006). While these parasite proteins share some characteristics with venom components, they are typically referred to as immunomodulatory or immunoregulatory proteins rather than traditional venom (Goodswen et al. 2022).

2.4.5 Viruses

Venom glands may support replication of some viruses or virus-like entities (Asgari and Rivers 2011). When taking into consideration the high rates of replication and the high mutation rates during the replication process and the behavior of viruses as quasispecies, especially those with RNA genomes, it is not surprising to find a high diversity of viruses in venom glands (Sanjuán 2010; Domingo et al. 2012). The first study on the virome of venom glands in a venomous animal, the Golden Orb-Weaver spider, *Nephila clavipes*, showed four new RNA viruses that were discovered to exist exclusively in the venom glands (Debat 2017).

Numerous investigations revealed a diverse range of viruses and virus-like particles (VLPs) in the venom glands of various venomous animals (Morales et al. 2005; Asgari and Rivers 2011; Gueguen et al. 2011; Gatti et al. 2012; Debat 2017). A recent study suggested that the accumulation of viral RNA in venom glands might contribute to virus HGT (Debat 2017). These events are not dissimilar to prokaryotic adaptation at the genome level driven by phage systems, and the significance of this in the fight against antimicrobial resistance, let alone venom biology, has yet to be assessed (Weinbauer and Rassoulzadegan 2004). In parallel, endoparasitoid wasp venom-associated viral components present additional physiological roles, as VLPs injected by the parasitoid wasp *Leptopilina heterotoma* (Hymenoptera: Cynipidae) during the process of endoparasitism have been reported to destroy the cellular

Table 1 Methods and Approaches to Understanding Venom Microbiomes. An overview of traditional and emerging techniques to investigate microbial diversity and function within the venom microenvironment, including potential challenges and considerations when designing experiments

Focus	Technique	Taxonomic classification	Functional data	Advantages	Primary considerations
Venom Microbiome Exploration	Metagenomics	YES	NO	Biomarker discovery, Comparative analysis	Contamination risk, dissection, library preparation
Venom production and regulation	Transcriptomics	NO	YES	Gene expression pattern, Key genes, Comparatives analysis with microbial genes	Difficulty in RNA extraction and processing, transcript sorting
Protein composition, Protein family exploration	Proteomics	NO	YES	Detect protein interactions or levels, Functional potential	Complex biological samples, database searches, statistical analysis
Chemical profiling and pathways	Metabolomics	NO	YES	Metabolite profiling, Comprehensive, Functional	Interdisciplinary collaboration, compound identification
Host health, Ecology, Selection	Isolate Cultures	YES	NO	Selective, Strain identification, AMR testing	Technical expertise, careful planning, contamination risk

immunity of *Drosophila melanogaster*—an early indication of coevolutionary significance for venom viromes that might extend beyond wasps into other envenomating species (Rizki et al. 1990). Future research should attempt to determine the roles of these viral components, their coevolutionary relevance, and their broader implications for advancing our understanding of venom biology and therapeutic interventions.

3 Methods and approaches to understanding venom microbiomes

Investigating the host–pathogen dynamics and co-evolutionary mechanisms within venom microbiomes necessitates precise methodologies to mitigate contamination risks and decipher the complex microbial interactions within venomous organisms (Table 1). Microbiome studies require technical expertise and careful planning and controls, as sample contamination can easily occur during initial processing. This is central to the context of venom microbiomes, as any organisms identified, for instance by NGS, could potentially originate from other sources within the original collection, or dissection tools, buffers, or other anatomical structures/organs encountered during micro-dissections of small arthropods.

Microbes identified from milked snake or spider venoms might have been associated with the fangs, either externally or at the exit ducts. Microbes found in ant venom samples might have been associated with the stinger or even the hindgut, considering the proximity of the stinger tip to the cloaca. During dissections, microbe-rich adjacent tissues could easily impregnate collected venom apparatuses, potentially leading to erroneous subsequent conclusions. Cross-contamination during library preparation can also occur, specifically through PCR cycles resulting in tag-switches and chimeric sequences leading to distorted diversity measurements; a solution involves implementing modified library preparation protocols, strategic sample labeling, and utilizing information from both samples and negative controls (Fountain-Jones et al. 2023). Exploring the integration of site-occupancy modeling in venom microbiome studies could enhance the quantification of measurement uncertainty, address imperfect detection issues, and provide a basis for robust statistical predictions by incorporating both biological and technical PCR replicates, thus improving study design and confidence in biodiversity assessments (Fountain-Jones et al. 2023).

Transcriptomic methods can be applied to discover differences or similarities in the microbial composition and function of microbes residing within venom glands by analyzing the RNA transcripts of the microbial genes.

This involves extracting RNA from the venom glands, converting it to complementary DNA (cDNA), and then using high-throughput sequencing techniques. The resulting data would provide information about the active genes and metabolic pathways of the microbes present in the venom glands, allowing researchers to compare the microbial profiles between different species and understand their potential roles in venom production or other physiological processes. However, in transcriptome analyses, there are major obstacles which are intrinsic to venom research, not often mentioned by published literature (an overview of RNA-Seq is given in Wu 2018). For instance, the typical venom transcriptome manuscript will qualitatively describe the diversity and characteristics of assembled transcripts recovered from RNA (either total or enriched in mRNA) isolated from a pool of venom apparatuses dissected off their model organism. Accurate and clean dissections are imperative to avoid the inclusion of other tissues like tracheae, muscles, venom reservoirs, and mitochondria in the analysis, as each carries specific transcripts. Downstream in the analysis, there is currently no established approach to reliably sort between venom-associated transcripts and unrelated transcripts, where the latter usually represents a major part of the observed diversity (Baek et al. 2013).

Metabolomics as applied to venomics has been more frequently applied to vertebrate venom analysis, however invertebrate studies have also increased in frequency, for instance with scorpions (Hu et al. 2011), spiders (Schroeder et al. 2008), and ants (Fox et al. 2018). In principle, interdisciplinary collaboration and unrestricted access to raw data are essential for producing invaluable results and conclusions. A main challenge still exists in the proper identification of compounds obtained through mass spectrometry, although emerging databases and analysis platforms such as the Global Natural Product Social Molecular Networking (GNPS) (Wang et al. 2016) and VenoMS (Forster et al. 2020) are facilitating identifying and tracing structural relationships between metabolites. Thus, integrating genomics, transcriptomics and proteomics results with metabolomics relies on interdisciplinary collaboration, which can then help to build a more comprehensive understanding of the multilayered, complex biochemical interactions between microbes and venomous hosts.

4 Conclusions and future directions

This review predominantly focuses on venomous organisms, emphasizing venom diversity and integrating microbiomic analyses to enhance understanding of venom composition, ecological interactions, and microbial symbionts. The composition and functionality of venom-associated microbiomes exhibit remarkable diversity, contributing to the unique characteristics of venoms. The examples provided in this review highlight the burgeoning understanding of connections between microbes and venomous animals, suggesting that microbial communities may contribute to shaping venom composition and function.

The emerging field of ecological venomics (genomics, transcriptomics, metabolomics, and proteomics) is beginning to show that ecological processes are influencing venom composition and diversity. Drawing parallels, as environmental stressors can impact our gut microbiome, similar ecological processes likely influence venom-associated microbial communities. In both cases, external factors influence biological compositions: venoms adapt to prey and habitat, while microbial assemblages can reflect environmental stressors such as changes in pH or temperature. Progress in both basic research and technological advancements are required for fully understanding venom microbiomes. Techniques such as metagenomics, proteomics, and comparative transcriptomics have undergone substantial enhancements, offering deep insight into the relationships between toxins and microbes within venom glands, organs, or ducts. Moreover, tissue or cell-specific sequencing, particularly in eukaryotic organisms, may reveal complex dynamics within venomous systems. A notable example is the cultivation of ant venom glands and their associated microbiota, which holds potential to enhance our understanding of the interdependence between venomous organisms and their microbial symbionts.

The microbiomes associated with the venom systems of hematophagous animals, including leeches, ticks, mosquitoes, and others, deserve dedicated inquiry and discussion due to their potential role in disease transmission and host adaptation. These animals serve as vectors for serious diseases like malaria and Lyme disease, with their venoms providing attractive environments for microbial colonization. However, the impact of venom-associated microbes on human and veterinary health remains poorly understood, necessitating further research to assess their contribution to envenomation pathology. The ecological and evolutionary pressures governing the nature of microbiomes in blood-feeding and predatory venom glands could differ significantly, and in important ways, presenting a need for focused investigation into this area of venomous host-microbe evolution and adaptation.

The role of viruses such as bacteriophages, which can influence the abundance of bacterial populations should be recognized in brief. Consideration of viruses' involvement in the biology and maintenance of ecological niches, such as venom and poison glands, where microbial communities are diverse, is not unwarranted. By forming the

foundation for future research in unifying environmental biology, host ecology, microbial ecology, and processes of venom action and delivery involving diverse microbial taxa, we establish a systematic framework to understand the diversity and roles of venom-associated microbiomes, possibly culminating in the discovery of compounds for pharmaceutical use. The venom-associated microbiome could have a significant impact on studies of venoms as therapeutics, biopesticides or other applied research where whole venom is used as a starting point. There are many published studies on venom activity that have used whole venom where the contribution of the venom-associated microbiome is unknown. In the majority of these studies the venoms are lyophilised which has been shown to greatly reduce bacterial viability, whereas work with fractionated venoms will not contain viable microbes but could contain microbial metabolites. The symbiosis of venom-associated microbes and their hosts represents an evolutionary arms race within the venom gland environment, the products of which can be used for novel drug discovery.

In the current scientific context, there is a growing emphasis on the study of biological relationships through a multisectoral and transdisciplinary approach that acknowledges the interconnectedness of human health, animal health, and environmental health. The integration of open-source research platforms like iNaturalist (www.inaturalist.org), GNPS (www.gnps.ucsd.edu), Wikidata (www.wikidata.org), Vertnet (www.vertnet.org), and the Global Biodiversity Information Facility (www.gbif.org) can facilitate collaborative efforts in elucidating the relationships between microbes and venomous animals. Constructing a comprehensive network that establishes connections between disciplines is an ongoing challenge that requires innovative strategies. This involves a multi-disciplinary approach that encompasses fields ranging from microbiology to computational biology. The ultimate objective of iVAMP is to create a comprehensive understanding of host metadata and the interactions of microorganisms within venom. This information may be extrapolated to enhance a broader comprehension of biological phenomena encompassing ecological, evolutionary, and health-related subjects. In the field of venom microbiome research, it is imperative to prioritize meaningful engagement with local communities, fostering mutual dialogue between scientists and the community, promoting collaborative fieldwork initiatives, and ensuring diverse representation within the scientific community (Ramírez-Castañeda et al. 2022). Meaningful engagement with local communities in venom microbiome research acknowledges the valuable traditional knowledge they possess, fostering a more comprehensive and inclusive approach to understanding venomous organisms. This also aids compliance with international laws on bioprospecting such as The Nagoya Protocol on access and benefit sharing. Through a practical and inclusive approach, iVAMP advocates for the direct involvement of local community members across various research stages, encompassing project initiation, data collection, and result interpretation. This collaborative strategy respects and values the traditional knowledge of local communities, and leads to advancements in the exploration of venom microbiomes. This approach aligns with the overarching goals of a unified microbiome initiative (UMI), which seeks to comprehensively explore Earth's microbial ecosystems for scientific discovery and practical applications (Alivisatos et al. 2015). Due to the limited understanding of microbial interactions, UMI advocates for the exploration and utilization of Earth's microbial ecosystems to drive innovation across various fields. Similar to the iVAMP initiative, UMI underscores the importance of microbiomes in addressing challenges related to climate change, agriculture, biofuels, human health, and drug discovery. It promotes the rational management of microbial communities for disease prevention, treatment, and precision medicine.

The existing literature on venom microbiomes exhibits its biases, with significant gaps in knowledge regarding environmental sampling sites, host species representation, and microbial diversity, resulting in disparities in the representation of these factors. To comprehensively understand venom microbiomes, future research should extend beyond bacteria and consider the presence and roles of viruses, fungi and archaea in these microbiomes. The study of venom microbiomes involves investigating evolutionary relationships between organisms and their microbial associates, providing a more holistic perspective on the dynamics shaping venom composition and function. Fungi and archaea are not as extensively studied as bacteria in venom microbiomes because the methodologies for studying bacteria are more well-established, allowing for easier isolation and characterization. As techniques for studying these less explored microbial groups improve, it becomes increasingly important to address this bias and investigate the diverse roles of fungi and archaea in venom ecosystems. The diversity in venomous adaptations, the influence of extreme environments, the metabolic costs associated with venom production, and the potential synergies between technological advancements and basic research are important for future investigations involving venom microbiomes. While progress has been made to infer bacterial contributions to venom composition, our understanding of the underlying mechanisms remains limited. Much more research is needed to explore the possible roles of microbes in venom composition and evolution. Additional research will also elucidate how venoms and microbiomes interact and influence each other.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13199-024-01031-0.

Acknowledgements E.G.P.F. was funded by FAPEG/CNPq grant no. 317847/2021-0. C.J.N. acknowledges funding from the National Institutes of Health (NIH) National Institute of General Medical Sciences (NIGMS) award R35GM124594 and from the Kamangar family in the form of an endowed chair. V.H. was funded by the Australian Research Council (FT190100482). T.J.C. was supported by the National Science Foundation (awards DEB-2406685 and DBI 2334779) and the Office of Research at the University of Puerto Rico at Mayagüez. The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication. We thank Veronika Guillemard for her helpful comments on recent literature.

Declaration

Statements and Declarations Clarissa J. Nobile is a cofounder and acting CEO of BioSynesis, Inc., a company developing diagnostics and therapeutics for biofilm infections. Steven A. Trim was the founder and CSO at Venomtech Ltd., a company selling venom libraries for research.

References

Abderemane-Ali F, Rossen ND, Kobiela ME, Craig II RA, Garrison CE, Chen Z, Colleran CM et al (2021) Evidence that toxin resistance in poison birds and frogs Is not rooted in sodium channel mutations and may rely on 'toxin sponge' proteins. J Gen Physiol 153(9):e202112872. https://doi.org/10.1085/jgp.202112872

Abreu TF, Sumitomo BN, Nishiyama MY, Oliveira UC, Souza GHMF, Kitano ES, Zelanis A et al (2017) Peptidomics of *Acanthoscurria gomesiana* spider venom reveals new toxins with potential antimicrobial activity. J Proteomics 151(January):232–242. https://doi.org/10.1016/j.jprot.2016.07.012

Alivisatos AP, Blaser MJ, Brodie EL, Chun M, Dangl JL, Donohue TJ, Dorrestein PC et al (2015) A unified initiative to harness earth's microbiomes. Science 350(6260):507–508. https://doi.org/10.1126/science.aac8480

Amorim-Carmo B, Parente AM, Souza ES, Silva-Junior AA, Araújo RM, Fernandes-Pedrosa MF (2022) Antimicrobial peptide analogs from scorpions: modifications and structure-activity. Front Mol Biosci 9:887763. https://doi.org/10.3389/fmolb.2022.887763

Asgari S, Rivers DB (2011) Venom proteins from endoparasitoid wasps and their role in host-parasite interactions. Annu Rev Entomol 56:313–335. https://doi.org/10.1146/annurev-ento-120709-144849

Attygalle AB, David Morgan E (1984) Chemicals from the glands of ants. Chem Soc Rev 13(3):245–278. https://doi.org/10.1039/CS9841300245

Auffenberg W (1981) The behavioral ecology of the Komodo monitor. University Presses of Florida, Gainesville, Florida

Augustin R, Schröder K, Murillo AP, Rincón SF, Anton-Erxleben F, Herbst E-M, Wittlieb J et al (2017) A secreted antibacterial neuropeptide shapes the microbiome of *Hydra*. Nat Commun 8(1):698. https://doi.org/10.1038/s41467-017-00625-1

Baek JH, Jung Hun Oh, Kim YH, Lee SH (2013) Comparative transcriptome analysis of the venom sac and gland of social wasp vespa tropica and solitary wasp rhynchium *Chlorophyllum brunneum*. J Asia-Pacific Entomol 16(4):497–502. https://doi.org/10.1016/j.aspen.2013.08.003

Bakshani CR, Morales-Garcia AL, Althaus M, Wilcox MD, Pearson JP, Bythell JC, Grant Burgess J (2018) Evolutionary conservation of the antimicrobial function of mucus: a first defence against infection. Npj Biofilms and Microbiomes 4(1):1–12. https://doi.org/10.1038/s41522-018-0057-2

Bane V, Lehane M, Dikshit M, O'Riordan A, Furey A (2014) Tetrodotoxin: chemistry, toxicity, source, distribution and detection. Toxins 6(2):693–755. https://doi.org/10.3390/toxins6020693

Benli M, Yigit N (2008) Antibacterial activity of venom from funnel web spider *Agelena labyrinthica* (Araneae: Agelenidae). J Venomous Animals Toxins Including Tropical Dis 14:641–650. https://doi.org/10.1590/S1678-91992008000400007

Beran F, Köllner TG, Gershenzon J, Tholl D (2019) Chemical convergence between plants and insects: biosynthetic origins and functions of common secondary metabolites. New Phytol 223(1):52–67. https://doi.org/10.1111/nph.15718

Billen J (1991) Ultrastructural organization of the exocrine glands in Ants. Ethol Ecol Evol 3(sup1):67–73. https://doi.org/10.1080/03949370.1991.10721913

Blaylock RS (2001) Normal oral bacterial flora from some southern african snakes. Onderstepoort J Vet Res 68(3):175–182

Blum S, Hermann Jr HR (1978) Venoms and venom apparatuses of the Formicidae: Dolichoderinae and Aneuretinae. In: Arthropod venoms. Berlin, Heidelberg: Springer Berlin Heidelberg, pp 871–894. https://doi.org/10.1007/978-3-642-45501-8_26.

Bourne DG, Morrow KM, Webster NS (2016) Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. Annu Rev Microbiol 70(1):317–340. https://doi.org/10.1146/annurev-micro-102215-095440

Bridges AR, Owen MD (1984) The morphology of the honey bee (*Apis mellifera* L.) venom gland and reservoir. J Morphol 181(1):69–86. https://doi.org/10.1002/jmor.1051810107

Brodie ED (2009) Toxins and venoms. Curr Biol 19(20):R931–R935. https://doi.org/10.1016/j.cub.2009.08.011

Budnik BA, Olsen JV, Egorov TA, Anisimova VE, Galkina TG, Musolyamov AK, Grishin EV, Zubarev RA (2004) De novo sequencing of antimicrobial peptides isolated from the venom glands of the wolf spider *Lycosa singoriensis*. J Mass Spectrometry: JMS 39(2):193–201. https://doi.org/10.1002/jms.577

Bull JJ, Jessop TS, Whiteley M (2010) Deathly drool: evolutionary and ecological basis of septic bacteria in *Varanus komodoensis* dragon mouths. PLoS ONE 5(6):e11097. https://doi.org/10.1371/journal.pone.0011097

Calvete JJ (2017) Venomics: integrative venom proteomics and beyond. Biochem J 474(5):611–634. https://doi.org/10.1042/BCJ20160577

Calvete JJ, Juárez P, Sanz L (2007) Snake venomics. Strategy and applications. J Mass Spectrometry: JMS 42(11):1405–1414. https://doi.org/10.1002/jms.1242

Cao Z, Yao Yu, Yingliang Wu, Hao P, Di Z, He Y, Chen Z et al (2013) The genome of *Mesobuthuss martensii* reveals a unique adaptation model of arthropods. Nat Commun 4(1):2602. https://doi.org/10.1038/ncomms3602

Castañeda O, Sotolongo V, Amor AM, Stöcklin R, Anderson AJ, Harvey AL, Engström A, Wernstedt C, Karlsson E (1995) Characterization of a potassium channel toxin from the caribbean sea anemone *Stichodactyla helianthus*. Toxicon: 33(5):603–13. https://doi.org/10.1016/0041-0101(95)00013-c

Chau R, Kalaitzis JA, Neilan BA (2011) On the origins and biosynthesis of tetrodotoxin. Aquat Toxicol 104(1):61–72. https://doi.org/10.1016/j.aquatox.2011.04.001

Chen J, Zhao Y, Li X-C, Zhao J-H (2019) Pyridine alkaloids in the venom of imported fire ants. J Agric Food Chem 67(41):11388–11395. https://doi.org/10.1021/acs.jafc.9b03631

Cipriani V, Debono J, Goldenberg J, Jackson TNW, Arbuckle K, Dobson J, Koludarov I et al (2017) Correlation between ontogenetic dietary shifts and venom variation in Australian brown snakes (*Pseudonaja*). comparative biochemistry and physiology. Toxicol

Pharmacol: CBP 197:53–60. https://doi.org/10.1016/j.cbpc.2017.04.007
Clark RF, Selden BS, Furbee B (1993) The incidence of wound infection following crotalid envenomation. J Emerg Med 11(5):583–586. https://doi.org/10.1016/0736-4679(93)90313-V
Clinton M, Ferrier DEK, Martin SAM, Brierley AS (2021) Impacts of jellyfish on marine cage aquaculture: an overview of existing knowledge and the challenges to finfish health. ICES J Mar Sci 78(5):1557–1573. https://doi.org/10.1093/icesjms/fsaa254
Clooney AG, Fiona F, Sleator RD, O'Driscoll A, Stanton C, Cotter PD, Claesson MJ (2016) Comparing apples and oranges?: Next generation sequencing and its impact on microbiome analysis. PloS One 11(2):e0148028. https://doi.org/10.1371/journal.pone.0148028
Colston TJ, Jackson CR (2016) Microbiome evolution along divergent branches of the vertebrate tree of life: What is known and unknown. Mol Ecol 25(16):3776–3800. https://doi.org/10.1111/mec.13730
Copley TR, Jabaji SH (2012) Honeybee glands as possible infection reservoirs of *Nosema ceranae* and *Nosema apis* in naturally infected forager bees. J Appl Microbiol 112(1):15–24. https://doi.org/10.1111/j.1365-2672.2011.05192.x
Costello EK, Gordon JI, Secor SM, Knight R (2010) Postprandial remodeling of the gut microbiota in burmese pythons. ISME J 4(11):1375–1385. https://doi.org/10.1038/ismej.2010.71
Daly JW, Secunda SI, Garraffo HM, Spande TF, Wisnieski A, Cover JF (1994) An uptake system for dietary alkaloids in poison frogs (Dendrobatidae). Toxicon: 32(6):657–63. https://doi.org/10.1016/0041-0101(94)90335-2
Daly M, Brugler MR, Cartwright P, Collins AG, Dawson MN, Fautin DG, France SC, Opresko DM, Rodriguez E, Romano SL (2007) The Phylum Cnidaria: a review of phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa 1668(1):127–82. https://doi.org/10.11646/zootaxa.1668.1.11
Darst CR, Menéndez-Guerrero PA, Coloma LA, Cannatella DC (2005) Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): A Comparative Analysis. Am Nat 165(1):56–69. https://doi.org/10.1086/426599
Debat HJ (2017) An rna virome associated to the golden orb-weaver spider *Nephila clavipes*. Front Microbiol 8:2097.https://doi.org/10.3389/fmicb.2017.02097
Dehghani R, Sharif MR, Moniri R, Sharif A, Kashani HH (2016) The identification of bacterial flora in oral cavity of snakes. Comp Clin Pathol 25(2):279–283. https://doi.org/10.1007/s00580-015-2178-9
Dicke M, Cusumano A, Poelman EH (2020) Microbial symbionts of parasitoids. Annu Rev Entomol 65(January):171–190. https://doi.org/10.1146/annurev-ento-011019-024939
Dillon RJ, Charnley AK (1995) Chemical barriers to gut infection in the desert locust. in vivo production of antimicrobial phenols associated with the bacterium *Pantoea agglomerans*. J Invertebr Pathol 66(1):72–75. https://doi.org/10.1006/jipa.1995.1063
Domingo E, Sheldon J, Perales C (2012) Viral quasispecies evolution. Microbiol Mol Biol Rev 76(2):159–216. https://doi.org/10.1128/MMBR.05023-11
Dugon M (2015) Evolution, morphology and development of the centipede venom system. https://doi.org/10.1007/978-94-007-6727-0_1-1.
Dumbacher JP, Wako A, Derrickson SR, Samuelson A, Spande TF, Daly JW (2004) Melyrid beetles (Choresine): a putative source for the batrachotoxin alkaloids found in poison-dart frogs and toxic passerine birds. Proc Natl Acad Sci USA 101(45):15857–15860. https://doi.org/10.1073/pnas.0407197101
Dunaj SJ, Bettencourt BR, Garb JE, Brucker RM (2020) Spider phylosymbiosis: divergence of widow spider species and their tissues' microbiomes. BMC Evol Biol 20(1):104. https://doi.org/10.1186/s12862-020-01664-x
Dunbar JP, Khan NA, Abberton CL, Brosnan P, Murphy J, Afoullouss S, O'Flaherty V, Dugon MM, Boyd A (2020) Synanthropic spiders, including the global invasive noble false widow *Steatoda nobilis*, are reservoirs for medically important and antibiotic resistant bacteria. Sci Rep 10(1):20916. https://doi.org/10.1038/s41598-020-77839-9
Dunbar JP, Sulpice R, Dugon MM (2019) The kiss of (cell) death: can venom-induced immune response contribute to dermal necrosis following arthropod envenomations? Clin Toxicol 57(8):677–685. https://doi.org/10.1080/15563650.2019.1578367
Dunn AK, Stabb EV (2005) Culture-independent characterization of the microbiota of the ant lion *Myrmeleon mobilis* (Neuroptera: Myrmeleontidae). Appl Environ Microbiol 71(12):8784–8794. https://doi.org/10.1128/AEM.71.12.8784-8794.2005
Dutertre S, Jin A-H, Vetter I, Hamilton B, Sunagar K, Lavergne V, Dutertre V et al (2014) Evolution of separate predation- and defence-evoked venoms in carnivorous cone snails. Nat Commun 5(1):3521. https://doi.org/10.1038/ncomms4521
Dutta D, Clevers H (2017) Organoid culture systems to study host-pathogen interactions. Curr Opin Immunol 48(October):15–22. https://doi.org/10.1016/j.coi.2017.07.012
Dzik JM (2006) Molecules released by helminth parasites involved in host colonization. Acta Biochim Pol 53(1):33–64
Eliades SJ, Brown JC, Colston TJ, Fisher RN, Niukula JB, Gray K, Vadada J, Rasalato S, Siler CD (2021) Gut microbial ecology of the critically endangered fijian crested iguana (*Brachylophus vitiensis*): effects of captivity status and host reintroduction on endogenous microbiomes. Ecol Evol 11(9):4731–4743. https://doi.org/10.1002/ece3.7373
Esmaeilishirazifard E, Usher L, Trim C, Denise H, Sangal V, Tyson GH, Barlow A et al (2022) Bacterial adaptation to venom in snakes and Arachnida. Microbiology Spectrum 10(3):e02408-e2421. https://doi.org/10.1128/spectrum.02408-21
Fautin DG (2009) Structural diversity, systematics, and evolution of Cnidae. Toxicon: 54(8):1054–64. https://doi.org/10.1016/j.toxicon.2009.02.024
Forster YM, Reusser S, Forster F, Bienz S, Bigler L (2020) VenoMS—A website for the low molecular mass compounds in spider venoms. Metabolites 10(8):327. https://doi.org/10.3390/metabo10080327
Fountain-Jones NM, Giraud T, Zinger L, Bik H, Creer S, Videvall E (2023) Molecular ecology of microbiomes in the wild: common pitfalls, methodological advances and future directions. Mol Ecol 33(2):e17223. https://doi.org/10.1111/mec.17223
Fox EGP, Meng Xu, Wang L, Chen Li, Yong-Yue Lu (2018) Gas-chromatography and uv-spectroscopy of Hymenoptera venoms obtained by trivial centrifugation. Data Brief 18(June):992–998. https://doi.org/10.1016/j.dib.2018.03.101
Fox EGP, Adams RM (2022) On the biological diversity of ant alkaloids. Annual Rev Entomol 67(1):367–385. https://doi.org/10.1146/annurev-ento-072821-063525
Fry BG, Vidal N, Norman JA, Vonk FJ, Holger Scheib SF, Ramjan R, Kuruppu S et al (2006) Early evolution of the venom system in lizards and snakes. Nature 439(7076):584–588. https://doi.org/10.1038/nature04328
Fry BG, Wroe S, Teeuwisse W, van Osch MJP, Moreno K, Ingle J, McHenry C et al (2009) A central role for venom in predation by *Varanus komodoensis* (Komodo dragon) and the extinct giant *Varanus (megalania) priscus*. Proc Natl Acad Sci USA 106(22):8969–8974. https://doi.org/10.1073/pnas.0810883106
García-Santibañez T, Rosenblueth M, Bolaños LM, Martínez-Romero J, Martínez-Romero E (2022) The divergent genome of scorpion group 1 (SG1) intracellular bacterium from the venom glands of *Vaejovis smithi*. Syst Appl Microbiol 45(6):126358. https://doi.org/10.1016/j.syapm.2022.126358
Gardiner M, Weldon A, Poindexter SA, Gibson N, Anna K, Nekaris I (2018) Survey of practitioners handling slow lorises (Primates:

Nycticebus): an assessment of the harmful effects of slow loris bites. J Venom Res 9:1–7
Garg A, Sujatha S, Jaya Garg N, Acharya S, Parija SC (2009) Wound infections secondary to snakebite. J Infect Dev Ctries 3(3):221–223. https://doi.org/10.3855/jidc.39
Gatti JL, Schmitz A, Colinet D, Poirie M (2012) Diversity of virus-like particles in parasitoids' venom: viral or cellular origin?. Parasitoid viruses 181-92.https://doi.org/10.1016/B978-0-12-384858-1.00015-1.
Gaver-Wainwright MM, Zack RS, Foradori MJ, Lavine LC (2011) misdiagnosis of spider bites: bacterial associates, mechanical pathogen transfer, and hemolytic potential of venom from the hobo spider, *Tegenaria agrestis* (Araneae: Agelenidae). J Med Entomol 48(2):382–388. https://doi.org/10.1603/me09224
Geffeney SL, Williams BL, Rosenthal JJC, Birk MA, Felkins J, Wisell CM, Curry ER, Hanifin CT (2019) Convergent and parallel evolution in a voltage-gated sodium channel underlies ttx-resistance in the greater blue-ringed octopus: *Hapalochlaena lunulata*. Toxicon Off J Int Society Toxinol 170(December):77–84. https://doi.org/10.1016/j.toxicon.2019.09.013
Ghosh T, Mohan KB, Pradipta R, Chiranjib G (2018) Short review of different microflora from the oral cavity of snakes. Uttar Pradesh J Zool 37(1–2):30 34. https://mbimph.com/index.php/UPJOZ/article/view/30
Gibbons SM, Gilbert JA (2015) Microbial diversity-exploration of natural ecosystems and microbiomes. Curr Opin Genet Dev 35(December):66–72. https://doi.org/10.1016/j.gde.2015.10.003
Gibson CM, Hunter MS (2009) Negative fitness consequences and transmission dynamics of a heritable fungal symbiont of a parasitic wasp. Appl Environ Microbiol 75(10):3115–3119. https://doi.org/10.1128/AEM.00361-09
Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE (2006) Metagenomic analysis of the human distal gut microbiome. Science 312(5778):1355–9. https://doi.org/10.1126/science.1124234
Goldstein EJC, Citron DM, Gonzalez H, Russell FE, Finegold SM (1979) Bacteriology of rattlesnake venom and implications for therapy. J Infect Dis 140(5):818–821. https://doi.org/10.1093/infdis/140.5.818
Goldstein EJC, Tyrrell KL, Citron DM, Cox CR, Recchio IM, Okimoto B, Bryja J, Fry BG (2013) Anaerobic and aerobic bacteriology of the saliva and gingiva from 16 captive Komodo dragons (*Varanus komodoensis*): new implications for the 'bacteria as venom' model. J Zoo Wildl Med 44(2):262–272
Goodswen SJ, Kennedy PJ, Ellis JT (2022) Compilation of parasitic immunogenic proteins from 30 years of published research using machine learning and natural language processing. Sci Rep 12(June):10349. https://doi.org/10.1038/s41598-022-13790-1
Grow NB, Nekaris KAI (2015) Does toxic defence in *Nycticebus* spp. relate to ectoparasites? the lethal effects of slow loris venom on arthropods. Toxicon 95:1–5. https://doi.org/10.1016/j.toxicon.2014.12.005
Gueguen G, Rajwani R, Paddibhatla I, Morales J, Govind S (2011) VLPs of Leptopilina *boulardi* share biogenesis and overall stellate morphology with VLPs of the heterotoma clade. Virus Res 160(1–2):159–165. https://doi.org/10.1016/j.virusres.2011.06.005
Harris RJ, Arbuckle K (2016) Tempo and mode of the evolution of venom and poison in tetrapods. Toxins 8(7):193. https://doi.org/10.3390/toxins8070193
Hauke TJ, Herzig V (2017) Dangerous arachnids—fake news or reality? Toxicon 138(November):173–183. https://doi.org/10.1016/j.toxicon.2017.08.024
Hernández-Elizárraga VH, Ocharán-Mercado A, Olguín-López N, Hernández-Matehuala R, Caballero-Pérez J, Ibarra-Alvarado C, Rojas-Molina A (2022) New insights into the toxin diversity and antimicrobial activity of the 'fire coral' *Millepora complanata*. Toxins 14(3):206. https://doi.org/10.3390/toxins14030206
Herzig V, Cristofori-Armstrong B, Israel MR, Nixon SA, Vetter I, King GF (2020) Animal toxins — nature's evolutionary-refined toolkit for basic research and drug discovery. Biochem pharmacol, pharmacol therapeutic potential venom peptides 181(November):114096. https://doi.org/10.1016/j.bcp.2020.114096
Hink WF, Butz JJ (1985) Primary culture of ant venom gland cells. In Vitro Cellular Dev Biol: J Tissue Culture Assoc 21(6):333–339. https://doi.org/10.1007/BF02691581
Hink WF Jr (1985) Process and medium for culturing ant venom gland cells. EP0141182A2, filed September 11, 1984, and issued May 15, 1985. https://worldwide.espacenet.com/patent/search/family/024191946/publication/EP0141182A2?q=pn%3DEP0141182A2. Accessed 10/21/2023
Holding ML, Strickland JL, Rautsaw RM, Hofmann EP, Mason AJ, Hogan MP, Nystrom GS et al (2021) Phylogenetically diverse diets favor more complex venoms in north american pitvipers. Proc Natl Acad Sci 118(17):e2015579118. https://doi.org/10.1073/pnas.2015579118
Hu H, Bandyopadhyay PK, Olivera BM, Yandell M (2012) elucidation of the molecular envenomation strategy of the cone snail *Conus geographus* through transcriptome sequencing of its venom duct. BMC Genomics 13(1):284. https://doi.org/10.1186/1471-2164-13-284
Hu Y, Zhiling Y, Yang ZJ, Zhu G, Fong W (2011) Comprehensive chemical analysis of venenum bufonis by using liquid chromatography/electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal 56(2):210–220. https://doi.org/10.1016/j.jpba.2011.05.014
Huang J, Chen J, Fang G, Pang L, Zhou S, Zhou Y, Pan Z et al (2021) Two novel venom proteins underlie divergent parasitic strategies between a generalist and a specialist parasite. Nat Commun 12(1):234. https://doi.org/10.1038/s41467-020-20332-8
Hug LA, Baker BJ, Anantharaman K, Brown CT, Probst AJ, Castelle CJ, Butterfield CN et al (2016) A new view of the tree of life. Nat Microbiol 1(5):1–6. https://doi.org/10.1038/nmicrobiol.2016.48
Hutson S (2010) Antivenoms needed, say officials, but companies won't bite. Nat Med 16(6):615. https://doi.org/10.1038/nm0610-615a
Hyde ER, Navas-Molina JA, Song SJ, Kueneman JG, Ackermann G, Cardona C, Humphrey G et al (2016) The oral and skin microbiomes of captive Komodo dragons are significantly shared with their habitat. mSystems 1(4):e00046-16. https://doi.org/10.1128/mSystems.00046-16
Isbister GK (2001) Venomous fish stings in Tropical Northern Australia. Am J Emerg Med 19(7):561–565. https://doi.org/10.1053/ajem.2001.28325
Isbister GK, Fan HW (2011) Spider bite. Lancet (London, England) 378(9808):2039–2047. https://doi.org/10.1016/S0140-6736(10)62230-1
Isbister GK, Gray MR (2003) Effects of envenoming by comb-footed spiders of the genera *Steatoda* and *Achaearanea* (Family Theridiidae: Araneae) in Australia. J Toxicol Clin Toxicol 41(6):809–819. https://doi.org/10.1081/CLT-120025346
Isbister GK, Gray MR (2004) Bites by australian mygalomorph spiders (*Araneae*, mygalomorphae), Including funnel-web spiders (Atracinae) and mouse spiders (*Actinopodidae*: *missulena* spp). Toxicon 43(2):133–140. https://doi.org/10.1016/j.toxicon.2003.11.009
Isbister GK, Hirst D (2003) A prospective study of definite bites by spiders of the family *Sparassidae* (huntsmen spiders) with Identification to species level. Toxicon: 42(2):163–71. https://doi.org/10.1016/s0041-0101(03)00129-6
Jayathilake JMNJ, Gunathilake KVK (2020) Cnidarian toxins: recent evidences for potential therapeutic uses. Eur Zoological J 87(1):708–713. https://doi.org/10.1080/24750263.2020.1837268
Ji M, Zhu T, Xing M, Luan N, Mwangi J, Yan X, Mo G et al (2019) An antiviral peptide from *Alopecosa nagpag* spider targets ns2b-ns3

protease of Flaviviruses. Toxins 11(10):584. https://doi.org/10.3390/toxins11100584

Jones TH, Torres JA, Spande TF, Garraffo HM, Blum MS, Snelling RR (1996) Chemistry of venom alkaloids in some *Solenopsis* (*Diplorhoptrum*) species from Puerto Rico. J Chem Ecol 22(7):1221–1236. https://doi.org/10.1007/BF02266962

Jones TH, Gorman JST, Snelling RR, Delabie JHC, Blum MS, Martin Garraffo H, Jain P, Daly JW, Spande TF (1999) Further alkaloids common to ants and frogs: Decahydroquinolines and a Quinolizidine. J Chem Ecol 25(5):1179–1193. https://doi.org/10.1023/A:1020898229304

Jouiaei M, Sunagar K, Gross AF, Scheib H, Alewood PF, Moran Y, Fry BG (2015) Evolution of an ancient venom: recognition of a novel family of cnidarian toxins and the common evolutionary origin of sodium and potassium neurotoxins in sea anemone. Mol Biol Evol 32(6):1598–1610. https://doi.org/10.1093/molbev/msv050

Kaas Q, Craik DJ (2015) Bioinformatics-aided venomics. Toxins 7(6):2159–2187. https://doi.org/10.3390/toxins7062159

Keenan SW, Engel AS, Elsey RM (2013) The alligator gut microbiome and implications for archosaur symbioses. Sci Rep 3(October):2877. https://doi.org/10.1038/srep02877

Kennedy B, Trim SA, Laudier D, LaDouceur EE, Cooper JE (2021) Arthropoda: Arachnida. Invertebrate Histology 221-46. https://doi.org/10.1002/9781119507697.ch8.

Kim C-H, Lee YJ, Go H-J, Hye Young Oh, Lee TK, Park JB, Park NG (2017) Defensin-neurotoxin dyad in a basally branching metazoan sea anemone. FEBS J 284(19):3320–3338. https://doi.org/10.1111/febs.14194

Klein AH, Ballard KR, Storey KB, Motti CA, Zhao M, Cummins SF (2019) Multi-Omics investigations within the phylum Mollusca, class Gastropoda: From ecological application to breakthrough phylogenomic studies. Brief Funct Genomics 18(6):377–394. https://doi.org/10.1093/bfgp/elz017

Knauf S, Abel L, Hallmaier-Wacker LK (2019) The Nagoya protocol and research on emerging infectious diseases. Bull World Health Organ 97(6):379. https://doi.org/10.2471/BLT.19.232173

Koludarov I, Jackson TN, Op B, den Brouw J, Dobson DD, Arbuckle K, Clemente CJ et al (2017) Enter the dragon: The dynamic and multifunctional evolution of Anguimorpha lizard venoms. Toxins 9(8):242. https://doi.org/10.3390/toxins9080242

Krishnankutty SP, Muraleedharan M, Perumal RC, MS, Benny J, Balan B, Kumar P et al (2018) Next-generation sequencing analysis reveals high bacterial diversity in wild venomous and non-venomous snakes from India. J Venomous Animals Toxins Tropical Dis 24:41. https://doi.org/10.1186/s40409-018-0181-8

Kuhn-Nentwig L, Stöcklin R, Nentwig W (2011) "Venom composition and strategies in spiders: is everything possible?" In *Advances in Insect Physiology*, edited by Jérôme Casas, 40:1–86. Academic Press, Spider Physiology and Behaviour. https://doi.org/10.1016/B978-0-12-387668-3.00001-5

Kvetkina A, Kostina E, Gladkikh I, Chausova V, Yurchenko E, Bakunina I, Pivkin M et al (2021) Deep-sea anemones are prospective source of new antimicrobial and cytotoxic compounds. Mar Drugs 19(12):654. https://doi.org/10.3390/md19120654

Lam KK, Crow P, Ng KHL, Shek KC, Fung HT, Ades G, Grioni A et al (2011) A Cross-sectional survey of snake oral bacterial flora from Hong Kong, SAR, China. Emergency Med J: EMJ 28(2):107–114. https://doi.org/10.1136/emj.2009.086694

Langenegger N, Nentwig W, Kuhn-Nentwig L (2019) Spider venom: components, modes of action, and novel strategies in transcriptomic and proteomic analyses. Toxins 11(10):611. https://doi.org/10.3390/toxins11100611

Lazarev VN, Polina NF, Shkarupeta MM, Kostrjukova ES, Vassilevski AA, Kozlov SA, Grishin EV, Govorun VM (2011) Spider venom peptides for gene therapy of *Chlamydia* infection. Antimicrob Agents Chemother 55(11):5367–5369. https://doi.org/10.1128/AAC.00449-11

Lebeck LM (1989) Extracellular symbiosis of a yeast-like microorganism within Comperia *merceti* (Hymenoptera: Encyrtidae). Symbiosis 7:51–56

Lenoir A, Devers S (2018) Alkaloid secretion inhibited by antibiotics in *Aphaenogaster* ants. CR Biol 341(6):358–361. https://doi.org/10.1016/j.crvi.2018.06.004

Lewis RJ, Dutertre S, Vetter I, Christie MJ (2012) *Conus* venom peptide pharmacology. Pharmacol Rev 64(2):259–98. https://doi.org/10.1124/pr.111.005322

Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI (2008) Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol 6(10):776–788. https://doi.org/10.1038/nrmicro1978

Liao Q, Feng Yu, Yang B, Lee S-Y (2019) Cnidarian peptide neurotoxins: a new source of various ion channel modulators or blockers against central nervous systems disease. Drug Discovery Today 24(1):189–197. https://doi.org/10.1016/j.drudis.2018.08.011

Liu JN, Wang TH, Jia QY, Gao XH, Wan H, Sun WY, Yang XL, Bao R, Liu JZ, Yu ZJ (2016) Characterization of the microbial communities in the ant lion *Euroleon coreanus* (Okamoto) (Neuroptera: Myrmeleontidae). Neotrop Entomol 45(4):397–403. https://doi.org/10.1007/s13744-016-0388-8

Logashina YA, Solstad RG, Mineev KS, Korolkova YV, Mosharova IV, Dyachenko IA, Palikov VA et al (2017) New disulfide-stabilized fold provides sea anemone peptide to exhibit both antimicrobial and TRPA1 potentiating properties. Toxins 9(5):154. https://doi.org/10.3390/toxins9050154

Lüddecke T, Herzig V, von Reumont BM, Vilcinskas A (2022) The biology and evolution of spider venoms. Biol Rev 97(1):163–178. https://doi.org/10.1111/brv.12793

Lukač M, Horvatek Tomić D, Mandac Z, Mihoković S, Prukner-Radovčić E (2017) Aerobna bakterijska i gljivična flora usne šupljine i kloake kravosasa (*Elaphe quatuorlineata*) u Hrvatskoj. Veterinarski arhiv 87(3):351–361. https://doi.org/10.24099/vet.arhiv.151216

Mackessy SP (2009) Handbook of venoms and toxins of reptiles. 1st Edition. Boca Raton: CRC Press. https://www.cabdirect.org/cabdirect/abstract/20113300380. Accessed 3/4/2024

Mackessy SP, Baxter LM (2006) Bioweapons synthesis and storage: the venom gland of front-fanged snakes. Zoologischer Anzeiger - J Comparative Zoology 245(3):147–159. https://doi.org/10.1016/j.jcz.2006.01.003

Mariottini GL, Grice ID (2016) Antimicrobials from cnidarians. a new perspective for anti-infective therapy? Mar Drugs 14(3):48. https://doi.org/10.3390/md14030048

Mariottini GL, Pane L (2014) Cytotoxic and cytolytic cnidarian venoms. A review on health implications and possible therapeutic applications. Toxins 6(1):108–151. https://doi.org/10.3390/toxins6010108

Marshall J, Kelley WP, Rubakhin SS, Bingham J-P, Sweedler JV, Gilly WF (2002) Anatomical correlates of venom production in *Conus californicus*. Biol Bull 203(1):27–41. https://doi.org/10.2307/1543455

Martiny JBH, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. Nature Rev Microbiol 4(2):102–112. https://doi.org/10.1038/nrmicro1341

Mitra A (2013) Function of the Dufour's gland in solitary and social Hymenoptera. J Hymenopt Res 35(October):33–58

"MolluscaBase Eds" (2024) https://doi.org/10.14284/448.

Monteiro CLB, Rubel R, Cogo LL, Mangili OC, Gremski W, Veiga SS (2002) Isolation and identification of *Clostridium perfringens* in the venom and fangs of *Loxosceles intermedia* (brown spider): enhancement of the dermonecrotic lesion in loxoscelism. Toxicon 40(4):409–418. https://doi.org/10.1016/s0041-0101(01)00209-4

Montgomery JM, Gillespie D, Sastrawan P, Fredeking TM, Stewart GL (2002) Aerobic salivary bacteria in wild and captive Komodo

dragons. J Wildl Dis 38(3):545–551. https://doi.org/10.7589/0090-3558-38.3.545

Morales J, Chiu H, Oo T, Plaza R, Hoskins S, Govind S (2005) Biogenesis, structure, and immune-suppressive effects of virus-like particles of a *Drosophila* parasitoid, (*Leptopilina victoriae*). J Insect Physiol 51(2):181–195. https://doi.org/10.1016/j.jinsphys.2004.11.002

Moran Y, Genikhovich G, Gordon D, Wienkoop S, Zenkert C, Özbek S, Technau U, Gurevitz M (2012) Neurotoxin localization to ectodermal gland cells uncovers an alternative mechanism of venom delivery in sea anemones. Proceedings Royal Society b: Biol Sci 279(1732):1351–1358. https://doi.org/10.1098/rspb.2011.1731

Nekaris K-I, Moore RS, Johanna Rode E, Fry BG (2013) Mad, bad and dangerous to know: the biochemistry, ecology and evolution of slow loris venom. J Venomous Animals Toxins Including Trop Dis 19(September):1–10. https://doi.org/10.1186/1678-9199-19-21

Nishiwaki H, Ito K, Otsuki K, Yamamoto H, Komai K, Matsuda K (2004) Purification and functional characterization of insecticidal sphingomyelinase c produced by *Bacillus cereus*. Eur J Biochem 271(3):601–606. https://doi.org/10.1111/j.1432-1033.2003.03962.x

Nishiwaki H, Ito K, Shimomura M, Nakashima K, Matsuda K (2007a) Insecticidal bacteria isolated from predatory larvae of the antlion species *Myrmeleon bore* (Neuroptera: Myrmeleontidae). J Invertebr Pathol 96(1):80–88. https://doi.org/10.1016/j.jip.2007.02.007

Nishiwaki H, Nakashima K, Ishida C, Kawamura T, Matsuda K (2007b) Cloning, functional characterization, and mode of action of a novel insecticidal pore-forming toxin, sphaericolysin, produced by *Bacillus sphaericus*. Appl Environ Microbiol 73(10):3404–3411. https://doi.org/10.1128/AEM.00021-07

Nixon SA, Robinson SD, Agwa AJ, Walker AA, Choudhary S, Touchard A, Undheim EAB et al (2021) Multipurpose peptides: the venoms of amazonian stinging ants contain anthelmintic ponericins with diverse predatory and defensive activities. Biochem Pharmacol 192(October):114693. https://doi.org/10.1016/j.bcp.2021.114693

O'Brien PA, Webster NS, Miller DJ, Bourne DG (2019) Host-microbe coevolution: applying evidence from model systems to complex marine invertebrate holobionts. MBio 10(1):10–1128. https://doi.org/10.1128/mbio.02241-18.10.1128/mbio.02241-18

O'Hara EP, Wilson D, Seymour JE (2021) The influence of ecological factors on cnidarian venoms. Toxicon X 9–10(July):100067. https://doi.org/10.1016/j.toxcx.2021.100067

de Oliveira Domingos M, Neves IV, Vigerelli H, Pimenta DC, Távora BD, Lemos TJ, Franzolin MR, Marques VD, Barbaro KC (2018) The potential of *Loxosceles gaucho* spider venom to regulate *Pseudomonas aeruginosa* mechanisms of virulence. Toxicon. 15(152):78–83. https://doi.org/10.1016/j.toxicon.2018.07.017

Osmakov DI, Kozlov SA, Andreev YA, Koshelev SG, Sanamyan NP, Sanamyan KE, Dyachenko IA et al (2013) Sea anemone peptide with uncommon β-hairpin structure inhibits acid-sensing ion channel 3 (ASIC3) and reveals analgesic activity. J Biol Chem 288(32):23116–23127. https://doi.org/10.1074/jbc.M113.485516

Ozbek S (2011) The cnidarian nematocyst: a miniature extracellular matrix within a secretory vesicle. Protoplasma 248(4):635–640. https://doi.org/10.1007/s00709-010-0219-4

Pankewitz F, Hilker M (2008) Polyketides in insects: ecological role of these widespread chemicals and evolutionary aspects of their biogenesis. Biol Rev Camb Philos Soc 83(2):209–226. https://doi.org/10.1111/j.1469-185X.2008.00040.x

Peraud O, Biggs JS, Hughen RW, Light AR, Concepcion GP, Olivera BM, Schmidt EW (2009) Microhabitats within venomous cone snails contain diverse actinobacteria. Appl Environ Microbiol 75(21):6820–6826. https://doi.org/10.1128/AEM.01238-09

Peters RS, Krogmann L, Mayer C, Donath A, Gunkel S, Meusemann K, Kozlov A et al (2017) Evolutionary history of the *Hymenoptera*. Curr Biol 27(7):1013–1018. https://doi.org/10.1016/j.cub.2017.01.027

Poulin R, Morand S (2000) The diversity of parasites. Q Rev Biol 75(3):277–293. https://doi.org/10.1086/393500

Pulford CV, Wenner N, Redway ML, Rodwell EV, Webster HJ, Escudero R, Kröger C et al (2019) The diversity, evolution and ecology of *Salmonella* in venomous Snakes. PLoS Negl Trop Dis 13(6):e0007169. https://doi.org/10.1371/journal.pntd.0007169

Qin Z, Wang S, Guo D, Zhu J, Chen H, Bai Le, Luo X, Yin Y (2019) Comparative analysis of intestinal bacteria among venom secretion and non-secretion snakes. Sci Rep 9(1):6335. https://doi.org/10.1038/s41598-019-42787-6

Quezada M, Licona-Cassani C, Cruz-Morales P, Salim AA, Marcellin E, Capon RJ, Barona-Gomez F (2017) Diverse cone-snail species harbor closely related streptomyces species with conserved chemical and genetic profiles, including polycyclic tetramic acid macrolactams. Front Microbiol 8:2305. https://doi.org/10.3389/fmicb.2017.02305

Ramírez-Castañeda V, Westeen EP, Frederick J, Amini S, Wait DR, Achmadi AS, Andayani N et al (2022) A set of principles and practical suggestions for equitable fieldwork in biology. Proc Natl Acad Sci 119(34):e2122667119. https://doi.org/10.1073/pnas.2122667119

Rivera-Ortega J, Thomé PE (2018) Contrasting antibacterial capabilities of the surface mucus layer from three symbiotic cnidarians. Front Marine Sci 5. https://doi.org/10.3389/fmars.2018.00392.

Rizki TM, Rizki RM, Carton Y (1990) *Leptopilina heterotoma* and *l. Boulardi*: Strategies to avoid cellular defense responses of *Drosophila melanogaster*. Exp Parasitol 70(4):466–475. https://doi.org/10.1016/0014-4894(90)90131-U

Roberts AJ, Suttle CA (2023) Pathogens and passengers: roles for crustacean zooplankton viruses in the global ocean. Microorganisms 11(4):1054. https://doi.org/10.3390/microorganisms11041054

Rojas MG, Morales-Ramos JA, Chen J (2011) Ingested boric acid effect on the venom chemistry of *Solenopsis invicta buren* (Hymenoptera: Formicidae). J Entomological Sci 46(2):89–95. https://doi.org/10.18474/0749-8004-46.2.89

Rosenberg E, Zilber-Rosenberg I (2018) The hologenome concept of evolution after 10 years. Microbiome 6(1):78. https://doi.org/10.1186/s40168-018-0457-9

Samy PR, Gopalakrishnakone P, Stiles BG, Girish KS, Swamy SN, Hemshekhar M, Tan KS, Rowan EG, Sethi G, Chow VTK (2012) Snake venom phospholipases A2: a novel tool against bacterial diseases. Current Medicinal Chem 19(36):6150–62. https://doi.org/10.2174/092986712804485791

Salvia R, Scieuzo C, Boschi A, Pezzi M, Mistri M, Munari C, Chicca M et al (2023) An overview of ovarian calyx fluid proteins of *Toxoneuron nigriceps* (Viereck) (Hymenoptera: Braconidae): an integrated transcriptomic and proteomic approach. Biomolecules 13(10):1547. https://doi.org/10.3390/biom13101547

Sanjuán R (2010) Mutational fitness effects in rna and single-stranded DNA viruses: common patterns revealed by site-directed mutagenesis studies. Philosophical Trans Royal Society b: Biol Sci 365(1548):1975–1982. https://doi.org/10.1098/rstb.2010.0063

Saporito RA, Donnelly MA, Norton RA, Martin Garraffo H, Spande TF, Daly JW (2007) Oribatid mites as a major dietary source for alkaloids in poison frogs. Proc Natl Acad Sci USA 104(21):8885–8890. https://doi.org/10.1073/pnas.0702851104

Savage R-L, Maud JL, Kellogg CTE, Hunt BPV, Tai V (2023) Symbiont diversity in the eukaryotic microbiomes of marine crustacean zooplankton. J Plankton Res 45(2):338–359. https://doi.org/10.1093/plankt/fbad003

Savoca S, Di Fresco D, Alesci A, Capillo G, Spanò N (2022) Mucus secretions in cnidarian, an ecological, adaptive and evolutive tool. Advances in Oceanography and Limnology 13(2). https://doi.org/10.4081/aiol.2022.11054.

Schendel V, Rash LD, Jenner RA, Undheim EAB (2019) The diversity of venom: The importance of behavior and venom system morphology in understanding its ecology and evolution. Toxins 11(11):666. https://doi.org/10.3390/toxins11110666

Schonour RB, Huff EM, Holding ML, Claunch NM, Ellsworth SA, Hogan MP, Wray K et al (2020) Gradual and discrete ontogenetic shifts in rattlesnake venom composition and assessment of hormonal and ecological correlates. Toxins 12(10):659. https://doi.org/10.3390/toxins12100659

Schroeder FC, Taggi AE, Gronquist M, Malik RU, Grant JB, Eisner T, Meinwald J (2008) NMR-spectroscopic screening of spider venom reveals sulfated nucleosides as major components for the brown recluse and related species. Proc Natl Acad Sci 105(38):14283–14287. https://doi.org/10.1073/pnas.0806840105

Shafiee F, Aucoin MG, Jahanian-Najafabadi A (2019) Targeted diphtheria toxin-based therapy: a review article. Front Microbiol 10:2340. https://doi.org/10.3389/fmicb.2019.02340

Shek KC, Tsui KL, Lam KK, Crow P, Ng KH, Ades G, Yip KT, Grioni A, Tan KS, Lung DC, Lam TS (2009) Oral bacterial flora of the Chinese cobra (*Naja atra*) and bamboo pit viper (*Trimeresurus albolabris*) in Hong Kong SAR, China. Hong Kong Med J 15(3):183

Shimwell C, Atkinson L, Graham MR, Murdoch B (2023) A first molecular characterization of the scorpion telson microbiota of *Hadrurus arizonensis* and *Smeringurus mesaensis*. PLoS ONE 18(1):e0277303. https://doi.org/10.1371/journal.pone.0277303

Sierra NC, Gold DA (2024) The evolution of cnidarian stinging cells supports a precambrian radiation of animal predators. Evol Dev 26(2):e12469. https://doi.org/10.1111/ede.12469

Silva LM, Lages CP, Venuto T, Lima RM, Diniz MV, Lage CL, Valentim EH, Baba PF, Pimenta P, Fortes-Dias CL (2008) Primary culture of venom glands from the brazilian armed spider, *Phoneutria nigriventer* (Araneae, Ctenidae). Toxicon: 51(3):428–34. https://doi.org/10.1016/j.toxicon.2007.10.018

Singhal S, Colston TJ, Grundler MR, Smith SA, Costa GC, Colli GR, Craig Moritz R, Pyron A, Rabosky DL (2021) Congruence and conflict in the higher-level phylogenetics of squamate reptiles: an expanded phylogenomic perspective. Syst Biol 70(3):542–557. https://doi.org/10.1093/sysbio/syaa054

Smith SN, Colston TJ, Siler CD (2021) Venomous snakes reveal ecological and phylogenetic factors influencing variation in gut and oral microbiomes. Front Microbiol 12:657754. https://doi.org/10.3389/fmicb.2021.657754

Swanson DL, Vetter RS (2005) Bites of brown recluse spiders and suspected necrotic arachnidism. N Engl J Med 352(7):700–707. https://doi.org/10.1056/NEJMra041184

Tarcha EJ, Olsen CM, Probst P, Peckham D, Muñoz-Elías EJ, Kruger JG, Iadonato SP (2017) Safety and pharmacodynamics of dalazatide, a Kv1.3 channel Inhibitor, in the treatment of plaque psoriasis: A Randomized Phase 1b Trial. PLoS ONE 12(7):e0180762. https://doi.org/10.1371/journal.pone.0180762

The UniProt Consortium (2019) UniProt: A worldwide hub of protein knowledge. Nucleic Acids Res 47(D1):D506–D515. https://doi.org/10.1093/nar/gky1049

Ting PT, Freiman A (2004) The story of *Clostridium botulinum*: from food poisoning to Botox. Clin Med 4(3):258–261. https://doi.org/10.7861/clinmedicine.4-3-258

Torabi E, Behdani M, Chafi MH, Moazzami R, Sabatier J-M, Khalaj V, Shahbazzadeh D, Bagheri KP (2017) Characteristics and lethality of a novel recombinant dermonecrotic venom phospholipase d from *Hemiscorpius* Lepturus. Toxins 9(3):102. https://doi.org/10.3390/toxins9030102

Torres JP, Tianero MD, Robes JMD, Kwan JC, Biggs JS, Concepcion GP, Schmidt EW (2017) *Stenotrophomonas*-like bacteria are widespread symbionts in cone snail venom ducts. Applied Environ Microbiol 83(23):e01418-17. https://doi.org/10.1128/AEM.01418-17

Torres-Larios A, Gurrola GB, Zamudio FZ, Possani LD (2000) Hadrurin, a new antimicrobial peptide from the venom of the scorpion *Hadrurus aztecus*. Eur J Biochem 267(16):5023–5031. https://doi.org/10.1046/j.1432-1327.2000.01556.x

Touchard A, Aili SR, Fox EGP, Escoubas P, Orivel J, Nicholson GM, Dejean A (2016) The biochemical toxin arsenal from ant venoms. Toxins 8(1):30. https://doi.org/10.3390/toxins8010030

Ul-Hasan S, Rodríguez-Román E, Reitzel AM, Adams RM, Herzig V, Nobile CJ, Colston TJ (2019) The emerging field of venom-microbiomics for exploring venom as a microenvironment, and the corresponding initiative for venom associated microbes and parasites (iVAMP). Toxicon: X 4:100016. https://doi.org/10.1016/j.toxcx.2019.100016

Ul-Hasan, S (2019) The utility of marine neogastropod *Californiconus californicus* as a model system for investigation of the venom microbiome [Doctoral dissertation, University of California Merced]. ProQuest

Undheim EAB, Jenner RA (2021) Phylogenetic analyses suggest centipede venom arsenals were repeatedly stocked by horizontal gene transfer. Nat Commun 12(1):818. https://doi.org/10.1038/s41467-021-21093-8

Undheim EAB, Jenner RA, King GF (2016) Centipede venoms as a source of drug leads. Expert Opin Drug Discov 11(12):1139–1149. https://doi.org/10.1080/17460441.2016.1235155

Vaelli PM, Theis KR, Williams JE, O'Connell LA, Foster JA, Eisthen HL (2020) The skin microbiome facilitates adaptive tetrodotoxin production in poisonous newts. Elife 9(April):e53898. https://doi.org/10.7554/eLife.53898

Valderrama-Vernaza M, Ramírez-Pinilla MP, Serrano-Cardozo VH (2009) Diet of the andean frog *Ranitomeya virolinensis* (Athesphatanura:Dendrobatidae). J Herpetol 43(1):114–123. https://doi.org/10.1670/07-247R1.1

Van Marle J, Piek T (1986) Venoms of the Hymenoptera: Biochemical, pharmacological and behavioural aspects. London, UK: Academic Press. https://shop.elsevier.com/books/venoms-of-the-hymenoptera/piek/978-0-12-554770-3

Vetter R, Isbister G, Bush S, Boutin L (2006) Verified bites by yellow sac spiders (Genus *Cheiracanthium*) in the United States and Australia: where is the necrosis? Am J Trop Med Hyg 74(July):1043–1048. https://doi.org/10.4269/ajtmh.2006.74.1043

Vetter RS, Cushing PE, Crawford RL, Royce LA (2003) Diagnoses of brown recluse spider bites (loxoscelism) greatly outnumber actual verifications of the spider in four western American states. Toxicon 42(4):413–418. https://doi.org/10.1016/S0041-0101(03)00173-9

Viswanathan MS, Shekar MC, Arularasan S, Nathani NM, Arumugam S, Patel AK, Gadhvi IR, Joshi CG (2018) In vitro primary culturing of cells and explant tissue of *Conus cumingii* venom duct: Cytotoxicity assessment of their culture supernatant on HEK 293T." Indian J Geo Marine Sci 47(05):1096 1102. https://nopr.niscpr.res.in/bitstream/123456789/44407/1/IJMS%2047%285%29%201096-1102.pdf

Vitt LJ, Caldwell JP (2013) Herpetology: An introductory biology of amphibians and reptiles. Academic Press.

Vogt N (2020) Venomous organoids. Nat methods 17(4):360–361. https://doi.org/10.1038/s41592-020-0806-z

Walker AA, Robinson SD, Hamilton BF, Undheim EAB, King GF (2020) Deadly proteomes: a practical guide to proteotranscriptomics of animal venoms. Proteomics 20(17–18):1900324. https://doi.org/10.1002/pmic.201900324

Walker AA, Robinson SD, Merritt DJ, Cardoso FC, Goudarzi MH, Mercedes RS, Eagles DA et al (2023) Horizontal gene transfer underlies the painful stings of asp caterpillars (Lepidoptera: Megalopygidae). Proc Natl Acad Sci 120(29):e2305871120. https://doi.org/10.1073/pnas.2305871120

Walker AA, Robinson SD, Yeates DK, Jin J, Baumann K, Dobson J, Fry BG, King GF (2018) Entomo-venomics: the evolution, biology and biochemistry of insect venoms. Toxicon 1(154):15–27. https://doi.org/10.1016/j.toxicon.2018.09.004

Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, Nguyen DD et al (2016) Sharing and community curation of mass spectrometry data with global natural products social molecular networking. Nat Biotechnol 34(8):828–837. https://doi.org/10.1038/nbt.3597

Weinbauer MG, Rassoulzadegan F (2004) Are viruses driving microbial diversification and diversity? Environ Microbiol 6(1):1–11. https://doi.org/10.1046/j.1462-2920.2003.00539.x

Whitelaw BL, Cooke IR, Finn J, Zenger K, Strugnell JM (2019) The evolution and origin of tetrodotoxin acquisition in the blue-ringed octopus (Genus *Hapalochlaena*). Aquatic Toxicol (Amsterdam, Netherlands) 206(January):114–122. https://doi.org/10.1016/j.aquatox.2018.10.012

Wigger E, Kuhn-Nentwig L, Nentwig W (2002) The venom optimisation hypothesis: a spider injects large venom quantities only into difficult prey types. Toxicon 40(6):749–752. https://doi.org/10.1016/S0041-0101(01)00277-X

Wilson D, Daly NL (2018) Venomics: A mini-review. high-throughput 7(3):19. https://doi.org/10.3390/ht7030019

Wu DC, Yao J, Ho KS, Lambowitz AM, Wilke CO (2018) Limitations of alignment-free tools in total RNA-seq quantification. BMC Genomics 19(1):510. https://doi.org/10.1186/s12864-018-4869-5

Yan L, Adams ME (1998) Lycotoxins, antimicrobial peptides from venom of the wolf spider *Lycosa carolinensis*. J Biol Chem 273(4):2059–2066. https://doi.org/10.1074/jbc.273.4.2059

Yang, Fan, Jin-Yong Yu, Si-Qi Chen, Dong-Dong Ning, Babar Hassan, and Yi-Juan Xu (2021) Identification and diversity analysis of bacteria in the venom glands of the fire ant, *Solenopsis invicta*, and two other ants (Hymenoptera: Formicidae). Acta Entomologica Sinica 64(9): 1061–69. https://doi.org/10.16380/j.kcxb.2021.09.006.

Yanoviak SP, Kaspari M, Dudley R, Poinar G (2008) Parasite-induced fruit mimicry in a tropical canopy ant. Am Nat 171(4):536–544. https://doi.org/10.1086/528968

Yoshida N, Oeda K, Watanabe E, Mikami T, Fukita Y, Nishimura K, Komai K, Matsuda K (2001) Chaperonin turned insect toxin. Nature 411(6833):44–44. https://doi.org/10.1038/35075148

Zakaria ME, Mohammed AZ (2010) Influence of honey bee diseases on bee venom efficiency. Egyptian J Agricultural Res 88(4):1037–1046

Authors and Affiliations

Marina E. De León[1,2] · Eduardo G. P. Fox[3] · Sara Dunaj[4] · Ronald A. Jenner[5] · Carl N. Keiser[6] · Jason Macrander[7] · Samantha A. Nixon[8,9] · Clarissa J. Nobile[10,11] · Daniel Petras[12] · Eduardo Rodriguez-Roman[13,14] · Anthony J. Saviola[15] · Steven A. Trim[16] · Natascha S. Varona[17] · Justin Yeager[18] · Sabah Ul-Hasan[19] · Volker Herzig[20,21] · Timothy J. Colston[22,23]

✉ Volker Herzig
vherzig@usc.edu.au

✉ Timothy J. Colston
tim@maddreptiles.com

1 University of California Davis, Genome Center, 451 Health Sciences Drive, Davis, CA 95616, USA

2 Smithsonian Tropical Research Institute, Roosevelt Ave. Tupper Building – 401, Panama City, Panamá Province 0843-03092, Panama

3 Programa de Pós-Graduação em Ambiente e Sociedade (PPGAS), Universidade Estadual de Goiás (UEG), Avenida Brasil 435, Quirinópolis GO 75864-970, Brazil

4 Department of Biological Sciences, University of Massachusetts Lowell, Lowell, MA, USA

5 Natural History Museum, Cromwell Road, London SW7 5BD, UK

6 Department of Biology, University of Florida, Gainesville, FL 32611, USA

7 Department of Biology, Florida Southern College, 111 Lake Hollingsworth Drive, Lakeland, FL 33801, USA

8 Cardiovascular Research Institute, University of California, San Francisco, 525 Mission Bay Boulevard, San Francisco, CA 94158, USA

9 Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, Institute for Molecular Bioscience, The University of Queensland, 306 Carmody Road, St Lucia 4067, Australia

10 Department of Molecular and Cell Biology, School of Natural Sciences, University of California Merced, 5200 North Lake Road, Merced, CA 95343, USA

11 Health Sciences Research Institute, University of California Merced, 5200 North Lake Road, Merced, CA 95343, USA

12 Department of Biochemistry, University of California Riverside, Riverside, CA, USA

13 Center for Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Cientificas, Caracas 1020A, Venezuela

14 Department of Biology, Emory University, 1510 Clifton Rd NE, Atlanta, GA 30322, USA

15 Department of Biochemistry and Molecular Genetics, University of Colorado Anschutz Medical Campus, 12801 East 17Th Avenue, Aurora, CO, USA

16 Venomtech Ltd, Discovery Park, Sandwich, Kent CT13 9FE, UK

17 Biology Department, University of Miami, 1301 Memorial Dr, Coral Gables, FL 33133, USA

18 Facultad de Ingenierías y Ciencias Aplicadas, Grupo de Biodiversidad Medio Ambiente y Salud, Universidad de Las Américas, Quito, Ecuador

19 Hologic, Inc, San Diego, CA, USA

20 Centre for Bioinnovation, University of the Sunshine Coast, Sippy Downs, QLD 4556, Australia

21 School of Science, Technology and Engineering, University of the Sunshine Coast, Sippy Downs, QLD 4556, Australia

22 Department of Biology, University of Puerto Rico at Mayagüez, Call Box 9000, Mayagüez, Puerto Rico 00681

23 Genomic Resources Collection, University of Puerto Rico at Mayagüez, Call Box 9000, Mayagüez, Puerto Rico 00681