

In-depth biochemical mapping of *Pyrodinium bahamense* highlights the contribution of associated microorganisms to critical cellular processes

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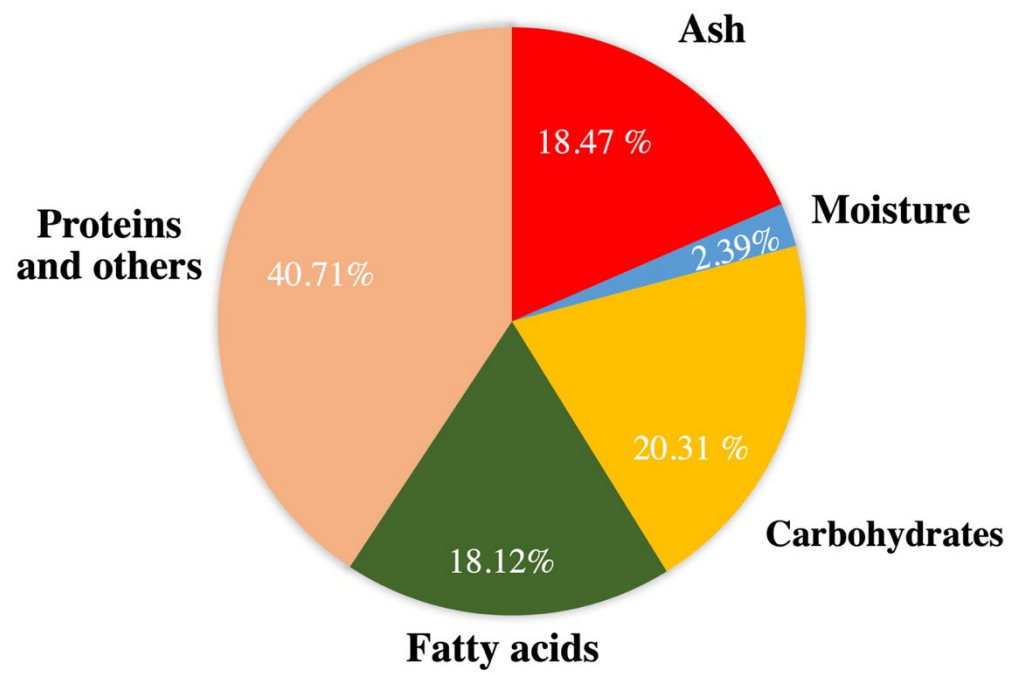
Supplemental Datasets

Data S1. MASCOT_NCBInr database search

The file contains a tabulated protein identification and annotation using MASCOT NCBInr database search.

Data S2. Protein identification across five reference taxa

The file contains a tabulated protein identification and annotation using various reference proteomes/transcriptomes of *Marinovum algicola*, *Alexandrium*, *Cylindrospermopsis raciborskii*, *Symbiodinium kawagutii* and *Pyrodinium bahamense*.



First Biological Replicate

Second Biological Replicate

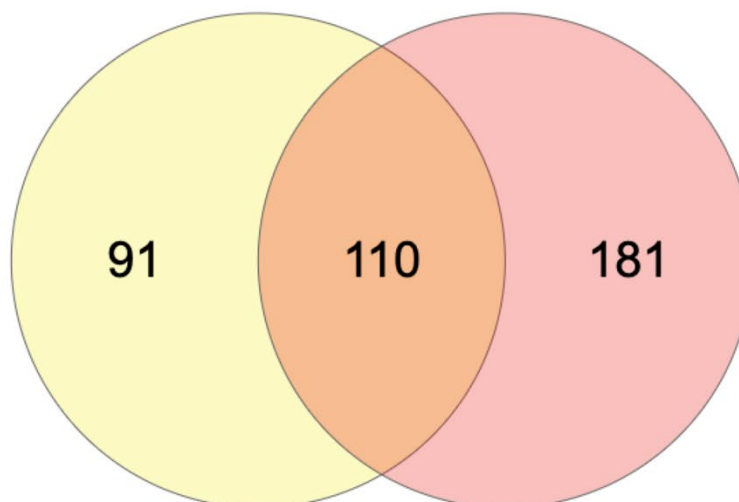
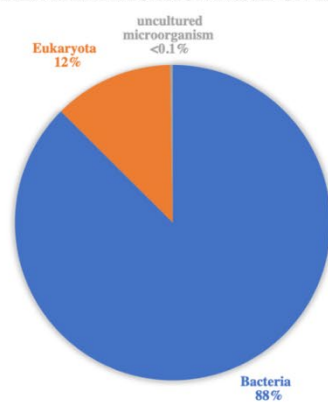
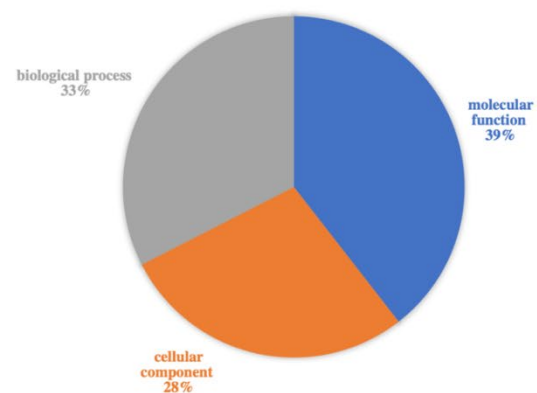


Figure S2. Summary of unique and similar GeneInfo identifier (GI) numbers for the two biological replicates of *P. bahamense*. 110 GI were common for both replicates while there were 91 and 181 unique GI numbers for the first and second biological replicate, respectively.

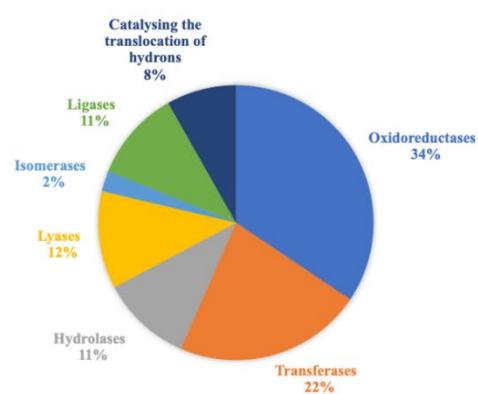
A PROTEIN IDENTIFICATION BASED ON TAXONOMY

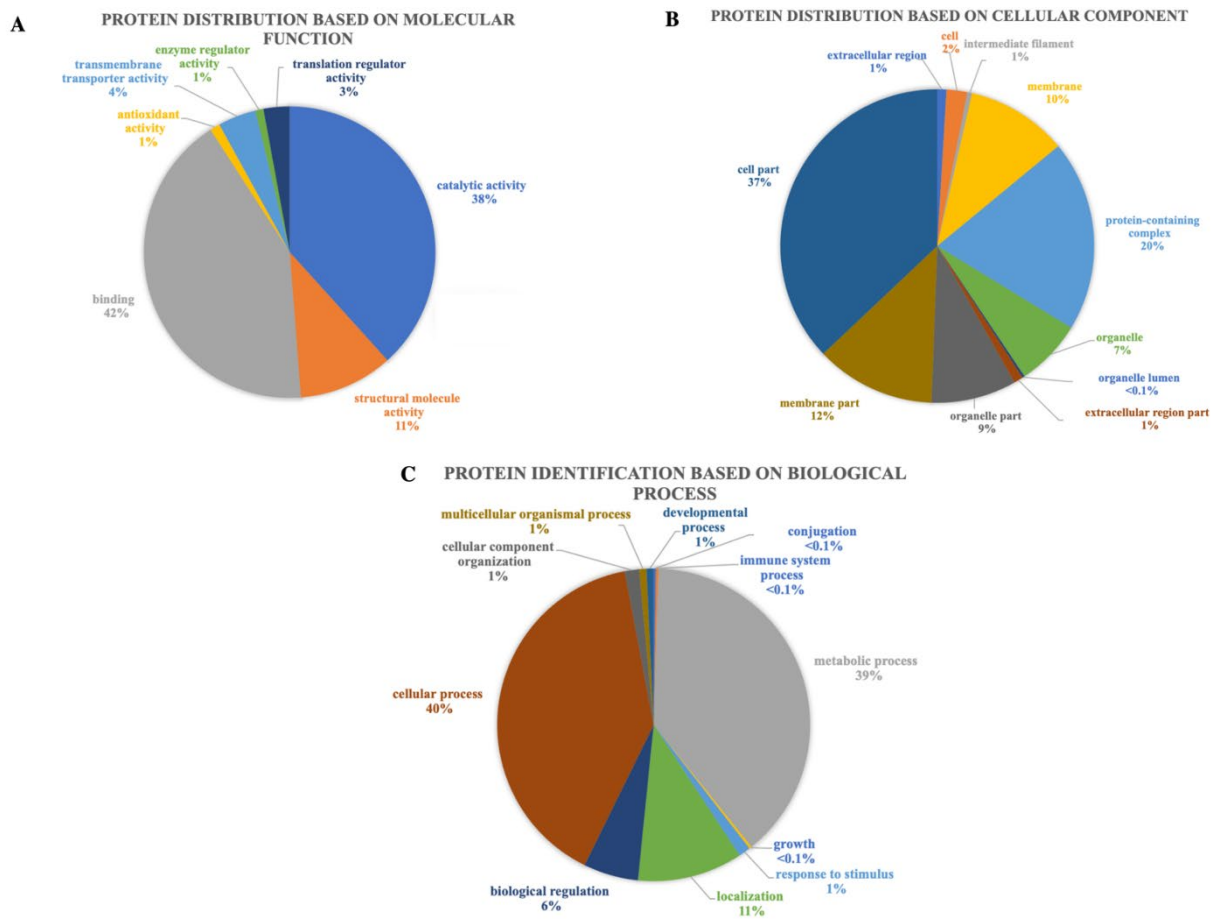


B PROTEIN DISTRIBUTION BASED ON ONTOLOGY



C PROTEIN DISTRIBUTION BASED ON ENZYME CLASS





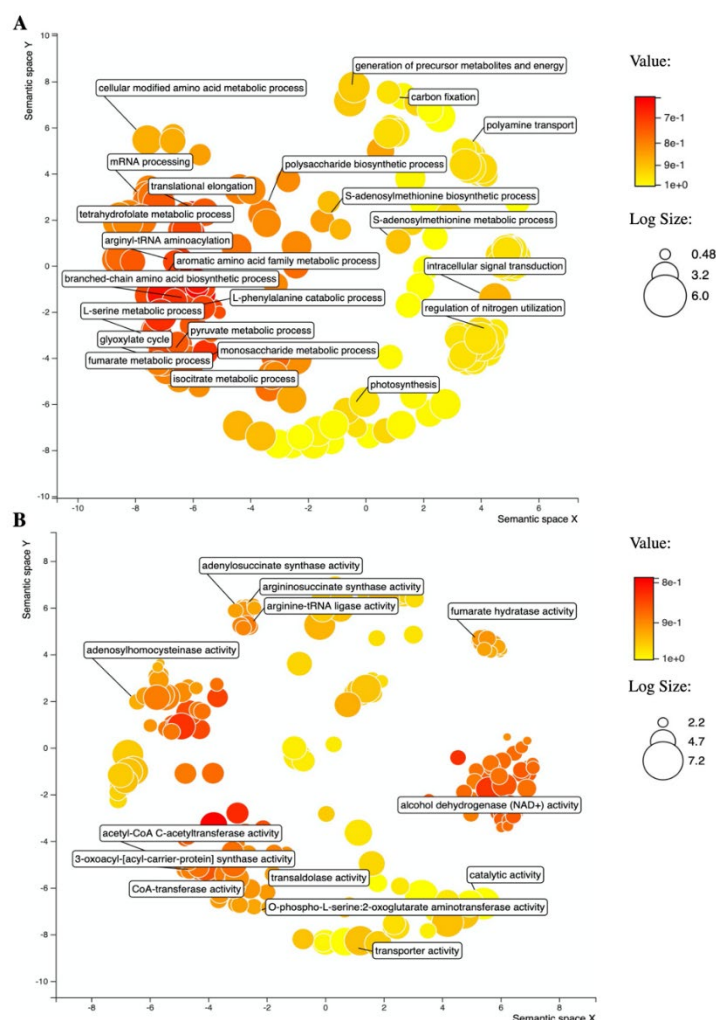


Figure S5. REVIGO analysis of combined GO identifiers of proteins identified using various reference databases. The GO identifiers obtained from proteins identified across different reference databases were visualized using REVIGO to map the various processes for *Pyrodicticum bahamense*. **A.)** Notable biological processes include amino acid biosynthesis and degradation, polysaccharide biosynthesis, S-adenosylmethionine biosynthesis and metabolism, glycolysis, and photosynthesis. **B.)** Meanwhile, important molecular functions include adenosylsuccinate synthase and adenosyl homocysteinase activity, glycolysis-related activities, catalysis and transporter activities. Bubble color indicates the p-values for the false discovery rates while the circle log size indicates the logarithmic frequency of the GO term.

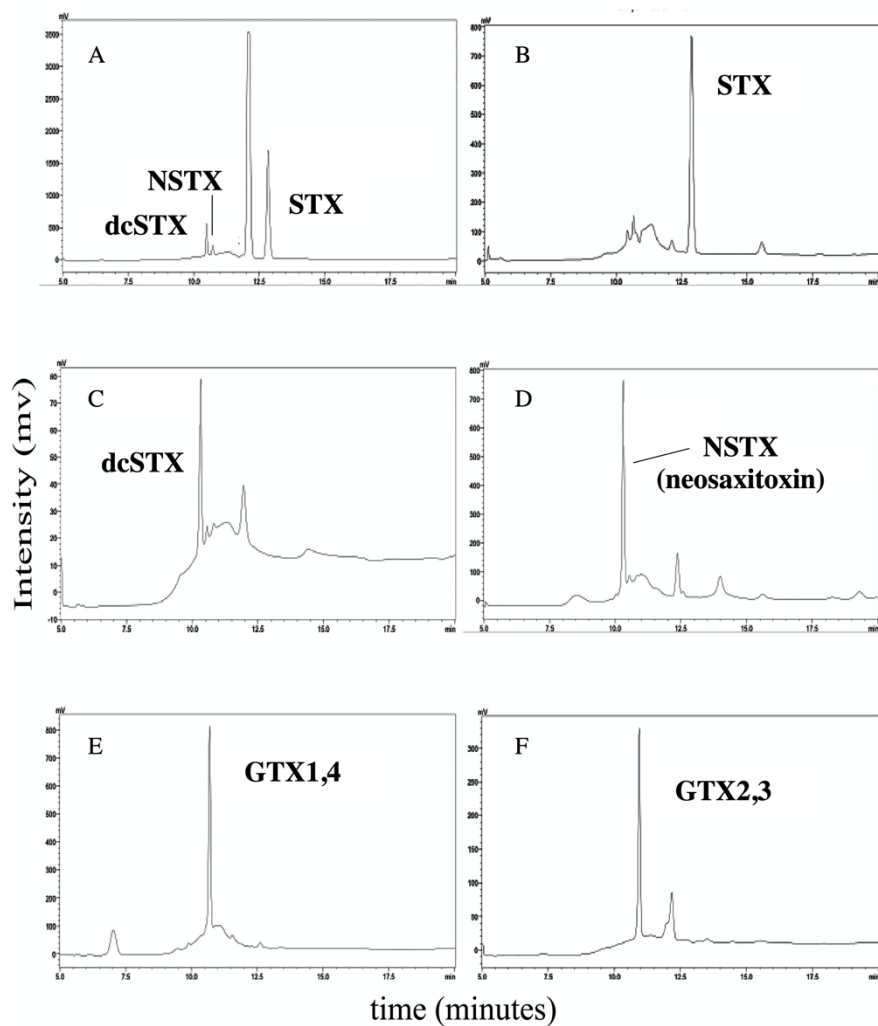


Figure S6. Toxin content of *P. bahamense*. A. A pre-column oxidation method for toxin content using available toxin standards for quantification was conducted based on the procedures of Subong, et al. 2017 and Subong, et al. 2021. Subcultured *P. bahamense* used for proteomic analysis showed toxin content of 200-215 fmol STX equiv/cell with toxin profiles consist of STX, neoSTX and dcSTX (A). Toxin content and profile was compared to standard B. saxitoxin (STX); C. decarbamoylsaxitoxin; D. neosaxitoxin (NSTX); E. gonyautoxin 1,4; F. gonyautoxin 2,3.

Supplementary Tables

Table S1. Optimized MRM Transitions for Monosaccharide Standards

Standard	Transition	Product m/z	CE
Arabinose	194.90 > 89.05	89.05	11
	194.90 > 149.20	149.20	9
	194.90 > 59.05	59.05	20
Fructose	224.95 > 89.05	89.05	13
	224.95 > 179.15	179.15	6
	224.95 > 59.10	59.10	23
Galactose	224.90 > 89.05	89.05	12
	224.90 > 179.15	179.15	7
	224.90 > 59.10	59.10	22
Glucose	224.95 > 179.15	179.15	7
	224.95 > 89.05	89.05	12
	224.95 > 59.05	59.05	23
Mannose	224.95 > 179.15	179.15	7
	224.95 > 119.10	119.10	10
	224.95 > 89.10	89.10	13
Ribose	194.90 > 149.20	149.20	8
	194.90 > 89.05	89.05	12
	194.90 > 59.10	59.10	20
Xylose	194.90 > 89.05	89.05	11
	194.90 > 149.20	149.20	9
	194.90 > 59.10	59.10	17

Table S2. Proteins from Potential Associated Bacterial Interactions

Protein	Number of peptides	Sequence Coverage (%)	Score	q-value	Function
amino acid ABC transporter-substrate binding proteins**	1	2.3	132	0	solute active transporter
Sequence: NTTWTFSRDTSMGLYF [Cylindrospermopsis raciborskii]					
elongation Tu factor	10	32.5	262	0	catalysts for aminoacyl-tRNA (aa-tRNA) binding to the ribosome
[Alexandrium]					
RHS-repeat associated core-containing protein	67	38.1	323	0	bacterial exotoxin
[Marinovum algicola]					
bifunctional folylpolyglutamate synthase/dihydrofolate synthase**	1	2.9	1.6*	0	synthesis of folate
Sequence: VGAGQMSRVDSALIAA [Marinovum algicola]					
propionyl-CoA synthetase**	1	8.7	1.2*	0.007	propanoate metabolism
Sequence: TFAELQTRVATLAGAL [Marinovum algicola]					

acryloyl-CoA reductase	2	7.9	0.98*	0.007	L-alanine fermentation
[<i>Marinovum algicola</i>]					

* denotes MaxQuant Score; otherwise, MASCOT score is reported

** unique peptide

Table S3. Proteins involved in Toxin Biosynthesis

Protein	Number of peptides	Sequence Coverage (%)	Score	q-value	Function
chlorophyll a/c- binding protein, partial (<i>Alexandrium tamarense</i>)	2	9.1	22*	0	photosynthesis
RHS repeat-associated core domain-containing protein [<i>Marinovum algicola</i>]	67	38.1	323	0	bacterial exotoxin
contig07438 adenosylhomocysteinase [Pbc transcript]	3	16.4	2.5*	0	methylation
methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase activity** Sequence: GLITLNPKEAVSSLA [<i>Symbiodinium kawagutii</i> transcript]	1	0.7	5.8*	0.0175	L-methionine biosynthesis
acetyl-CoA acetyltransferase [<i>Alexandrium</i>]	2	15	17*	0	transferase activity
contig00523 methionine S-adenosyl transferase [Pbc transcript]	2	6.9	5.00*	0	cysteine and methionine metabolism
periplasmic sensor hybrid histidine kinase ** Sequence: GDLMVVSRPGHGATFF [<i>Alexandrium/ Mameiliella alba</i>]	1	2.5	5.9*	0	integral membrane component

iron-containing alcohol dehydrogenase **	1	2.2	6.2*	0	redox reaction
Sequence: TDVKMMMKHPSFTPQV [Alexandrium/ Bacillus sp.]					
contig06470 ferredoxin-binding protein II [Pbc transcript]	8	42	125*	0	photosynthesis
ferredoxin-nitrite reductase **	1	2.5	6.0*	0	nitrogen metabolism
Sequence: IPLNVVVKPEEVVSL [Cylindrospermopsis raciborskii]					
* denotes MaxQuant Score; MASCOT score if not indicated ** unique peptide					

Table S4. Proteins involved in Arginine Biosynthesis

Protein	Number of peptides	Sequence Coverage (%)	Score	q-value	Function
ornithine decarboxylase [<i>Marinovum algicola</i>]	3	4.1	5.3*	0	polyamine synthesis
argininosuccinate synthase [<i>Marinovum algicola</i>]	7	21	28*	0	<i>argininosuccinate</i> synthesis
ornithine-acyl-ACP acyltransferase** Sequence: LAEDQIDRRRAMLQVP [<i>Alexandrium</i>]	1	6.8	5.8*	0	transferase activity
bifunctional ornithine acetyltransferase/N-acetylglutamate synthase** Sequence: VGAGQMSRVDSALIAA [<i>Marinovum algicola</i>]	1	2.9	1.6*	0	arginine biosynthesis

* denotes MaxQuant Score; otherwise, MASCOT score is reported

** unique peptide

Table S5. Polyketide Synthesis-related Proteins

Protein	Number of peptides	Sequence Coverage (%)	Score	q-value	Function
type I polyketide synthase mixed NRPS/PKS (CyrB) [<i>Cylindrospermopsis raciborskii</i>]	2	1.0	12*	0	polyketide synthesis and non-ribosomal peptide synthesis
beta-ketoacyl-ACP reductase [<i>Marinovum algicola</i>]	2	13	34*	0	type II Fatty acid biosynthesis
enoyl-[acyl-carrier-protein] reductase [<i>Marinovum algicola</i>]	3	12	3.6*	0	type II fatty acid biosynthesis
ornithine-acyl-ACP acyltransferase **	1	6	6.8*	0	ornithine synthesis
Sequence: LAEDQIDRRRAMLQVP					
[<i>Alexandrium</i>]					
beta-ketoacyl-[acyl-carrier-protein] synthase II	9	50	20*	0	fatty acid biosynthesis
[<i>Marinovum algicola</i>]					
3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabA	4	34	3.8*	0	lipid A biosynthesis
[<i>Marinovum algicola</i>]					

* denotes MaxQuant Score; otherwise, MASCOT score is reported

** unique peptide

Table S6. Proteins Involved in Photosynthesis and Bioluminescence

Protein	Number of peptides	Sequence Coverage (%)	Score	q-value	Function
Rubisco [<i>Symbiodinium</i>]	6	7.7	130*	0	carbon fixation
peridinin chlorophyll a-binding protein precursor [Pbc transcript]	13	47.9	253*	0	photosynthesis
luciferin binding protein** Sequence: VAQETFTKMVQLARKL [Pbc transcript]	1	3.7%	1.0*	0	bioluminescence

* denotes MaxQuant Score; otherwise, MASCOT score is reported

** unique peptide

Table S7. Proteins Involved in Circadian Rhythm and Growth

Protein	Number of peptides	Sequence Coverage (%)	Score	q-value	Function
citrate synthase [<i>Marinovum algicola</i>]	10	29.9	57*	0	citric acid cycle
pyruvate kinase [<i>Marinovum algicola</i>]	2	7.5	2.4*	0	glycolysis
glyceraldehyde dehydrogenase**	1	4.3	6.7*	0	glycolysis
Sequence: PTSTGAAKAVGLVLPE					
[<i>Marinovum algicola</i>]					
2-oxoglutarate dehydrogenase**	1	2.3	7.0*	0	citric acid cycle
Sequence: VDGSGDVKYHLGASSD [<i>Alexandrium</i>]					
succinyl-CoA-3-ketoacid COA transferase**	1	7.7	24*	0	valine, leucine, isoleucine degradation
Sequence: DTGNLVFRKTARNFNP					
[<i>Marinovum algicola</i>]					
tricarboxylate transporter**	1	9.4%	2.0*	0.005	citrate transporter
Sequence: QGNLSIPRRMCAGLVG					
[Pbc transcript]					

* denotes MaxQuant Score; otherwise, MASCOT score is reported; ** unique peptide

References:

- Subong, B.J.J.; Benico, G.A.; Sulit, A.K.L.; Mendoza, C.O.; Cruz, L.J.; Azanza, R. V.; Jimenez, E.C. Toxicity and protein expression of *Alexandrium* species collected in the philippine waters. *Philipp. J. Sci.* **2017**, *146*, 425–436.
- Subong, B.J.J.; Lluisma, A.O.; Azanza, R. V.; Salvador-Reyes, L.A. Differentiating two closely related *Alexandrium* species using comparative quantitative proteomics. *Toxins (Basel)*. **2020**, *13*, DOI: 10.3390/toxins13010007, doi:10.3390/toxins13010007