The following supplement accompanies the article

Expression analysis and identification of antimicrobial peptide transcripts from six North American frog species

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Supplement 1. Supplementary figures show the location of frog capture sites (Fig. S1), primers used to sequence putative antimicrobial peptide sequences (Fig. S2), alignment of predicted antimicrobial peptides identified in this study (Fig. S3), and the mRNA abundance of antimicrobial peptide precursor temporin-1BYa in *Rana boylii*, by sex (Fig. S4)

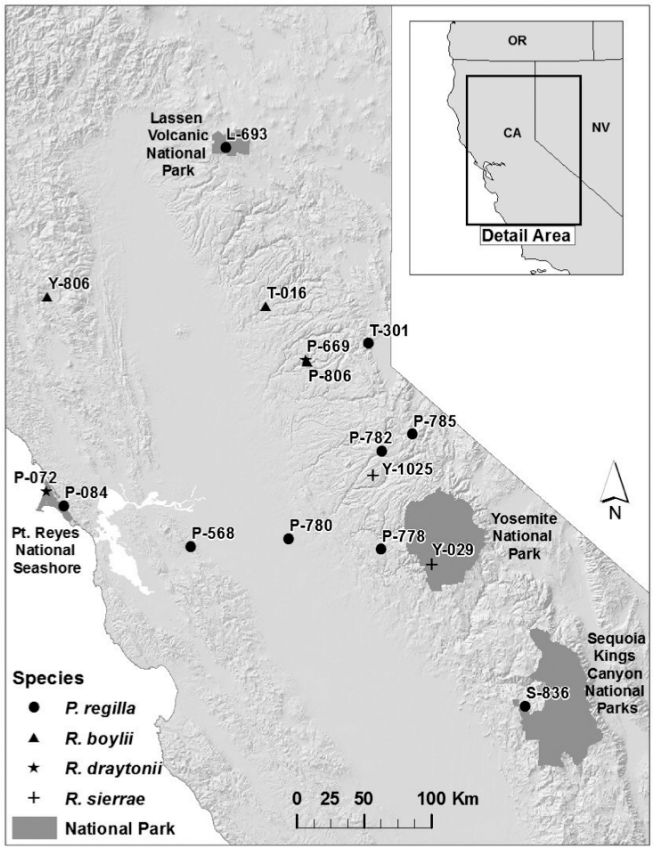

Fig. S1. Sites where *Pseudacris regilla*, *Rana boylii*, *R. draytonii*, and *R. sierrae* were captured to collect skin secretions

A:

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LRO-335[a]                                                                                                                ATG TTC ACC TTG
LRO-209                                                                                                             G AGC CCA AAG ATG TTC ACC WTG
LRO-208                                                                                                  AC CAA CTG AAC YAC CCG AGC
     1    GCT TTG TAG GAT AGA CCT GCA CTG AAG TCT TCC AGC CGT CTA CAT TCT GAG CAC CAA CTG AAC TAC CCG AGC CCA AAG ATG TTC ACC TTG   90
                                                                                                                           M   F   T   L    4


LRO-335[a]   AAG AAA TCC CTT TTA C
LRO-213                                                                                                 G GAA GAG AGA RAT GCH GAT G
LRO-212                                                                  C ATT TCC TTA TCT CTC TGT GAA C
LRO-210                                                      C CTT GGG ACC ATC AAC TTA TC
     91   AAG AAA TCC CTG TTA CTC CTC TTT TTC CTT GGG ACC ATC AAC TTA TCT CTC TGT GAG GAA GAG AGA AAT GCA GAA GAA GAA AGA AGA GAT  180
      5    K   K   S   L   L   L   L   F   F   L   G   T   I   N   L   S   L   C   E   E   E   R   N   A   E   E   E   R   R   D    34

    181   GAA CCA GAT GAA AGG GAT GTT CAA GTG GAA AAA CGA CTT TCA CCA AAC CTG CTC AAG AGC TTG TTG GGA AAA TAA CCA AAA ATG TTA AGA  270
     35    E   P   D   E   R   D   V   Q   V   E   K   R   L   S   P   N   L   L   K   S   L   L   G   K   *                   58


LRO-214[b]       GAA TTG GAA RTC ATC TGA TGT G
LRO-216[b]                                   GA ATA TCA TTT AGC TAA ATG CTA AAT G
     271  ATG GAA TTG GAA ATC ATC TGA TGT GGA ATA TCA TTT AGC TAA ATG CGC AAC AGA TGT CTT ATT TAA AAA ATA AAT ATG TTG CAT
354
```

B:

Forward primers:		**Reverse primers:**	
LRO-208	ACCAACTGAAC**Y**ACC**C**GAGC	LRO-214	**C**ACATCAGATGA**Y**TTCCAATTC
LRO-333	ACCAACTGAAC**T**ACC**T**GAGC	LRO-215	**C**ACATCA**D**ATG**RY**TTCCAATT**B**
LRO-525	ACCAACTGAAC**T**ACC**C**GAGC	LRO-330	**C**ACATCAGATGA**C**TTCCAATTC
		LRO-331	**C**ACATCAGATGA**T**TTCCAATTC
LRO-209	GAGCCCAAAGATGTTCACC**W**TG	LRO-332	**T**ACATCAGATGA**T**TTCCAATTC
LRO-526	GAGCCCAAAGATGTTCACC**T**TG		
		LRO-216	**CATTTA**GCA**T**TTAGCTAAATGATATTC
LRO-210	**C**CTTGGGACCATC**AA**CTTATC	LRO-217	**TGR**T**GT**GCA**T**TTAGCTAAATGATATTC
LRO-211	**T**CTTGGGACCATC**TC**CTTATC	Antisense primer	GACATC**TGGTGT**GCAATTAGCT
LRO-212	CATTTCCTTATCTCTCTGTGAAC		
LRO-213	GGAAGAGAGARATGCHGATG		
LRO-334	GTATGTTCACCTTGAA**T**AAATT**T**CC		
LRO-335	ATGTTCACCTTGAA**G**AAAT**C**CCTTTTAC		
Sense primer	ATGT_CACCT_GAA**G**AAAT**C**CCTC		

Fig. S2. Primers used to sequence antimicrobial peptide genes from amphibian skin secretions. (A) The locations of several primers are indicated relative to the putative protein coding sequence for *Rana temporaria* mRNA for temporin H precursor (GenBank accession number Y09394). (B) The sequences (5' to 3') for all primers used to identify antimicrobial peptide genes are listed, grouped according to location relative to the putative protein coding sequence. Nucleotides that differ within each group of primers are underlined in **bold**. The alignment of 1 primer from each group is shown in (A)

[a]Primer sequence is divided between 2 lines

[b]Reverse complement of primer sequence is shown

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                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|..
                          10        20        30        40        50        60        70        80
brevinin-1Pb      MFTLNKFLLL LFFLGTINLS LCEEER-NAE EERRDEPDET DVEVEKRFLP IIAGIAAKVF PKIFCAISKK C
brevinin-1BY      MFTLKKSLLL LFFLGTINLS LCEEERDADE EERRDDPDET NVEVEKRFLP ILASLAAKFG PKLFCLVTKK C
brevinin-1DR      MFTLKKSLLL LFFLGTINLS LCEEERDADE EERRDDPDES NVEVEKRFLP ILAGLATKIV PKVFCLITKK C
esculentin-1Pa    MFTLKKSLLL IVLLGIISLS LCEQERNADE DEESEIKRGI FPKIIGKGIK TGIVNGIKSL VKGVGMKVFK AGLNNIGNTG CNED-EC
esculentin-1Pb    MFTLKKSLLL IVLLGIISLS LCEQERNADE DEESEIKRGI FPKIIGKGIK TGIVNGIKNL VKGVGMKVFK AGLSNIGNTG CNED-EC
esculentin-1Pc    MFTLKKSLLL IVLLGIISLS LCEQERNADE DEESETKRGL FTKINKKKAK TGVFNIIKTI GKEAGMDVIR AGIDTIS--- CKIKGEC
esculentin-2BY    MFTLKKSLLL LFFLGTISLS LCEQERDADE EDGEKEVKRS IFSLLTAGAK LLGKTLFKMA GKAGAEHLAC KATNQC
esculentin-2DR    MFTLKKSMLL IFFLGTISLS LCEQERDADE EDGEKEVKRG ILSLITTGAK LLGKTLFKMA GKAGAEHLAC KATNQC
ranacyclin-P      MFTLKKSLLL LFFLGTISLS LCEQERDSDD EDQGEVTEQV VKRLVRGCWT KSYPPKPCFV RG
ranacyclin-1BY    MFTLKKSLLL LFFLGTISLS LCEEERDADE EDGGEVTEEV VKRVLKGCWT KSYPPKPCFG KR
ranalexin-1C      ---------- ---LGTINLS LCEEERNAEE ERRDNPDERD VEVEKRFLGG LMKAFPALIC AVTKKC
ranatuerin-2PRa   MFTMKKSLLL FFFLGTISLS LCEEERGADE DDGVELTEEE VKRGLLSSFK GVAKGVAKDL AGKLLEKLKC KITG-C
ranatuerin-2PRb   MFTLEKSLLL FFFLGTISLS LCEEERGADE DDDVEMTEEE VKGGIMDSVK ----GVAKNL AAKLLEKLKC KITG-C
ranatuerin-2PRc   MFTMKKSLLL FFFLGTISLS LCEEERDADD DQG-EVVKKE VKRAFFTTVK ----NLVTNV AGTVIDKMKC KLTGQC
ranatuerin-2BYa   MFTLKKSLLL LFFLGTINLS LCEEERDAGD DQG-EVVKQE VKRAFFTTFK ----NLVTNV AGTVIDKMKC KLTGEC
ranatuerin-2BYb   MFTLKKSLLL FFFLGTISLS LCEEERGADE DDGVELTEEE VKRGILSTFK GLAKGVAKDL AGKLLDKFKC KITG-C
ranatuerin-2DR    MFTLKKSLLL FFFLGTISLS LCEEERGADE DDGVELTEEE VKRGIMDTFK GIAKGVAKNL AGKLLDELKC KMTG-C
ranatuerin-2SRa   MFTMKKSLLL FFFLGTISLS LCEEERGADE DDGVELTEEE VKRGLLSSFK GVAKGVAKDL AGKLLEKLKC KITG-C
ranatuerin-2SRb   MFTLKKSLLL FFFLGTISLS LCEEERGADE DDDVEMTEEE VKRGIMDSVK ----GVAKNL AAKLLEKLKC KITG-C
temporin-1C       ---------- ---LGTINLS LCEEERDSDQ EERRDDPGER NVEVEKR-FL PLFASLIGKL LG
temporin-1Pb      MFTLKKSLLL LFFLGTINLS LCEEERNADE EERRDDPDEM NVEVEKR-FL PLVGKILSGL IGK
temporin-1PR      MFTMKKSLLL LFFLGTINLS LCEEERDADE EERRDDPEER NVEVEKR-FL PIIAKVLGNL LGK
temporin-1BYa     MFTLKKSLLL LFFLGTINLS LCEEERNADE DERRDDPEER NVEVEKR-FL PIIAKVLSGL LGK
temporin-1DRa     MFTLKKSLLL LFFLGTINLS LCEEERDADE EERRDDPEER NVEVEKR-FL PIIAKVLGNL LGK
temporin-1DRb     MFTLKKSLLL LFFLGTINLS LCEEERNADE EERRDDPEER AVEVEKRNFL GTLVNLAKKI LGK
temporin-1SR      MFTMKKSLLL LFFLGTINLS LCEEERDADE EERRDDPEER NVEVEKR-FL PIIAKVLGNL LGK
temporin-2SR      ---LKKSLLL LFFLGTINLS LCEEERNA-E EERRDDPEEI NVEVEKR-FP ELSEDALASL LGK
temporin-3C       MFTLKKSLLL LFFLGTINLS LCEEERDADQ EERRDDPGER NVEVEKR-FF PFFGKILSPA FGK
temporin-3PR      MFTLKKSLLL LFFLGTINLS LCEEERDADQ EERRDDPGER NVEVEKR-FF PFFGKILSPA FGK
odorranain-M-Pa   MFTLKKLLLL LFFLGVAFSS PCYRKREADE EGNDGEAKTE GIKRATAWGP RHGLLPIRPI RIRPLCGNDK S
odorranain-M-Pb   MFTLKKLLLL LFFLGVAFSS PCYRKREADE EGNDGEAKTE GIKRGIPWRP PHGLKPRPPT R-KLFCGKDK S
odorranain-M-Pc   MFTLKKLLLL LFFLGVAFSP PCYRKREADE EGNDGEAKTE GIKRGLPWRP HHVLKPLPPS RMKPFCGKDK S
odorranain-M-PR   MFTLKKLLLL LFFLGVAFSS PCYRKREADE EGNDGEAKTE GIKRGIPWRP PHGLKPRPPT R-KLFCGKDK S
odorranain-M-DR   MFTLKKLLLL LFFLGVAFSP PCYRKREADE EGNDGEAKTE GIKRGLPWRP HHVLKPLPPS RMKPFCGKDK S
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Fig. S3. Predicted antimicrobial peptide precursor sequences, listed by name of the predicted mature peptide and aligned within each mature peptide group. Species for each antimicrobial peptide precursor is indicated by capital letters at the end of each mature peptide name: C = *Lithobates clamitans*, P = *L. pipiens*, PR = *Pseudacris regilla*, BY = *Rana boylii*, DR = *R. draytonii*, SR = *R. sierrae*. Putative cleavage site for release of mature peptide (KR) is shaded. Partial coding sequences that lack the amino terminus were identified for 3 predicted antimicrobial peptide precursors: ranalexin-1C, temporin-1C, and temporin-2SR

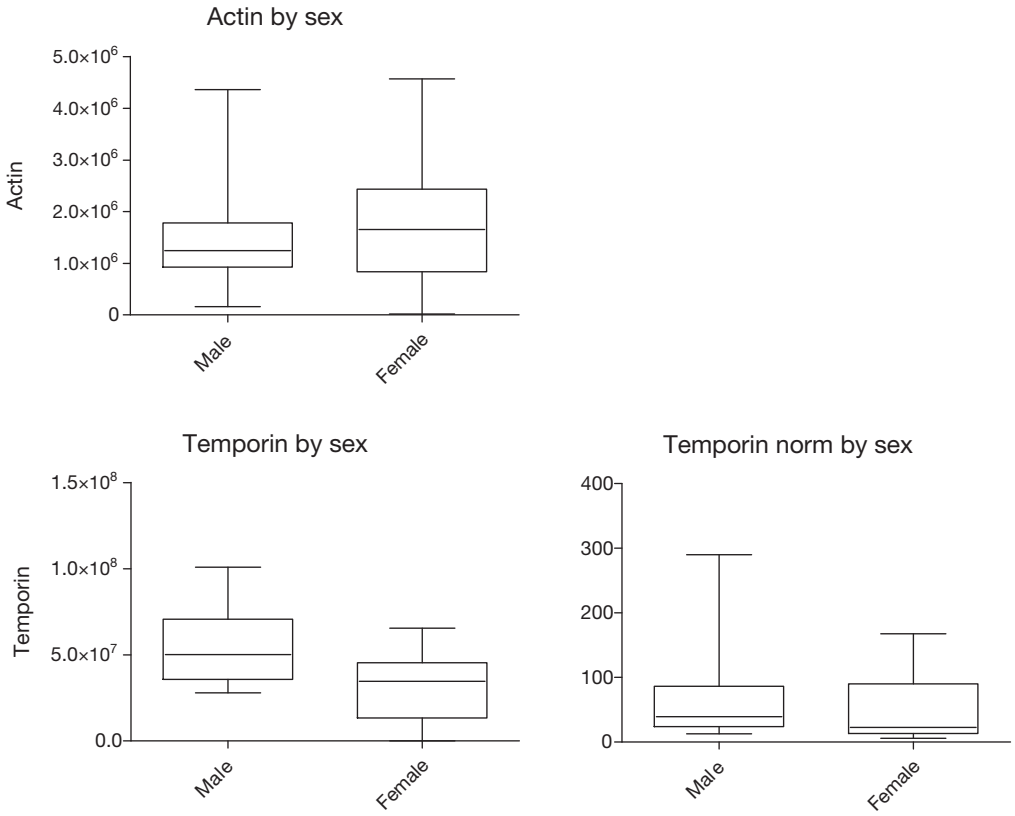

Fig. S4. *Rana boylii*. mRNA abundance of actin, mRNA abundance of antimicrobial peptide precursor temporin-1BYa, and mRNA abundance of antimicrobial peptide precursor temporin-1BYa normalized to actin in frog skin secretions by sex. Box plots show lower quartile, median, and upper quartile; whiskers indicate minimum and maximum values