

Characterization of the Venom Delivery Mechanism and Proteome for the Wandering Spider, *Ctenus hibernalis* (Aranea:Ctenidae)

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Introduction

- Spider venom is a multicomponent protein mixture that includes a rich blend of neurotoxic peptides (Saez et al. 2012).
- The venom of only a small percentage of the currently classified spiders has been categorized.
- The species *Ctenus hibernalis* belongs to the Ctenidae family which also contains *Phoneutria nigriventer*, the most venomous spider in the world (Herzig et al. 2002).
- The venom of *C. hibernalis* is not considered to be harmful to humans (Howell & Jenkins 2004).
- The aim of this study was to characterize the venom composition of *C. hibernalis* using mass spectrometry (MS) proteomics, as well as describe its venom apparatus using Scanning Electron Microscopy (SEM).



Fig 1. *Ctenus hibernalis* of Northern Alabama. Source: bugguide.net

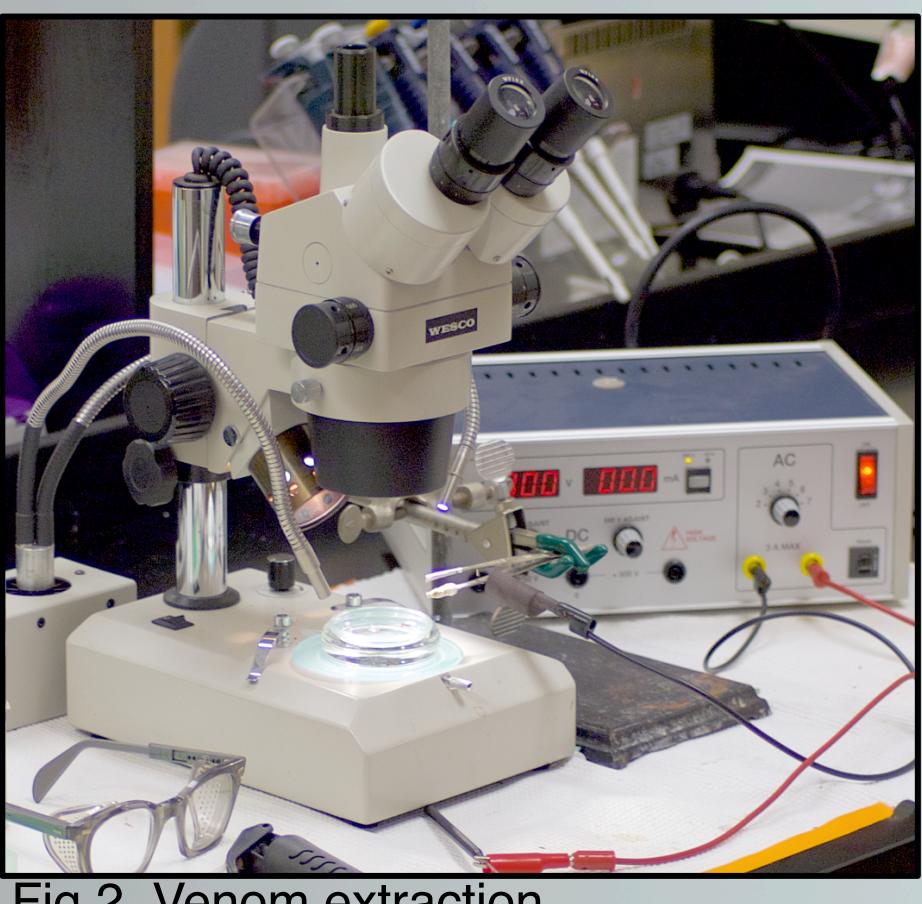


Fig 2. Venom extraction electrostimulatory apparatus.

Methods

- Adult female *Ctenus hibernalis* were collected in September 2015 in the Homewood Forest Preserve in Homewood, Alabama.
- Specimens were anesthetized and venom was collected using electrostimulation.
- Venom protein concentration was analyzed using BCA protein assay.
- Venom was analyzed using tryptic digests of 1D gel fractions using LTQ XL ion MS and HPLC at the Mass Spectrometry/Proteomics Core at the University of Alabama at Birmingham.
- MS data was then searched against Uniprot filtered down to Araneae venom peptides.
- Identified peptides were filtered, grouped, and quantified using Scaffold (Proteome Software, Portland Oregon).
- Gene ontology from sequences with at least 90% coverage was determined using Blast2GO (Conesa et al.).
- The anatomy and morphology of the venom delivery apparatus were visualized using scanning electron microscopy.

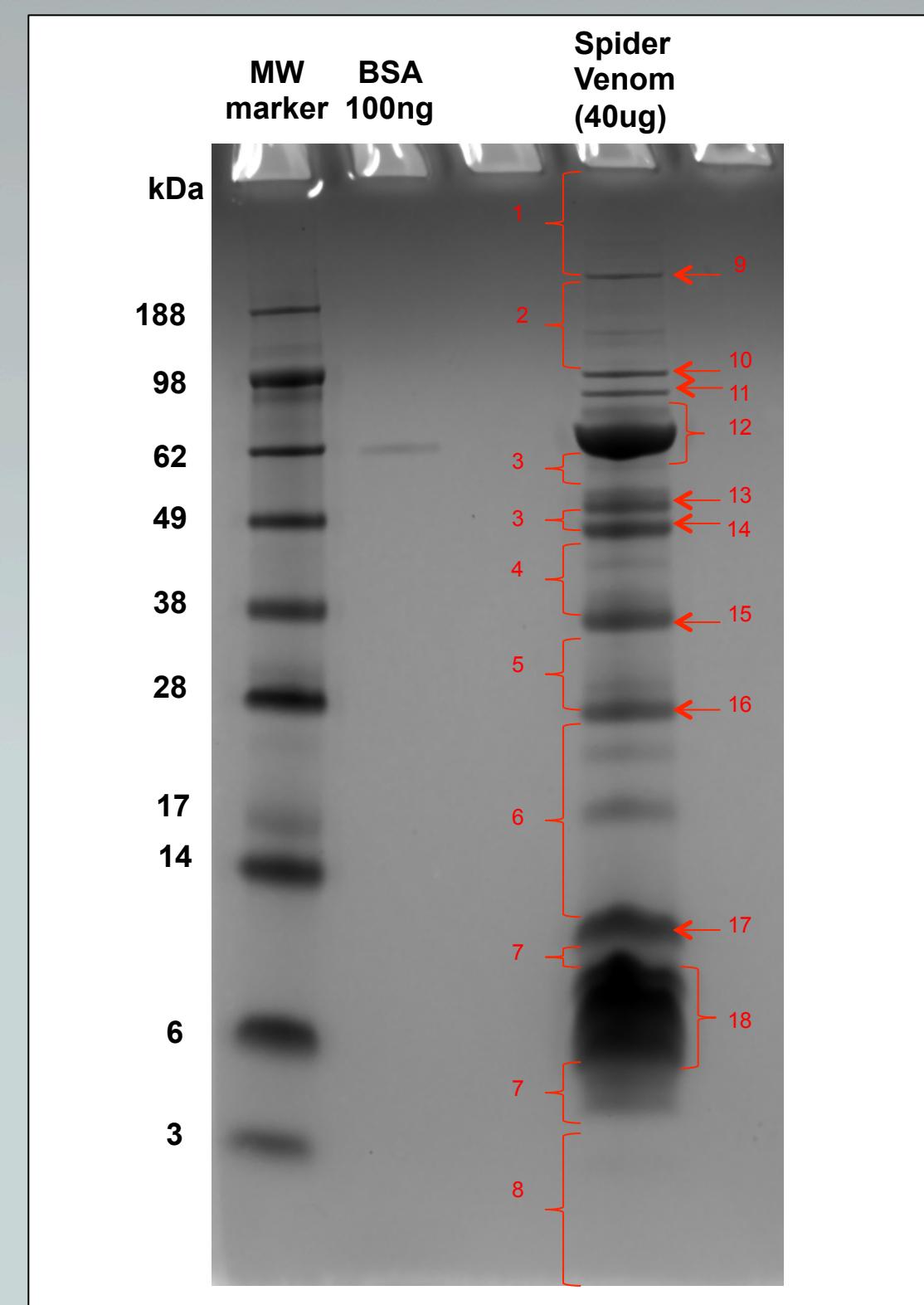


Fig 3. Long stacked 1D gel of pooled crude venom from 21 adult female *Ctenus hibernalis*, separated into 18 fractions compared to molecular weight marker.

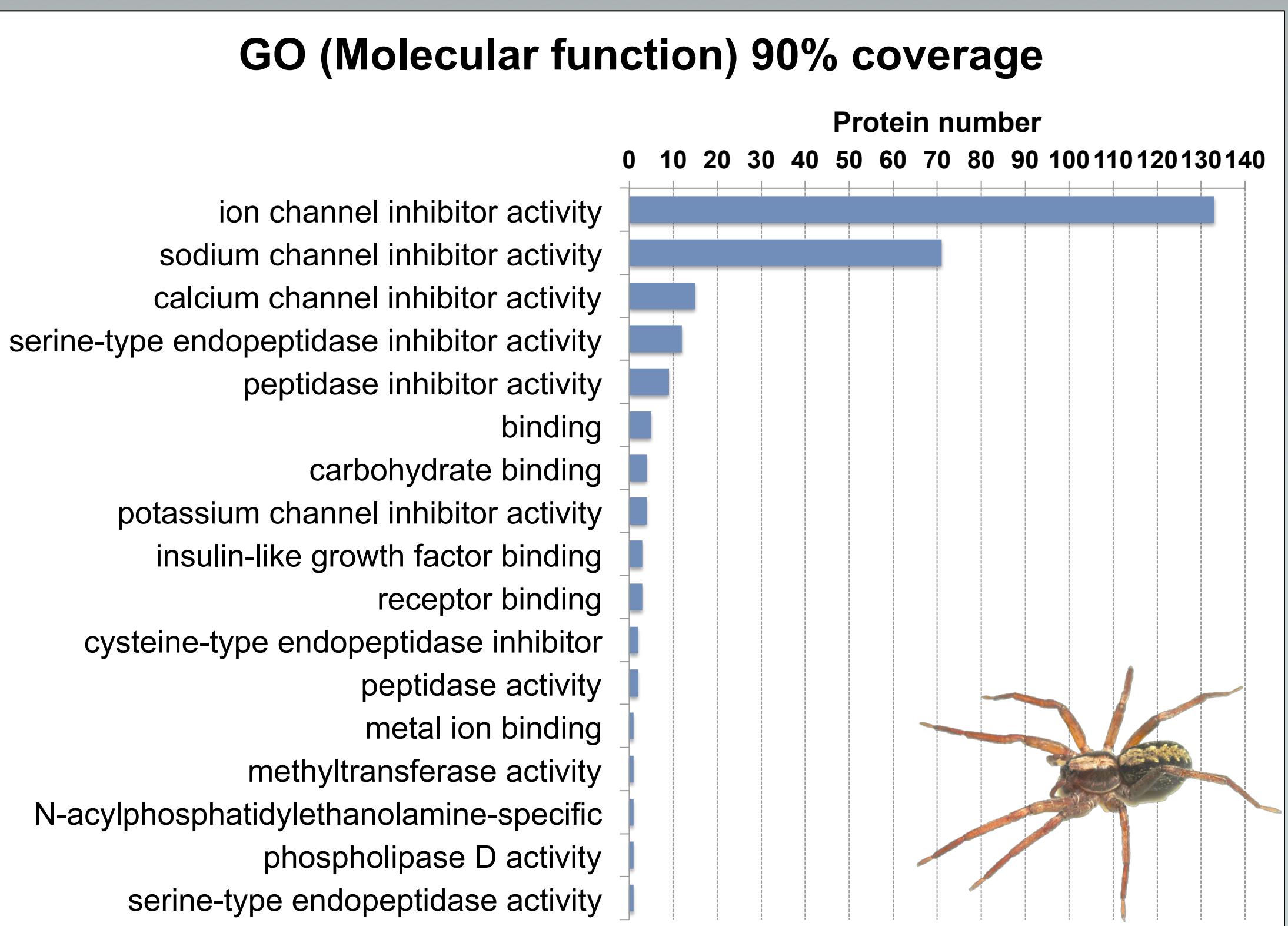


Fig 4. Molecular functions of venom protein components from 249 *C. hibernalis* sequence matches with 90% coverage from BLAST2GO. Cellular component and biological process GO terms (not pictured) were also determined for 479 and 248 sequences respectively with 90% coverage.

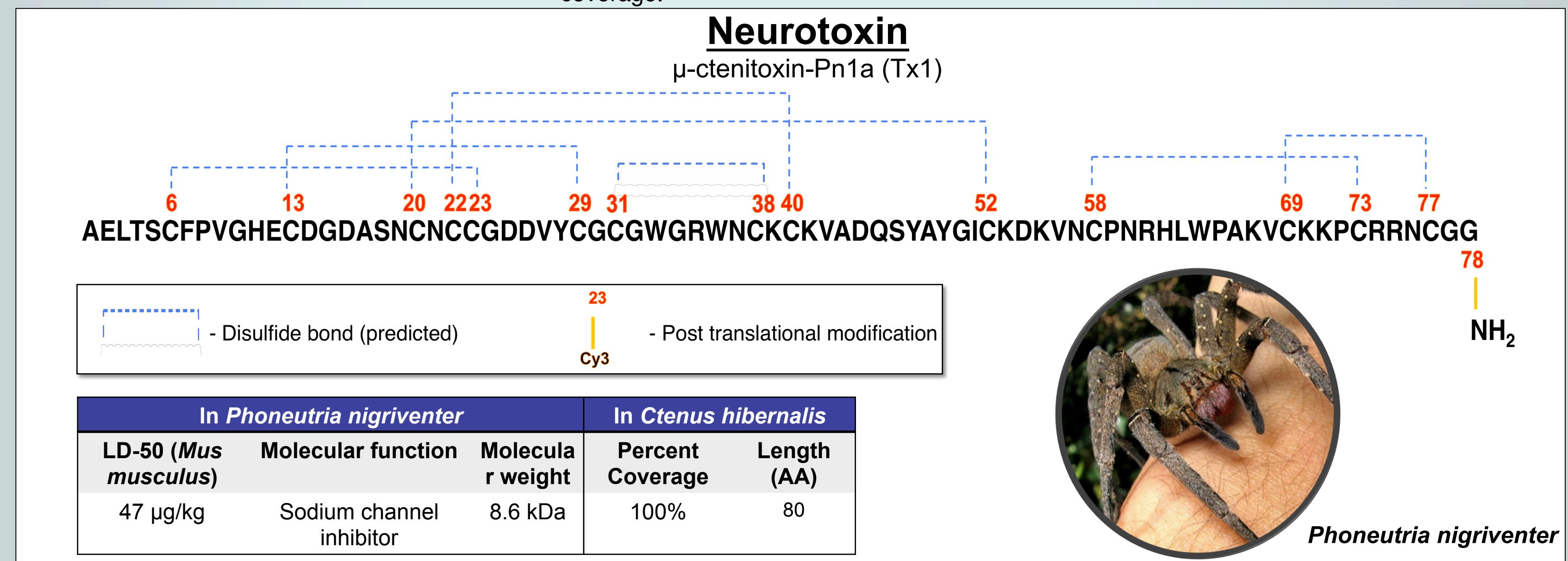


Fig 5. Neurotoxin mu-ctenitoxin-Pn1a (Tx1) from *Phoneutria nigriventer*, primary sequence confirmed to exist in *C. hibernalis*. Contains predicted disulfide bridges, as well as posttranslational modifications, LD-50, molecular function, and molecular weight found in *P. nigriventer* published on Arachnoserver. Image source: João P. Burini via WikiCommons.

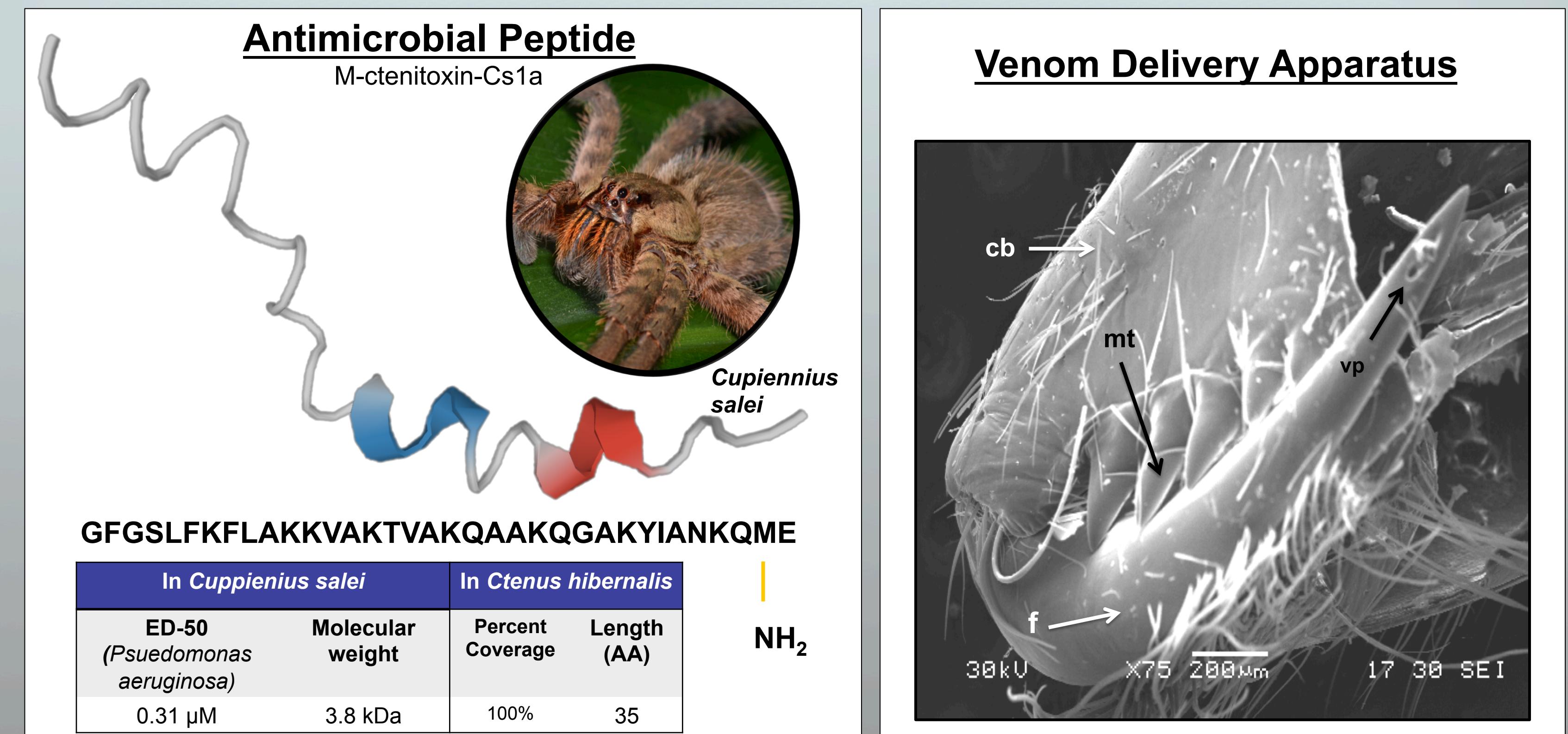


Fig 6. Antimicrobial peptide M-ctenitoxin-Cs1a primary sequence from *Cupiennius salei* confirmed to exist in *C. hibernalis*. Contains posttranslational modifications, ED-50, and molecular weight found in *C. salei* published in Arachnoserver. Also displayed is 3D structure from Protein Database (PDB: 2K38). Image source: H. Höffer

Fig 7. View of the cheliceral base (cb) and movable fang (f) of female *Ctenus hibernalis*. The venom pore (vp) is located superior to the fang at its terminus. The fang lies nested on five marginal teeth (mt) along the cheliceral retro margin.

Results

- The proteins were separated into 18 fractions in the 1D gel ranging from 3-188kDa (Fig. 3).
- From the tryptic digests of the 18 fractions, 1,180 peptides matched with published spider venom peptide sequences in Uniprot database with a protein threshold of 99% confidence. Of those matches, 631 had at least 90% sequence coverage.
- Of those 631 peptides, gene ontology was determined for 484 sequences that represented an extensive range of molecular functionality (Fig. 4).
- A 100% match occurred with the sequence of the mature peptide sequence of the highly lethal Tx1 toxin found in *Phoneutria nigriventer* (Fig. 5), as well as the antimicrobial peptide Cs1a found in *Cupiennius salei* (Fig. 6).

Discussion

- This study marks the first attempt to characterize the proteome found within the venom of a U.S native member of the Ctenidae family.
- The images generated represent the first SEM morphological characterization of *C. hibernalis*.
- This sample may contain novel peptides yet to be characterized in Uniprot that are entirely unique to *C. hibernalis*.
- Generating a transcriptomic database is necessary to discover these novel peptides, as well as to generate entire sequences, and to further elucidate gene ontology information.
- Further exploration of the expression levels of Tx1 as well as structural and functional differences will be necessary to understand why drastic toxicity differences.

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