Supplementary Material

The *Rana sylvatica* skin-secreted antimicrobial peptide (AMP) gene repertoire highlights broader patterns in anuran AMP evolution

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Contents:

Supplementary Methods

Animal Capture and Care 2021

In late March and early April 2021, frogs were captured from two vernal ponds spatially separated by ~200 m and transported to the University of Waterloo in insulated opaque containers containing sphagnum moss moistened with well water and the container kept cool with a covered ice pack. While the University of Waterloo WATER Facility was undergoing renovations, frogs were housed in an isolated area of the Katzenback Lab. Frogs were housed communally in ventilated opaque containers containing sphagnum moss moistened with spring water and were held at a temperature approximating their natural environment (8-12 °C) for 9-14 days, and offered crickets periodically, although feeding was not observed. Frogs were either used experimentally under spring conditions, as members of the “Spring” group or transferred to terrariums to simulate summer conditions to serve as members of the “Captive Summer” group. Members of the “Captive Summer” treatment group were housed communally in terrariums containing coconut coir substrate and sphagnum moss moistened with spring water, as well as wood chips, an artificial log hide, and a shallow dish containing spring water. Terrariums were held at room temperature18-22 °C with indirect natural light provided by a nearby window. Terrariums were inspected daily to observe animal health and remove waste, and mist with spring water if substrate appeared dry. Individuals were fed to satiation with gut-loaded crickets twice weekly, calcium and vitamin D3 were supplemented by dusting crickets with Repti Calcium with D3 (Zoomed) on every second feeding, and terrariums were cleaned and prepared with fresh substrate monthly. Members of the “Captive Summer” treatment group were held in terrariums for 7 - 56 days prior to experimental use.

In August 2021, wood frogs were captured from the forest floor. Frogs were transported to the Katzenback Lab at the University of Waterloo as described above and were held for a maximum of 24 h prior to experimental use.

Plasmid Isolation by Alkaline Denaturation

Aliquots of 1.5 mL were taken from each culture and pelleted by centrifugation at 10,000 × g for 5 min. Pellets were resuspended in 100 µL of ice-cold 50 mM glucose, 10 mM EDTA and 25 mM Tris at pH 8. Cells were lysed by the addition of 200 µL of room temperature 0.2 M NaOH, 1% sodium dodecyl sulfate and gentle inversion to mix. The reaction was neutralized by the addition of 150 µL of ice-cold 3 M KOAc at pH 6 and gentle inversion to mix. Lysed cultures were centrifuged at 15,500 × g for 30 min at 4 °C, after which 200 µL of supernatant was removed, combined with 20 µL of 1 mg/mL RNase A (BioBasic, Cat#: RB0473) and incubated at 37 °C for 20 min to remove RNA. Plasmid DNA was pelleted by adding 550 µL of ice-cold isopropanol, thoroughly mixing by inversion, and centrifuging for 15,500 × g for 30 min at 4 °C. Supernatant was removed and pellets were washed twice with 1 mL of ice-cold 70% ethanol. Pellets were air-dried prior to resuspension in molecular grade water.

Phylogenetic analysis of predicted AMP precursors

Using Clustal Omega [1], the predicted *Rana sylvatica* AMP precursor amino acid sequences were aligned with all skin secreted AMP precursor sequences derived from Ranid frogs found in the UniprotKB/Swiss-Prot database, as well as two skin secreted AMP precursor sequences derived from hylid frogs which served as an outgroup. While the evolutionary relationship between Ranid and hylid AMPs is not firmly established at present, these precursors included signal peptides which aligned closely with Ranid AMP precursors but consistently formed an outgroup. Phylogenetic analysis of the aligned sequences was performed in IQ-TREE v2.0.7 [2]. Using the ModelFinder [3] option, the Jones Taylor Thornton (JTT) substitution model [4] with four components of gamma rate heterogeneity (+G4) was determined to be optimal according to the Bayesian information criterion. Maximum likelihood analysis was performed with 1000 ultrafast bootstrap replicates [5]. Visualization and annotation of the resulting tree was performed in MEGA11 [6].


R. sylvatica Chr. 3:624120768-624120834 | brevinin-1SYb
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R. sylvatica Chr. 3:617334923-617334989 | temporin-1SYb
R. sylvaticaChr. 3:617257260-617257194 | temporin-1SYd
R. sylvatica Chr. 3:624155221-624155287
99
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R. sylvatica Chr. 3:615302201-615302135 | esculentin-2SY
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R. muscosa Chr. 8:381169307-381169373
R. muscosa Chr. 8:381379940-381379874
R. muscosa Chr. 8:384654495-384654429
R. temporaria Chr. 8:167457461-167457527 | bradykinin precursor
R. muscosa Chr. 8:382044809-382044743
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R. temporaria Chr. 8:160194777-160194843 | temporin-C
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R. temporaria Chr. 8:167429273-167429339 | temporin-H
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R. muscosa Chr. 8:378741632-378741698
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R. muscosa Chr. 8:384426012-384425946
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R. muscosa Chr. 8:378663501-378663567
R. muscosa Chr. 8:378851302-378851368
R. temporaria Chr. 8:165898672-165898738
R. temporaria Chr. 8:165929049-165929115 | temporin-T
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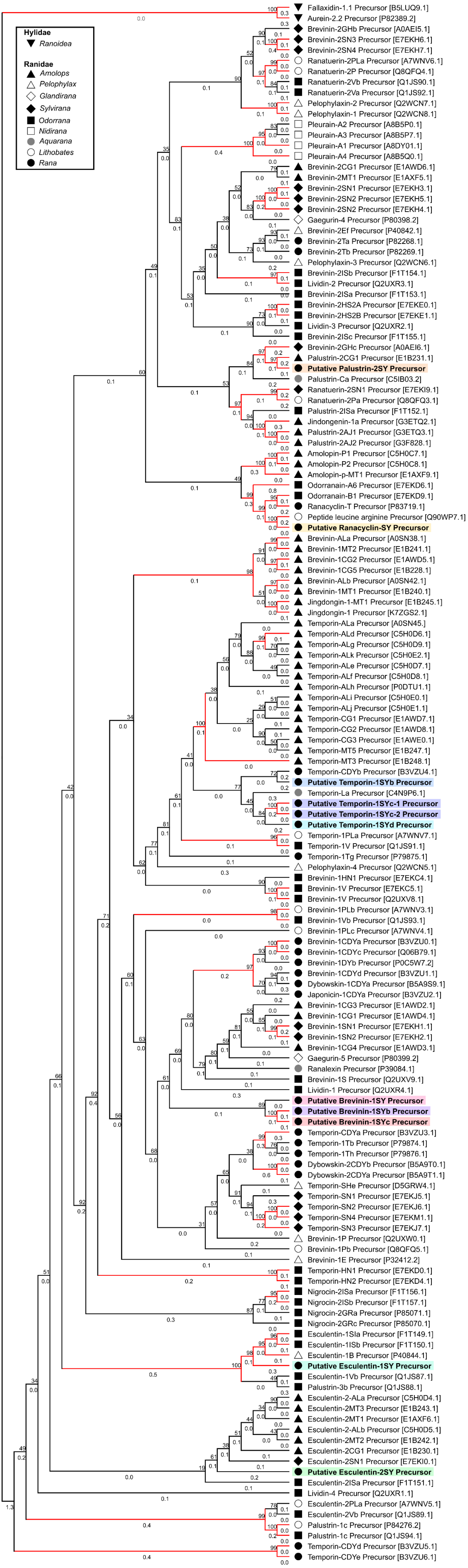
R. kukunoris Chr. 8:172029474-172029408
0.050

Figure S1. Phylogenetic relationships between predicted skin-secreted AMP signal peptides encoded in ranoid genomes. Sequences with high similarity to the *Rana sylvatica* signal peptide consensus sequence were identified in *R. sylvatica* chromosome 3 (GenBank Accession CM053028.1), *Rana kukunoris* chromosomes 2, 7 and 8 (GenBank Accessions CM056050.1, CM056055.1 and CM056056.1), *Rana muscosa* chromosome 8 (GenBank Accession CM055123.1), *Rana temporaria* chromosome 8 (RefSeq Accession NC_053496.1) and *Pyxicephalus adspersus* chromosome 10 (GenBank Accession CM016425.1). For predicted signal peptides associated with an annotated gene that encodes an identified AMP, the AMP is listed beside the species and genome coordinates. An unrooted phylogram was generated using the maximum likelihood method. Values above branches indicate ultrafast bootstrap values. A scale bar indicating relative distance is given at the bottom. The most expansive clades supported by ultrafast bootstrap values >95% are bolded and coloured. Tree annotation was performed using MEGA11.


Hylidae
Ranoidea
Ranidae
Amolops
Pelophylax
Glandirana
Sylvirana
Odorrana
Nidirana
Aquarana
Lithobates
Rana
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Brevinin-2SN4 Precursor [E7EKH7.1]
Ranatuerin-2PLa Precursor [A7WNV6.1]
Ranatuerin-2P Precursor [Q8QFQ4.1]
Ranatuerin-2Vb Precursor [Q1JS90.1]
Ranatuerin-2Va Precursor [Q1JS92.1]
Pelophylaxin-2 Precursor [Q2WCN7.1]
Pelophylaxin-1 Precursor [Q2WCN8.1]
Pleurain-A2 Precursor [A8B5P0.1]
Pleurain-A3 Precursor [A8B5P7.1]
Pleurain-A1 Precursor [A8DY01.1]
Pleurain-A4 Precursor [A8B5Q0.1]
Brevinin-2CG1 Precursor [E1AWD6.1]
Brevinin-2MT1 Precursor [E1AXF5.1]
Brevinin-2SN1 Precursor [E7EKH3.1]
Brevinin-2SN2 Precursor [E7EKH5.1]
Brevinin-2SN2 Precursor [E7EKH4.1]
Gaegurin-4 Precursor [P80398.2]
Brevinin-2Ef Precursor [P40842.1]
Brevinin-2Ta Precursor [P82268.1]
Brevinin-2Tb Precursor [P82269.1]
Pelophylaxin-3 Precursor [Q2WCN6.1]
Brevinin-2ISb Precursor [F1T154.1]
Lividin-2 Precursor [Q2UXR3.1]
Brevinin-2ISa Precursor [F1T153.1]
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Brevinin-2HS2B Precursor [E7EKE1.1]
Lividin-3 Precursor [Q2UXR2.1]
Brevinin-2ISc Precursor [F1T155.1]
Brevinin-2GHc Precursor [A0AEI6.1]
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Putative Palustrin-2SY Precursor
Palustrin-Ca Precursor [C5IB03.2]
Ranatuerin-2SN1 Precursor [E7EKI9.1]
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Brevinin-1HN1 Precursor [E7EKC4.1]
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Brevinin-1CDYc Precursor [Q06B79.1]
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Brevinin-1CDYd Precursor [B3VZU1.1]
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Japonicin-1CDYa Precursor [B3VZU2.1]
Brevinin-1CG3 Precursor [E1AWD2.1]
Brevinin-1CG1 Precursor [E1AWD4.1]
Brevinin-1SN1 Precursor [E7EKH1.1]
Brevinin-1SN2 Precursor [E7EKH2.1]
Brevinin-1CG4 Precursor [E1AWD3.1]
Gaegurin-5 Precursor [P80399.2]
Ranalexin Precursor [P39084.1]
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Putative Brevinin-1SYc Precursor
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Temporin-1Tb Precursor [P79874.1]
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Dybowskin-2CDYb Precursor [B5A9T0.1]
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Nigrocin-2GRa Precursor [P85071.1]
Nigrocin-2GRc Precursor [P85070.1]
Esculentin-1SIa Precursor [F1T149.1]
Esculentin-1ISb Precursor [F1T150.1]
Esculentin-1B Precursor [P40844.1]
Putative Esculentin-1SY Precursor
Esculentin-1Vb Precursor [Q1JS87.1]
Palustrin-3b Precursor [Q1JS88.1]
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Esculentin-2MT1 Precursor [E1AXF6.1]
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Esculentin-2SN1 Precursor [E7EKI0.1]
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Temporin-CDYe Precursor [B3VZU6.1]

Figure S2. Phylogenetic relationships between putative *Rana sylvatica* AMP precursors and previously identified Ranid AMP precursors. A cladogram was generated using the maximum likelihood method and rooted using two skin-secreted AMPs derived from hylid frogs as an outgroup. Values above branches indicate ultrafast bootstrap values, while values below branches indicate relative distance. Branches are coloured in accordance with ultrafast bootstrap values, with those supported by values >95% in red. The genus from which each AMP precursor was identified is indicated by a symbol adjacent to its name. Tree annotation was performed using MEGA11.

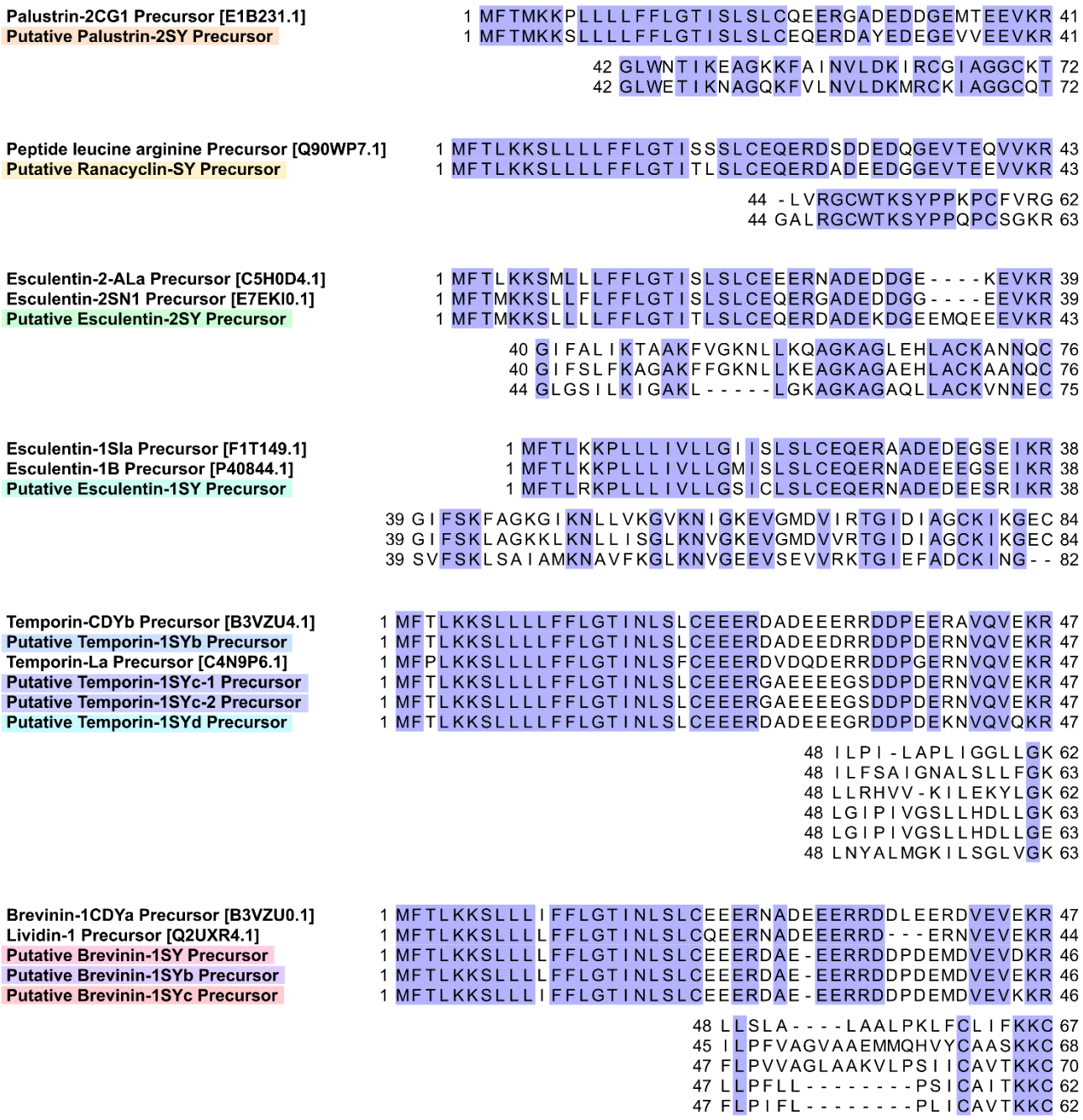
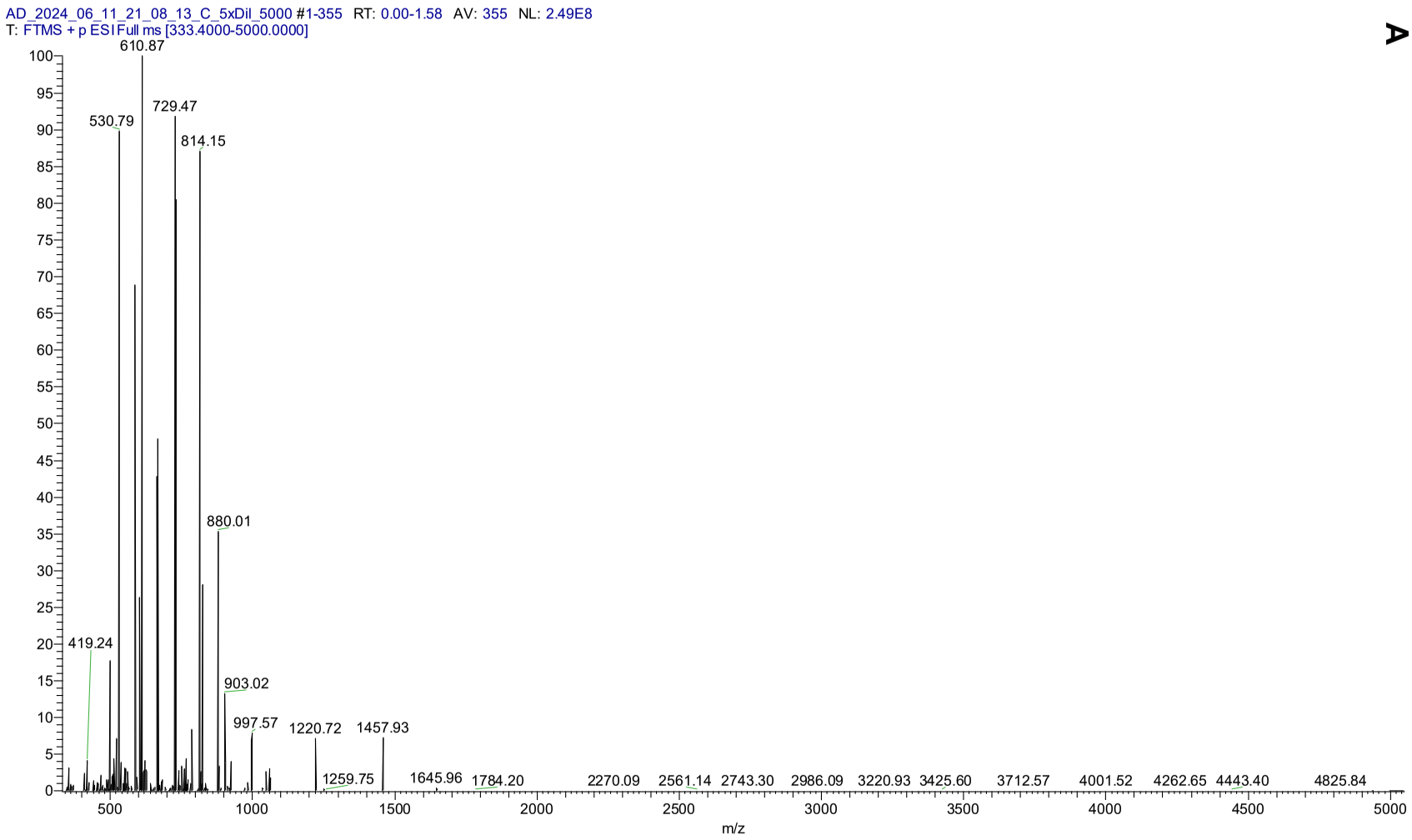


Figure S3. Clustal Omega alignments of putative *Rana sylvatica* AMP precursors with related AMP precursors. Aligned precursors were selected based on phylogenetic analysis of all Ranid AMP precursors in the Uniprot / Swissprot database. In cases where a single closest relative was not identified two AMP precursors which spanned the most closely related clade were used. Representatives of established AMP families are aligned together. Residues with 100% conservation among the aligned sequences are highlighted in blue. Sequences are divided into their preproregion (above) and mature AMP region (below).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1259.75
1645.96
1784.20
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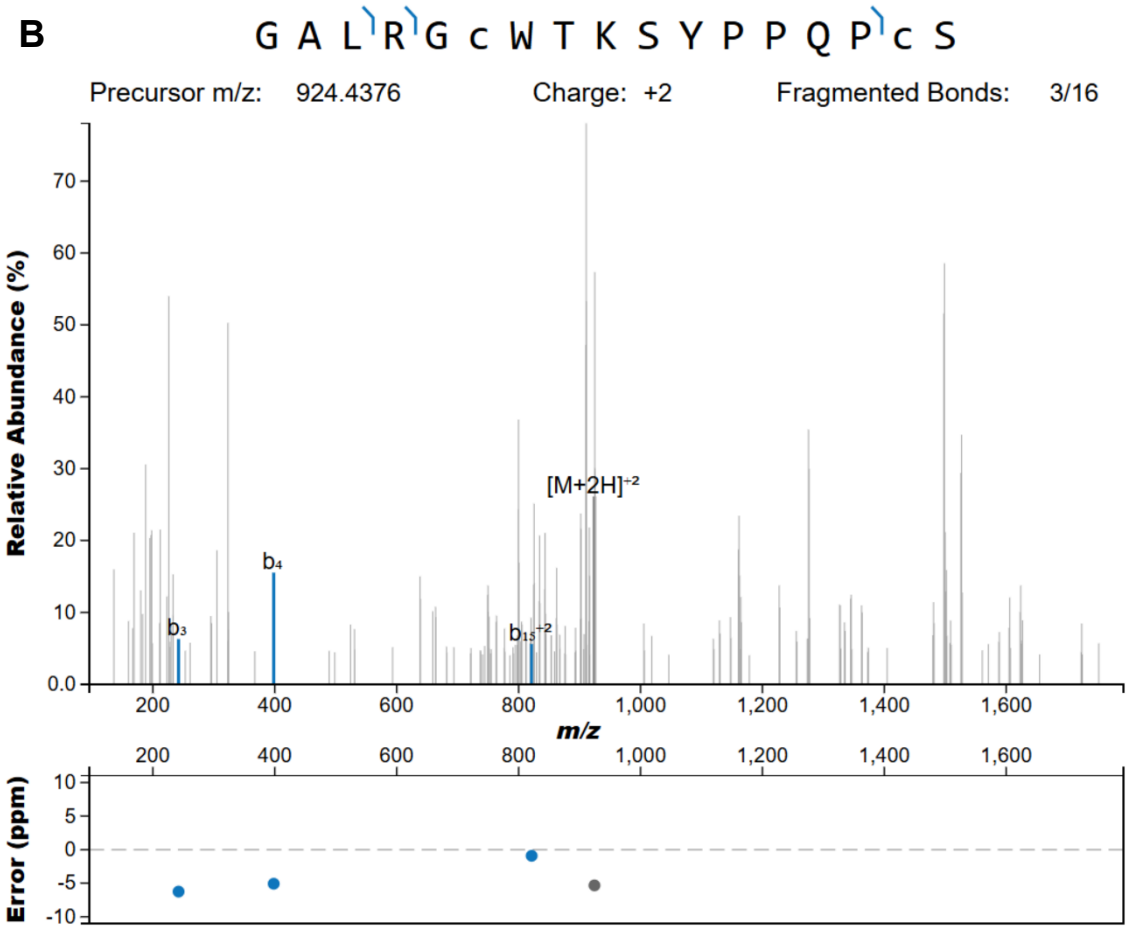
4443.40
4825.84
m/z

A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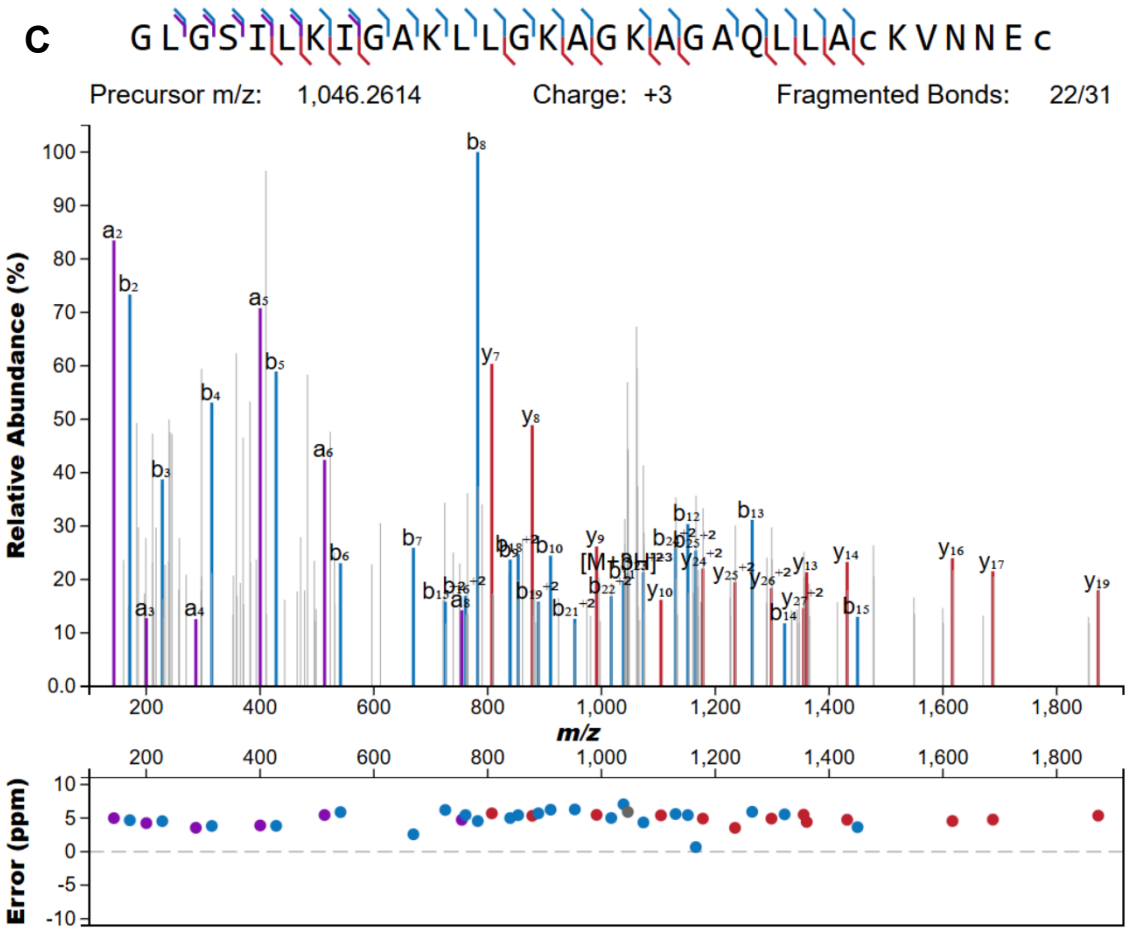
b3
b15+2
m/z
Error (ppm)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Relative Abundance (%)
m/z
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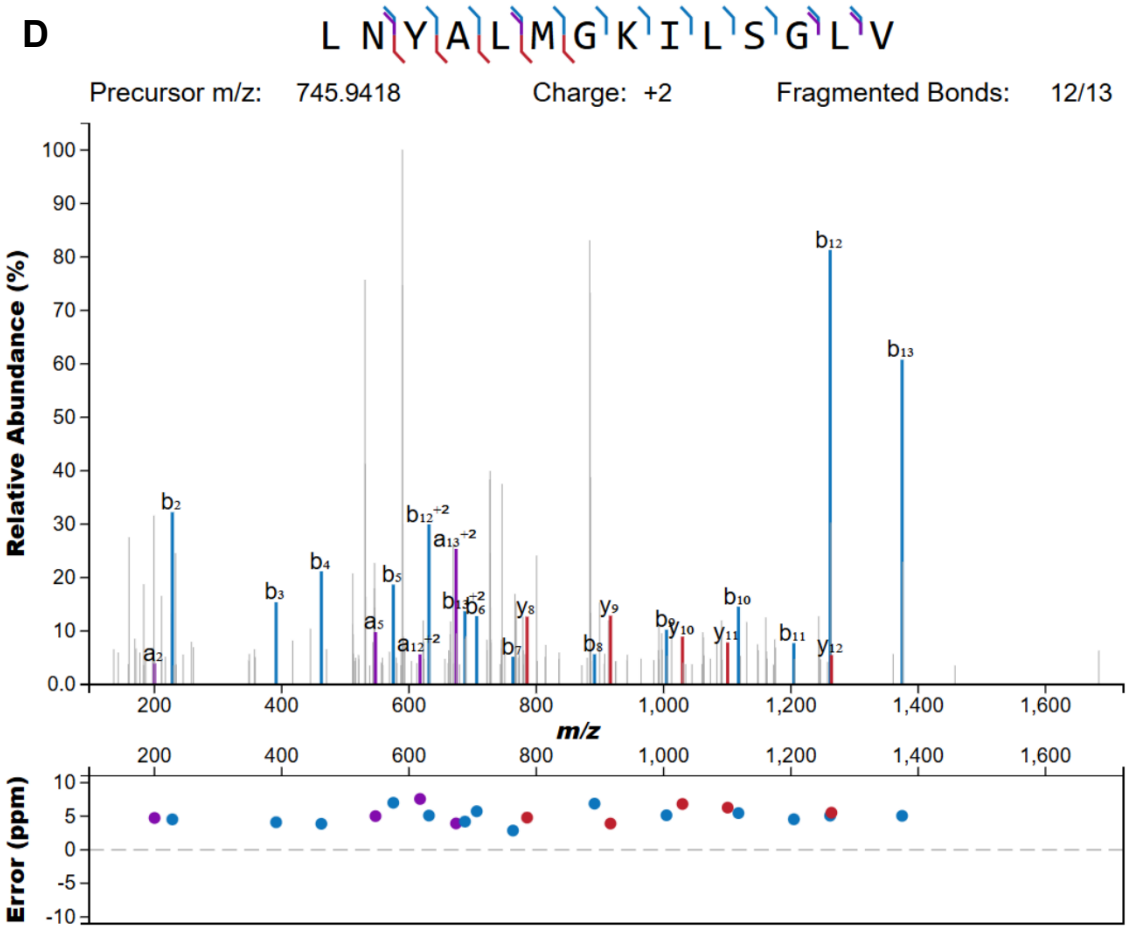
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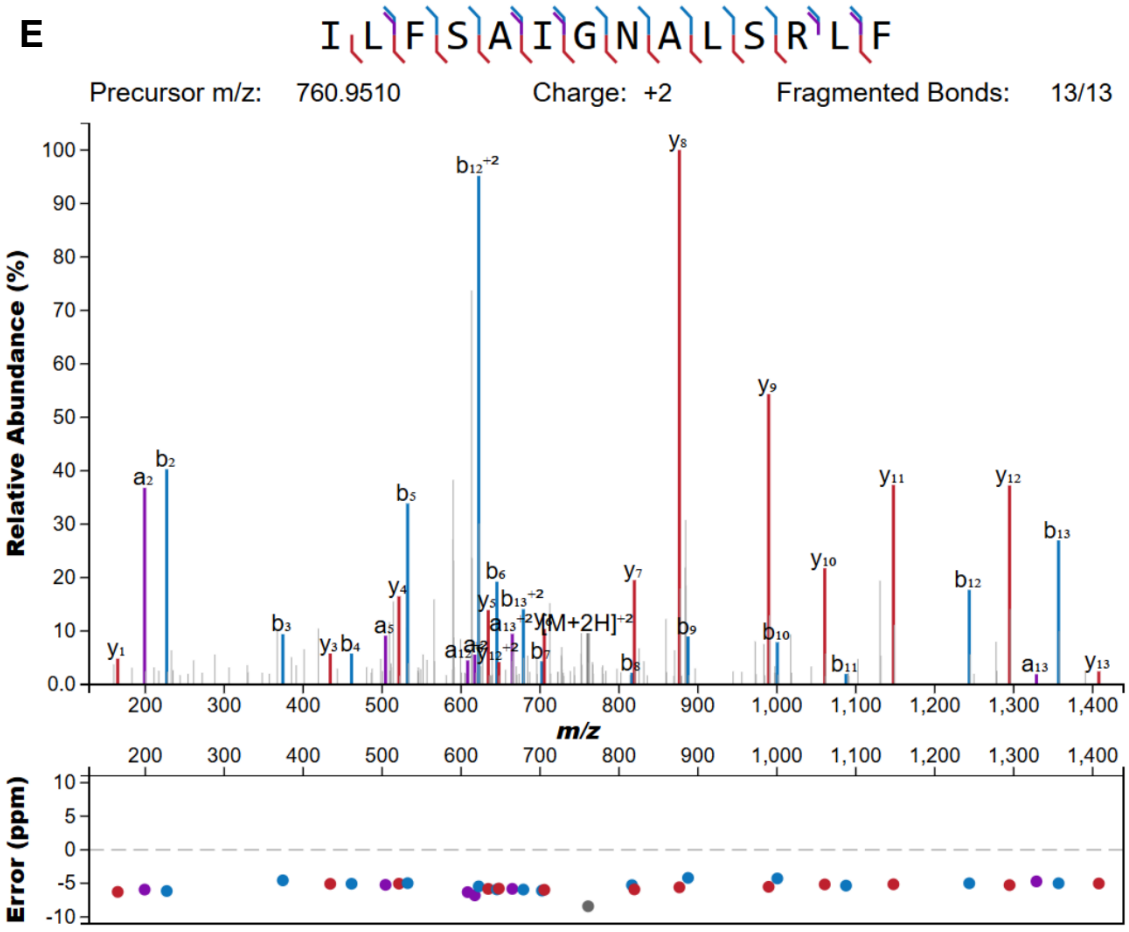
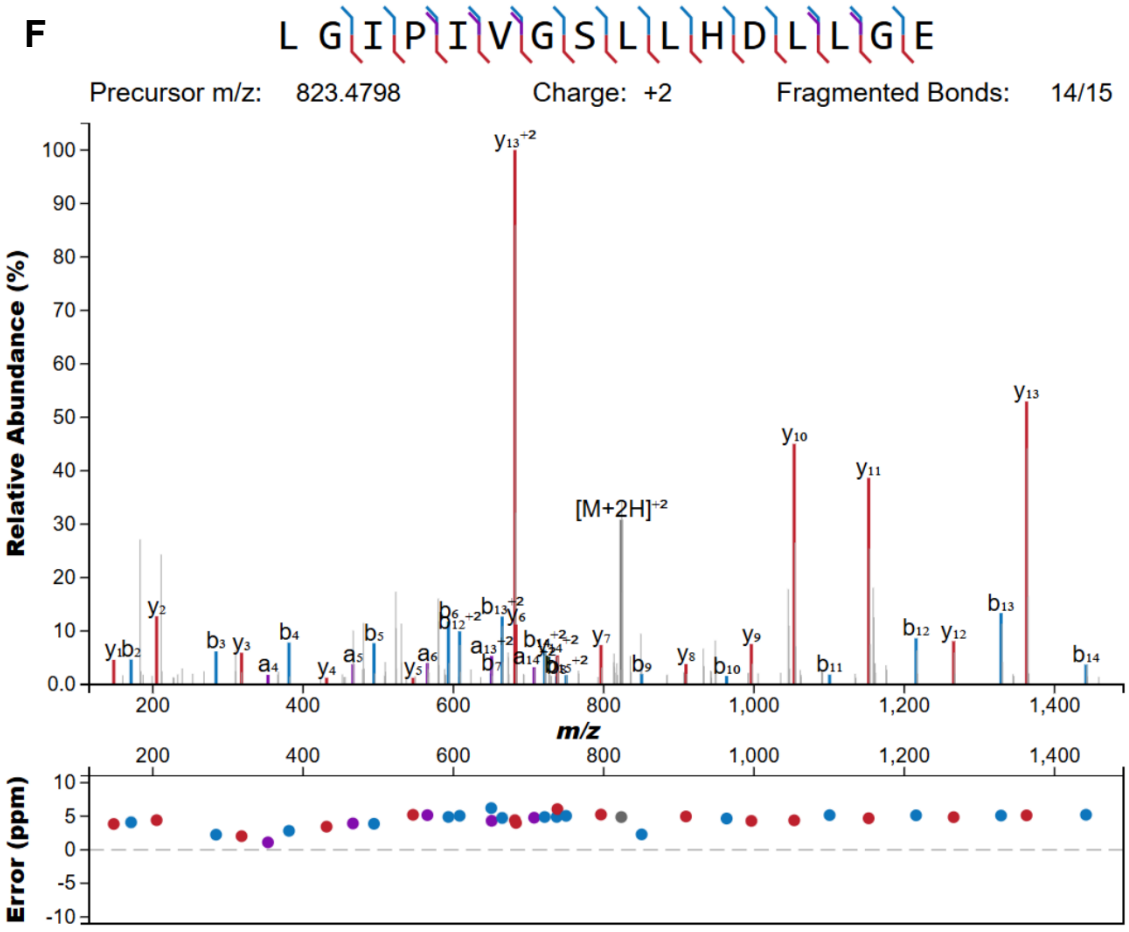
Relative Abundance (%)
m/z
Error (ppm)

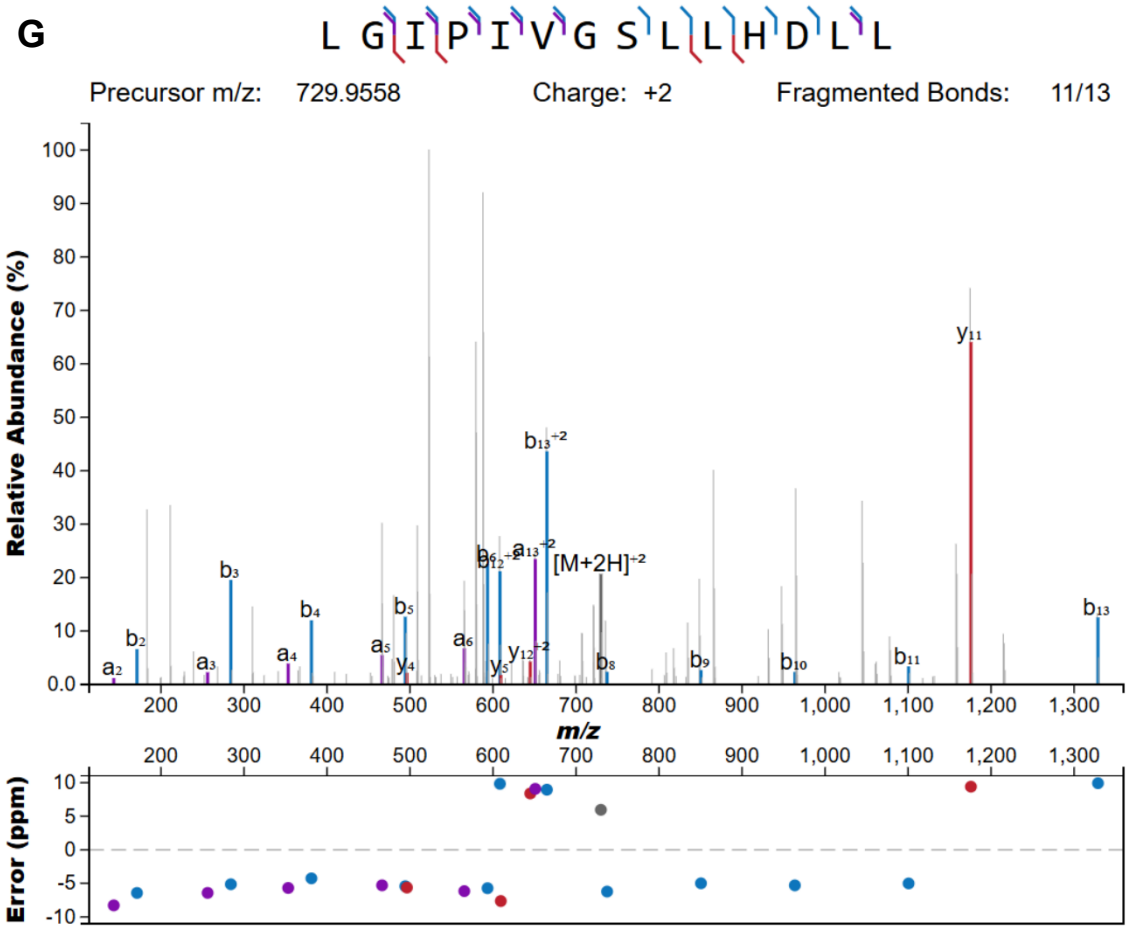
E


I L F S A I G N A L S R L F
Precursor m/z: 760.9510
Charge: +2
Fragmented Bonds: 13/13
Relative Abundance (%)
m/z
Error (ppm)

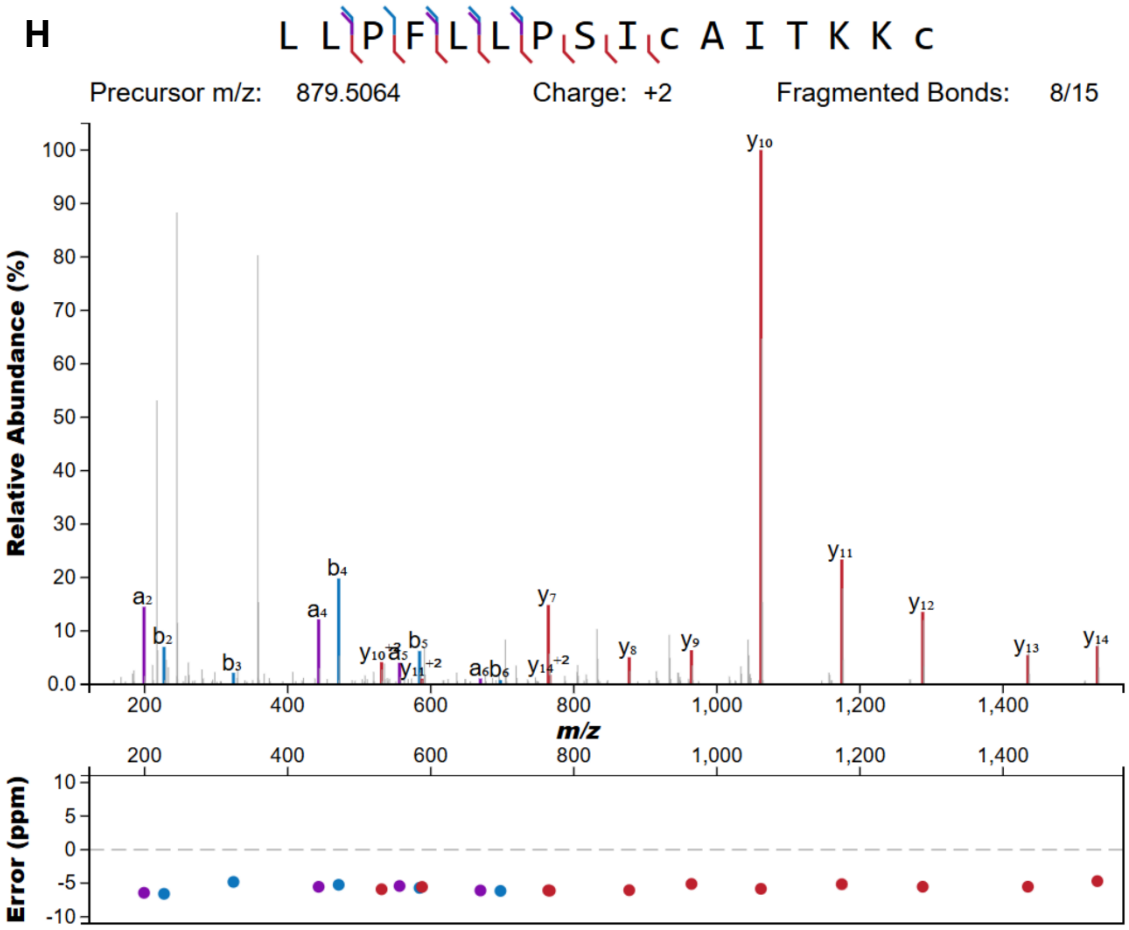
F


L G I P I V G S L L H D L L G E
Precursor m/z: 823.4798
Charge: +2
Fragmented Bonds: 14/15
Relative Abundance (%)
m/z
Error (ppm)
[M+2H]+2

G

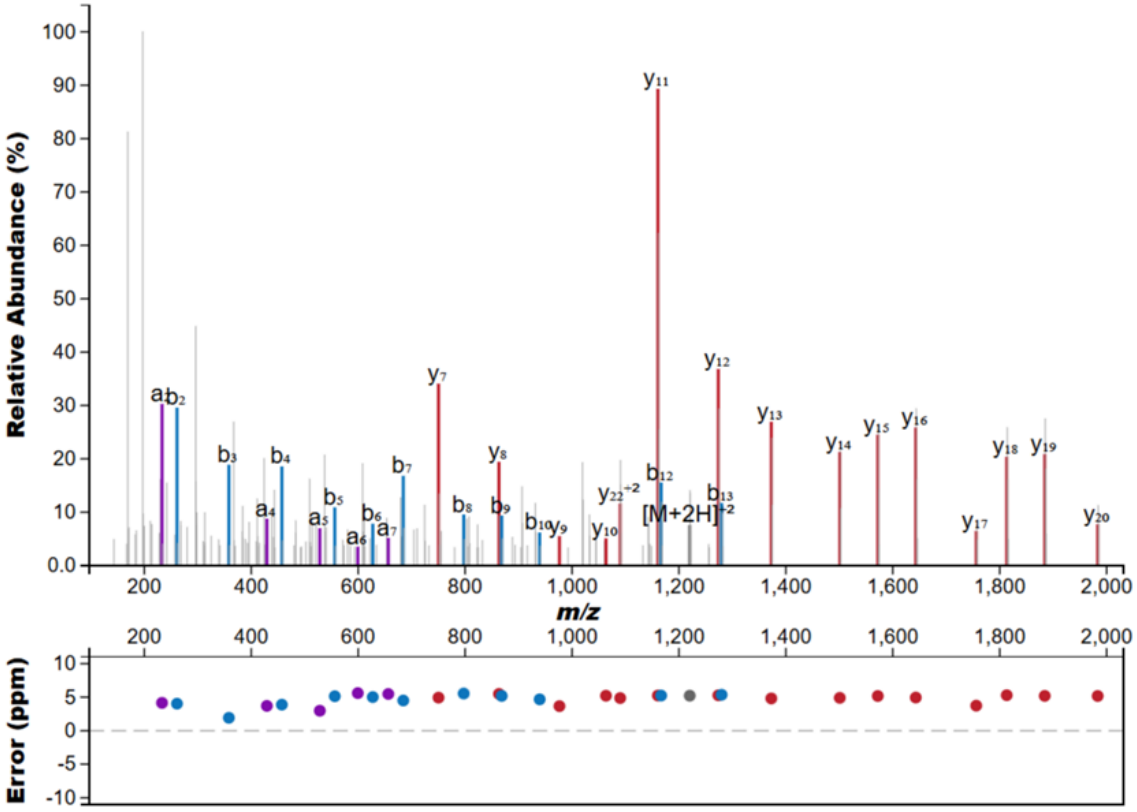

L G I P I V G S L L H D L L
Precursor m/z: 729.9558
Charge: +2
Fragmented Bonds: 11/13
Relative Abundance (%)
m/z
Error (ppm)
[M+2H]+2

H


L L P F L L P S I c A I T K K c
Precursor m/z: 879.5064
Charge: +2
Fragmented Bonds: 8/15
Relative Abundance (%)
m/z
Error (ppm)

I F L P V V A G L A A K V L P S I I c A V T K K c

Precursor m/z: 1,220.2151 Charge: +2 Fragmented Bonds: 16/23



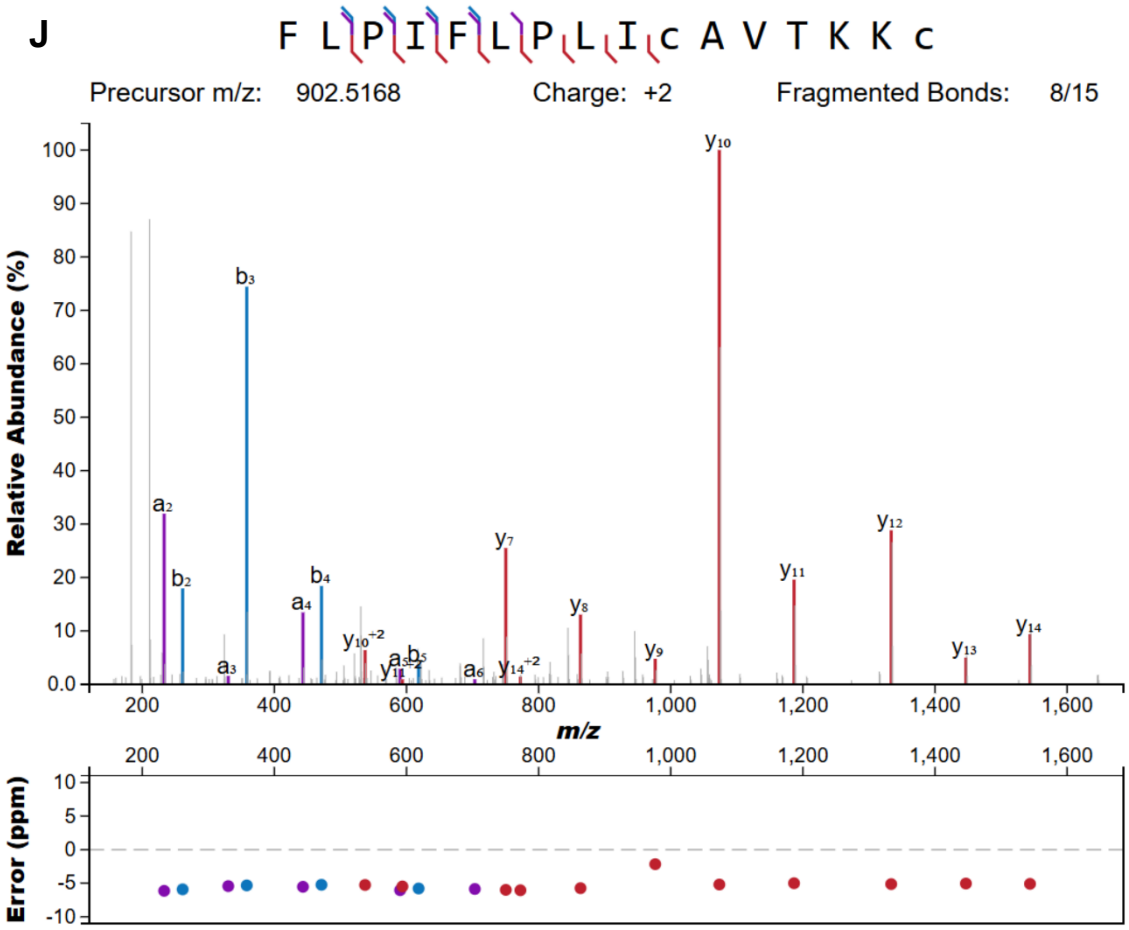
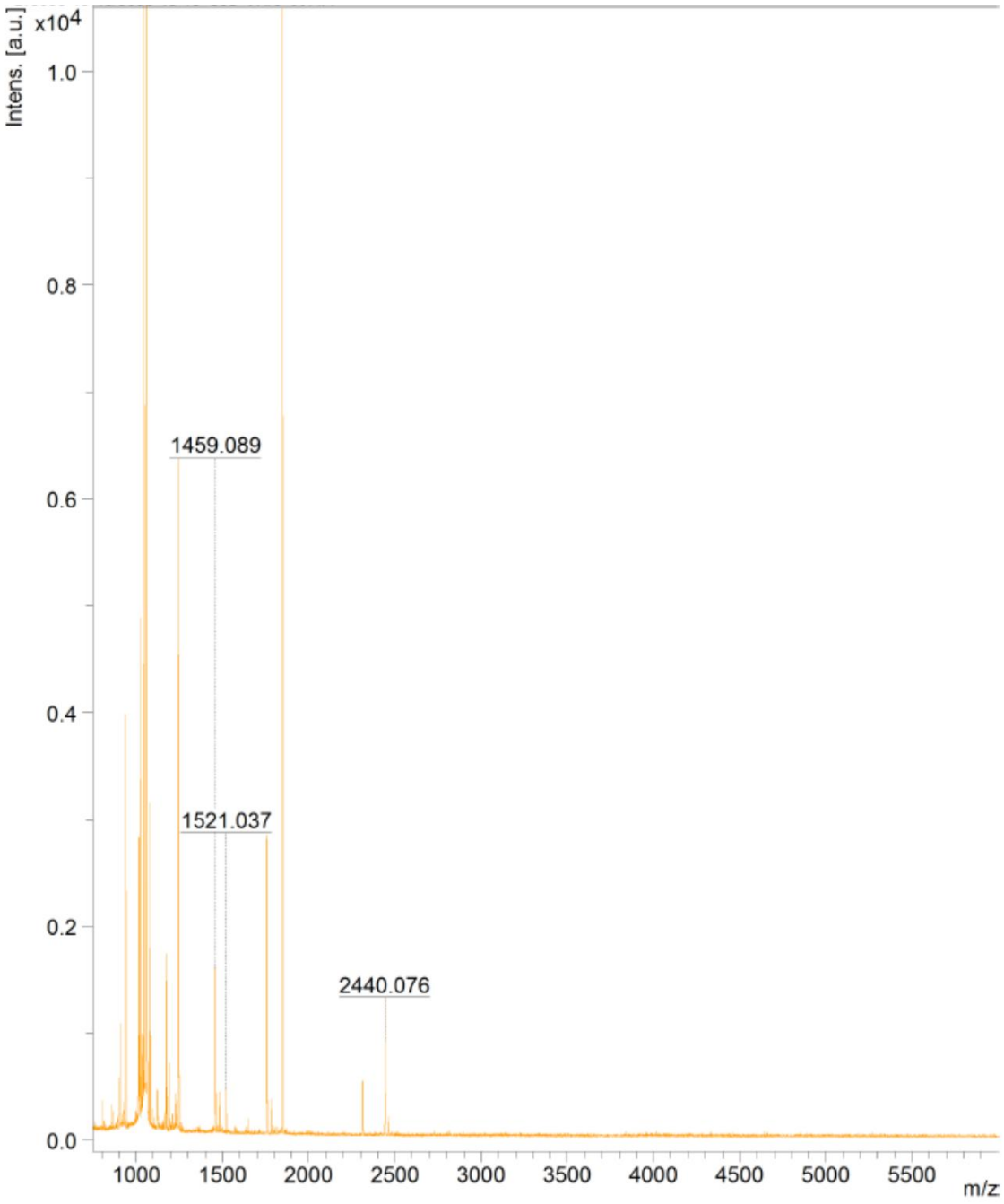
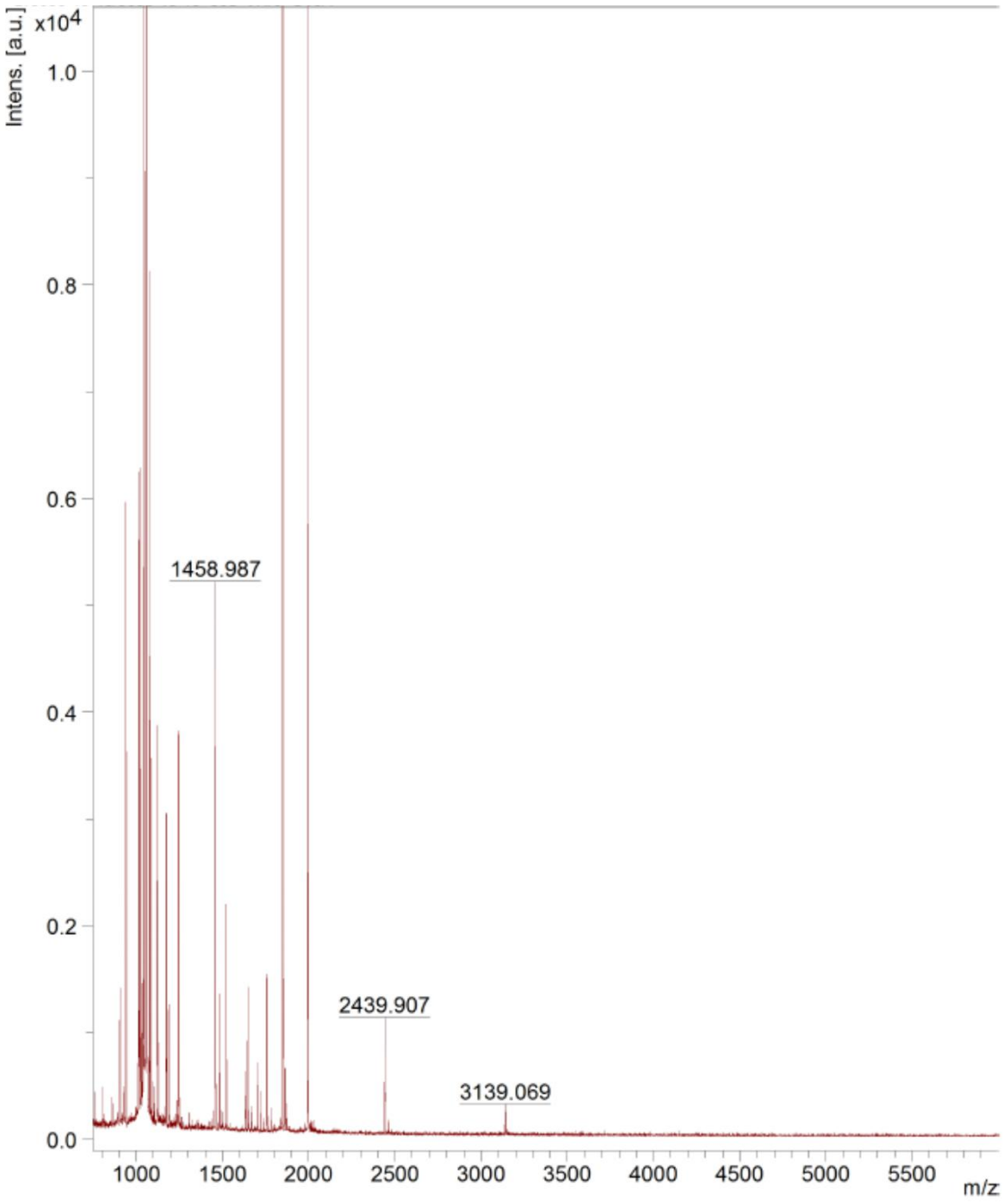


Figure S4. Electrospray ionization Fourier transform mass spectra of enriched *Rana sylvatica* skin secretions. (A) An example of a full mass spectrum obtained from an enriched skin secretion sample, including ions with mass to charge ratios ranging from 333.4 – 5000, which spans all singly and doubly charged ions of the predicted mature AMPs. (B – J) Annotated individual tandem mass spectra of mature AMP peaks. Tandem mass spectra were obtained from peaks matching the predicted mass to charge ratios of (B) ranacyclin-SY, (C) esculentin-2SY, (D) temporin-1SYd, (E) temporin-1SYb, (F) temporin-1SYc1, (G) temporin-1SYc2, (H) brevinin-1SYb, (I) brevinin-1SY and (J) brevinin-1SYc. Fragment ion peaks which support the predicted mature AMP sequence are labelled and highlighted in purple (a ions), blue (b ions) and red (y ions). The relative error associated with each peak is given below the annotated spectrum.

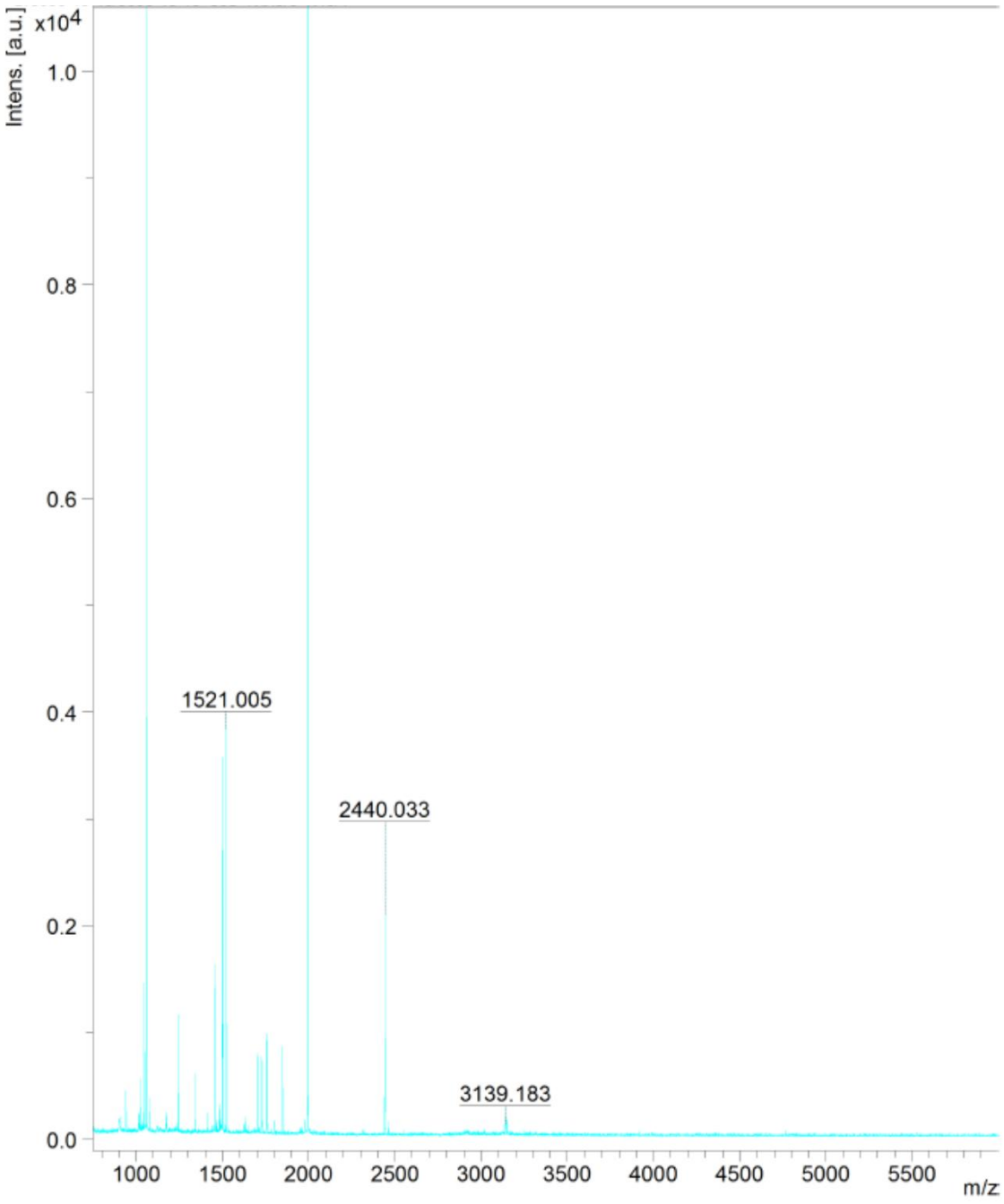
A


Intens. [a.u.]
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
1459.089
1521.037
2440.076
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
m/z

B


Intens. [a.u.]
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
1458.987
2439.907
3139.069
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
m/z

C


Intens. [a.u.]
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
1521.005
2440.033
3139.183
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
m/z

D

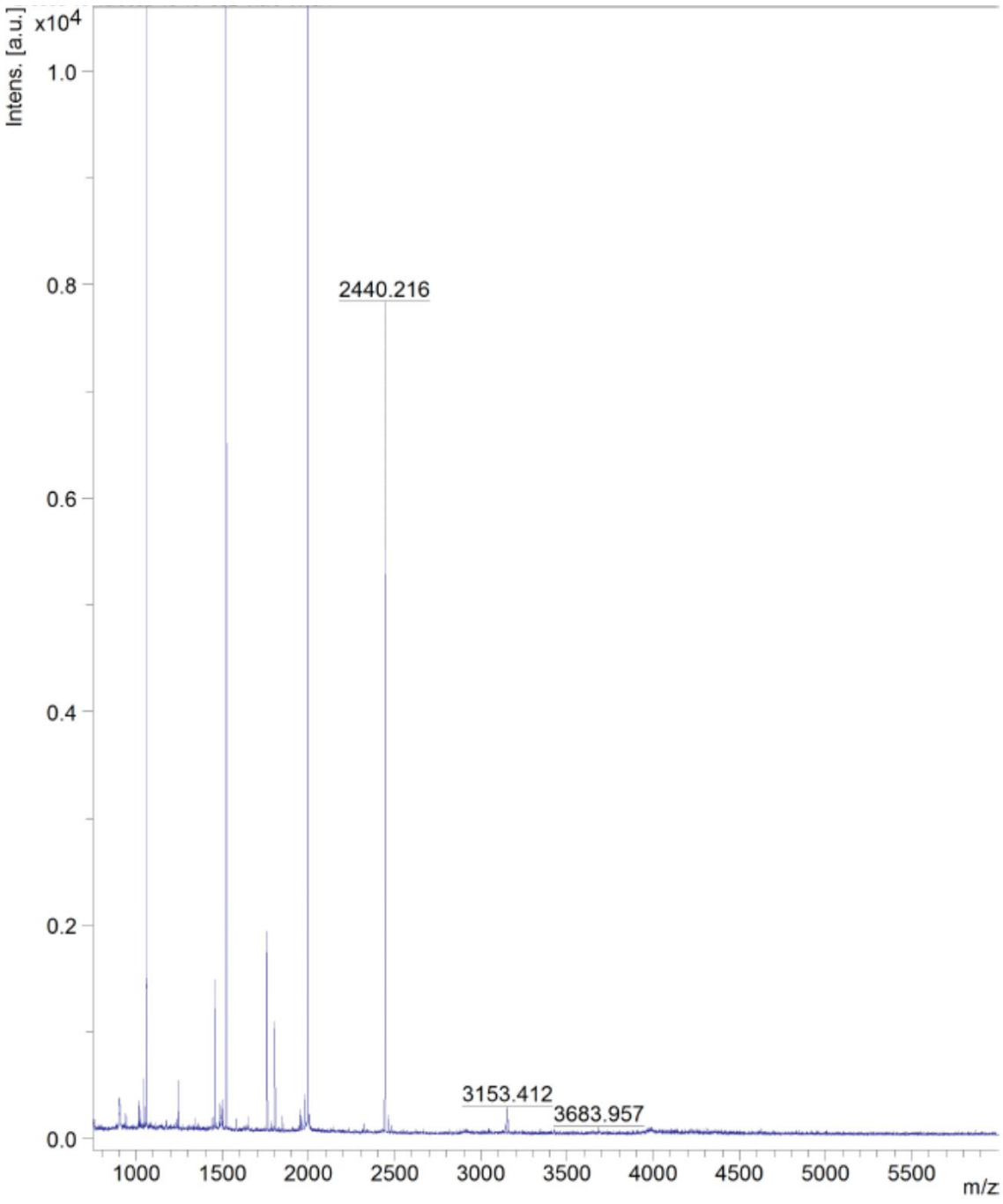


Figure S5. Representative matrix-assisted laser desorption/ionization time-of-flight mass spectra of enriched *Rana sylvatica* skin secretions. The spectrum with the highest number of identified AMP peaks is given for each seasonal group. Spectra are as follows: (A) Spring Sample 4, (B) Captive Summer Sample 4, (C) Wild Summer Sample 3, (D) Fall Sample 3.

Table S1. RNA sequencing datasets used in transcriptome assembly and transcript abundance estimation. All RNA seq. datasets were generated by a previously published study[7].

BioProject	BioSample	Run	Treatment	Days Post Treatment	Used In Transcriptome Assembly	Used in Transcript Abundance Estimation
PRJNA392411	SAMN07299155	SRR5810438	Control	10	Y	Y
PRJNA392411	SAMN07299156	SRR5810439	Section Line Bd exposure	3	Y	Y
PRJNA392411	SAMN07299157	SRR5810440	Control	3	Y	Y
PRJNA392411	SAMN07299158	SRR5810441	Carter Meadow Bd exposure	3	Y	Y
PRJNA392411	SAMN07299159	SRR5810442	Section Line Bd exposure	7	Y	Y
PRJNA392411	SAMN07299160	SRR5810443	Control	3	Y	Y
PRJNA392411	SAMN07299161	SRR5810444	Section Line Bd exposure	3	Y	Y
PRJNA392411	SAMN07299162	SRR5810445	Section Line Bd exposure	3	Y	Y
PRJNA392411	SAMN07299163	SRR5810446	Control	10	Y	Y
PRJNA392411	SAMN07299164	SRR5810447	Control	7	Y	Y
PRJNA392411	SAMN07299174	SRR5810448	Section Line Bd exposure	10	Y	Y
PRJNA392411	SAMN07299173	SRR5810449	Carter Meadow Bd exposure	10	Y	Y
PRJNA392411	SAMN07299170	SRR5810450	Carter Meadow Bd exposure	3	Y	Y
PRJNA392411	SAMN07299169	SRR5810451	Carter Meadow Bd exposure	3	Y	Y
PRJNA392411	SAMN07299172	SRR5810452	Control	7	Y	Y
PRJNA392411	SAMN07299171	SRR5810453	Carter Meadow Bd exposure	10	Y	Y
PRJNA392411	SAMN07299166	SRR5810454	Carter Meadow Bd exposure	7	Y	Y
PRJNA392411	SAMN07299165	SRR5810455	Section Line Bd exposure	3	Y	Y
PRJNA392411	SAMN07299168	SRR5810456	Section Line Bd exposure (previously exposed)	3	Y	N
PRJNA392411	SAMN07299167	SRR5810457	Carter Meadow Bd exposure	7	Y	Y
PRJNA392411	SAMN07299149	SRR5810458	Carter Meadow Bd exposure	10	Y	Y
PRJNA392411	SAMN07299150	SRR5810459	Control	3	Y	Y
PRJNA392411	SAMN07299151	SRR5810460	Control	7	Y	Y
PRJNA392411	SAMN07299152	SRR5810461	Control	3	Y	Y
PRJNA392411	SAMN07299145	SRR5810462	Control	10	Y	Y
PRJNA392411	SAMN07299146	SRR5810463	Carter Meadow Bd exposure	7	Y	Y
PRJNA392411	SAMN07299147	SRR5810464	Section Line Bd exposure	10	Y	Y
PRJNA392411	SAMN07299148	SRR5810465	Section Line Bd exposure	7	Y	Y
PRJNA392411	SAMN07299153	SRR5810466	Carter Meadow Bd exposure	10	Y	Y
PRJNA392411	SAMN07299154	SRR5810467	Carter Meadow Bd exposure	3	Y	Y
PRJNA392411	SAMN07299183	SRR5810468	Control	7	Y	Y
PRJNA392411	SAMN07299184	SRR5810469	Carter Meadow Bd exposure	10	Y	Y
PRJNA392411	SAMN07299181	SRR5810470	Section Line Bd exposure	7	Y	Y
PRJNA392411	SAMN07299182	SRR5810471	Control	3	Y	Y
PRJNA392411	SAMN07299179	SRR5810472	Section Line Bd exposure (previously exposed)	7	Y	N
PRJNA392411	SAMN07299180	SRR5810473	Section Line Bd exposure (previously exposed)	7	Y	N
PRJNA392411	SAMN07299177	SRR5810474	Section Line Bd exposure	7	Y	Y
PRJNA392411	SAMN07299178	SRR5810475	Control	10	Y	Y
PRJNA392411	SAMN07299175	SRR5810476	Section Line Bd exposure (previously exposed)	3	Y	N

BioProject	BioSample	Run	Treatment	Days Post Treatment	Used In Transcriptome Assembly	Used in Transcript Abundance Estimation
PRJNA392411	SAMN07299176	SRR5810477	Section Line Bd exposure	3	Y	Y
PRJNA392411	SAMN07299194	SRR5810478	Control	7	Y	Y
PRJNA392411	SAMN07299193	SRR5810479	Control	10	Y	Y
PRJNA392411	SAMN07299188	SRR5810480	Section Line Bd exposure	7	Y	Y
PRJNA392411	SAMN07299187	SRR5810481	Section Line Bd exposure	10	Y	Y
PRJNA392411	SAMN07299186	SRR5810482	Section Line Bd exposure (previously exposed)	3	Y	N
PRJNA392411	SAMN07299185	SRR5810483	Section Line Bd exposure (previously exposed)	3	Y	N
PRJNA392411	SAMN07299192	SRR5810484	Carter Meadow Bd exposure	7	Y	Y
PRJNA392411	SAMN07299191	SRR5810485	Carter Meadow Bd exposure	3	Y	Y
PRJNA392411	SAMN07299190	SRR5810486	Section Line Bd exposure (previously exposed)	7	Y	N
PRJNA392411	SAMN07299189	SRR5810487	Carter Meadow Bd exposure	7	Y	Y
PRJNA392411	SAMN07299195	SRR5810492	Section Line Bd exposure	10	Y	Y
PRJNA392411	SAMN07299196	SRR5810493	Section Line Bd exposure	10	Y	Y

Table S2. Primers used in RACE-PCR.

Target Gene	Primer	Sequence (5'-3')
palustrin-2sy	Rs_palu2_5RACE	CCCGCATTCTTGATGGTTTCCCAGAGAT
	Rs_palu2_3RACE	AGAGGTCTCTGGGAAACCATCAAGAATGCG
ranacyclin-sy	Rs_racy_5RACE	GGTGGATAACTCTTGGTCCAGCACCCTCTG
	Rs_racy_3RACE	AGACCTCCACTTAAGACTTCCAGCTGTCTA
esculentin-2sy	Rs_escu2_5RACE	CCTGCCTTGCCAAGCAACTTTGCTCCA
	Rs_escu2_3RACE*	AATGCAAGAGGAAGAAGTAAAAAGAGGTCT
esculentin-1sy	Rs_escu1_5RACE	CGCACAACCTCGCTAACTTCCTCGCCTA
	Rs_escu1_3RACE	GTTACTGATTGTCCTTCTTGGGAGCATCTG
temporin-1syd	Rs_temp1d_5RACE	CCCACCAAACCACTGAGAATCTTTCCCAT
	Rs_temp1c/d_3RACE_1*	AAAGAATGTTCAAGTGCAAAAACGACTTAA
temporin-1syb	Rs_temp1b_5RACE	TCGACTGAGAGCATTTCCAATAGCTGAA
	Rs_temp1b_3RACE	AAGGAATGTTCAAGTGGAAAAACGAATTCT
temporin-1syc-1	Rs_temp1c_5RACE	CCAACAAATCATGGAGAAGACTTCCAACAA
	Rs_temp1c/d_3RACE_1*	AAAGAATGTTCAAGTGCAAAAACGACTTAA
temporin-1syc-2	Rs_temp1c_5RACE	CCAACAAATCATGGAGAAGACTTCCAACAA
	Rs_temp1c/d_3RACE_2*	ATGTTGAAGTGAAAAAACGATTTTTACCAA
brevinin-1syb	Rs_panbrev_5RACE	TTTCCAATTCAAGTTTCCAAAGTTTCAACA
	Rs_brev1b_5RACE _nested‡	ATTGCACAAATTGACGGCAATAAA
	Rs_brev1b_3RACE	ATGTTGAAGTGGAAAAACGATTGTTACCAT
brevinin-1sy	Rs_brev1a_5RACE	CAAGACCTTAGCGGCCAGACCTGCAACA
	Rs_brev1a_3RACE	ATGTTGAAGTGGATAAACGATTTTTACCAG
brevinin-1syc	Rs_panbrev_5RACE	TTTCCAATTCAAGTTTCCAAAGTTTCAACA
	Rs_brev1c_5RACE _nested‡	GGTTACTGCACAAATTAACGGCAAA
	Rs_brev1c_3RACE*	ATGTTGAAGTGAAAAAACGATTTTTACCAA

All primers used in RACE-PCR reactions were appended with the sequence "GATTACGCCAAGCTT" on the 5' end to allow for cloning with the included pRACE vector.
‡ Indicates the use of a nested primer.
* Indicates that primer specificity was inadequate for direct amplicon sequencing and cloning was performed.

Table S3. Genomic resources used for AMP gene context comparison.

Genome Accession	Chromosome Accession	Name	Species
GCA_028564925.1	CM053028.1	aRanSyl1.merge	*Rana sylvatica*
GCA_905171775.1	NC_053496.1	aRanTem1.1	*Rana temporaria*
GCA_029206835.1	CM055123.1	Rmu.v1	*Rana muscosa*
GCA_029574335.1	CM056056.1	ASM2957433v1	*Rana kukunoris*
GCA_004786255.1	CM016425.1	Pads_1.0	*Pyxicephalus adspersus*
GCA_036250615.1	CM070037.1	ASM3625061v1	*Ptychadena robeensis*

Dataset S1 (separate file). A FASTA file providing *Rana sylvatica* AMP gene cDNA sequences.

Dataset S2 (separate file). A GFF3 file annotating *Rana sylvatica* AMP genes, signal peptide exon loci, and neighbouring genes in the aRanSyl1.merge genome assembly (Genbank: GCA_028564925.1). Entries for mRNA of neighbouring genes are named after their best blastn match in the NCBI core_nt database.

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1. Sievers, F. & Higgins, D. G. Clustal Omega for making accurate alignments of many protein sequences. *Protein Science* **27**, 135–145 (2018).
2. Minh, B. Q. *et al.* IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* **37**, 1530–1534 (2020).
3. Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., Von Haeseler, A. & Jermiin, L. S. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**, 587–589 (2017).
4. Jones, D. T., Taylor, W. R. & Thornton, J. M. The rapid generation of mutation data matrices from protein sequences. *Bioinformatics* **8**, 275–282 (1992).
5. Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q. & Vinh, L. S. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution* **35**, 518–522 (2018).
6. Tamura, K., Stecher, G. & Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* **38**, 3022–3027 (2021).
7. Eskew, E. A. *et al.* Gene expression differs in susceptible and resistant amphibians exposed to *Batrachochytrium dendrobatidis*. *Royal Society Open Science* **5**, 170910 (2018).