Venomics approach reveals a high proportion of *Lactrodectus*-like toxins in *Steatoda nobilis* venom - First link to post-bite symptomology -

John P. Dunbar[1], Antoine Fort[2], Damien Redureau[3], Aiste Vitkauskaite[1], Ronan Sulpice[2], Michel Dugon[1], Loïc Quinton[3]

1 - Venom Systems & Proteomics Lab, School of Natural Sciences, Ryan Institute, National University of Ireland Galway, Ireland
2 - Plant Systems Biology Lab, Plant and AgriBiosciences Research Centre, School of Natural Sciences, Ryan Institute, National University of Ireland Galway, Ireland
3 - Mass Spectrometry Laboratory, MolSYS RU, University of Liège

CONTEXT

The Noble false widow spider *Steatoda nobilis* has expanded its range globally and may represent a potential threat to native ecosystems and public health **[1,2,3,4,5,6]**. Envenomations can result in local and systemic neurotoxic symptoms, similar to true black widows (genus *Latrodectus*) **(Fig 1).** We used transcriptomic and proteomic cutting-edge approaches to deeply characterise *S. nobilis* venom. Among the toxins, the most represented in numbers are α-latrotoxins, δ-latroinsectotoxins and latrodectins, which were first characterised from black widow venoms. Approximately two-thirds of the venom is composed of *Latrodectus-like* toxins. We present symptomology from 23 cases (15 unpublished) of *S. nobilis* envenomations confirming necrosis and *Latrodectus-like* symptoms such as debilitating pain, tremors, fatigue, nausea and hypotension. The continued rising numbers of *S. nobilis* will undoubtedly result in further bites and this study will help provide the medical community with a better understanding of the potential medical outcomes from bites by this species and alert them to the possibility of medically important outcomes.

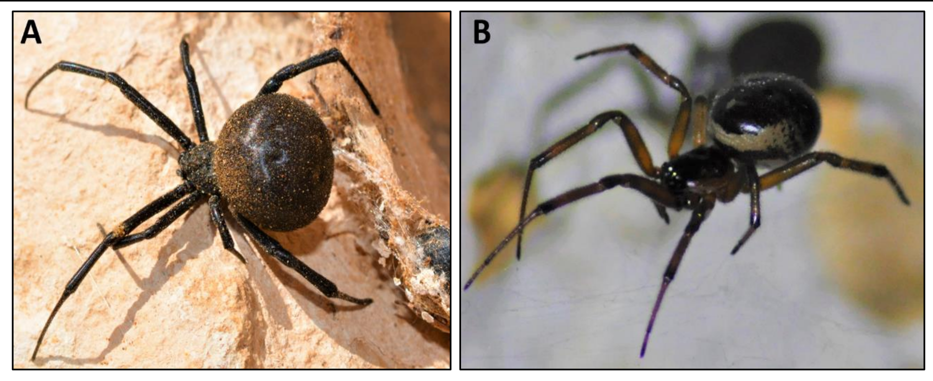


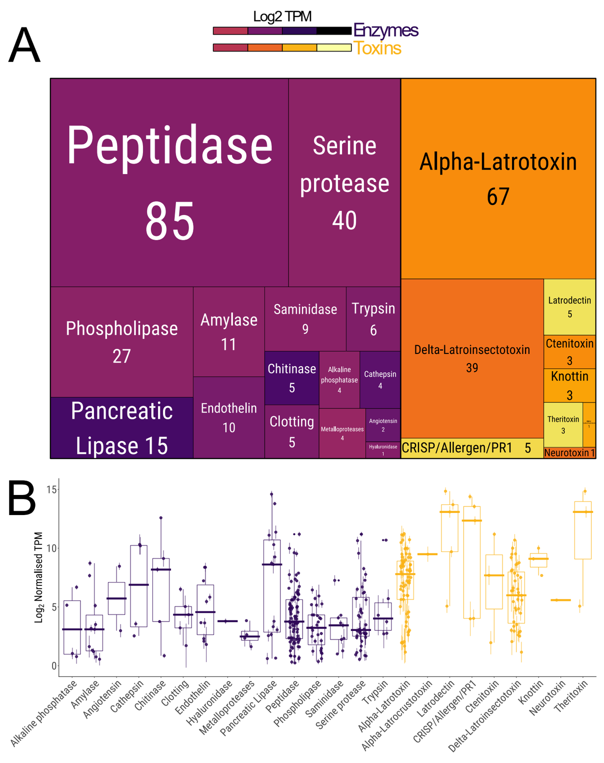
Figure 1. Similarities between Black widow and False widow spiders. **A)** Mature female Black widow *Latrodectus lilianae*, Morocco (Photo taken by M. Dugon), **B)** Mature female False widow *Steatoda nobilis*, Ireland (Photo taken by JP. Dunbar).

METHODS

RNA was isolated from venom glands of 25 adult female *S. nobilis* and sequenced using Illumina technology and transcriptome assembly using the Trinity pipeline. Raw venom was reduced, alkylated and digested with trypsin before being separated using micro-HPLC hyphenated to Q-Exactive. Bioinformatic analysis of the proteomics data was done using PEAKS Studio X+. A detailed Materials and Methods section is available in the article **Dunbar et al., (2020) [6].** 11 adult females, two adult males, and parents of two male minors reported envenomation symptoms caused by *S. nobilis*. In all cases, the victims provided the authors with either photographs, live specimens that were confirmed by the authors, or physical remains of the offending spiders which were identified using genetic sequencing.

MAIN RESULTS

Figure 2 TRANSCRIPTOMICS



We found 228 and 127 genes encoding for enzymes and toxins. Peptidases and serine proteases are the most abundant enzymes (85 and 40 genes), with pancreatic lipases and chitinases most expressed. Toxin genes mainly comprised of α-latrotoxin and δ-latroinsectotoxins (67 and 39), with higher expression for latrodectin, CRISP/Allergen/PR1 and theritoxin genes.

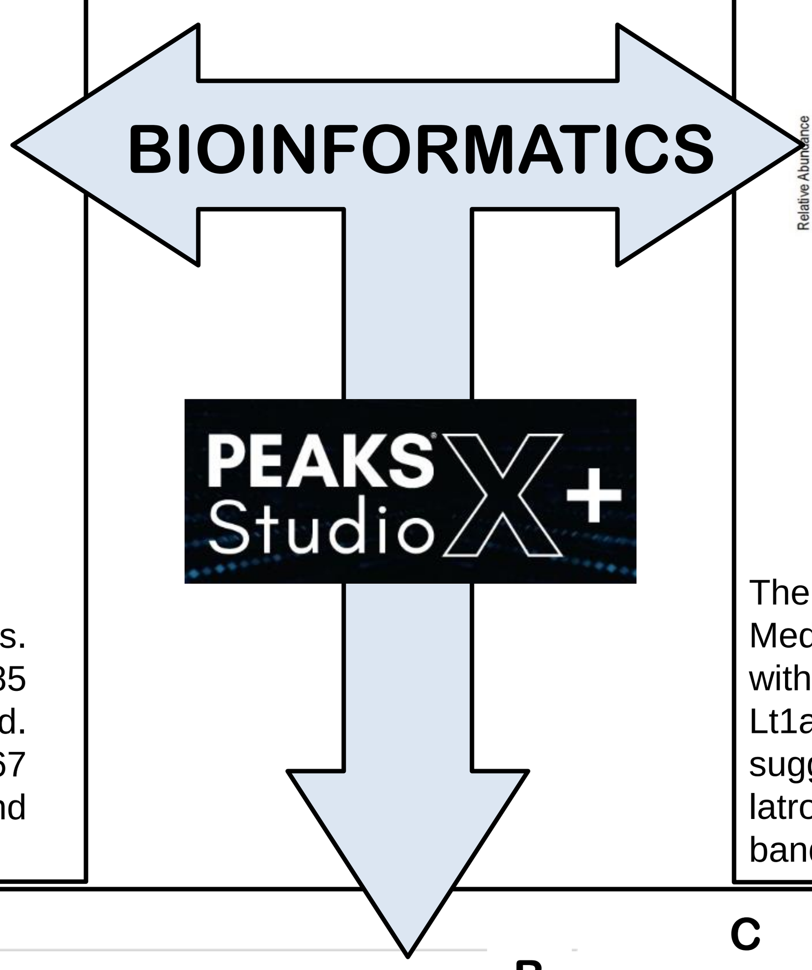
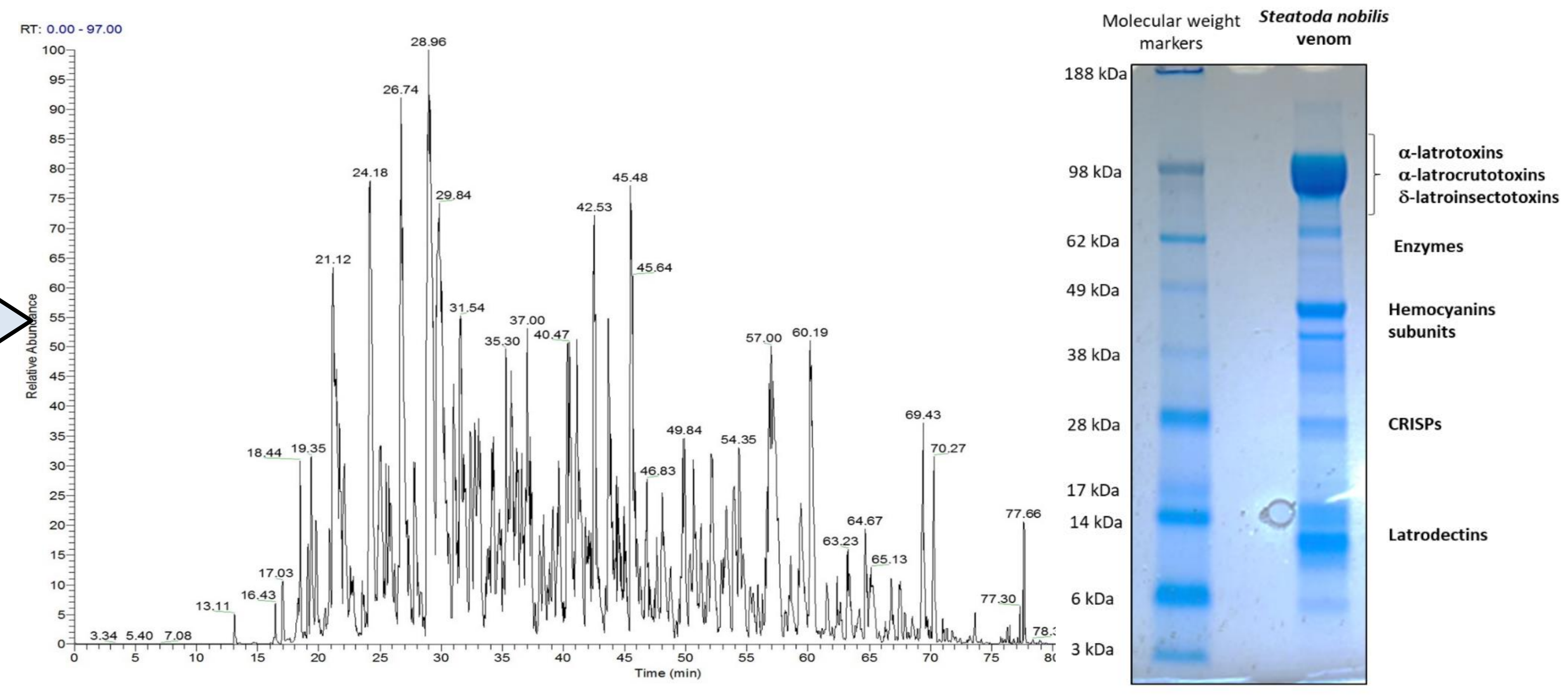
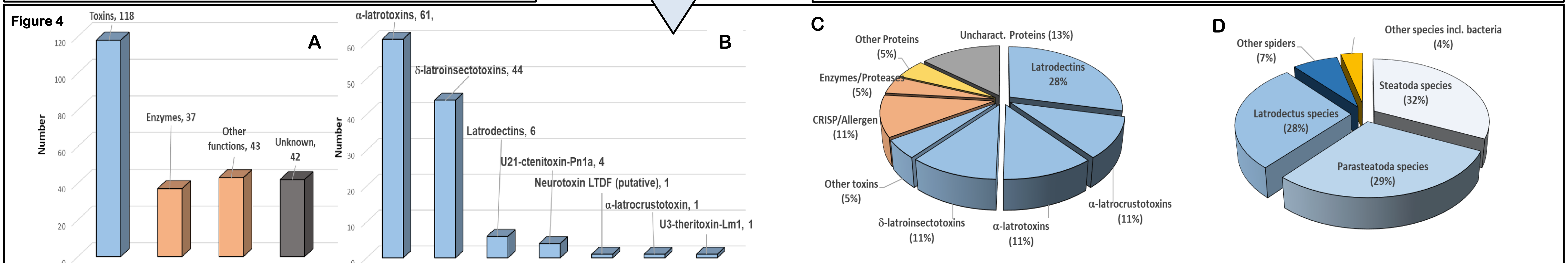


Figure 3 SHOTGUN PROTEOMICS



The best identification from proteomics was a δ-latroinsectotoxin-Lt1a from *L. tredecimguttatus*, the Mediterranean black widow. U11-Theriditoxin-Lha1d, from the Australian black widow *L. hasselti* followed within the best matches. Two isoforms of δ-latroinsectotoxin-Lt1a, an α-latrocrustotoxin-Lt1a, an α-latrotoxin-Lt1a, an α-latrotoxin-Lh1a and a Toxin 21 (from *Cupennius salei)* was additionally identified. SDS-PAGE suggests the most intense bands (90 and 140 kDa) corresponded to the presence of α-latrotoxin, δ-latroinsectotoxins and α-latrocrustotoxin. The intensity of the bands indicates that these toxins constitute the most concentrated group of proteins in the venom.

Figure 4



Integration of transcriptomics and proteomics data highlight 240 proteins. BLAST identified 118 toxins, 37 enzymes, 43 proteins with other activities and 42 proteins had unknown functions **(Fig 4A)**. The 118 toxin-annotated sequences were classified into 9 different families **(Fig 4B)**. Numerous α-latrotoxins, δ-latroinsectotoxins and latrodectins (in common with *Latrodectus*), were clearly identified. Importantly, the 118 identified toxins using proteomic data to mine transcript data closely matched the 127 predicted toxin transcripts from the venom gland transcriptome (identified against SwissProt and TrEMBL databases), highlighting the power of the approach. **Fig 4C** presents quantified proteins and classified to their families. The figure clearly shows that two thirds of the venom is composed of toxins (66% of the total intensity of the signal). Interestingly, the most concentrated toxins in the venom of *S. nobilis* are *Latrodectus*-like toxins. This finding is in good agreement with the transcriptomic data, suggesting that venom composition is largely controlled at gene expression level. **Fig 4D** displays the genera from which the 198 proteins are the closest. While *Steatoda* and *Parasteatoda* are the two closest (32% and 29%, in relative signal intensity), the genus *Latrodectus* represents more than a quarter of the whole venom, thus confirming a close relationship between the "false" and the "true" widow spiders.

SYMPTOMOLOGY

Figure 5

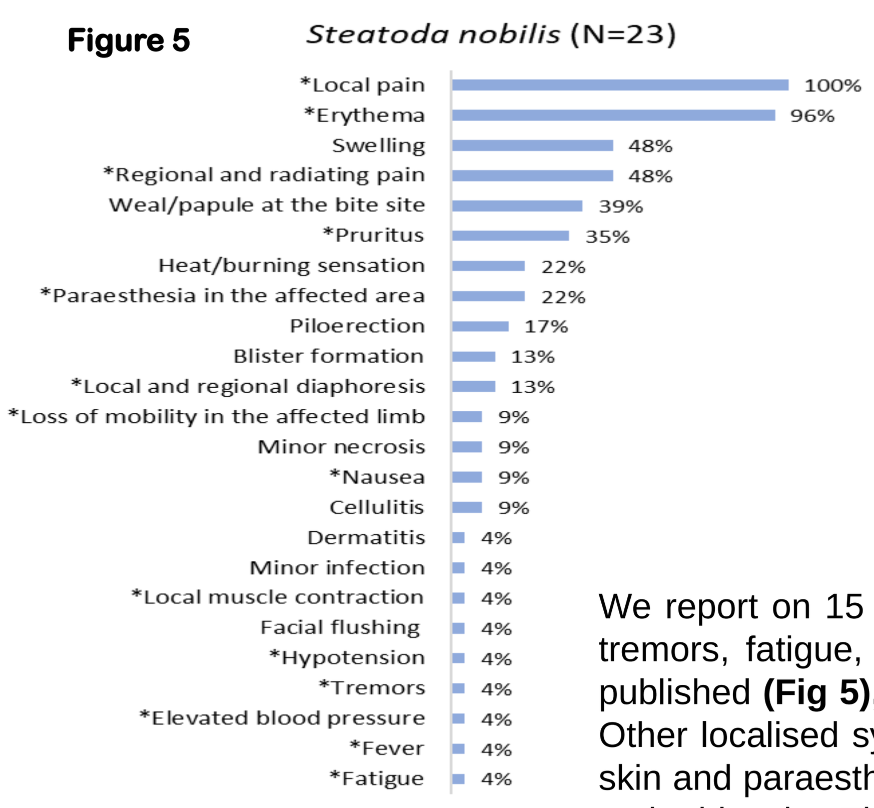


Figure 6

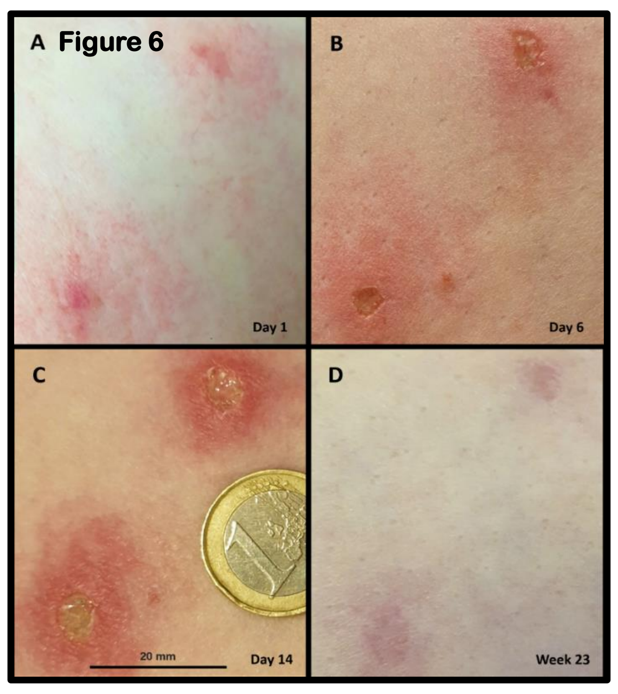


Figure 7

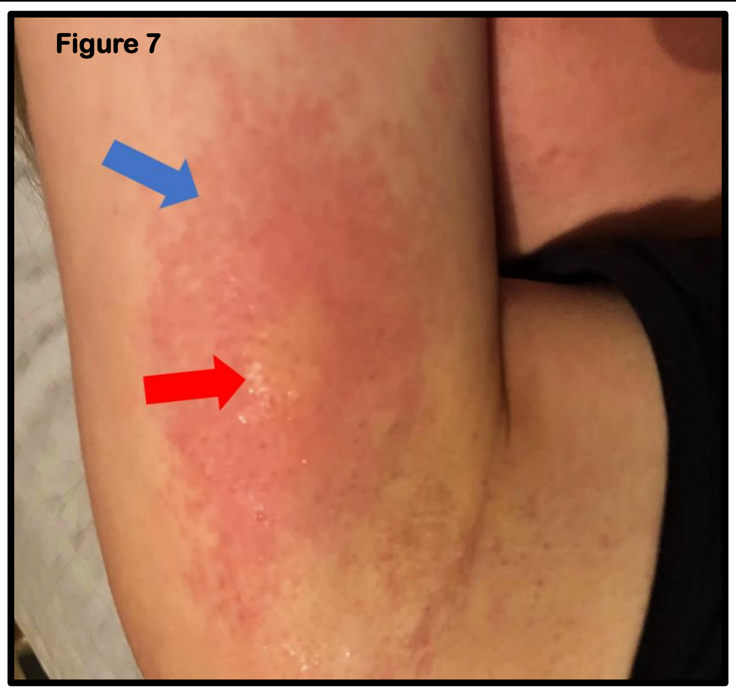


Figure 8

We report on 15 new cases of bites by *S. nobilis* revealing a range of pathologies including necrosis **(Fig 5, 6)**, *Latrodectus-like* symptoms: debilitating pain, tremors, fatigue, nausea and hypotension **(Fig 5)**, and vectored bacterial infections including cellulitis and dermatitis **(Fig 5, 8)**. Including cases previously published **(Fig 5),** the most common symptoms are pain, erythema **(Fig 7 blue arrow)**, swelling, a weal at the bite site **(Fig 7 red arrow)** and pruritus **(Fig 5)**. Other localised symptoms include radiating pain from the bite site, local sweating, a sensation of heat, tenderness, piloerection, inflammation, irritation, raised skin and paraesthesia around the bite site, muscle contractions in the affected area, reduced mobility in the affected limb, and lump/pimple and blister formation at the bite site. The venom of *S. nobilis* has the ability to induce systemic effects, such as facial flushing, feverishness, elevated and low blood pressure.

BIBLIOGRAPHY

[1] Warrell, D., et al., Toxicon, 1991. 29(10): p. 1263-1265.
[2] Bauer, T., et al., NeoBiota, 2019. 42: p. 19.
[3] Dugon, M.M., et al. in Biology and Environment: Proceedings of the Royal Irish Academy. 2017. JSTOR.
[4] Dunbar, J.P., et al., Clinical Toxicology, 2018. 56(6): p. 433-435.
[5] Hambler, C., The'Noble false widow'spider *Steatoda nobilis* is an emerging public health and ecological threat. 2019.
[6] Dunbar, J.P., et al., Toxins, 2020. 12(6): p. 402

ACKNOWLEDGMENTS

- GIGA-R proteomics platform of ULiège
- Nanou Tanteliarisoa for her technical help during the preparation of the samples
- Sam Afoullouss for lyophilizing venom samples

CONCLUSIONS

Venomics approach reveals the striking similarity between *S. nobilis* and black widows venoms **(Fig 4)**. The most powerful toxin classes and the enzymatic machinery allowing the venom to more easily spread into the prey are present in large quantities. As *S. nobilis* continues to expand its range, its impact on native wildlife needs to be monitored and its potential invasiveness assessed. While the potency of the toxin isoforms have yet to be determined from LD50 models, it should be noted that α-latrotoxins, the highly potent neurotoxins that effect the nervous system of vertebrates, are more highly concentrated in *S. nobilis* venom than in *Latrodectus*. These case reports confirm that the venom is evidently highly active and can potentially induce medically important symptoms. Severe debilitating pain was experienced by some victims. One victim felt intense pain radiating from her hip to the neck and jaw line and down to her ankle for 24 hrs. Another victim experienced debilitating pain requiring strong analgesics that target directly the central nervous system, and a third victim **(Fig 8)** required weeks of opioid medication to manage the pain. More concerning is the severe debilitating pain, tremors, hypotension, nausea, muscle contractions and impaired mobility of the affected limb which are *Latrodectus*-like symptoms. These and some of the other symptoms reported in this case series share close similarities to the neurotoxic symptoms that are consistent with Latrodectism **(Fig 5)**. For this reason, even though most cases are not considered dangerous and symptoms remain mild to moderate, envenomations by *S. nobilis* should not be trivialised, especially when *Latrodectus* venom occasionally induces severe symptoms with fatal outcomes. It is important to fully understand the full potential range of symptoms before dismissing them, especially considering the increase in *S. nobilis* bite incidents and their global range expansion. Given the composition of the venom depicted in this study, in addition to the symptoms presented by victims, *S. nobilis* should be considered as a species of medical importance and there is no doubt that *S nobilis* (with *L. tredecimguttatus*) is one of the most dangerous spiders in Western Europe. Steatodism is a rare, but potentially severe, clinical syndrome when caused by the Noble false widow spider *S. nobilis*.