



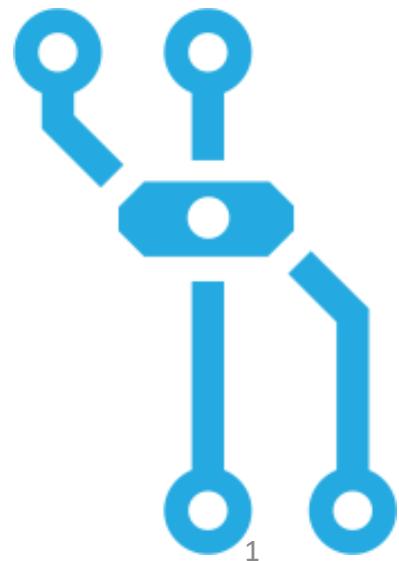
Discovering antimicrobial peptides in amphibian and insect transcriptomes



BioTalk – Exit Seminar
Friday, July 16, 2021

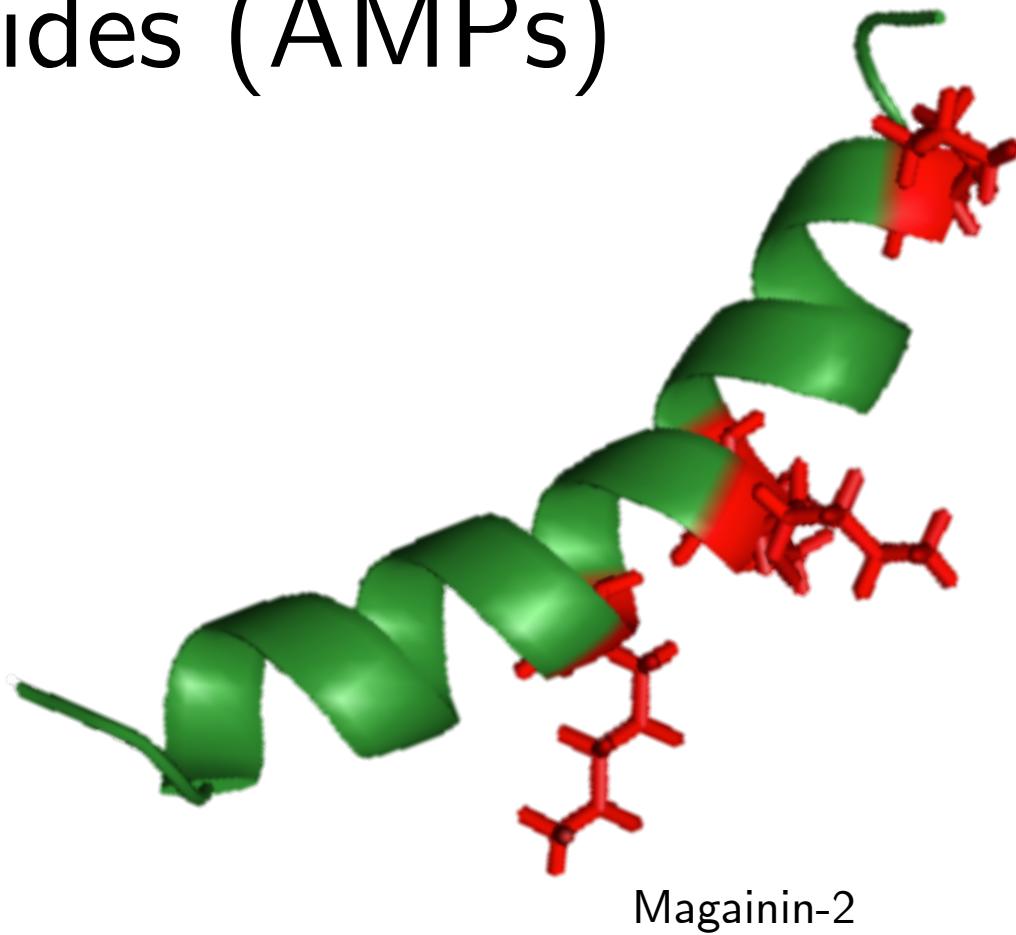
Diana Lin

MSc Student, Bioinformatics Graduate Program, UBC
Dr. Inanc Birol Lab, Genome Sciences Centre (GSC), BC Cancer

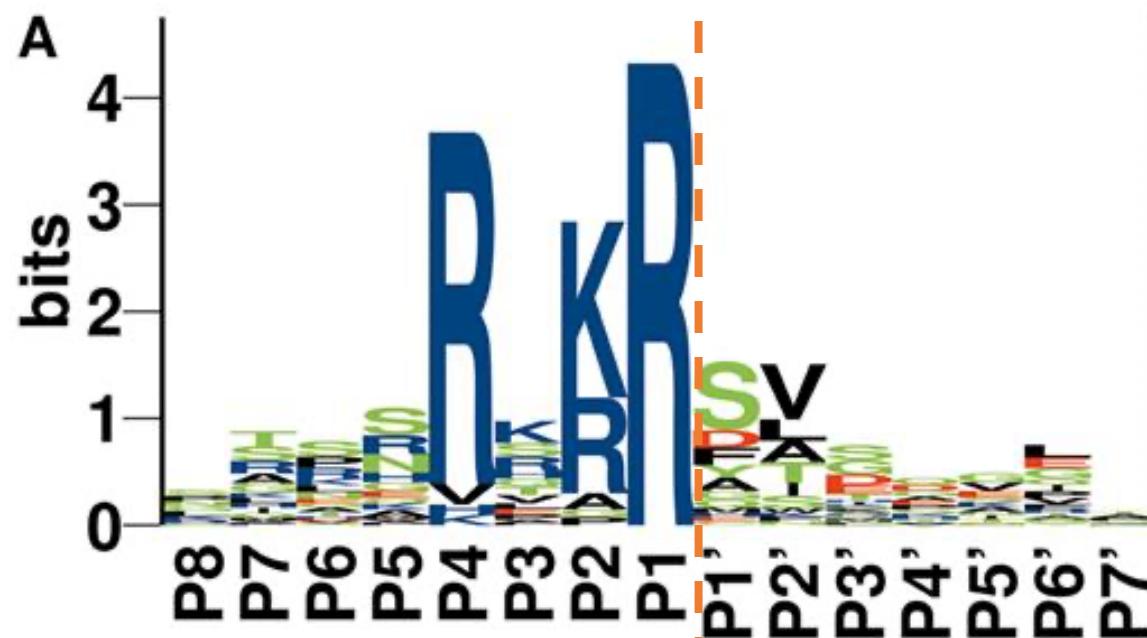
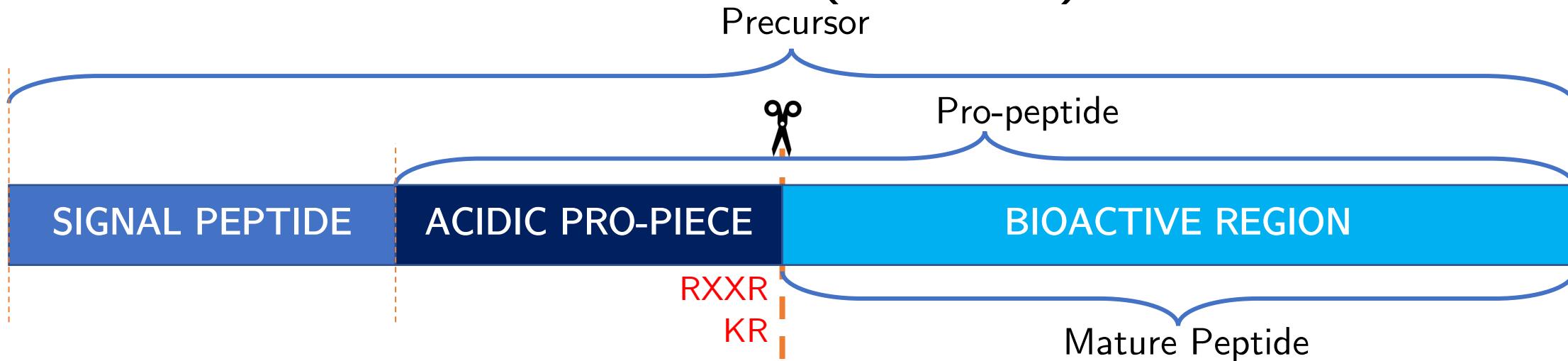


Antimicrobial Peptides (AMPs)

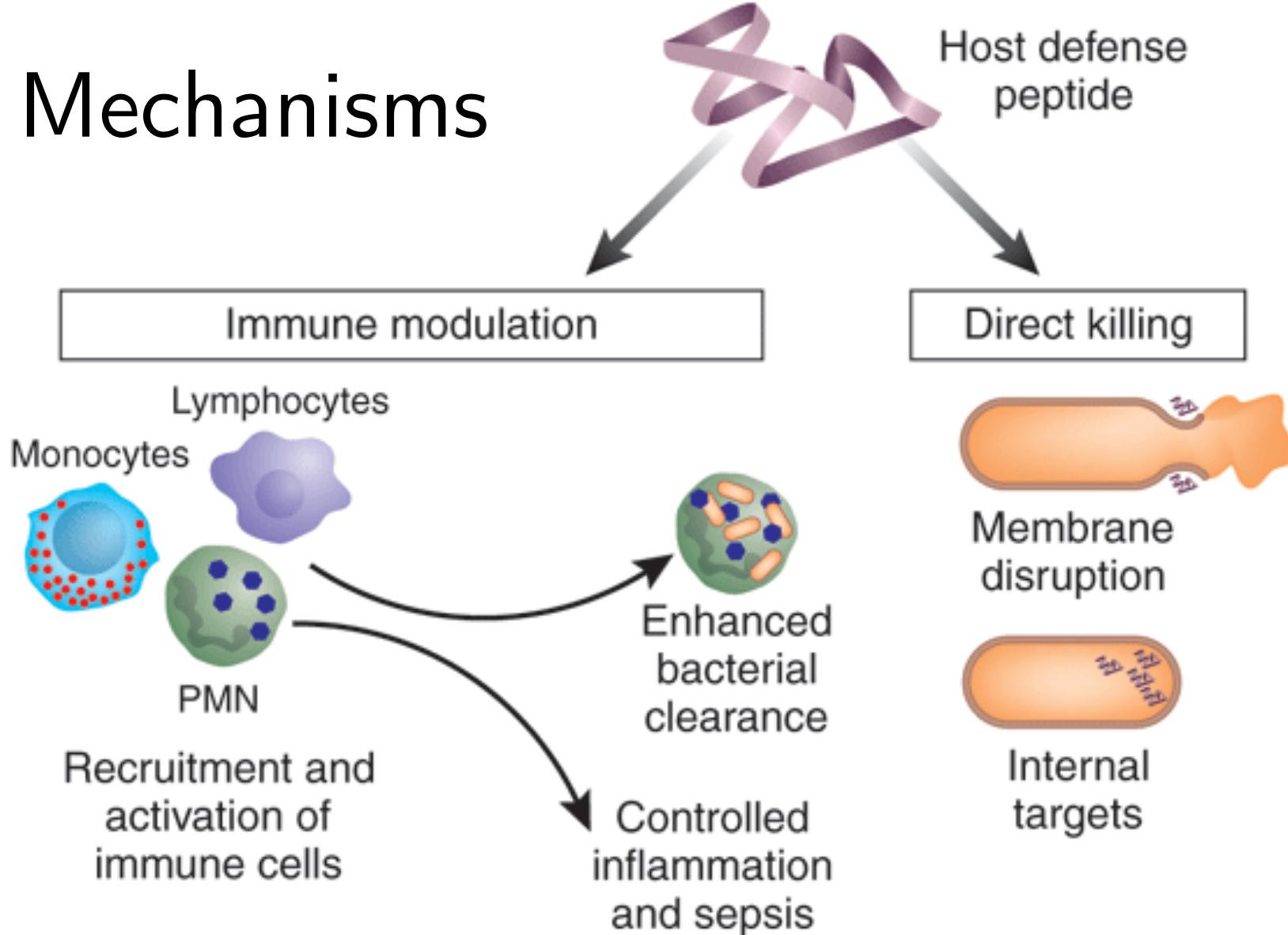
- Short peptide sequences
(5 to 50 amino acids)
- Often positively charged
- Amphipathic
- Produced by all life forms
- Part of the innate
immune system



Antimicrobial Peptides (AMPs)



AMP Mechanisms



Motivation

“Infectious Arms Race”: Rise of antimicrobial resistance creates a problem that requires a novel method to fight pathogens

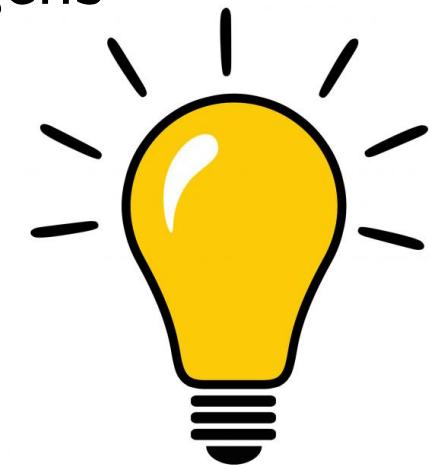
PROBLEM

Antibiotic
Resistance



SOLUTION

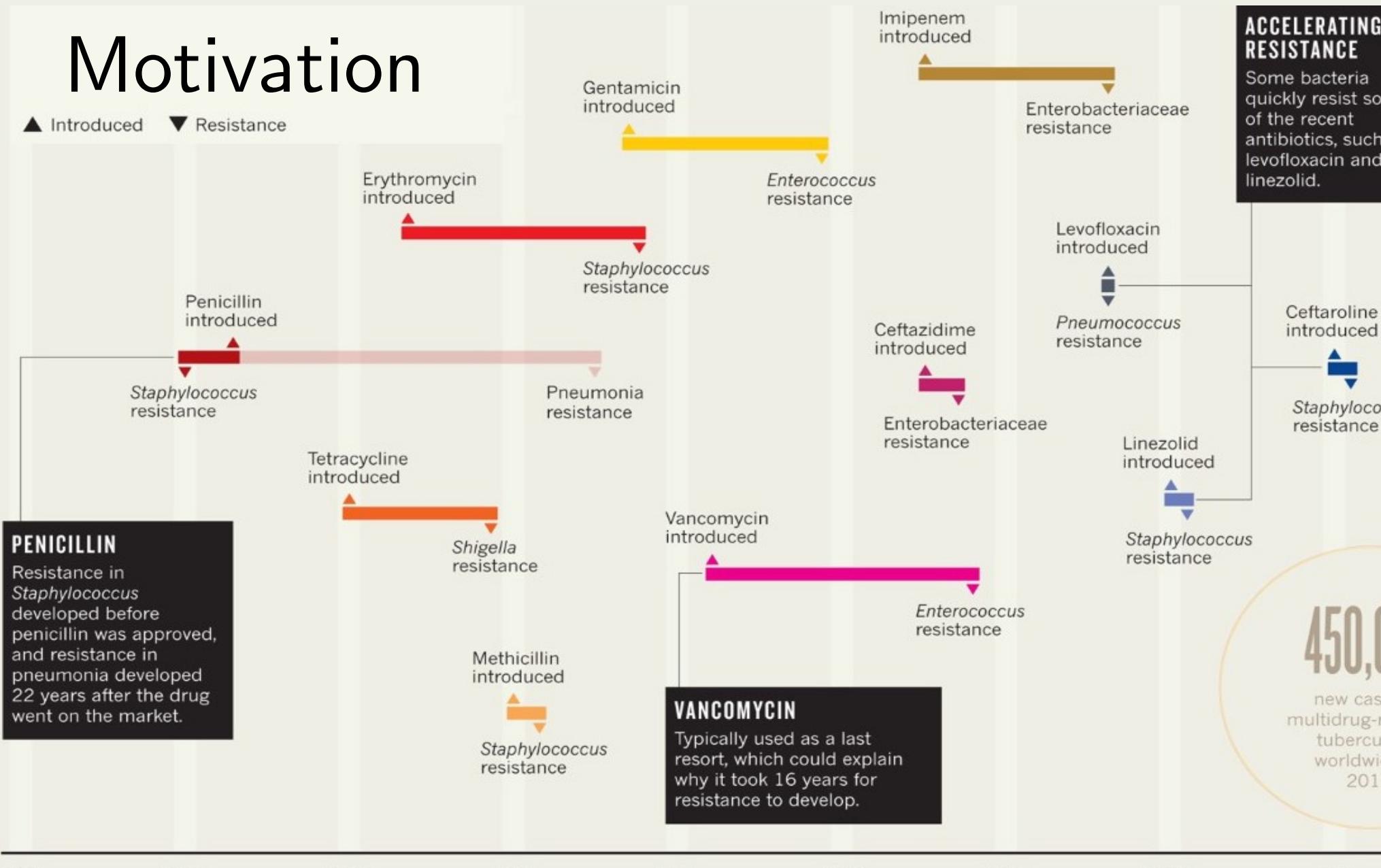
Antimicrobial
Peptides!



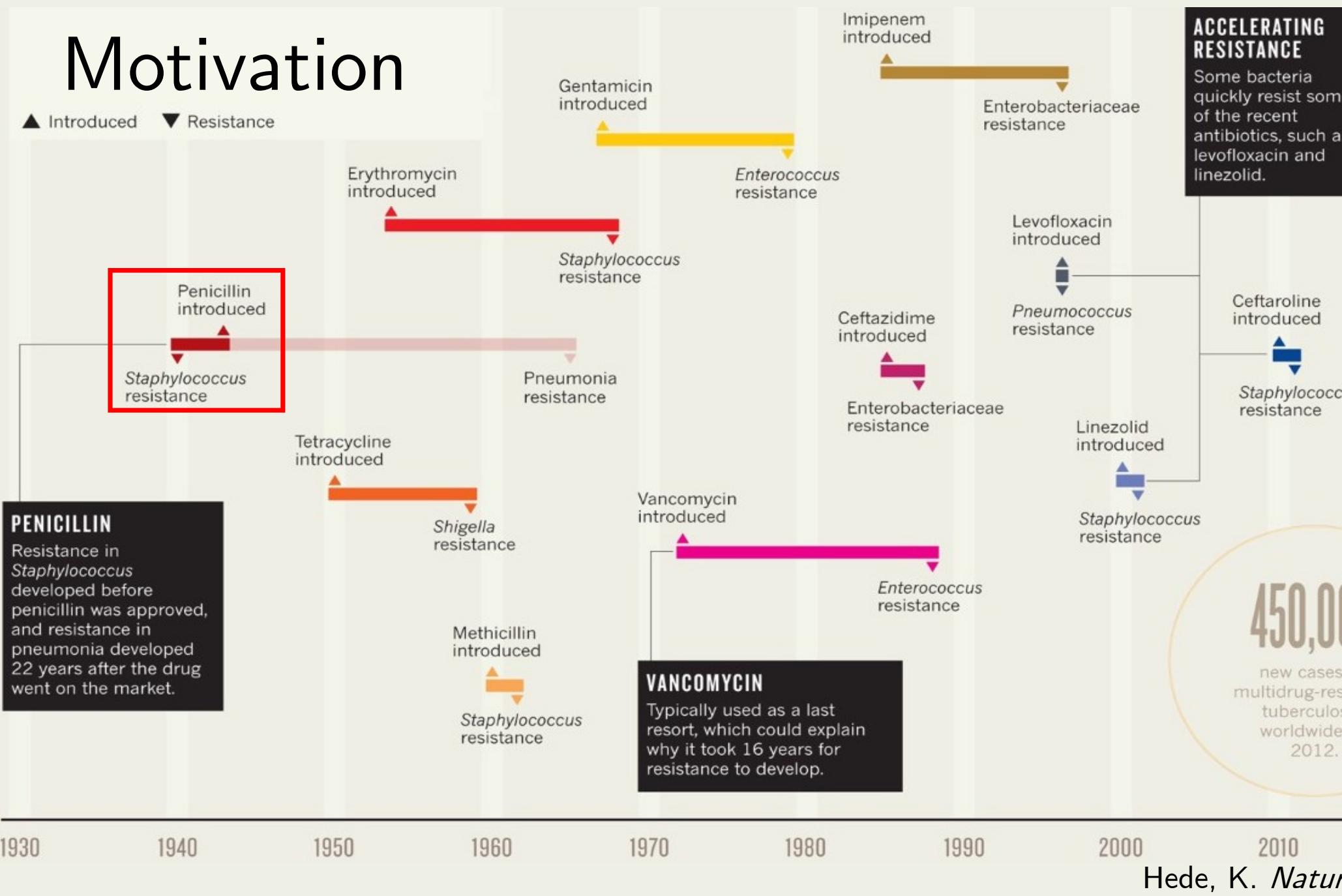
“Winning the fight against infectious bacteria requires staying ahead of the organisms' uncanny ability to flank our frontal assaults.”

-Karyn Hede, Nature (2014)

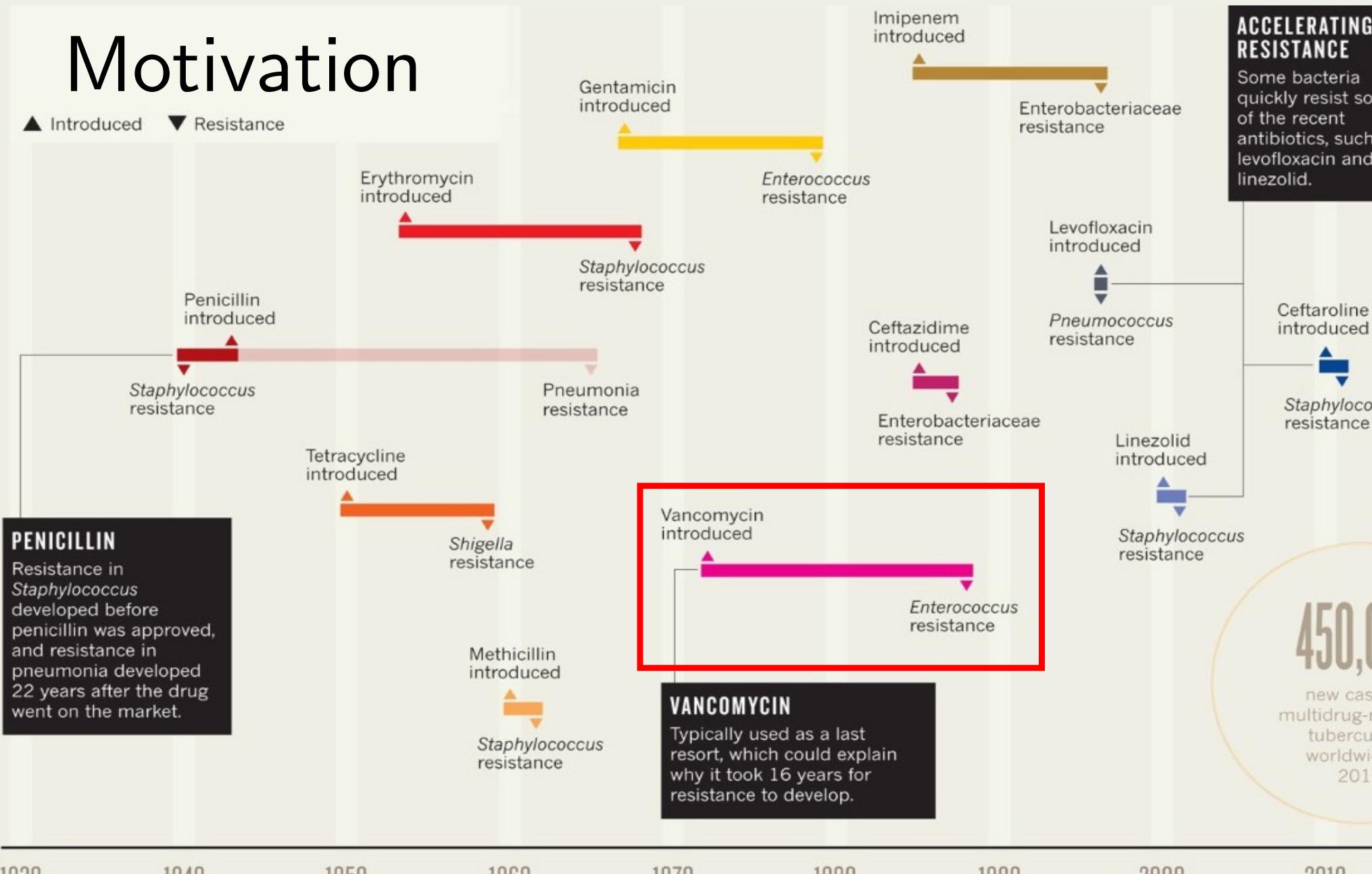
Motivation



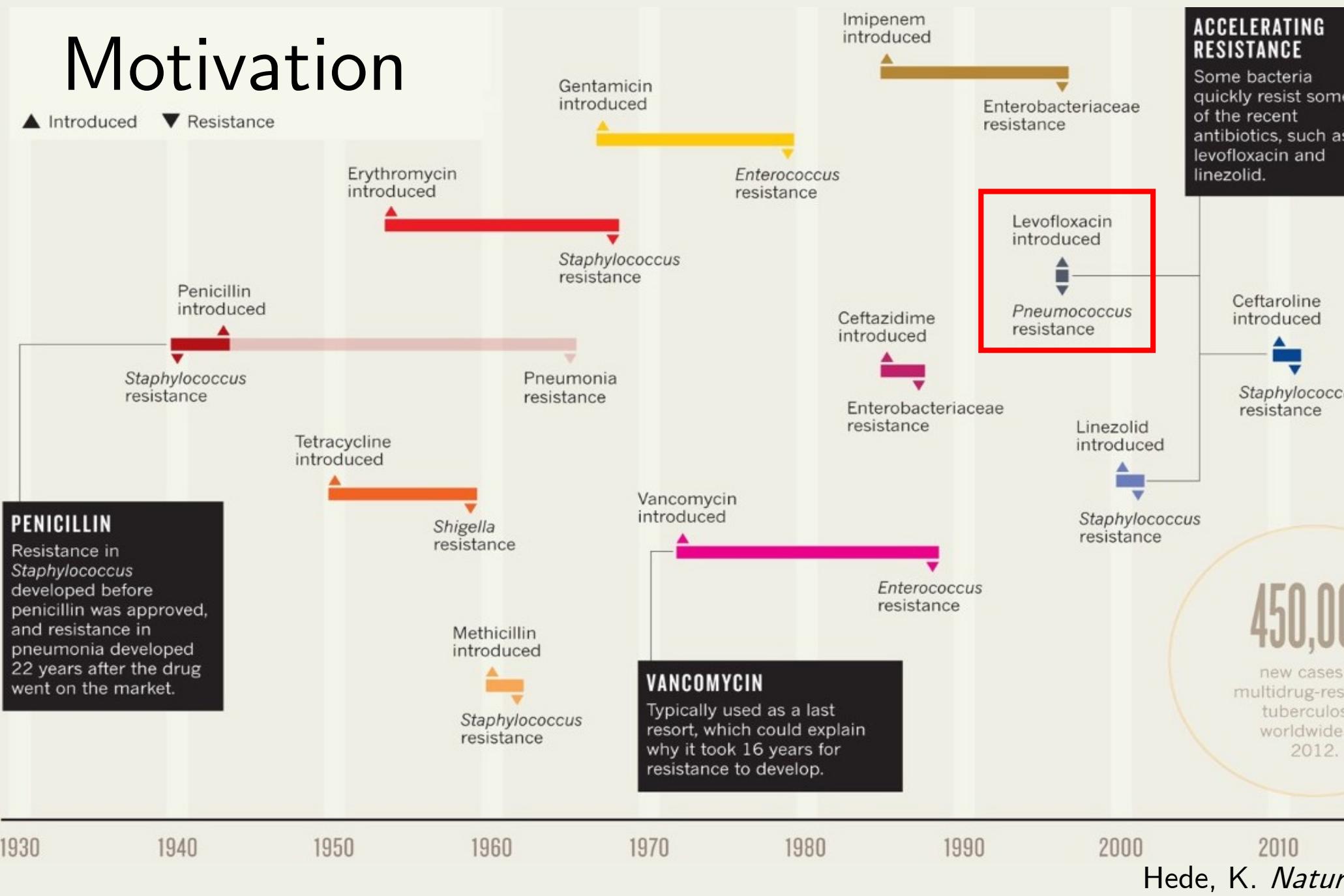
Motivation



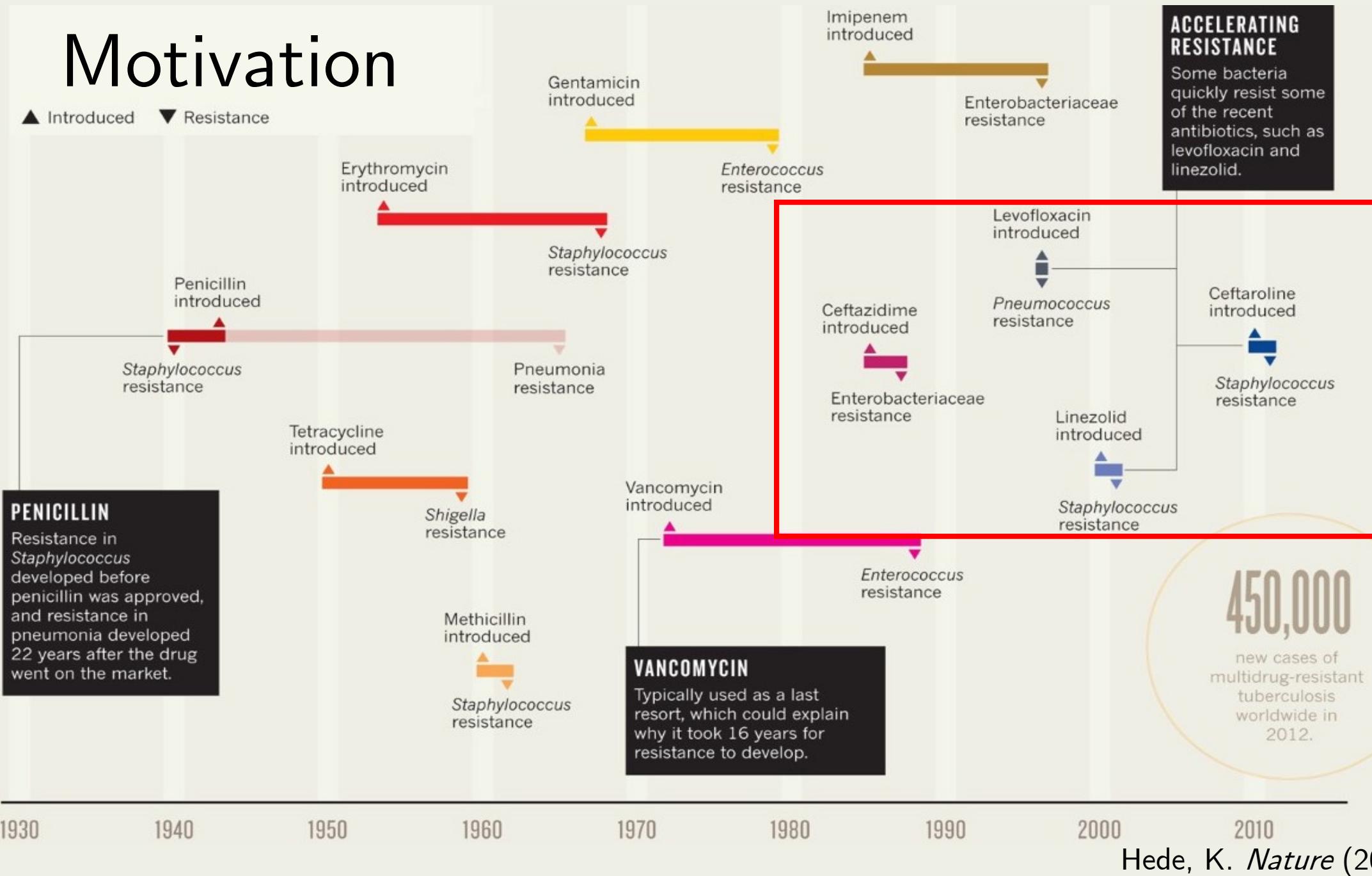
Motivation



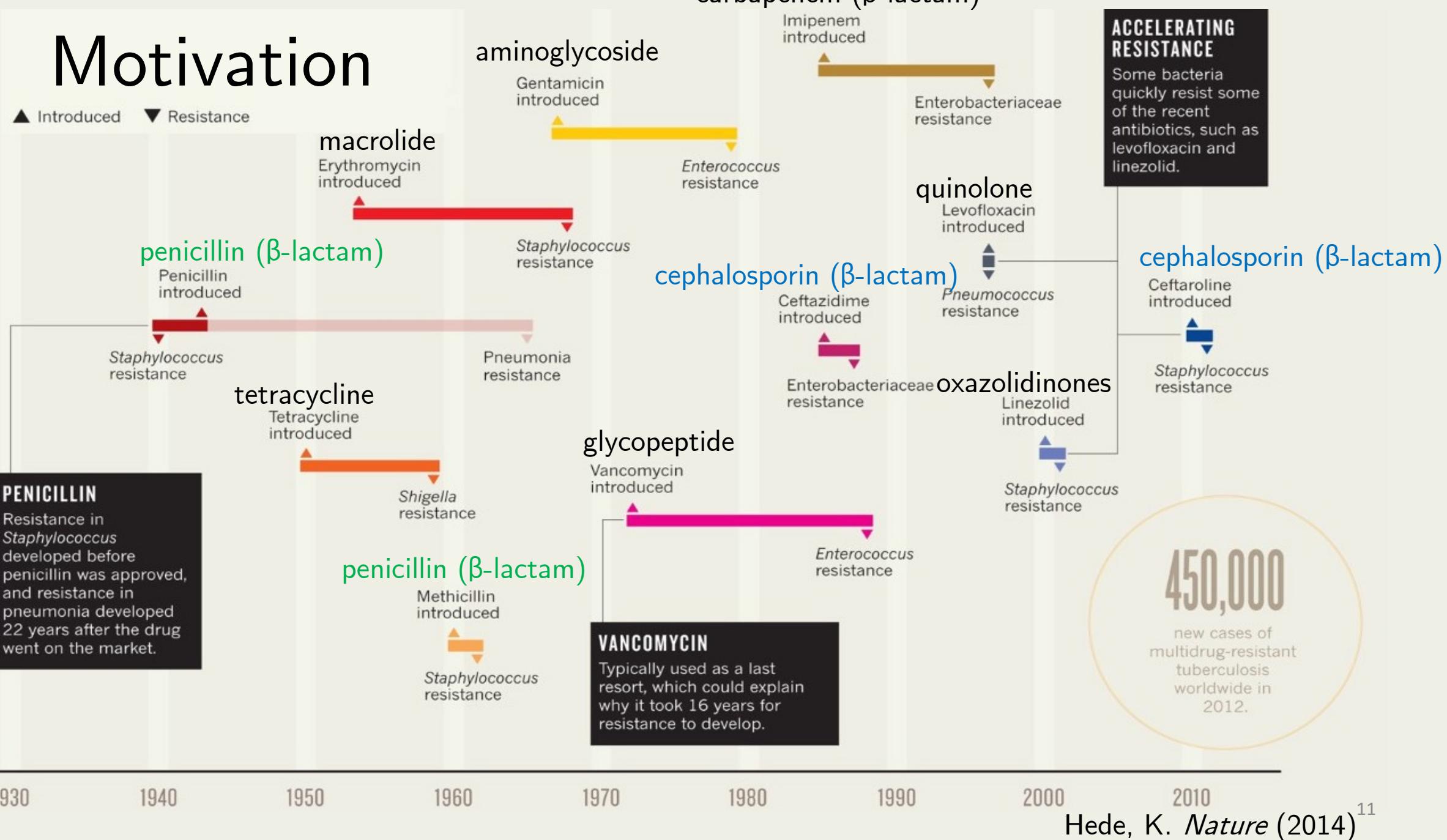
Motivation



Motivation

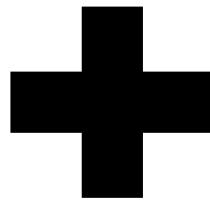


Motivation



FAST

emergence and spread
of multi-drug
resistance



SLOW

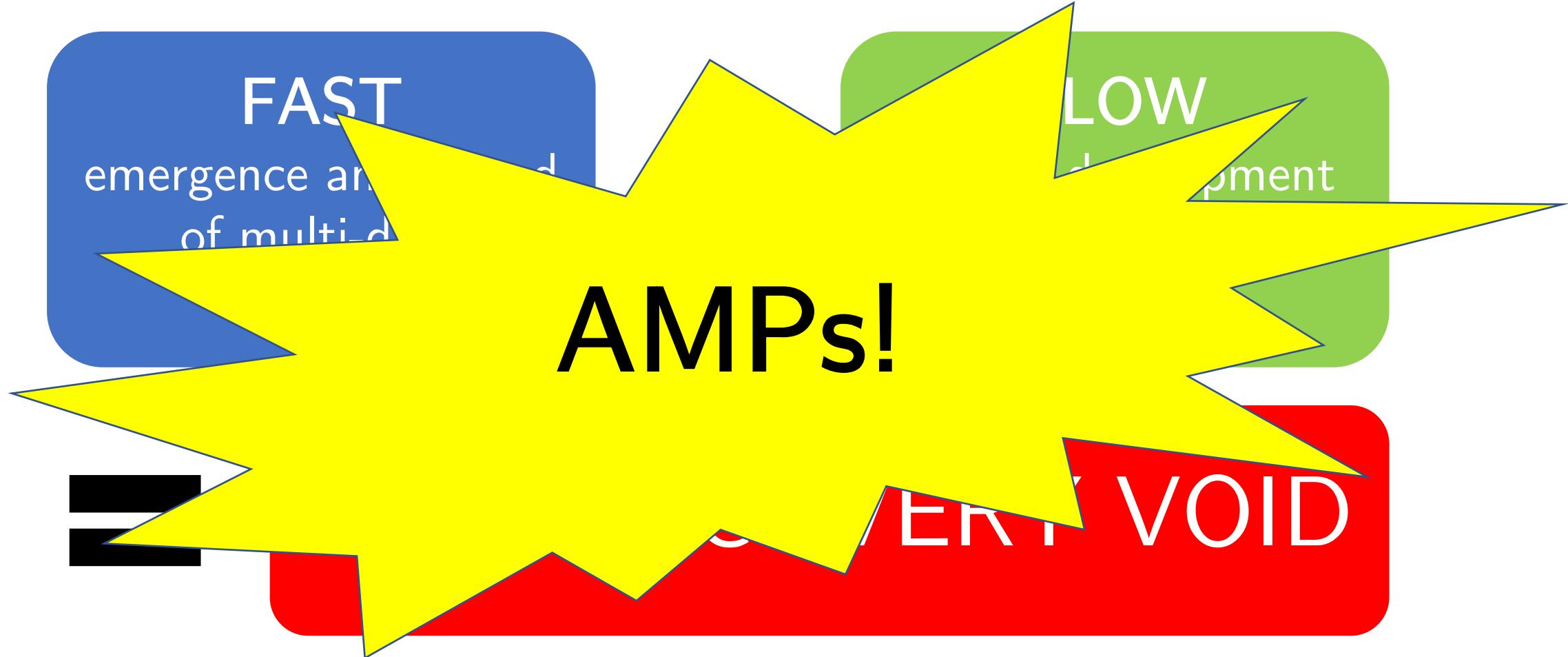
pace of development
of *novel* classes of
antibiotics



THE DISCOVERY VOID

“The [current antibiotic discovery] pipeline is insufficient to counter the rising resistance in these priority bacteria.”

Dr. Peter Beyer, Senior Advisor,
WHO Antimicrobial Resistance Division



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Objectives

- To develop **and** execute an AMP discovery pipeline to mine for AMP sequences in publicly available genomic resources
- To find candidate AMPs suitable for synthesis and wet lab validation
- To improve existing AMP annotation in AMP databases





rAMPPage: Rapid Anti-microbial Peptide Annotation and Gene Estimation

bcgsc / rAMPPage Private Watch 4

Code Issues Pull requests Actions Projects Wiki Security Insights Settings

main 4 branches 0 tags Go to file Add file Code

dy-lin update rAMPPage description 8300e2f 20 days ago 283 commits

amp_seqs	update APD3 website	last month
scripts	add version number	20 days ago
src	move warning to the top	6 months ago
Flowchart.png	update flow chart	2 months ago
README.md	update rAMPPage description	20 days ago
multi-input.txt	add stAMPede and documentation	5 months ago
rAMPPage.png	add rAMPPage logo	last month

README.md

rAMPPage: Rapid AMP Annotation and Gene Estimation

Written by Diana Lin.

About

rAMPPage: Rapid AMP Annotation and Gene Estimation

Readme

Releases

No releases published Create a new release

Packages

No packages published Publish your first package

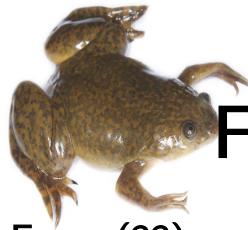
Contributors 2

dy-lin Diana Lin

saninta0212 Sambina Islam Aninta

15

Datasets



Frogs & Toads (38)



Frogs (33)

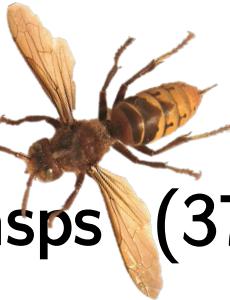
<i>A. femoralis</i>	<i>P. adspersus</i>	<i>R. sirensis</i>
<i>A. mantzorum</i>	<i>P. amboli</i>	<i>R. sylvatica</i>
<i>A. petersi</i>	<i>P. megacephalus</i>	<i>R. temporaria</i>
<i>C. alboguttata</i>	<i>P. microps</i>	<i>S. ruber</i>
<i>D. auratus</i>	<i>P. nigromaculatus</i>	<i>X. allofraseri</i>
<i>D. leucomelas</i>	<i>P. toftae</i>	<i>X. borealis</i>
<i>D. tinctorius</i>	<i>Q. boulengeri</i>	<i>X. laevis</i>
<i>H. pugnax</i>	<i>R. catesbeiana</i>	<i>X. largeni</i>
<i>L. verreauxii</i>	<i>R. dennysi</i>	<i>X. tropicalis</i>
<i>O. margaretae</i>	<i>R. imitator</i>	
<i>O. sylvatica</i>	<i>R. omeimontis</i>	
<i>O. tormota</i>	<i>R. pipiens</i>	

Toads (5)

<i>A. minuta</i>
<i>B. gargarizans</i>
<i>L. Boringii</i>
<i>M. sangzhiensis</i>
<i>O. rhodostigmatus</i>



Ants, Bees, & Wasps (37)



Ants (8)

<i>A. echinatior</i>
<i>C. castaneus</i>
<i>C. obscurior</i>
<i>M. gulosa</i>
<i>O. monticola</i>
<i>P. barbatus</i>
<i>T. bicarinatum</i>
<i>T. rugulatus</i>

Bees (5)

<i>A. cerana</i>
<i>A. mellifera</i>
<i>B. ardens</i>
<i>B. consobrinus</i>
<i>B. ussurensis</i>

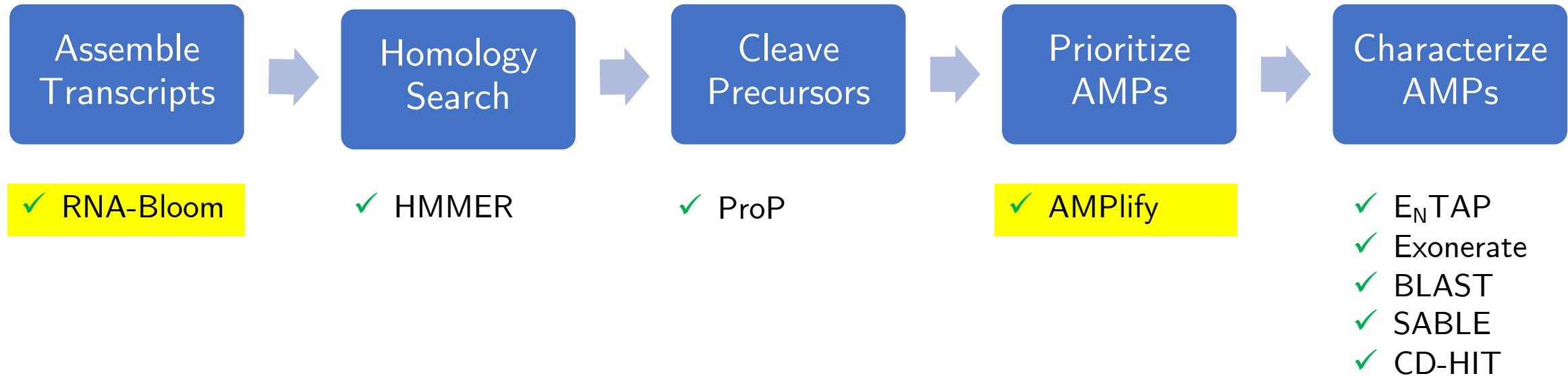
Wasps (24)

<i>A. compressa</i>	<i>P. snelleni</i>
<i>A. flavomarginatum</i>	<i>P. turionellae</i>
<i>B. nigricans</i>	<i>P. varia</i>
<i>C. vestalis</i>	<i>P. vindemmiae</i>
<i>D. collaris</i>	<i>S. deformae</i>
<i>D. longicaudata</i>	<i>S. kj8906</i>
<i>M. demolitor</i>	<i>T. sarcophagae</i>
<i>N. giraulti</i>	<i>U. rufipes</i>
<i>N. vitripennis</i>	<i>V. analis</i>
<i>N. vitripennis x N. giraulti</i>	<i>V. crabro</i>
<i>O. decorates</i>	<i>V. dybowskii</i>
<i>P. rothneyi</i>	<i>V. similis</i>

Why Amphibians and Insects?



rAMPage Pipeline



Assembly: RNA-Bloom



- *De novo* transcriptome assembly with single and paired-end reads
- Reference-guided assembly if reference or draft transcriptome available

Screenshot of the GitHub repository page for bcgsc / RNA-Bloom.

Key statistics shown:

- Code: 1,356 commits, 1 branch, 0 packages, 12 releases, 1 contributor.
- Issues: 0
- Pull requests: 0
- Actions: 0
- Projects: 0
- Wiki: 0
- Security: 0
- Insights: 1
- Unwatch releases: 11
- Star: 23
- Fork: 1

Tags: denovo-assembly, rna-seq, single-cell-rna-seq, bioinformatics-tool, nanopore-sequencing

Description: lightweight transcriptome assembly for short reads and nanopore reads

Homology Search: Databases

May 10, 2021

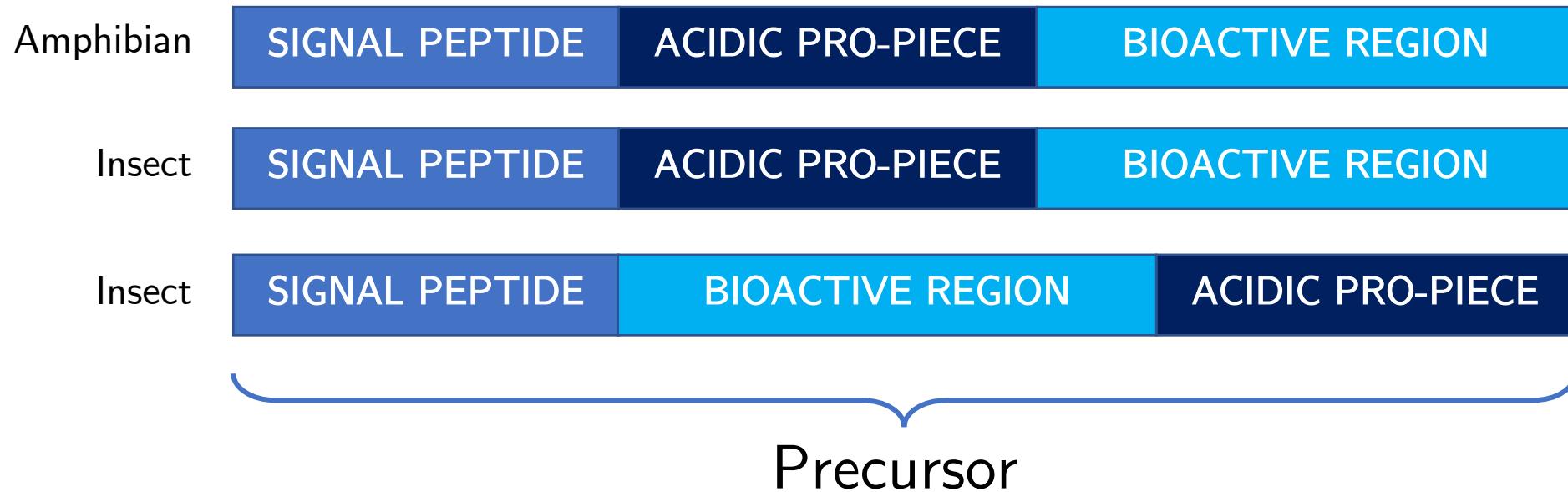
Database	Amphibians (nr)	Insects (nr)	All (nr)
APD3 Sep. 2020	1,075 +34	310 +13	3,125 +97
DADP Sep. 2020	1,921	0	1,921
NCBI Jul. 2020	2,850 +41	985 +57	185,967
“Reference” AMPs	4,663 +73	1,204 +62	N/A

APD3: Antimicrobial Peptide Database 3, <https://wangapd3.com/main.php>

DADP: Database of Anuran Defense Peptides, <http://split4.pmfst.hr/dadp/>

Homology Search

- 4,663 (nr) amphibians; 1,204 insect (nr) known “reference” AMPs



Cleaving Precursor AMPs: Amphibians

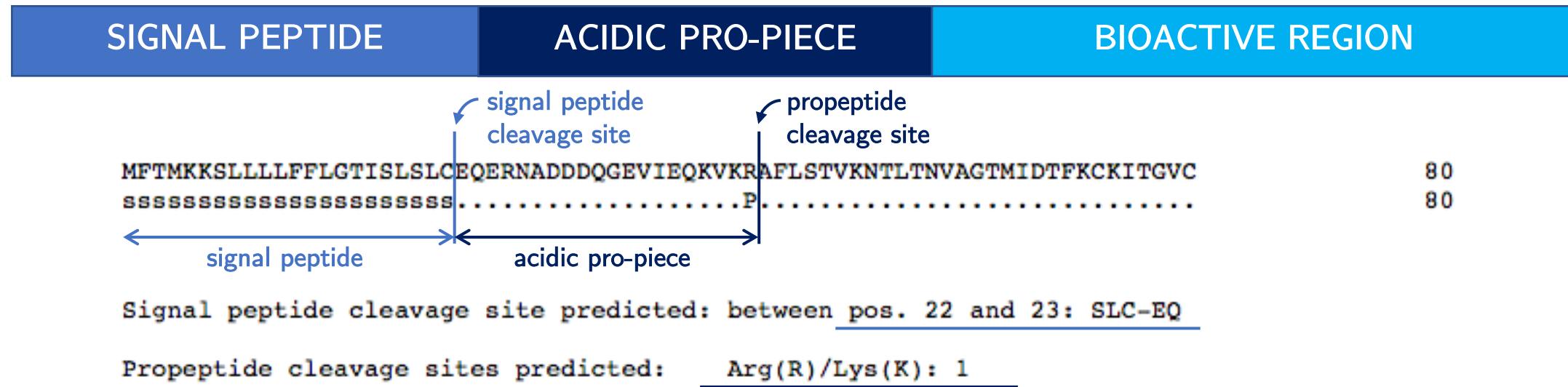


R. catesbeiana

American bullfrog

>rcatesbeiana-back skin-139086.p2

MFTMKKSLLLLFFLGTISLSCEQERNADDDQGEVIEQKV**KR**AFLSTVKNTLTNVAGTMIDTFKCKITGVC



Cleaving Precursor AMPs: Insects



M. gulosa red bulldog ant

>mgulosa-venom-325.p1

MRTFIVLIALAIICAVIEA

GRNPVNTKPTPYP**RLRR**

EAEAEAETEPGRPQNWNKPTPNP**RGRR**

EAEANAVAASPLGTIQTRPTGPPRPRREAEPHAEALMPGAPSJVVKPTYAPPPRGRREAEPGRLNPVNNKPTPHP**RLRR**
EAEAEAEPGRPNPVNNKPTPHP**RLRR** EAETEAEEAEPGRPQNWNNKPTPNPR

SIGNAL PEPTIDE

ACIDIC PRO-PIECE?

BIOACTIVE REGION?

SIGNAL PEPTIDE

BIOACTIVE REGION?

ACIDIC PRO-PIECE?

The diagram illustrates the primary structure of a protein precursor. The sequence starts with a signal peptide (MRTFIVLIALAI) followed by a signal peptide cleavage site (indicated by a vertical line and bracket). The next segment is the propeptide (QTRPTGPPRPRREAEPHAEALMPGAPS), which ends at another signal peptide cleavage site. A horizontal arrow labeled "propeptide cleavage site" points to the boundary between the propeptide and the acidic pro-piece. The acidic pro-piece is labeled "acidic pro-piece?" and contains a series of negatively charged amino acids (ssssssssssssssssss). Below the sequence, a dotted line indicates the C-terminal end of the protein. Molecular weights are indicated on the right side of the sequence: 80, 160, 240, 80, 160, and 240.

Signal peptide cleavage site predicted: between pos. 19 and 20: IEA-GR

Propeptide cleavage sites predicted: Arg(R)/Lys(K): 4

Prioritizing AMPs: AMPlify

Sequence → **AMPlify** → score ≥ 0.50 → AMP non-AMP

Criteria

- ✓ AMPlify score ≥ 0.50
- ✓ Length $\leq 50\text{aa}$
- ✓ Charge $\geq +2$

Sequence ID	Sequence	Prediction	Score	Length	Charge	Criteria Met?
rcatesbeiana-back_skin-139086.p2-sig_mature-2	AFLSTVKNTLTNVAGTMIDTFKCKITGVC	AMP	0.9999962	29	2	
mgulosa-venom-325.p1-sig_mature-1	GRPNPVNTKPTPYPRLRR	AMP	0.9907686	18	5	
mgulosa-venom-325.p1-sig_mature-2	EAEAEAETEPGRPQNWNNKPTPNPRGRREA EANAVAASPLGTIQTRPTGPPRPRREAEPAH EALMPGAPSVHVVKPTYAPPPRGRR	AMP	0.9987987	86	3	
mgulosa-venom-325.p1-sig_mature-3	EAEPGRLNPVNNKPTPHPRLRR	AMP	0.50080854	22	3	
mgulosa-venom-325.p1-sig_mature-4	EAEAEAEPGRPNPVNNKPTPHPRLRR	non-AMP	0.25241083	26	1	
mgulosa-venom-325.p1-sig_mature-5	EAETEAEEAEPGRPQNWNNKPTPNPR	non-AMP	0.017407548	27	-3	

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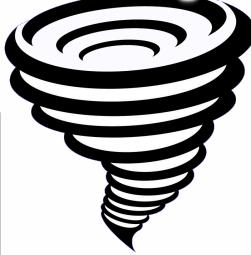
Novelty Criteria

✓ Alignment < 100%

Characterizing AMPs

- Alignment to our “reference” AMPs
- Alignment to orthology, domain, and protein databases
- Secondary structure prediction

Sequence_ID	AMPlify Score	Length	Charge	Top Precursor	Top Mature	GO Term	IPScan	Pfam	Secondary Structure	Criteria Met?
rcatesbeiana-back_skin-139086.p2-sig_mature-2	0.9999962	29	2	ABB89058.1: ranatuerin 2CHb precursor, partial [Rana chiricahuensis] (75.86%)	AP00617: Palustrin-2 (UCLL1a; XXU; 1S=S, frog, amphibians, animals) (75.86%)	GO:0005576 (extracellular region) GO:0006952 (defense response)	IPR012521 (Frog antimicrobial peptide, brevinin-2/esculentin type)	PF08023 (Frog antimicrobial peptide)	CCEEHHHHHH CCCCCCCCCC EEEECCCC	✓
mgulosa-venom-325.p1-sig_mature-1	0.9907686	18	5	NA	P81437.1: Formaecin-2 [Myrmecia gulosa] (100%)	GO:0042381 (hemolymph coagulation) GO:0042742 (defense response to bacterium)	IPR012514 (Formaecin)	PF08106 (Formaecin family)	CCCCCCCCCC CCCCCCCCCC	✗



Final Output

- FASTA file

Criteria
✓ AMPlify score >= 0.90 / 0.80
✓ Length <= 30aa
✓ Charge >= +2

>rcatesbeiana-back_skin-139086.p2-sig_mature-2 length=29
score=0.9999962 prediction=AMP charge=2
AFLSTVKNTLTNVAGTMIDTFKCKITGVC

>mgulosa-venom-325.p1-sig_mature-1 length=18 score=0.9907686
prediction=AMP charge=5
GRPNPVNTKPTPYPRLRR

>rsylvatica-skin-357168.p1-no_sig_mature-2 length=28
score=0.9976821 prediction=AMP charge=10
SSKKKKCKFFCKLKKKINSIPIISIPFK

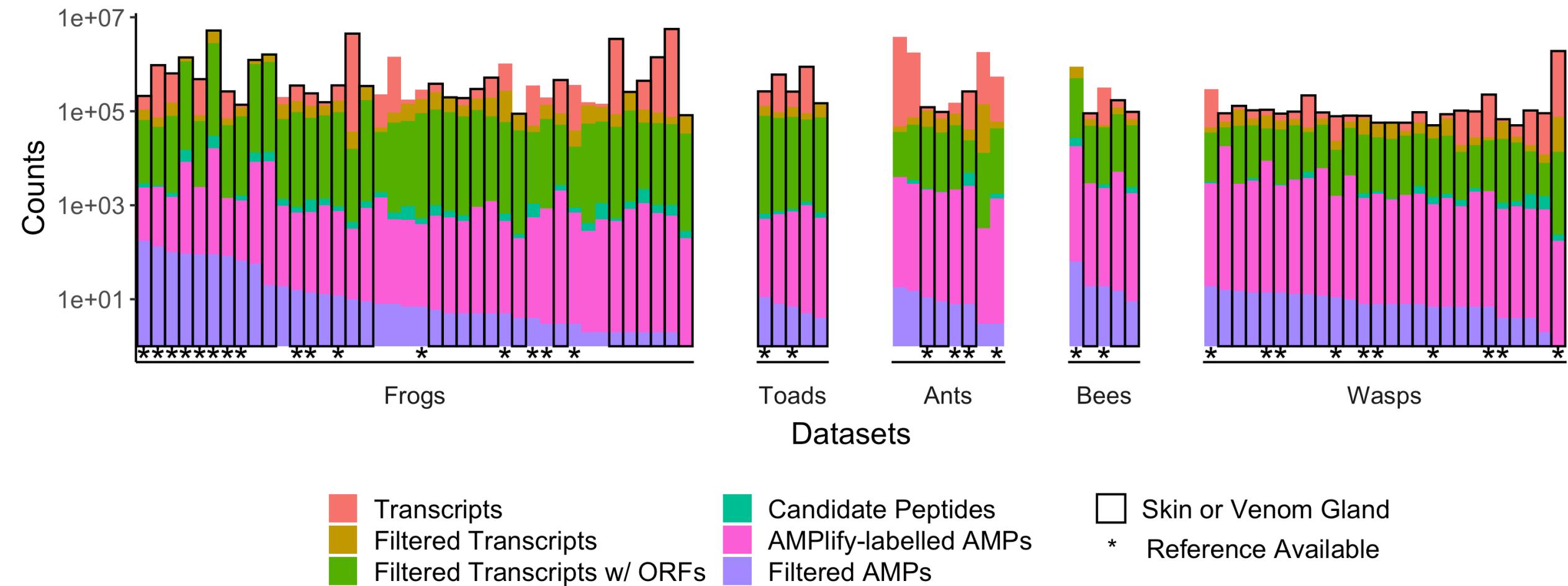
- TSV file

Selecting AMPs for Synthesis

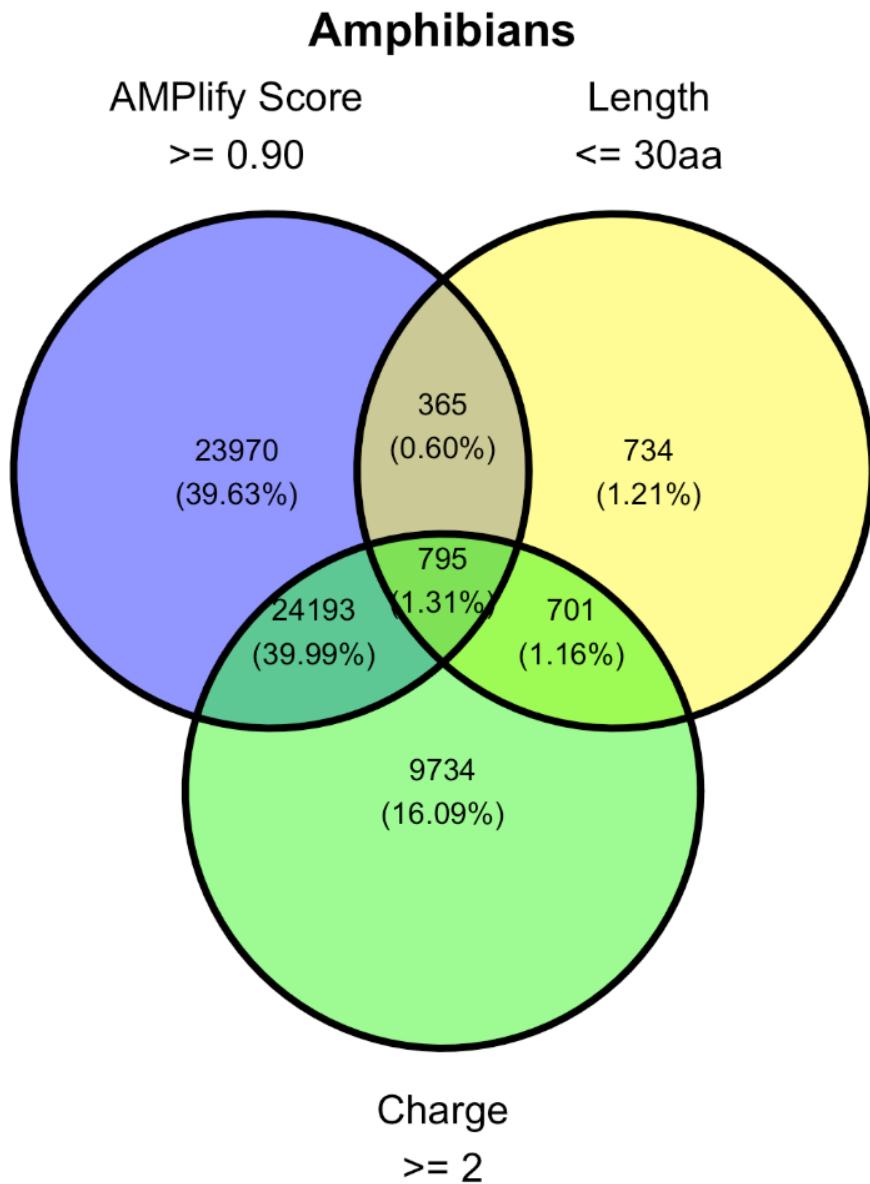
1. AMPs present in > 2 species
2. Insect AMPs with AMPlify score > 0.99 present in 1 species
3. Top-scoring sequences of 95% similarity clustering

Results: Summary

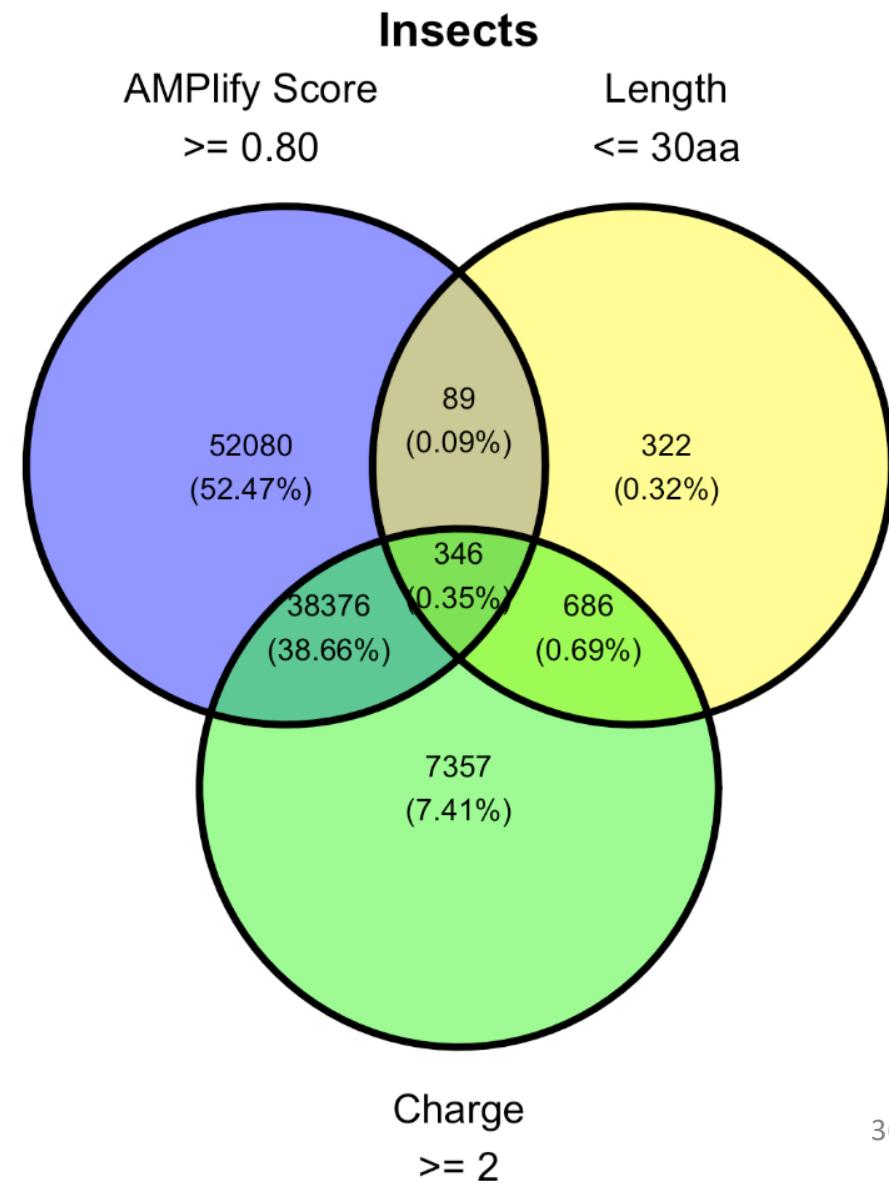
Criteria: AMPlify score ≥ 0.90 (amphibians), 0.80 (insects); Length $\leq 30\text{aa}$; Charge ≥ 2
Total Transcripts: 53,031,368
Total AMPs: 1,483



Results

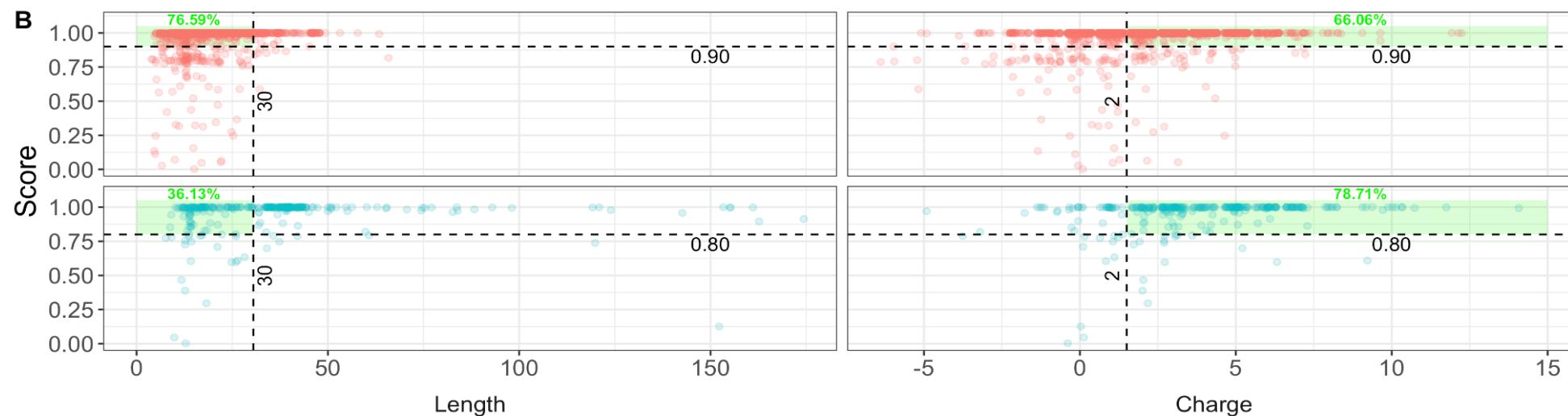
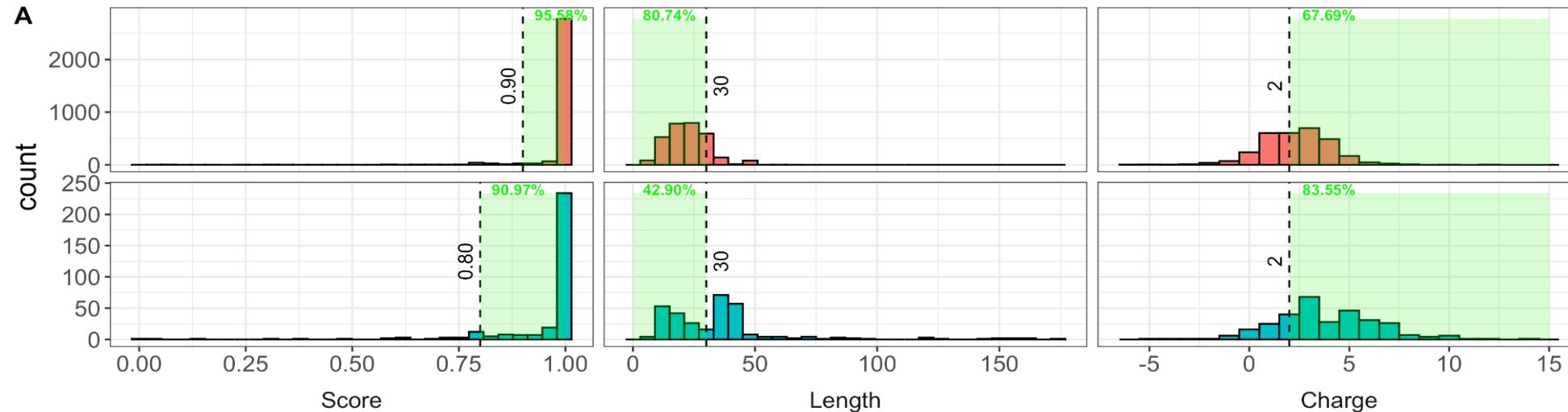


AMP Filters



Results

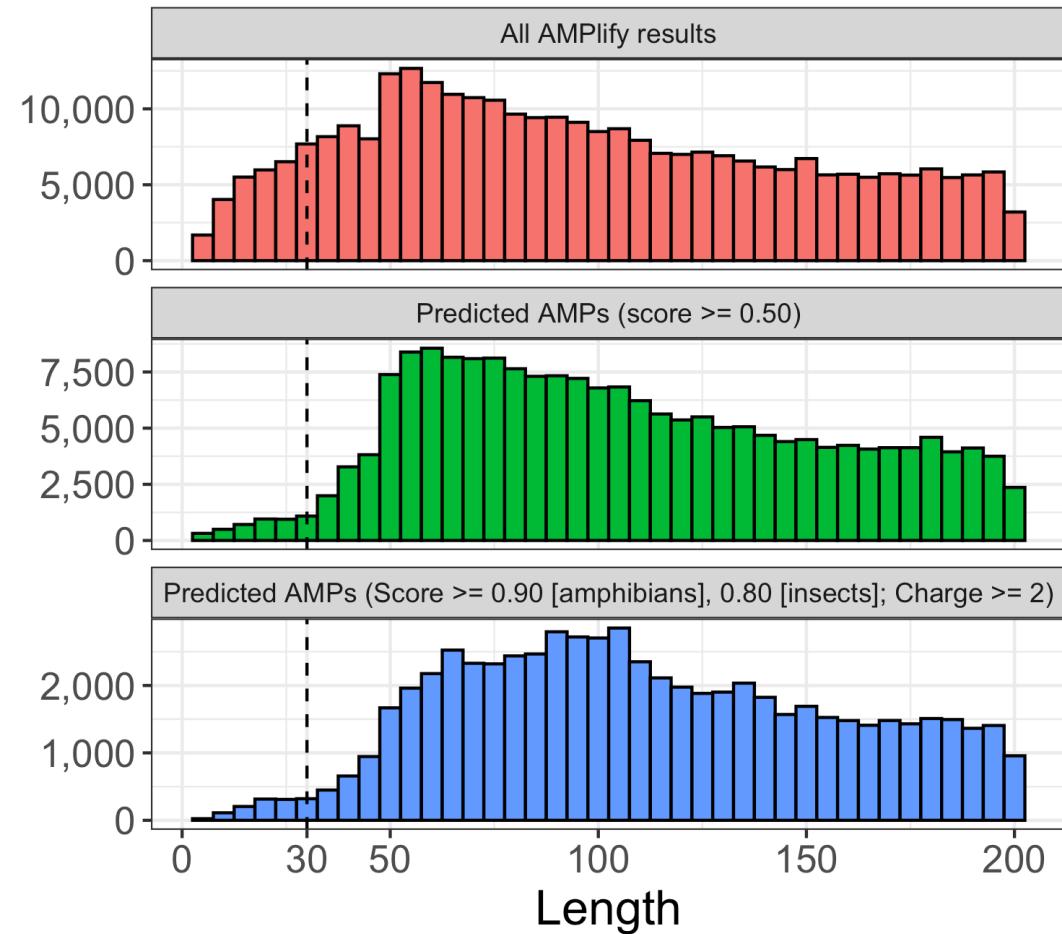
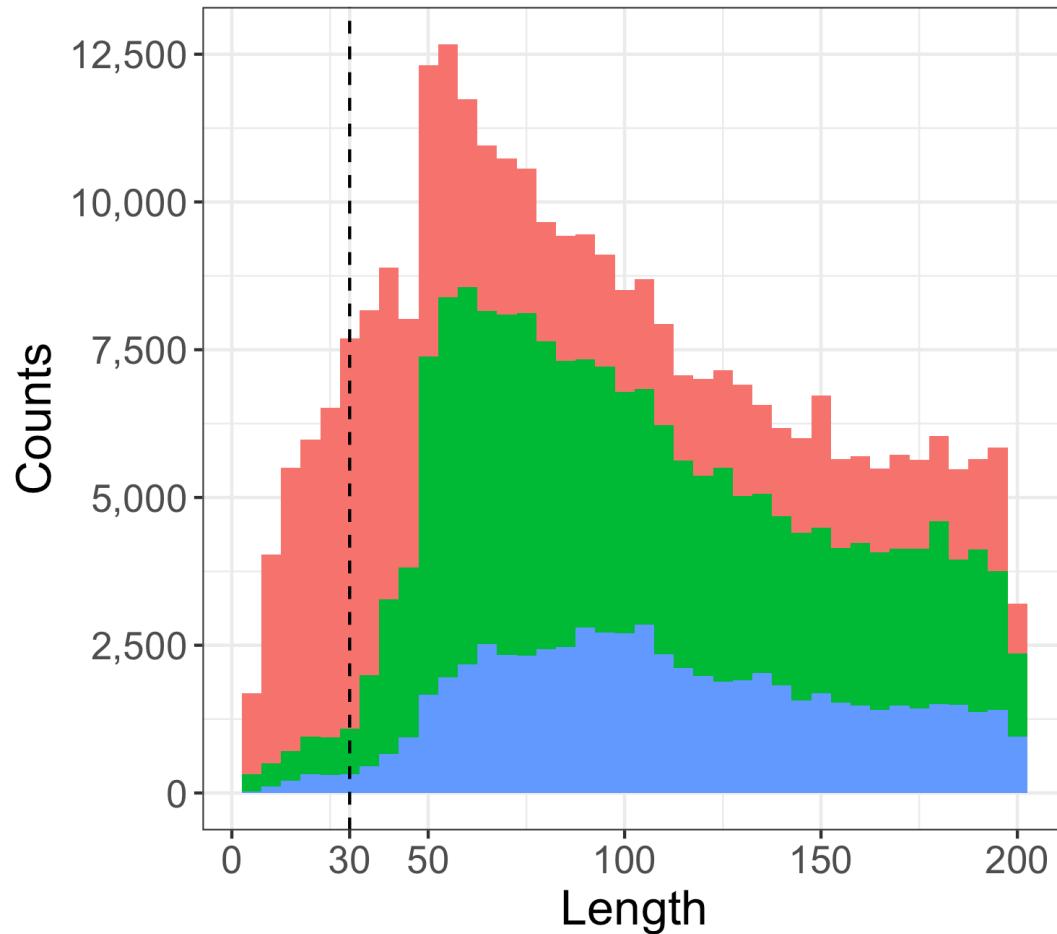
Distribution of Reference Mature AMPs



Class ■ Amphibians ■ Insects

Results

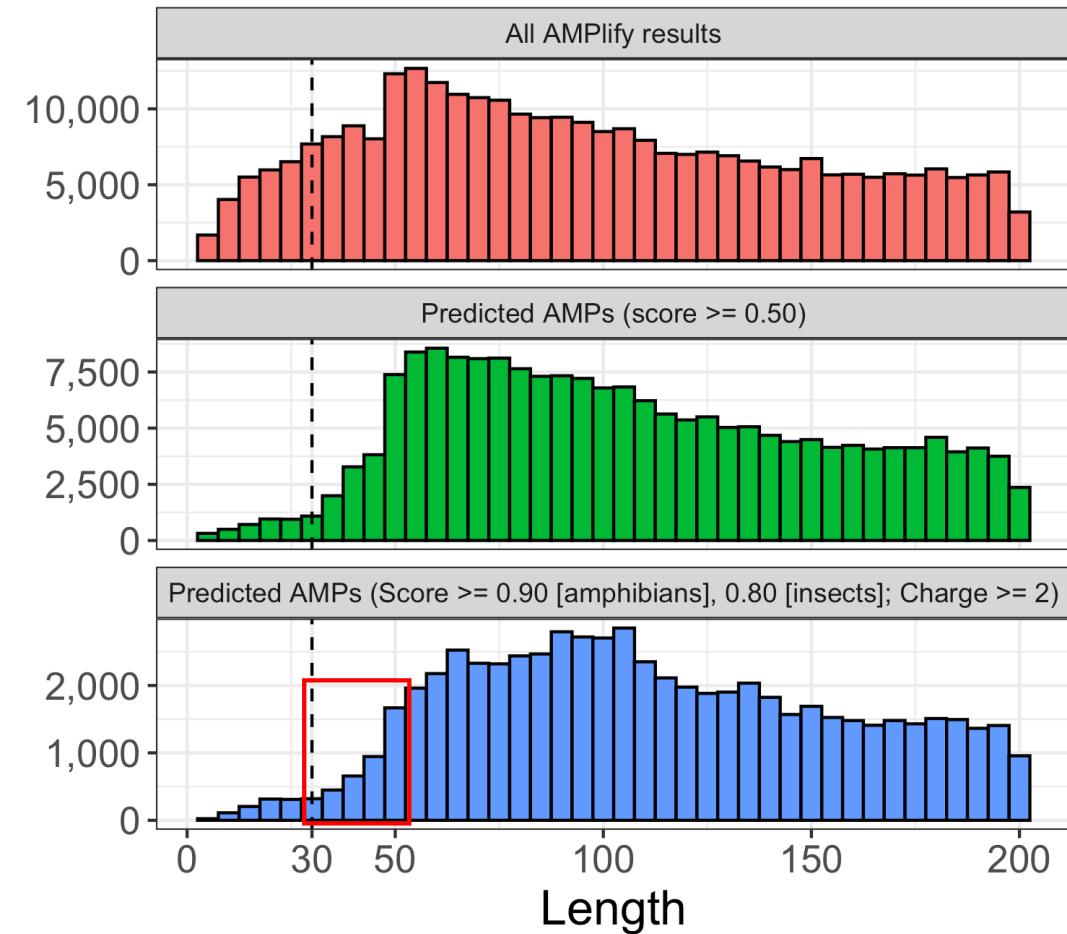
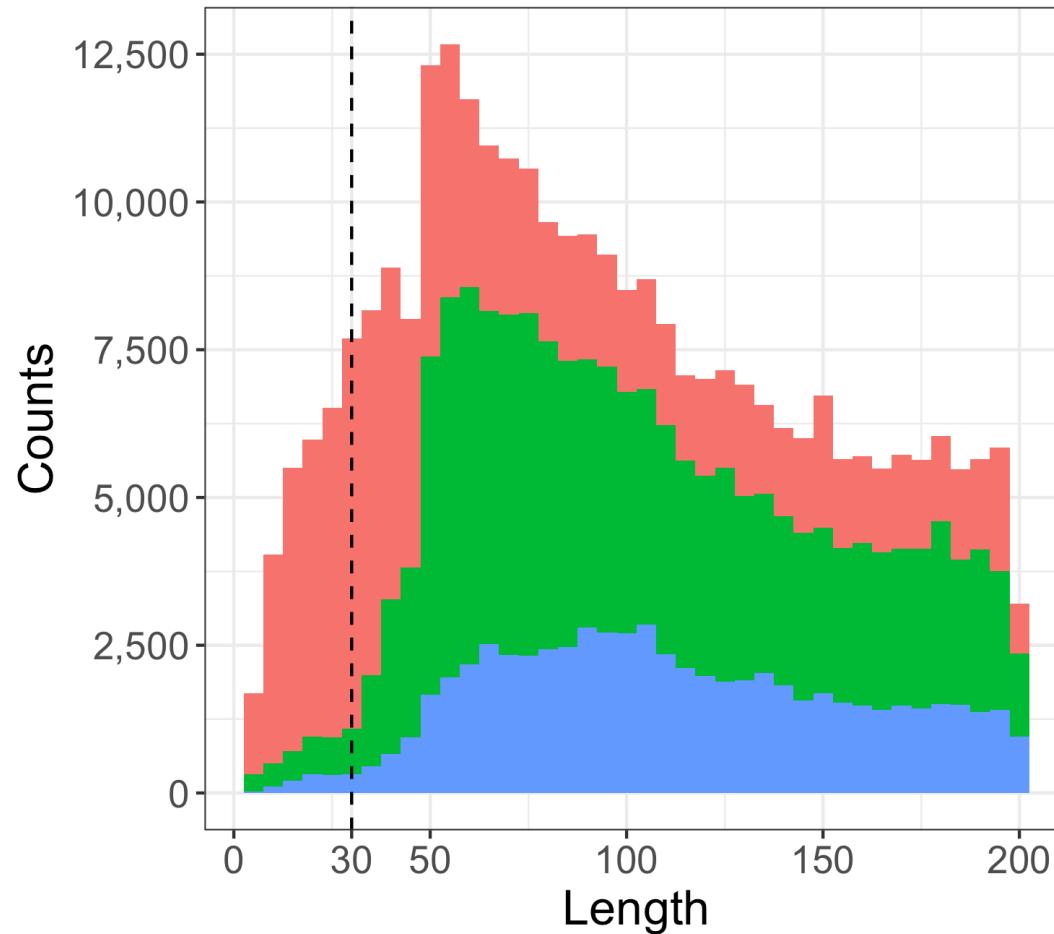
Length Distribution



binwidth = 5

Results

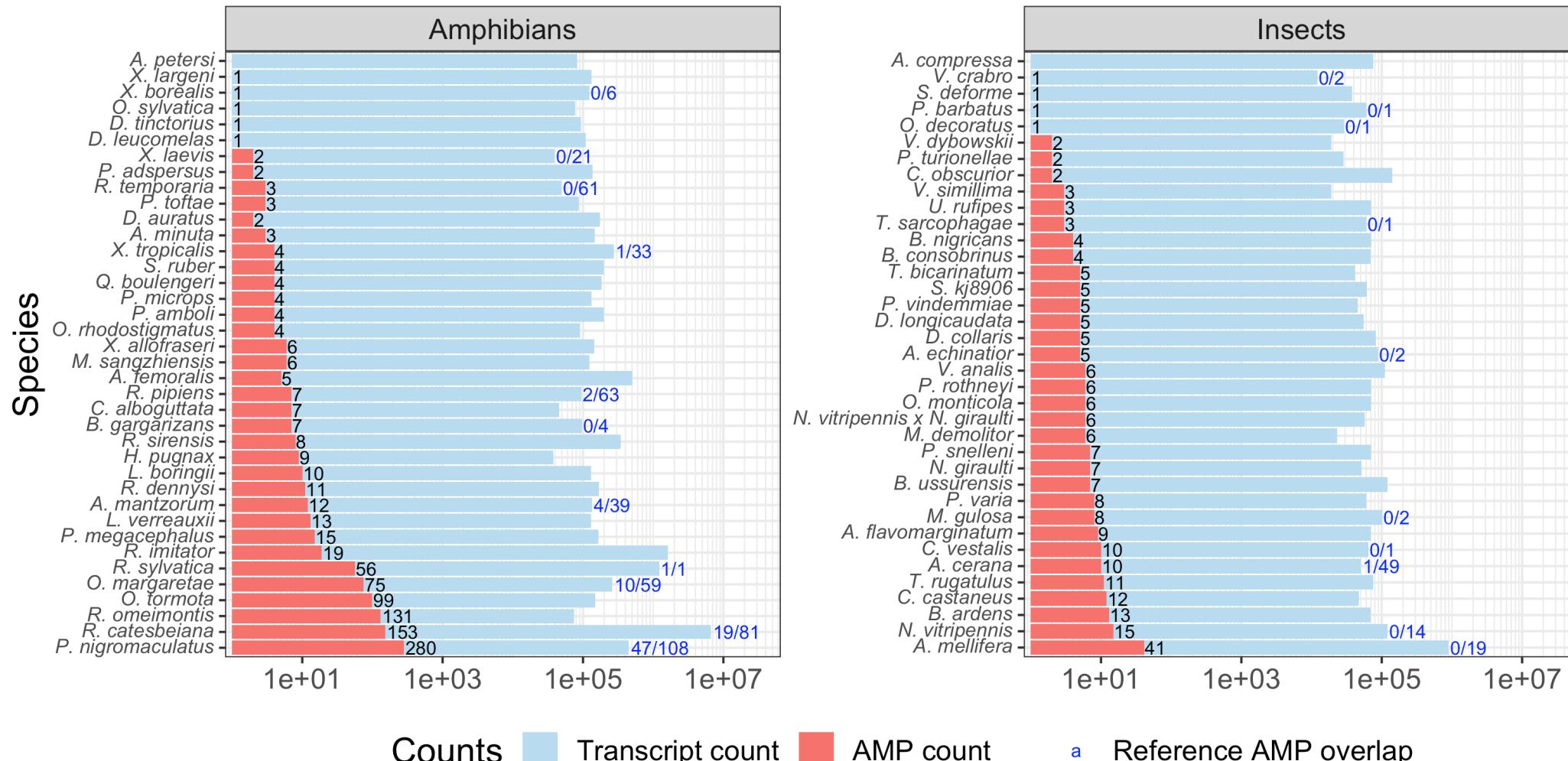
Length Distribution



binwidth = 5

Results

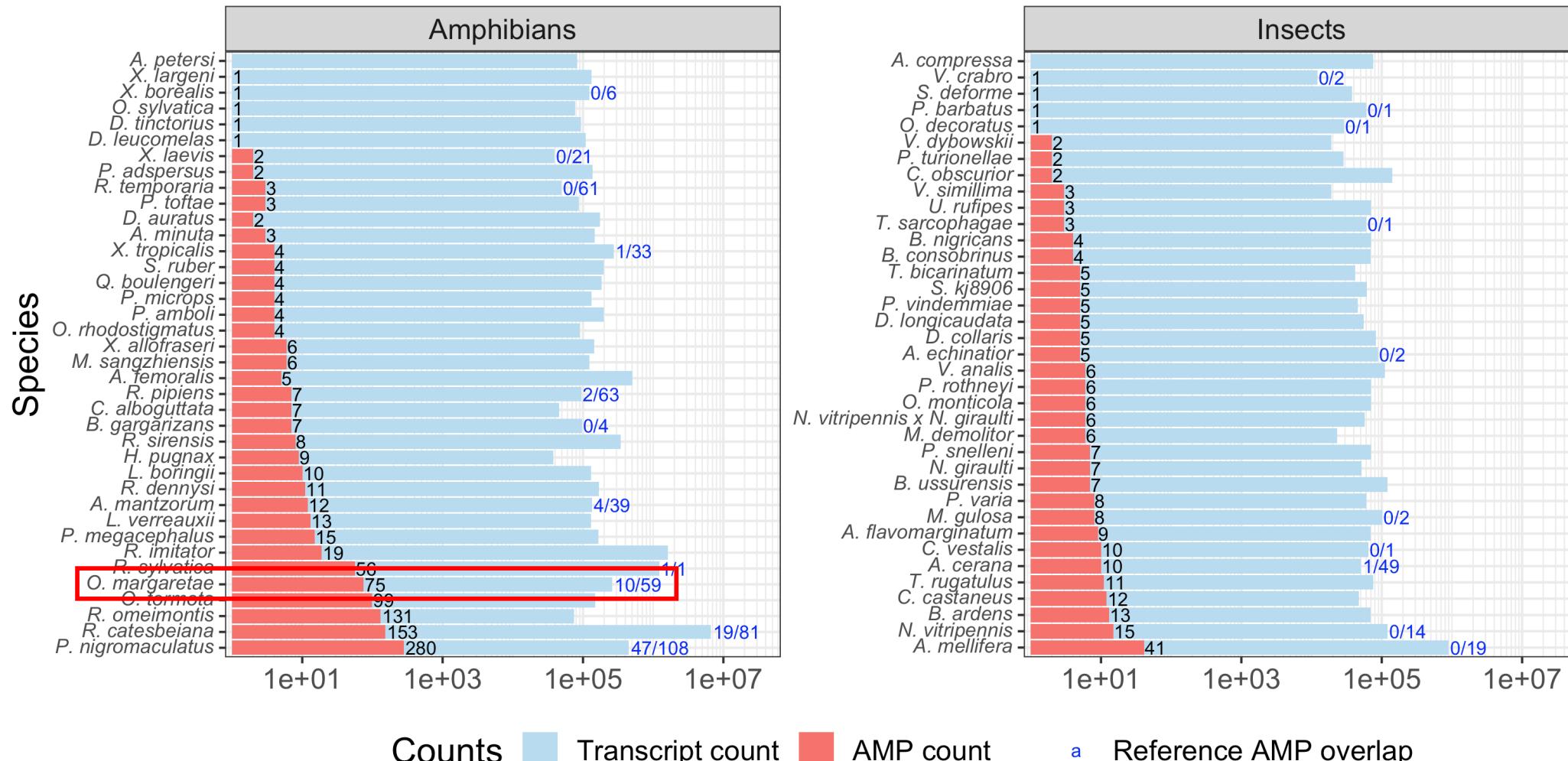
Species Distribution AMPlify Score >= 0.90, Length <= 30aa, Charge >= 2 (nr)



Results

Species Distribution

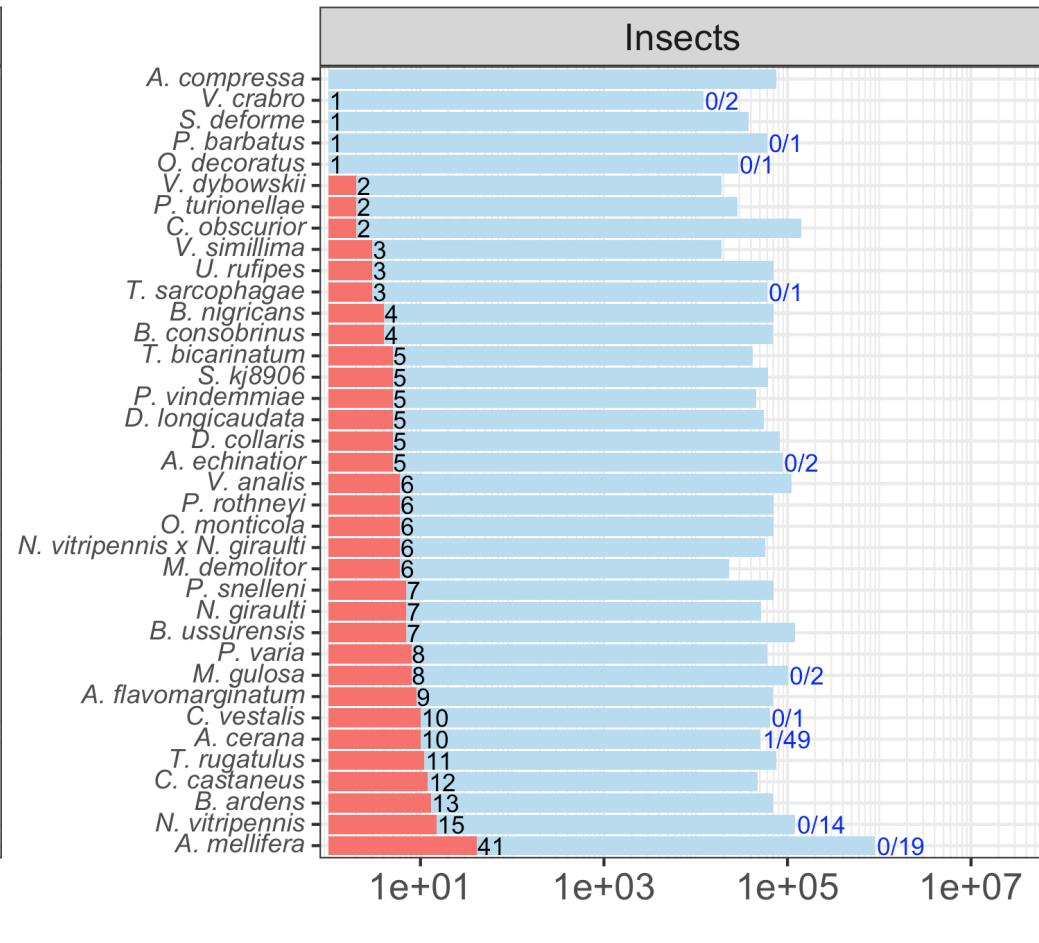
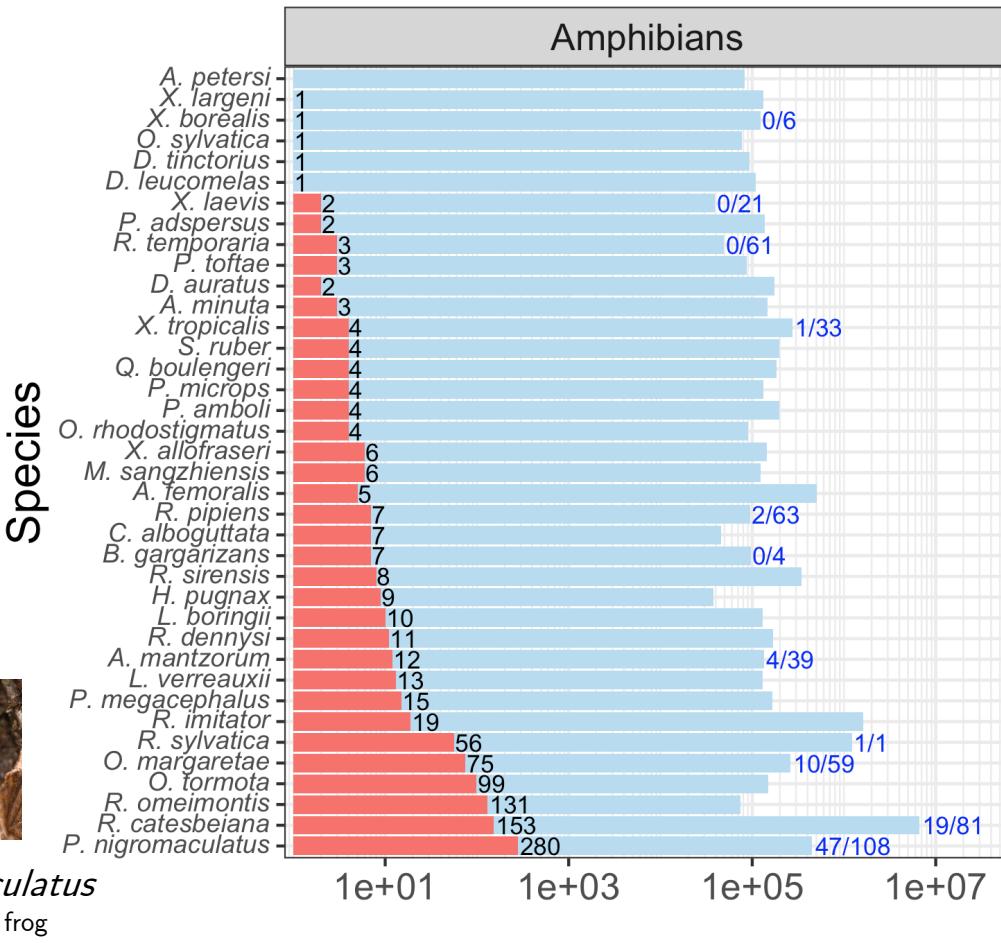
AMPLify Score >= 0.90, Length <= 30aa, Charge >= 2 (nr)



Results

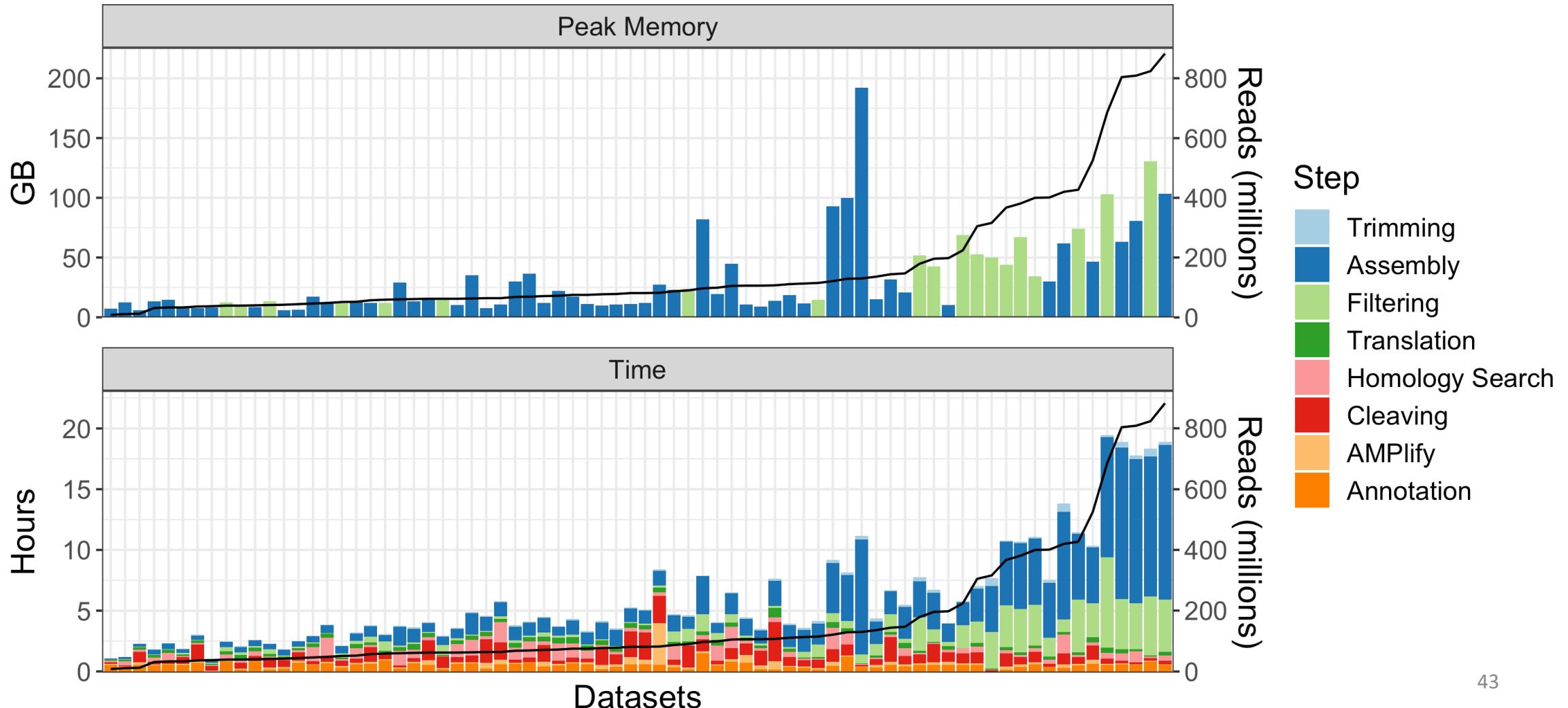
Species Distribution

AMPLify Score ≥ 0.90 , Length $\leq 30\text{aa}$, Charge ≥ 2 (nr)



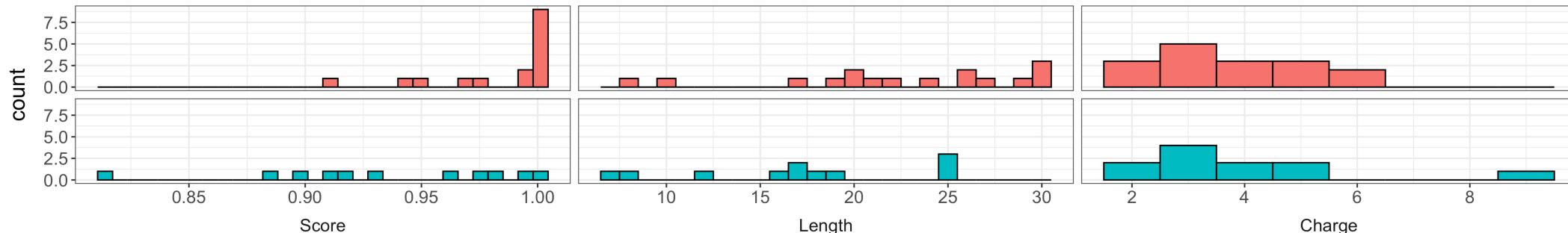
Results

rAMPage Benchmarking

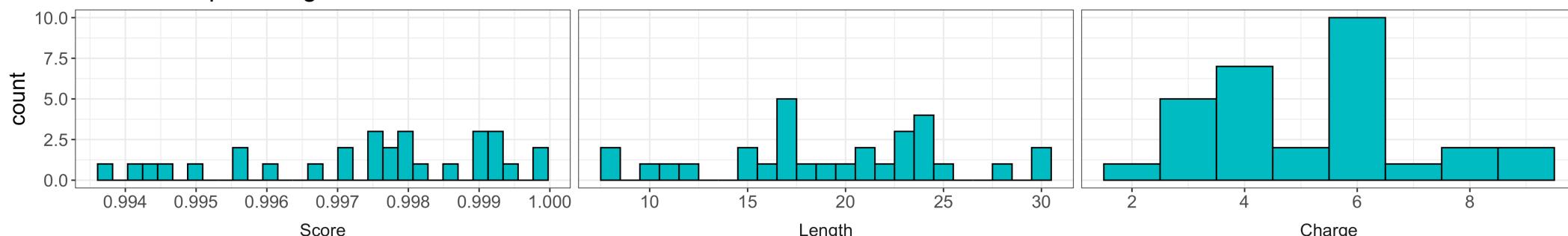


Results: Selected AMPs for Synthesis

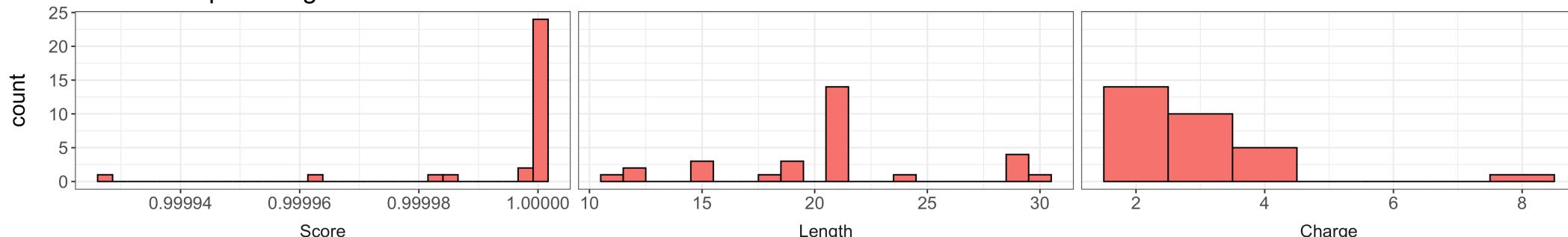
A Method: Top Species Count



B Method: Top-scoring Insects



C Method: Top-scoring Clusters



Class Amphibians Insects

Summary

- ✓ *rAMPage* pipeline runs all the tools from beginning to end, from reads to candidate AMPs
- ✓ 90 candidate AMPs from *rAMPage* selected for synthesis and wet lab validation

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Questions?