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The emerging field of venom-microbiomics for exploring venom as a microenvironment, and the corresponding Initiative for Venom Associated Microbes and Parasites (iVAMP)

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ABSTRACT

Venom is a known source of novel antimicrobial natural products. The substantial, increasing number of these discoveries have unintentionally culminated in the misconception that venom and venom-producing glands are largely sterile environments. Culture-dependent and -independent studies on the microbial communities in venom microenvironments reveal the presence of archaea, algae, bacteria, fungi, protozoa, and viruses. Venom-centric microbiome studies are relatively sparse to date with the adaptive advantages that venom-associated microbes might offer to their hosts, or that hosts might provide to venom-associated microbes, remaining largely unknown. We highlight the potential for the discovery of venom microbiomes within the adaptive landscape of venom systems. The considerable number of convergently evolved venomous animals, juxtaposed with the comparatively few known studies to identify microbial communities in venom, provides new possibilities for both biodiversity and therapeutic discoveries. We present an evidence-based argument for integrating microbiology as part of venomics (i.e., venom-microbiomics) and introduce iVAMP, the Initiative for Venom Associated Microbes and Parasites (<https://ivamp-consortium.github.io/>), as a growing collaborative consortium. We express commitment to the diversity, inclusion and scientific collaboration among researchers interested in this emerging subdiscipline through expansion of the iVAMP consortium.

1. Text

While scientific research in toxinology and microbiology has persisted for centuries, a cursory search of the literature reveals less than

150 studies overlap between these two fields despite each significantly advancing as a result of next generation sequencing technology (Fig. 1, Supplemental Table 1, Supplemental Code). The integration of genomics (Moran and Gurevitz, 2006), transcriptomics (Pahari et al., 2007),

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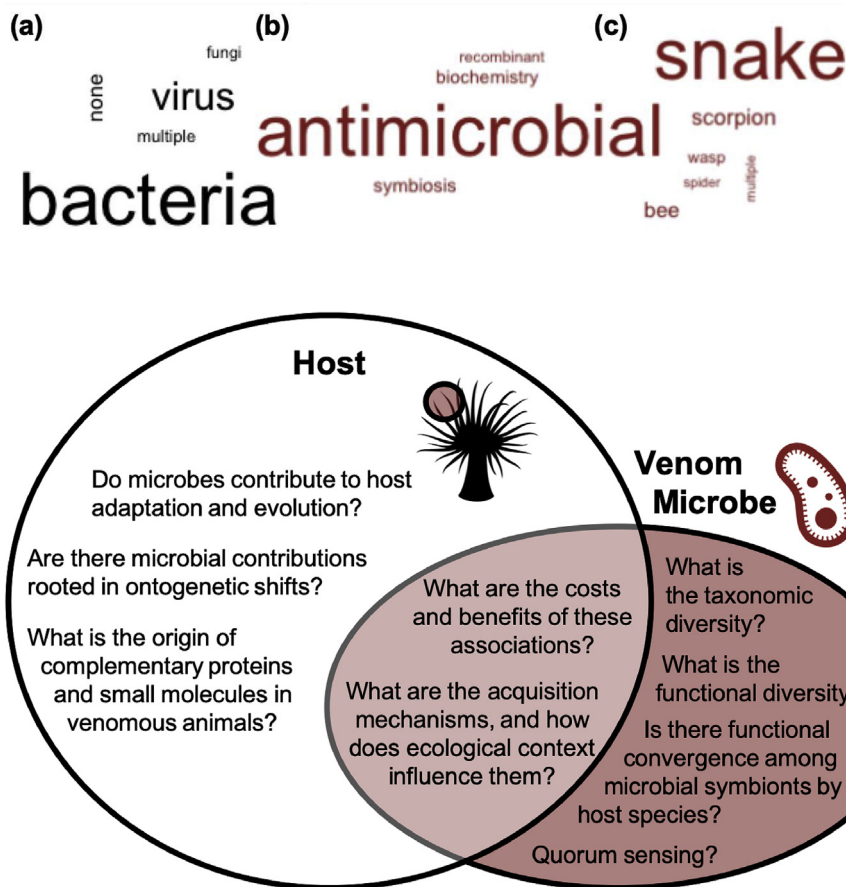


Fig. 1. Word clouds representative of Supplemental Table 1 content. A breakdown of 140 resultant articles from searching Web of Science for venom-microbe studies. (a) Most articles are either bacteria- or virus-specific, and a subset (16 articles) are not related to studies involving microbes. After removing these articles, investigation of the remaining 126 show (b) approximately 71% focus on venom toxins exhibiting antimicrobial properties with only about 11% focused on venom-microbe interactions. (c) Roughly 57% of the surveyed studies focus on snake venom, and the remaining studies are largely from arthropods.

Fig. 2. Proposed questions for venom-microbiome exploration of the ecology and evolution of venomous hosts and their microbial associates. A Venn diagram displaying the intersections of microbiology and venomics through an ecology and evolution focus. The questions presented are examples of possible areas of investigation to advance the field.

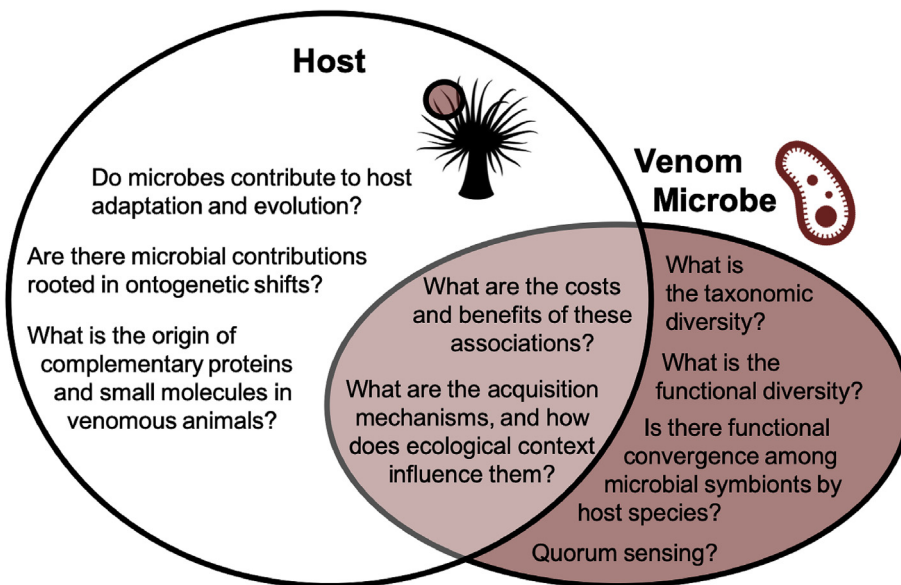


Table 1
Explicit Sequencing and Next-Generation venom microbiome studies, including published & in progress work within iVAMP (“+” denotes a collaboration formed because of access to the iVAMP network). Next-generation venom microbiome studies are comparatively recent, and few in number. Even so, the diversity of these host and microbial community studies highlight the potential benefits of integrating microbiology and venomics (Webb and Summers, 1990; Peraud et al., 2009; Goldstein et al., 2013; Debat, 2017; Torres et al., 2017; Esmailishirazifard et al., 2018).

Published Studies	Organism	Tissue	Wild/Captive	Approach
Webb and Summers, 1990	Wasp	Venom gland	Captive	Culture, Sanger Sequencing
Peraud et al. (2009)	Cone-snail (3 species)	Body, Hepatopancreas, Venom Duct	Wild	Culture, FISH, Sanger Sequencing
Goldstein et al. (2013)	Monitor Lizard	Saliva, Gingiva	Captive	Culture, Sanger Sequencing, 16S
Simmonds et al. (2016)	Parasitoid Wasp	Venom Gland	Wild	RNAseq/reverse transcriptomics
Debat, 2017	Spiders	Transcriptomes of the Body, Brain, Silk Gland	Wild	Data-mining (NGS)
Torres et al. (2017)	Cone-snail (8 species)	Venom Duct, Muscle, External Duct	Wild	16S, 454
Esmailishirazifard et al. (2018)	Snakes (5 species) Spiders (2 species)	Venom, Oral Cavity	Wild, Captive	Culture, 16S, WGS
iVAMP Projects in progress	Organism	Tissue	Wild/Captive	Approach
Colston	Snakes (multiple)	Venom, Venom Glands, Venom Ducts, Oral Cavity, Muscle, Stomach and GIT	Wild, Captive	16S, RNAseq transcriptomics, Proteomics
Harms + Macrander	Lionfish: <i>Pterois volitans</i>	venom glands, venom	Wild (Invasive)	Transcriptomics, Proteomics
Keiser + Colston	Spiders: <i>Stegodyphus</i>	venom glands, venom	Wild, Captive	16S, RNAseq transcriptomics, Proteomics
Stiers, Colston	Snake: <i>Crotalus scutulatus</i>	Venom, Venom Glands, Venom Ducts, Oral Cavity, Muscle, Stomach and GIT	Wild, Captive	16S, RNAseq transcriptomics, Proteomics
Ul-Hasan, Nobile, Petras	Cone-snail: <i>Californiconus californicus</i>	Venom, Venom Duct, Hepatopancreas, Shell, Egg	Wild, Captive	16S and 18S, Proteomics, Metabolomics

and proteomics (Fry, 2005) into the study of venom has contributed to new toxin discovery and associated biological activity (Oldrati et al., 2016; Calvete, 2017). Over the past 15 years, microbiome research has yielded breakthroughs in our knowledge of unculturable microbial “dark matter” (Bernard et al., 2018), the origins of life (Spang et al., 2017), and human health (Arnold et al., 2016; Clavel et al., 2016). Providing ecological and evolutionary context has enhanced both

microbiology (Boughner and Singh, 2016; Hird, 2017) and venomics (Prashanth et al., 2016; Sunagar et al., 2016; Calvete, 2017). We thus propose viewing venom as a microenvironment that occupies a unique niche in which microbes may adapt as a critical perspective for investigating the dynamics of venom-microbe interactions.

Researchers in the fields of both venomics and microbiology share common interests in natural products (Katz and Baltz, 2016; Robinson

et al., 2017) and adaptive evolution (Phuong et al., 2016; Hird, 2017). With more information on the presence and diversity of venom-associated microbiomes (Table 1), future research efforts can focus on how microbes colonize and thrive in venom glands as a starting point for integrating these fields (McFall-Ngai, 2014; Nunes-Alves, 2015). For example, examining the biology of the host using microscopy (Schlafer and Meyer, 2017) and biomechanics (Yevick and Martin, 2018) could result in translated predictive models (Biggs et al., 2015) for identifying the underlying mechanisms of toxin and metabolite function (Sapp, 2016; Adnani et al., 2017). Determining if and which venom micro-environments are truly sterile, and if microbes contribute to shaping the genetic architecture of the venom gland, will prove critical in our understanding of venom evolution (Conlin et al., 2014) and antimicrobial resistance (Adnani et al., 2017). Correlating microbial community profiles with functional characteristics of venom could provide yet another layer to the venomomics field that would deepen our insight on the mechanisms driving venom variation. Identifying microbial species that have adapted to these seemingly extreme environments (Rampelotto, 2013) will open new avenues of research, and emphasizes the need for phylogenetically representative venom host model systems to be bred axenically *in vivo* to allow researchers to test the functional roles of venom-associated microbes observed in the wild (Fig. 2).

The host-microbe interactions that naturally occur in the venom microenvironment remain largely unknown, and addressing this knowledge gap through directed microbiome sequencing experiments within a wildtype ecosystem framework will strengthen our understanding of animal associated microbes (McFall-Ngai et al., 2013). A variety of microbial studies have found tetrodotoxin-producing bacteria in venomous and poisonous animals (Hwang et al., 1989; Cheng et al., 1995; Pratheepa and Vasconcelos, 2013; Stokes et al., 2014) as well as a number of viruses with RNA genomes residing in venom (Debat, 2017). These studies contrast with the notion of the venom microenvironment as largely sterile in that the primary research on venom-gland derived toxin compounds focuses on antimicrobial properties (Fig. 1). However, (1) compounds derived from or contained within venom that demonstrate antimicrobial activity against clinical and/or reference strains (Almeida et al., 2018) may not reflect what occurs against wild-type strains that co-evolved within venom glands (Reis et al., 2018), and (2) cultured microbes can produce compounds in a lab setting that they may not produce in nature (McCoy and Clapper, 1979; Simmons et al., 2008; Peraud et al., 2009; Catalán et al., 2010; Quezada et al., 2017b, 2017a, 2017b; Silvestre et al., 2005; Yu et al., 2011). The captive environment, which is already known to affect the host venom profile (Willemse et al., 1979; Freitas-de-Sousa et al., 2015), may also influence microbial composition of the oral and venom microbiomes (Hyde et al., 2016), which has led to a call for microbiome studies to utilize wild-collected samples (Colston and Jackson, 2016; Hird, 2017). Studying the venom microbiome, and considering the adaptive traits of microbes under selection in an ecological context as it occurs in the wild, clarifies the evolutionary pressures for these antimicrobial compounds found in venom (Fig. 2). *In vitro*, *in vivo*, and natural venom microbiome experiments alongside culture-dependent and -independent techniques contribute to our understanding of mutual symbioses, with room for predictive modeling to identify novel niches for microbial adaptation and competition (Bull et al., 2010; Zhu et al., 2018).

An initial search shows approximately 100 papers per year have consistently been published on venom antimicrobial peptides (PubMed search term - antimicrobial AND peptide AND venom 14th Mar 2019) for the past 5 years. The few venom-microbiome studies in the literature to date (Table 1) indicate a clear need for an expansion of the subdiscipline of venom-microbiome research, and this has led to the formation of an international, collaborative cohort of researchers referred to as the Initiative for Venom Associated Microbes and Parasites (or iVAMP, <https://ivamp-consortium.github.io/>). A major goal of the iVAMP consortium is to provide a platform for the scientific community

to openly discuss areas of interest to the field. Fig. 2 outlines some examples of ongoing questions that may be of interest to iVAMP researchers. By emphasizing representation through practice, this consortium supports working with and for communities from which we sample rather than taking from them. Involving scientists across the globe through initiatives like iVAMP extends beyond the requirements of legislation, such as the Nagoya Protocol (Buck and Hamilton, 2011), to ensure that science is accessible to the public and inclusive of all parties involved. Overall, the approach taken by this initiative expands suggested practices (Weber and Schell Word, 2001; Cheng et al., 2018) for the benefit of scientific innovation and discovery.

As an organization, iVAMP has explicit goals and approaches for furthering the fields of microbiome research and venomics (Fig. 2) as well as specific aims for conducting ethical, inclusive, reproducible science. In doing so, our practices seek to prevent counterproductive competition and instead embrace interdisciplinary, collaborative scientific research. The broad scientific disciplines covered by iVAMP members provide a network that allows researchers access to a variety of technical platforms and key resources that otherwise may not be available in individual labs. This is especially important for those researchers who may want to enter the venomomics field, but lack accessibility to the necessary resources or instrumentation. Expansion of knowledge on microbes living in the many diverse venom host micro-environments additionally contributes to currently absent aspects of holobiont and coevolutionary theory (Faure Denis et al., 2018). Through iVAMP, researchers set an open-access tone for the sub-discipline of venom-microbiomics that will be useful well into the future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.toxcx.2019.100016>.

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Conflicts of interest

The authors declare no conflicts of interest.

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