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# Journal Pre-proof

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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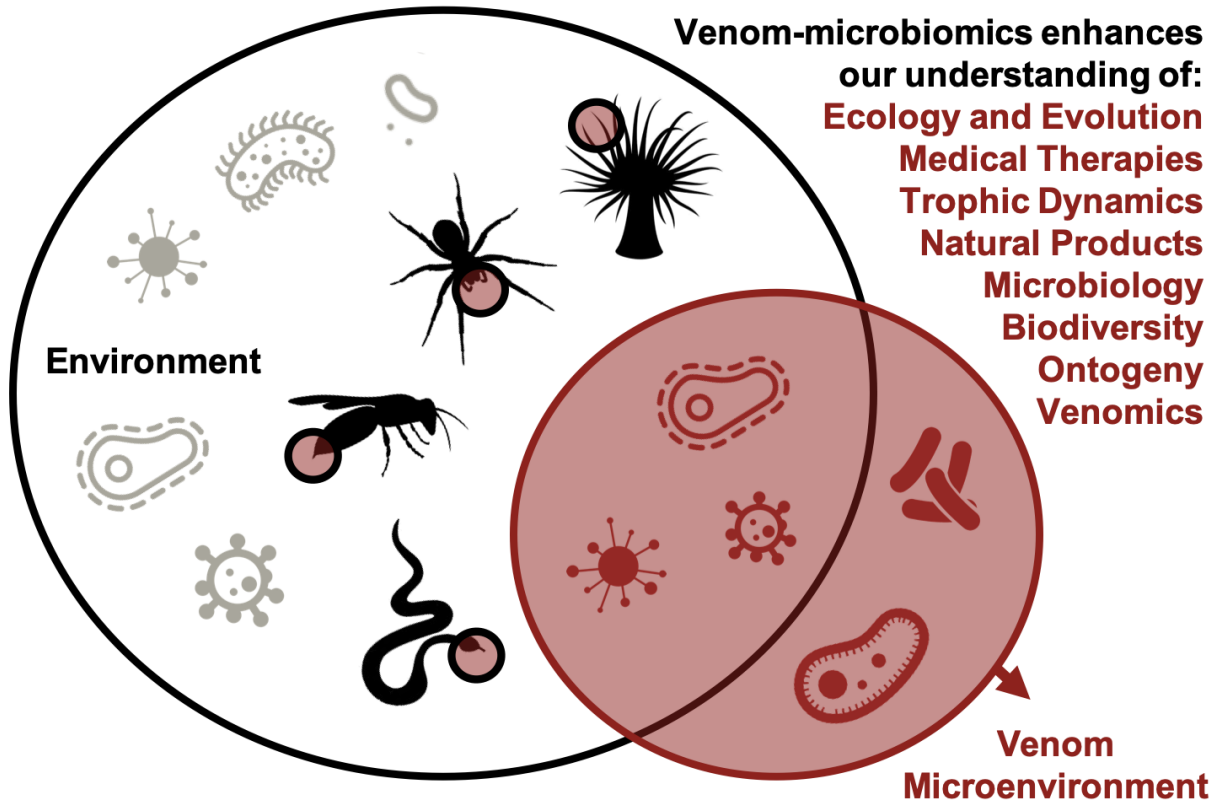
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Journal

1 Toxicon:X – Correspondence

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

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38 **Abstract**

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

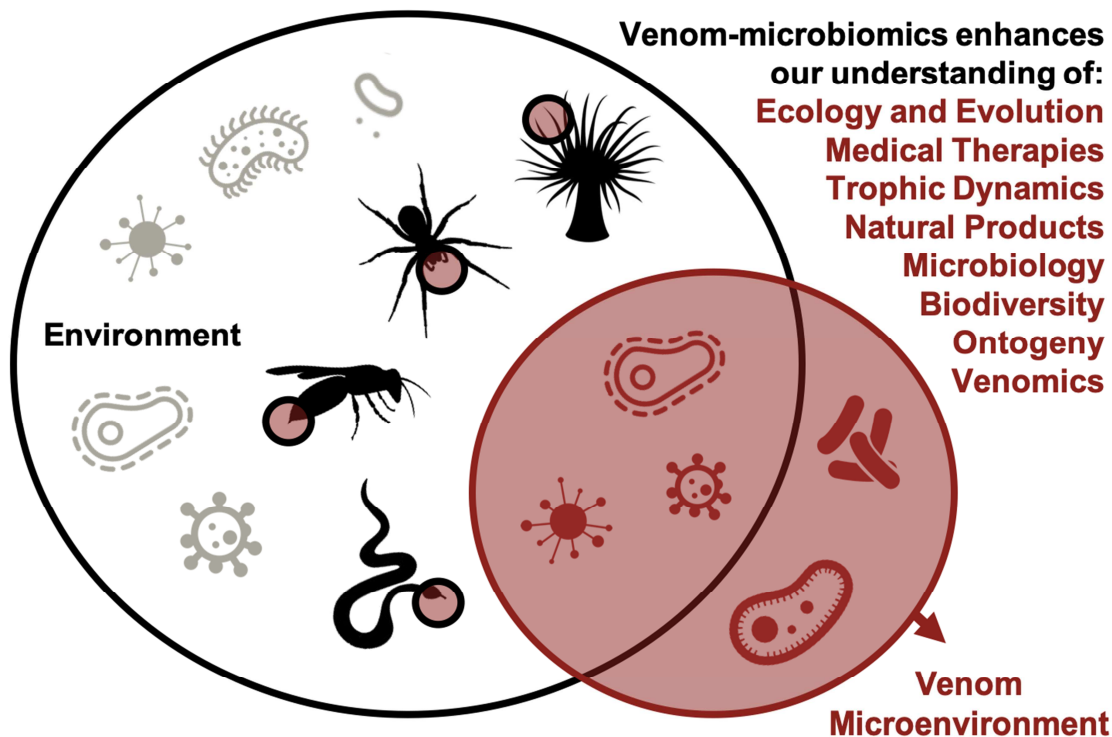
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57 **Keywords:** bacteria, coevolution, holobiont, microbiome, symbiont, virus

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59 **Highlights:**

- 60 • Venom-microbiome studies as an integrative field of venomics and microbiology
- 61 • Argument for multi-omics-based discovery through a microenvironment framework
- 62 • Introduction of a venom-microbiome research consortium (iVAMP)



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65 **Text**

66 While scientific research in toxinology and microbiology has persisted for centuries, a cursory  
67 search of the literature reveals less than 150 studies overlap between these two fields despite  
68 each significantly advancing as a result of next generation sequencing technology (Figure 1,  
69 Supplemental Table 1, Supplemental Code). The integration of genomics (Moran and Gurevitz,  
70 2006), transcriptomics (Pahari et al., 2007), and proteomics (Fry, 2005) into the study of venom  
71 has contributed to new toxin discovery and associated biological activity (Oldrati et al., 2016;  
72 Calvete, 2017). Over the past 15 years, microbiome research has yielded breakthroughs in our  
73 knowledge of unculturable microbial “dark matter” (Bernard et al., 2018), the origins of life  
74 (Spang et al., 2017), and human health (Arnold et al., 2016; Clavel et al., 2016). Providing  
75 ecological and evolutionary context has enhanced both microbiology (Boughner and Singh,  
76 2016; Hird, 2017) and venomics (Prashanth et al., 2016; Sunagar et al., 2016; Calvete, 2017). We  
77 thus propose viewing venom as a microenvironment that occupies a unique niche in which  
78 microbes may adapt as a critical perspective for investigating the dynamics of venom-microbe  
79 interactions.

80 Researchers in the fields of both venomics and microbiology share common interests in  
81 natural products (Katz and Baltz, 2016; Robinson et al., 2017) and adaptive evolution (Phuong et  
82 al., 2016; Hird, 2017). With more information on the presence and diversity of venom-associated  
83 microbiomes (Table 1), future research efforts can focus on how microbes colonize and thrive in  
84 venom glands as a starting point for integrating these fields (McFall-Ngai, 2014; Nunes-Alves,  
85 2015). For example, examining the biology of the host using microscopy (Schlafer and Meyer,  
86 2017) and biomechanics (Yevick and Martin, 2018) could result in translated predictive models  
87 (Biggs et al., 2015) for identifying the underlying mechanisms of toxin and metabolite function  
88 (Sapp, 2016; Adnani et al., 2017). Determining if and which venom microenvironments are truly  
89 sterile, and if microbes contribute to shaping the genetic architecture of the venom gland, will  
90 prove critical in our understanding of venom evolution (Conlin et al., 2014) and antimicrobial  
91 resistance (Adnani et al., 2017). Correlating microbial community profiles with functional  
92 characteristics of venom could provide yet another layer to the venomics field that would deepen  
93 our insight on the mechanisms driving venom variation. Identifying microbial species that have  
94 adapted to these seemingly extreme environments (Rampelotto, 2013) will open new avenues of  
95 research, and emphasizes the need for phylogenetically representative venom host model systems  
96 to be bred axenically *in vivo* to allow researchers to test the functional roles of venom-associated  
97 microbes observed in the wild (Figure 2).

98 The host-microbe interactions that naturally occur in the venom microenvironment  
99 remain largely unknown, and addressing this knowledge gap through directed microbiome  
100 sequencing experiments within a wildtype ecosystem framework will strengthen our  
101 understanding of animal associated microbes (McFall-Ngai et al., 2013). A variety of microbial  
102 studies have found tetrodotoxin-producing bacteria in venomous and poisonous animals (Hwang  
103 et al., 1989; Cheng et al., 1995; Pratheepa and Vasconcelos, 2013; Stokes et al., 2014) as well as  
104 a number of viruses with RNA genomes residing in venom (Debat, 2017). These studies contrast  
105 with the notion of the venom microenvironment as largely sterile in that the primary research on  
106 venom-gland derived toxin compounds focuses on antimicrobial properties (Figure 1). However,  
107 (1) compounds derived from or contained within venom that demonstrate antimicrobial activity  
108 against clinical and/or reference strains (Almeida et al., 2018) may not reflect what occurs  
109 against wild-type strains that co-evolved within venom glands (Reis et al., 2018), and (2)

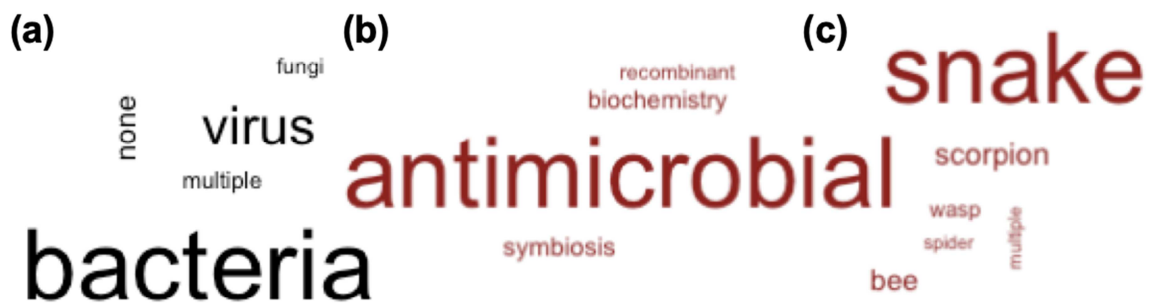
110 cultured microbes can produce compounds in a lab setting that they may not produce in nature  
111 (McCoy and Clapper, 1979; Simmons et al., 2008; Peraud et al., 2009; Catalán et al.,  
112 2010; Quezada et al., 2017b, 2017a, 2017b; Silvestre et al., 2005; Yu et al., 2011). The captive  
113 environment, which is already known to affect the host venom profile (Willemse et al., 1979;  
114 Freitas-de-Sousa et al., 2015), may also influence microbial composition of the oral and venom  
115 microbiomes (Hyde et al., 2016), which has led to a call for microbiome studies to utilize wild-  
116 collected samples (Colston & Jackson, 2016; Hird, 2017). Studying the venom microbiome, and  
117 considering the adaptive traits of microbes under selection in an ecological context as it occurs in  
118 the wild, clarifies the evolutionary pressures for these antimicrobial compounds found in venom  
119 (Figure 2). *In vitro*, *in vivo*, and natural venom microbiome experiments alongside culture-  
120 dependent and -independent techniques contribute to our understanding of mutual symbioses,  
121 with room for predictive modeling to identify novel niches for microbial adaptation and  
122 competition (Bull et al., 2010; Zhu et al., 2018).

123 An initial search shows approximately 100 papers per year have consistently been  
124 published on venom antimicrobial peptides (PubMed search term - antimicrobial AND peptide  
125 AND venom 14th Mar 2019) for the past 5 years. The few venom-microbiome studies in the  
126 literature to date (Table 1) indicate a clear need for an expansion of the subdiscipline of venom-  
127 microbiome research, and this has led to the formation of an international, collaborative cohort of  
128 researchers referred to as the Initiative for Venom Associated Microbes and Parasites (or  
129 iVAMP, <https://ivamp-consortium.github.io/>). A major goal of the iVAMP consortium is to  
130 provide a platform for the scientific community to openly discuss areas of interest to the field.  
131 Figure 2 outlines some examples of ongoing questions that may be of interest to iVAMP  
132 researchers. By emphasizing representation through practice, this consortium supports working  
133 with and for communities from which we sample rather than taking from them. Involving  
134 scientists across the globe through initiatives like iVAMP extends beyond the requirements of  
135 legislation, such as the Nagoya Protocol (Buck and Hamilton, 2011), to ensure that science is  
136 accessible to the public and inclusive of all parties involved. Overall, the approach taken by this  
137 initiative expands suggested practices (Weber et al., 2001; Cheng et al., 2018) for the benefit of  
138 scientific innovation and discovery.

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

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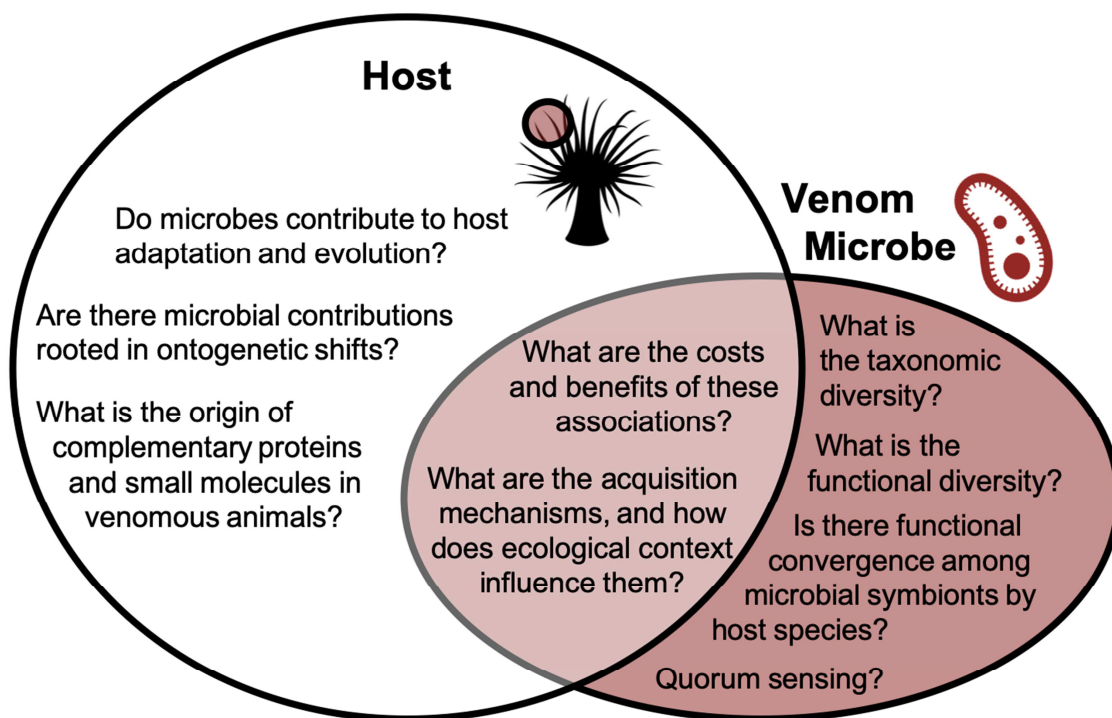


152 **Figures and Tables**

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**Figure 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.



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**Figure 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.

<b>PUBLISHED STUDIES</b>	<b>ORGANISM</b>	<b>TISSUE</b>	<b>WILD / CAPTIVE</b>	<b>APPROACH</b>
<b>WEBB B.A., SUMMERS M.D. 1990</b>	Wasp	Venom gland	Captive	Culture, Sanger Sequencing
<b>PERAUD ET AL. 2009</b>	Cone-snail (3 species)	Body, Hepatopancreas, Venom Duct	Wild	Culture, FISH, Sanger Sequencing
<b>GOLDSTEIN ET AL. 2013</b>	Monitor Lizard	Saliva, Gingiva	Captive	Culture, Sanger Sequencing, 16S
<b>SIMMONDS ET AL. 2016</b>	Parasitoid Wasp	Venom Gland	Wild	RNAseq/reverse transcriptomics
<b>DEBAT 2017</b>	Spiders	Transcriptomes of the Body, Brain, Silk Gland, Venom Gland	Wild	Data-mining (NGS)
<b>TORRES ET AL. 2017</b>	Cone-snail (8 species)	Venom Duct, Muscle, External Duct	Wild	16S, 454
<b>ESMAELISHIRAZIFARD ET AL. 2018</b>	Snakes (5 species) Spiders (2 species)	Venom, Oral Cavity	Wild, Captive	Culture, 16S, WGS
<b>IVAMP PROJECTS IN PROGRESS</b>	<b>ORGANISM</b>	<b>TISSUE</b>	<b>WILD / CAPTIVE</b>	<b>APPROACH</b>
<b>COLSTON</b>	Snakes (multiple)	Venom, Venom Glands, Venom Ducts, Oral Cavity, Muscle, Stomach and GIT	Wild, Captive	16S, RNAseq transcriptomics, Proteomics
<b>HARMS + MACRANDER</b>	Lionfish: <i>Pterois volitans</i>	venom glands, venom	Wild (Invasive)	Transcriptomics, Proteomics
<b>KEISER + COLSTON</b>	Spiders: <i>Stegodyphus</i>	venom glands, venom	Wild, Captive	16S, RNAseq transcriptomics, Proteomics
<b>STIERS, COLSTON</b>	Snake: <i>Crotalus scutulatus</i>	Venom, Venom Glands, Venom Ducts, Oral Cavity, Muscle, Stomach and GIT	Wild, Captive	16S, RNAseq transcriptomics, Proteomics
<b>UL-HASAN, NOBILE, PETRAS</b>	Cone-snail: <i>Californiconus</i>	Venom, Venom Duct,	Wild, Captive	16S and 18S, Proteomics,

<i>californicus</i>	Hepatopancreas, Shell, Egg	Metabolomics
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**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; Esmaeilshirazifard et al., 2018).

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## Conflicts of interest

The authors declare no conflicts of interest.

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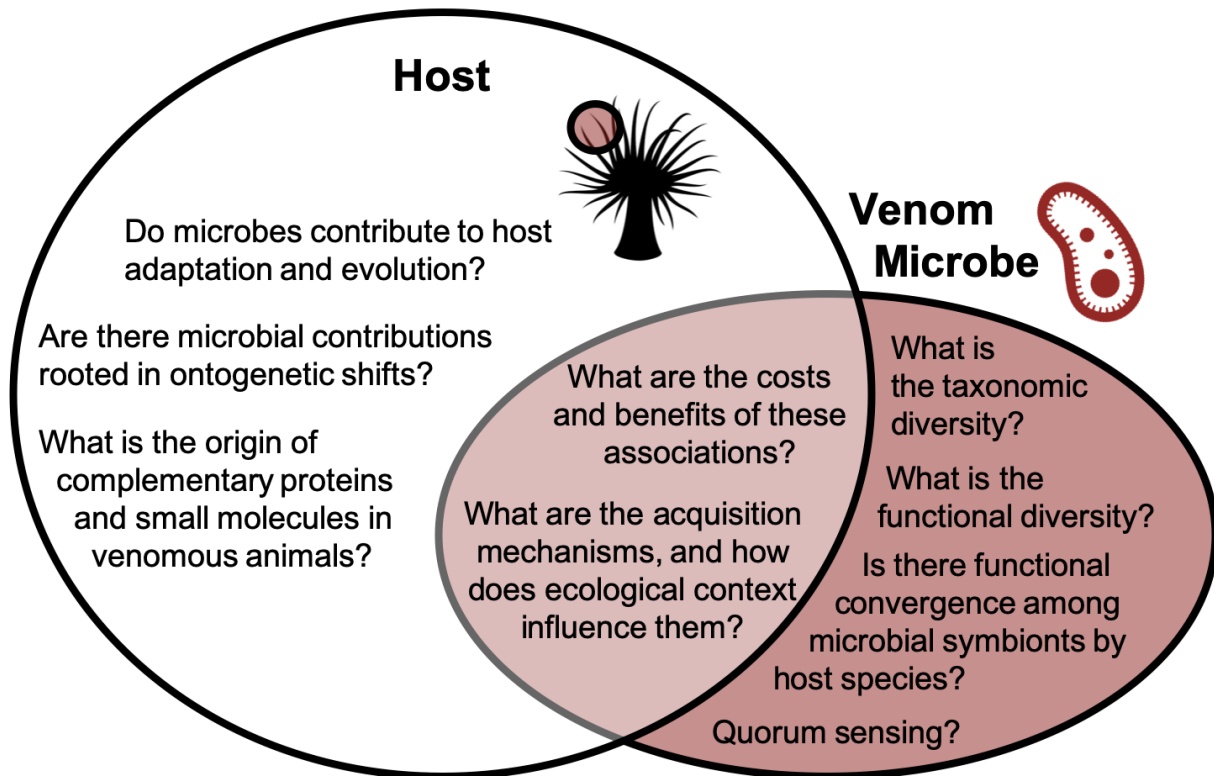
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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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