

What resistance hotspots are missing by not including functionally determined ARGs in our analyses?

A look into sewage resistome profiles and different reference sequence collections

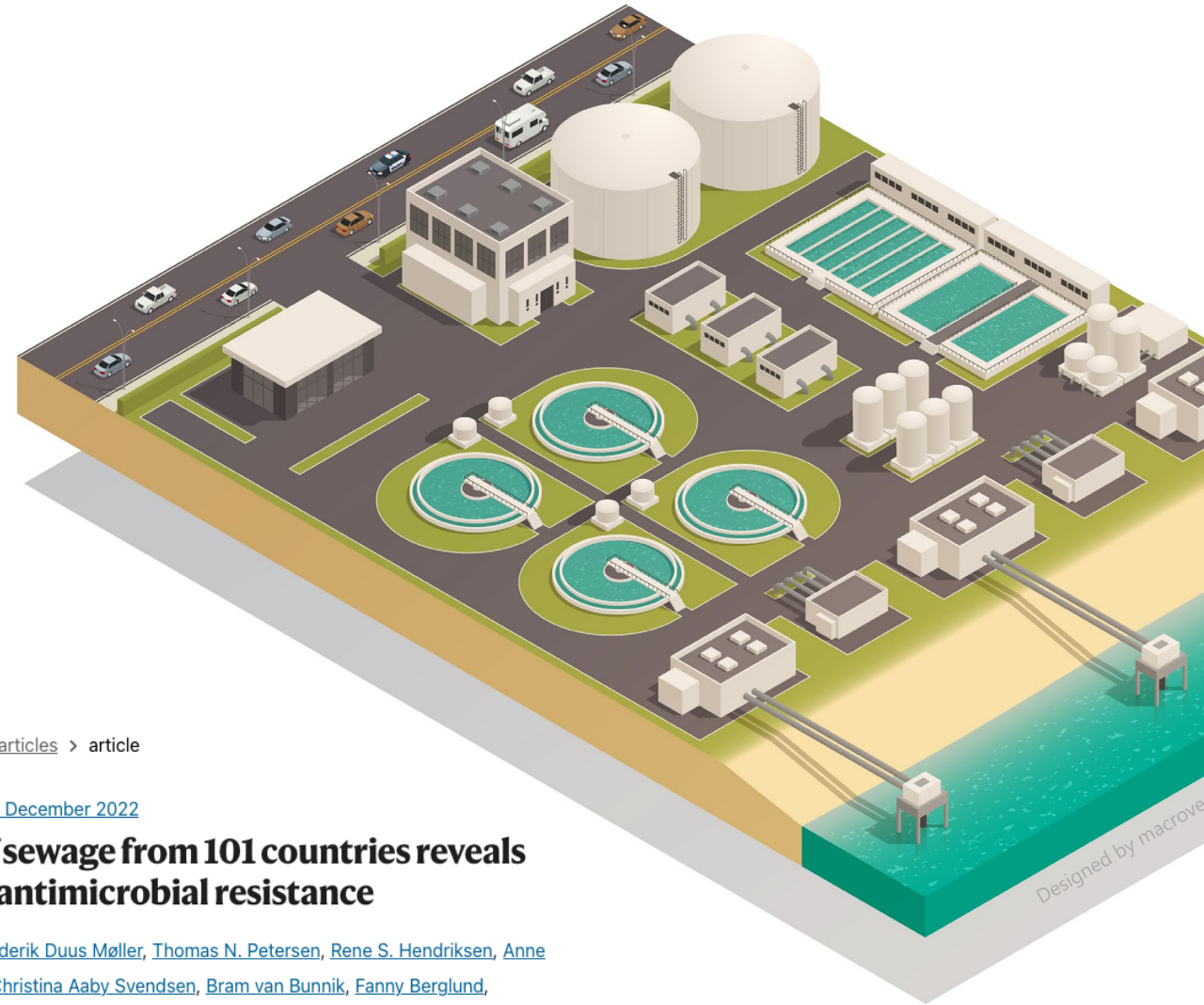
Hannah-Marie Martiny



Global Sewage

A collection of sewage samples


- from 371 cities in 111 countries
- collected between 2016 and 2022



[nature](#) > [nature communications](#) > [articles](#) > [article](#)

Article | [Open access](#) | [Published: 08 March 2019](#)

Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage

[Rene S. Hendriksen](#), [Patrick Munk](#), [Patrick Njage](#), [Bram van Bunnik](#), [Luke McNally](#), [Oksana Lukjancenko](#), [Timo Röder](#), [David Nieuwenhuijse](#), [Susanne Karlsmose Pedersen](#), [Jette Kjeldgaard](#), [Rolf S. Kaas](#), [Philip Thomas Lancken Conradsen Clausen](#), [Josef Korbinian Vogt](#), [Pimlapas Leekitcharoenphon](#), [Milou G. M. van de Schans](#), [Tina Zuidema](#), [Ana Maria de Roda Husman](#), [Simon Rasmussen](#), [Bent Petersen](#), [The Global Sewage Surveillance project consortium](#), [Clara Amid](#), [Guy Cochrane](#), [Thomas Sicheritz-Ponten](#), [Heike Schmitt](#), ... [Frank M. Aarestrup](#)  [+ Show authors](#)

[Nature Communications](#) **10**, Article number: 1124 (2019) | [Cite this article](#)

78k Accesses | **519** Citations | **422** Altmetric | [Metrics](#)

[nature](#) > [nature communications](#) > [articles](#) > [article](#)

Article | [Open access](#) | [Published: 01 December 2022](#)

Genomic analysis of sewage from 101 countries reveals global landscape of antimicrobial resistance

[Patrick Munk](#) , [Christian Brinch](#), [Frederik Duus Møller](#), [Thomas N. Petersen](#), [Rene S. Hendriksen](#), [Anne Mette Seyfarth](#), [Jette S. Kjeldgaard](#), [Christina Aaby Svendsen](#), [Bram van Bunnik](#), [Fanny Berglund](#), [Global Sewage Surveillance Consortium](#), [D. G. Joakim Larsson](#), [Marion Koopmans](#), [Mark Woolhouse](#) & [Frank M. Aarestrup](#)

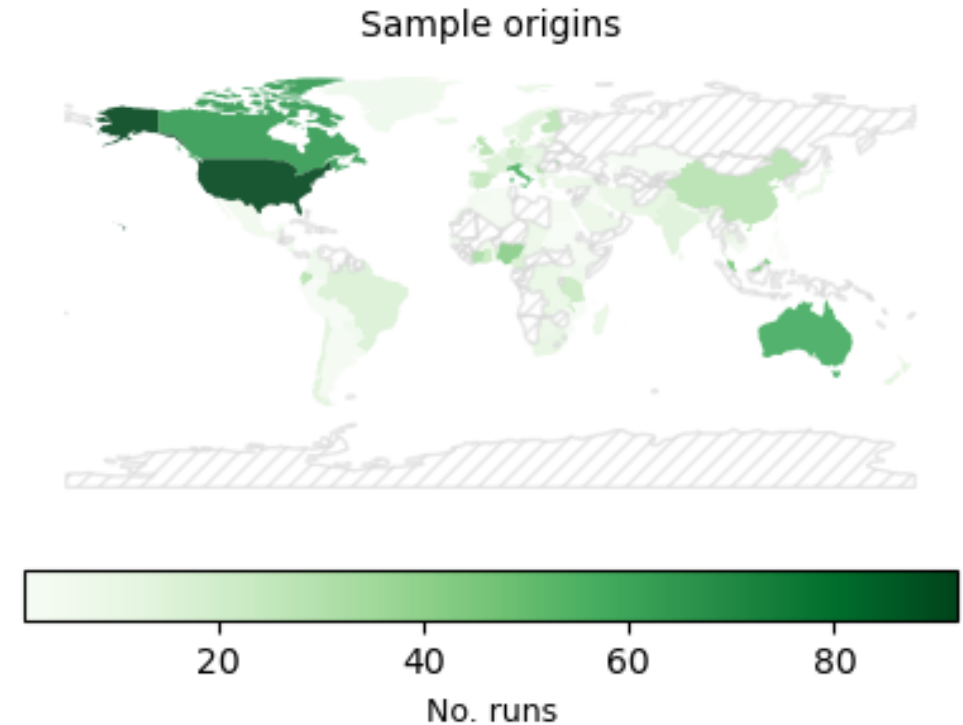
[Nature Communications](#) **13**, Article number: 7251 (2022) | [Cite this article](#)

46k Accesses | **48** Citations | **506** Altmetric | [Metrics](#)

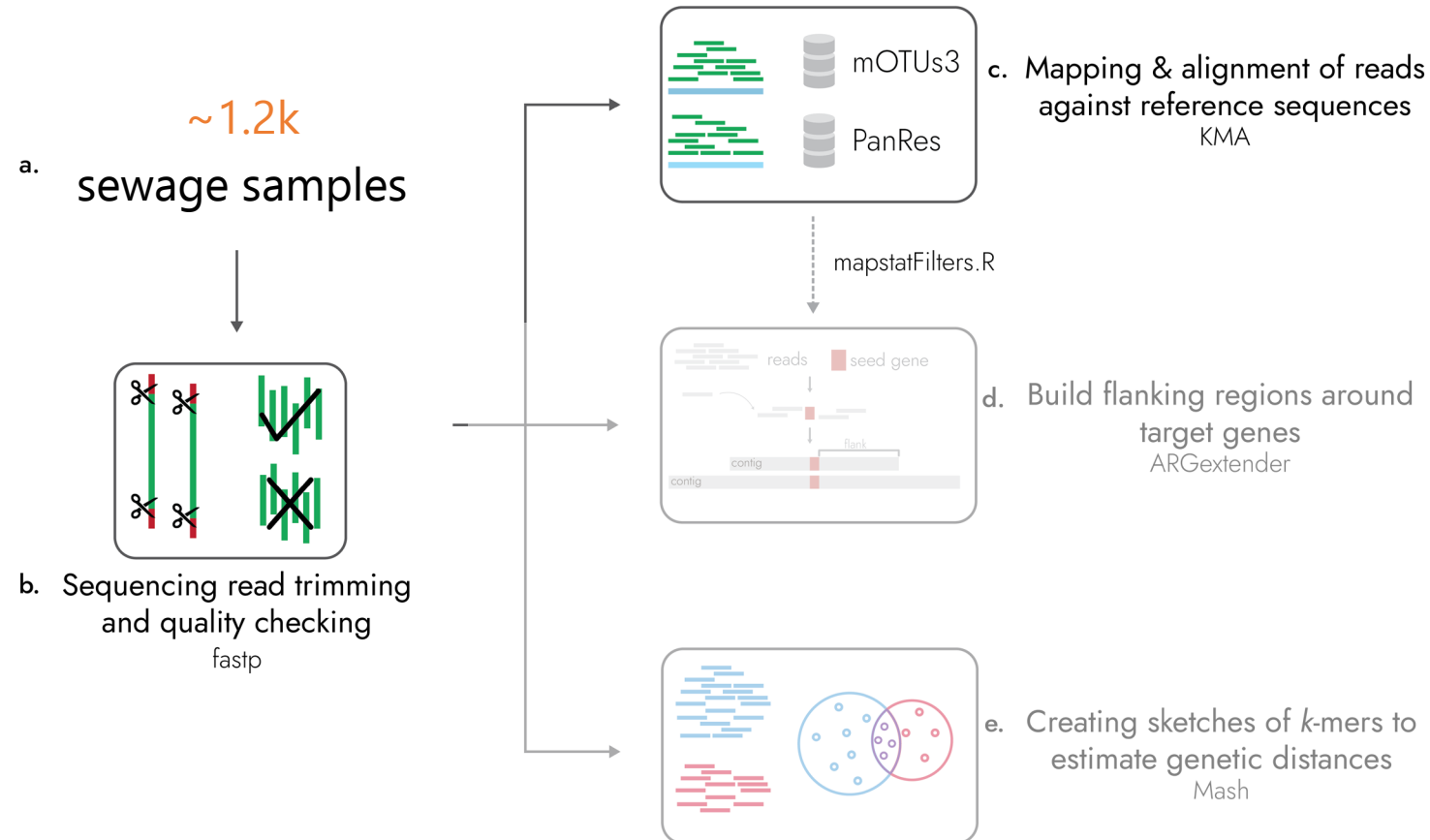
Global Sewage

A collection of sewage samples

- from 371 cities in 111 countries
- collected between 2016 and 2022

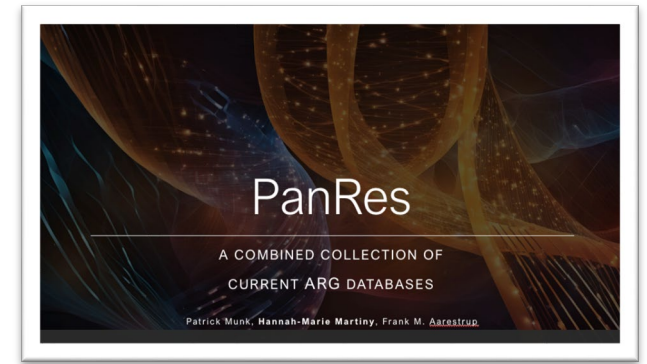
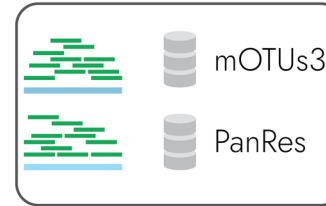


The ARGprofiler pipeline

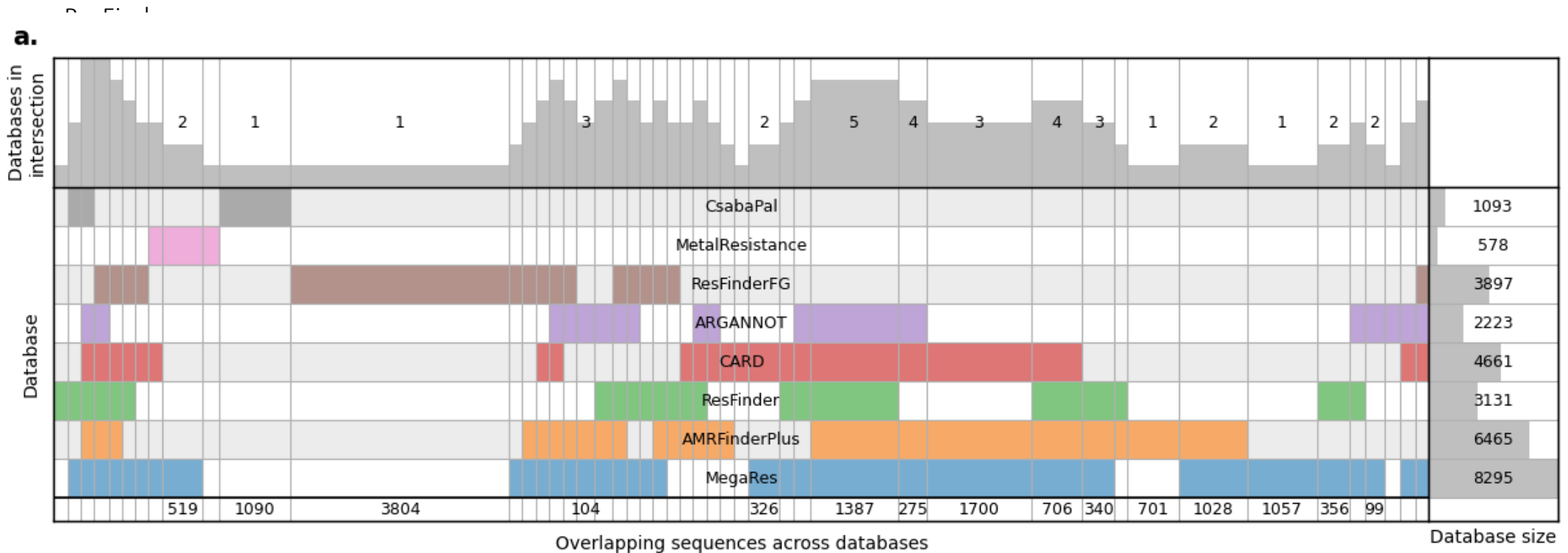


Hannah-Marie Martiny, Nikiforos Pyrounakis, Thomas N Petersen, Oksana Lukjančenko, Frank M Aarestrup, Philip T L C Clausen, Patrick Munk, ARGprofiler—a pipeline for large-scale analysis of antimicrobial resistance genes and their flanking regions in metagenomic datasets, *Bioinformatics*, 2024; btiae086, <https://doi.org/10.1093/bioinformatics/btae086>

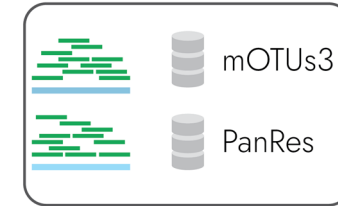
Focus on PanRes



From my last Monday presentation (20231116)



Focus on a subset of PanRes



ResFinder¹

ResFinderFG²

ResFinderNG³

¹ Bortolaia, V., Kaas, R. S., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., ... & Aarestrup, F. M. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. *Journal of Antimicrobial Chemotherapy*, 75(12), 3491-3500.

² Gschwind, R., Ugarcina Perovic, S., Weiss, M., Petitjean, M., Lao, J., Coelho, L. P., & Ruppé, E. (2023). ResFinderFG v2. 0: a database of antibiotic resistance genes obtained by functional metagenomics. *Nucleic Acids Research*, 51(W1), W493-W500.

³ Daruka, L., Czikkely, M. S., Szili, P., Farkas, Z., Balogh, D., Maharramov, E., ... & Pal, C. (2023). Antibiotics of the future are prone to resistance in Gram-negative pathogens. *bioRxiv*, 2023-07.

Focus on a subset of PanRes



ResFinder¹

3,131 ARGs

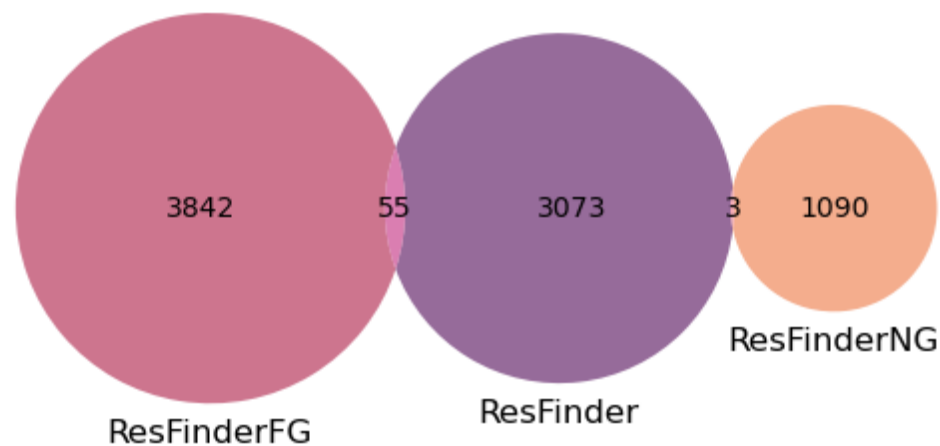
ResFinderFG²

3,897 ARGs

ResFinderNG³

1,093 ARGs

a. Overlap between genes



ResFinder

+

ResFinderFG

+

+

ResFinderNG

+

+

+

¹ Bortolaia, V., Kaas, R. S., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., ... & Aarestrup, F. M. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. *Journal of Antimicrobial Chemotherapy*, 75(12), 3491-3500.

² Gschwind, R., Ugarcina Perovic, S., Weiss, M., Petitjean, M., Lao, J., Coelho, L. P., & Ruppé, E. (2023). ResFinderFG v2. 0: a database of antibiotic resistance genes obtained by functional metagenomics. *Nucleic Acids Research*, 51(W1), W493-W500.

³ Daruka, I., Czikkely, M. S., Szili, B., Earkas, Z., Balogh, D., Maharramov, E., ... & Pal, C. (2023). Antibiotics of the future are prone to resistance in Gram-negative pathogens. *bioRxiv*, 2023-07-06.

Focus on a subset of PanRes

ResFinder¹

3,131 ARGs
2,117 found
(67.61%)

Sample-wise

FragmentCountAln:
median: 7,814
range: 128 - 105,182

Gene-wise

FragmentCountAln:
median: 54
range: 0 - 2,958,302

ResFinderFG²

3,842 ARGs
3,560 found
(92.66%)

Sample-wise

FragmentCountAln:
median: 4,909
range: 90 - 43,785

Gene-wise

FragmentCountAln:
median: 111
range: 0 - 738,891

ResFinderNG³

1,090 ARGs
980 found
(81.65%)

Sample-wise

FragmentCountAln:
median: 8,613
range: 113 - 223,712

Gene-wise

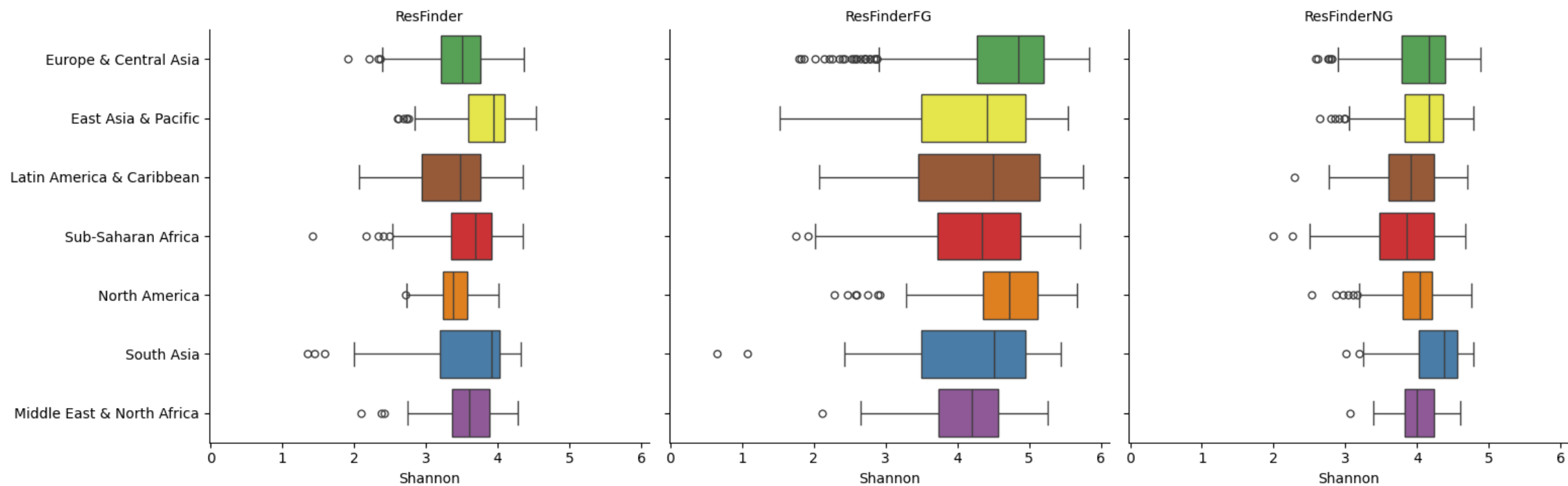
FragmentCountAln:
median: 225
range: 0 - 2,347,799

Focus on a subset of PanRes

ResFinder¹

ResFinderFG²

ResFinderNG³



Focus on a subset of PanRes

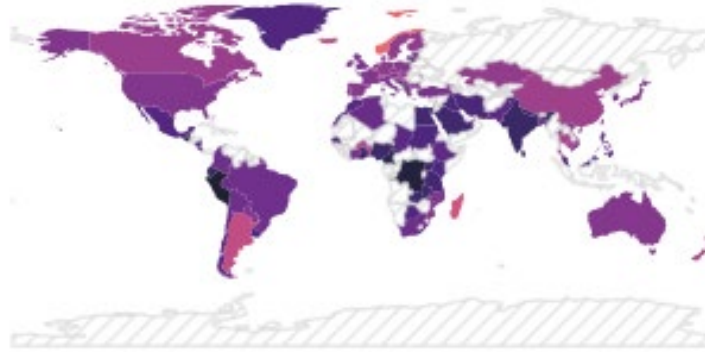
ResFinder¹

3,131 ARGs
2,117 found
(67.61%)



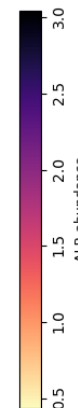
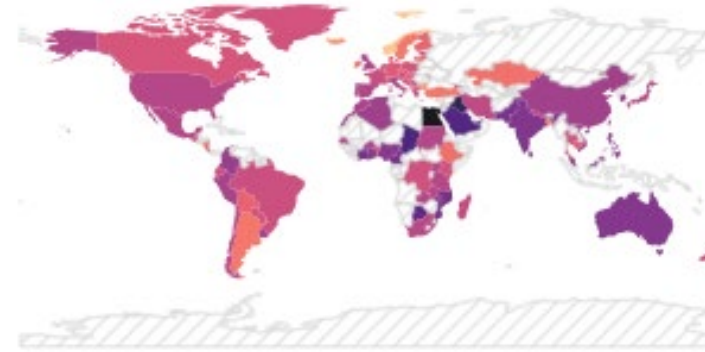
ResFinderFG²

3,842 ARGs
3,560 found
(92.66%)



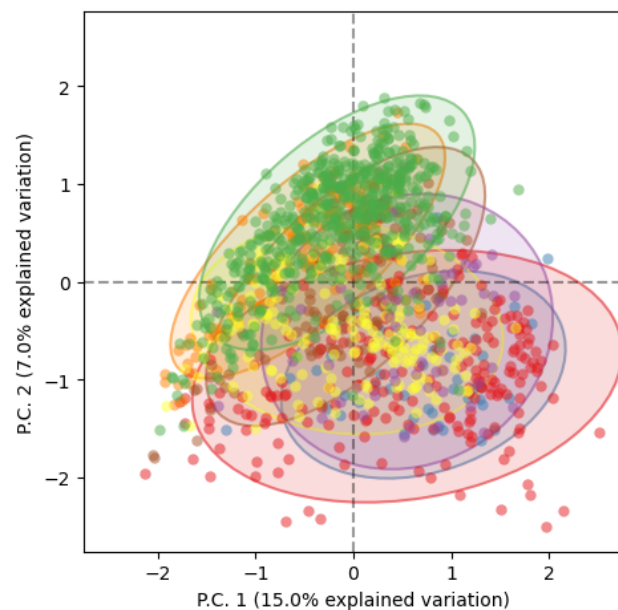
ResFinderNG³

1,090 ARGs
980 found
(81.65%)

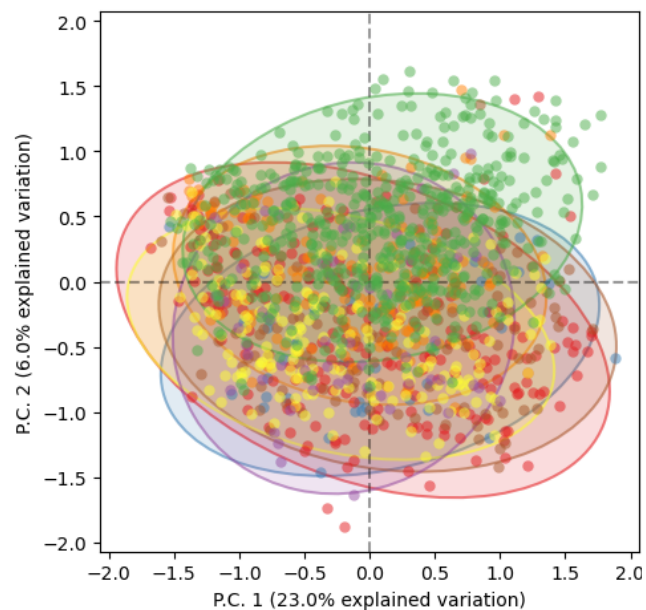
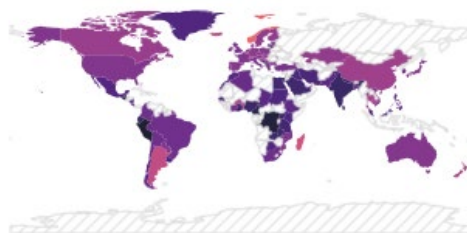


Focus on a subset of PanRes

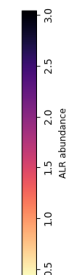
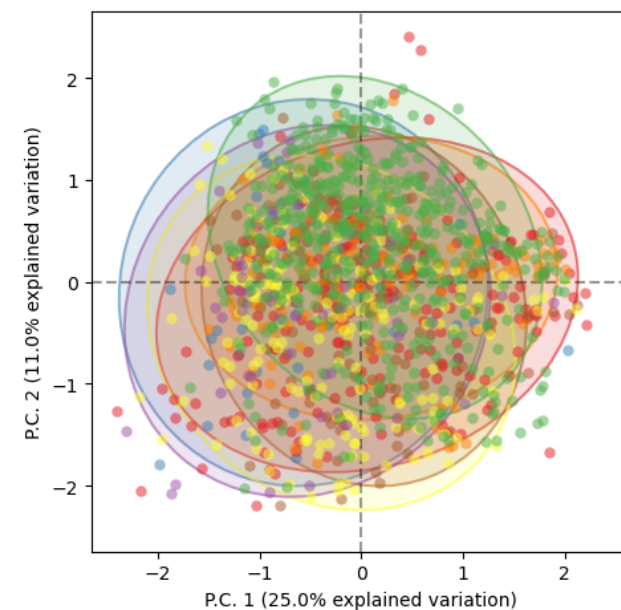
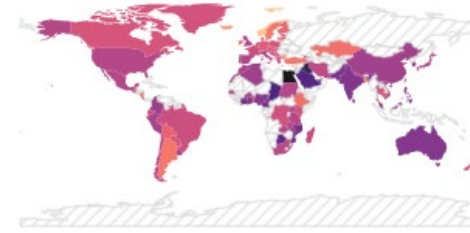
ResFinder¹



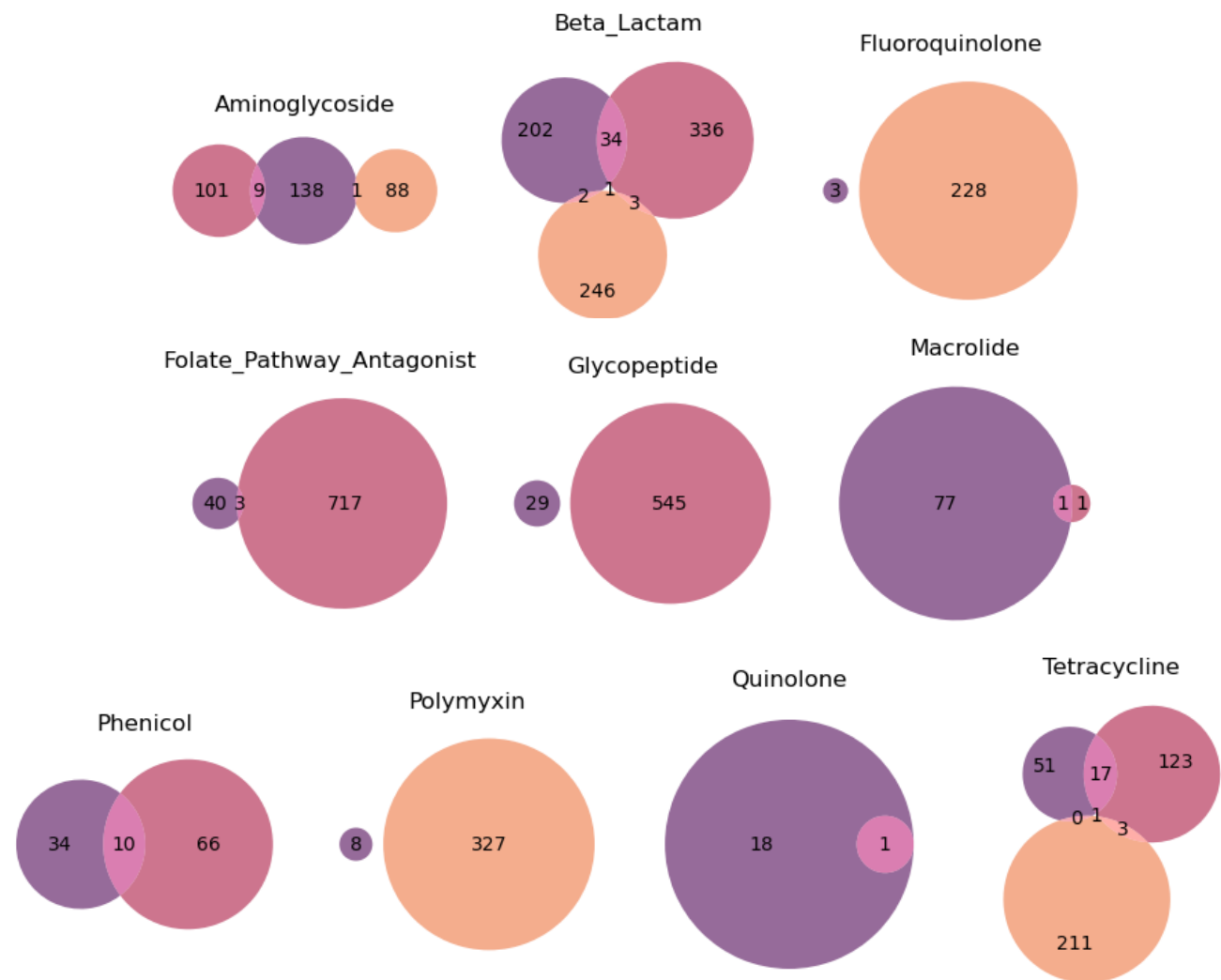
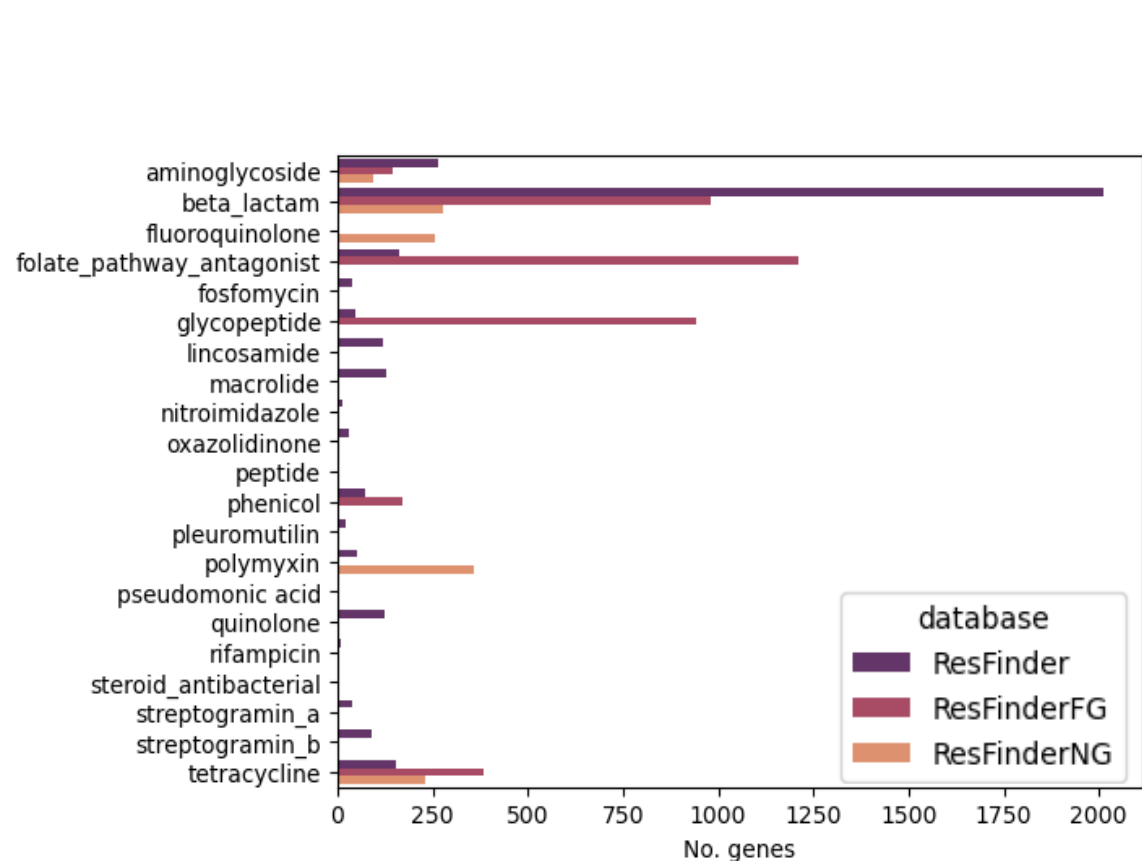
ResFinderFG²



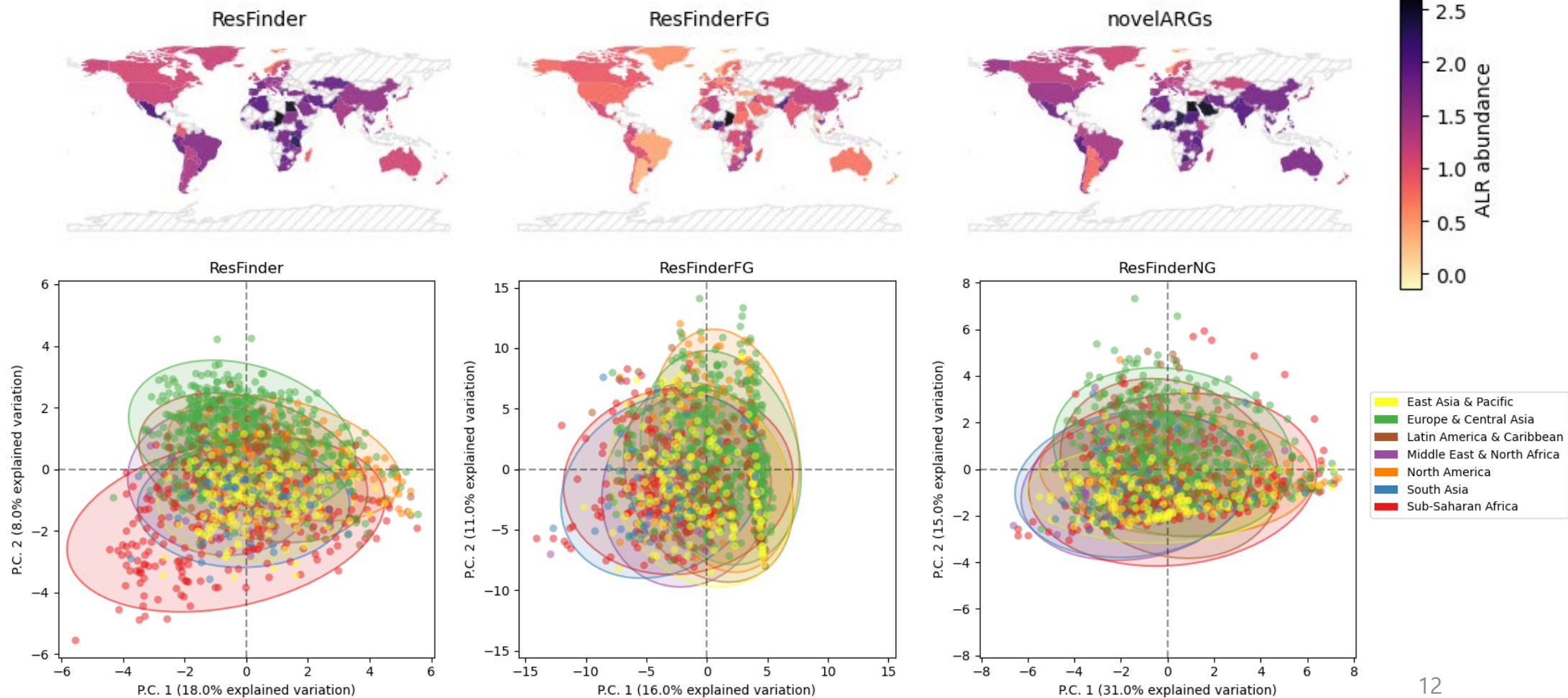
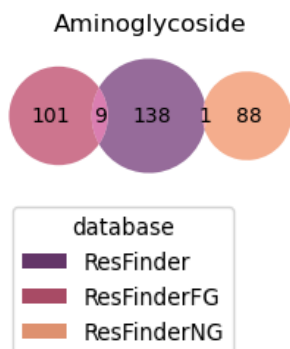
ResFinderNG³



Looking at the resistance classes

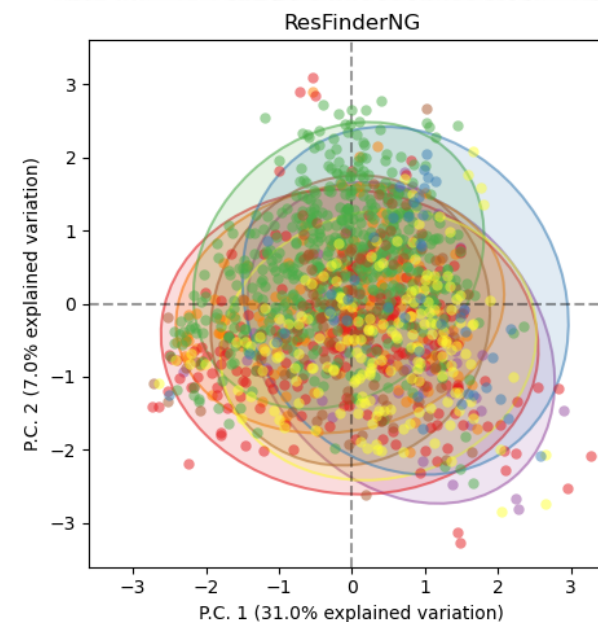
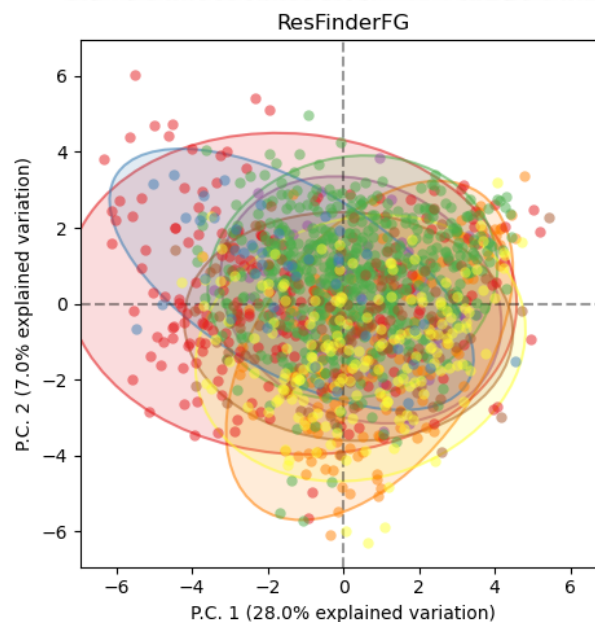
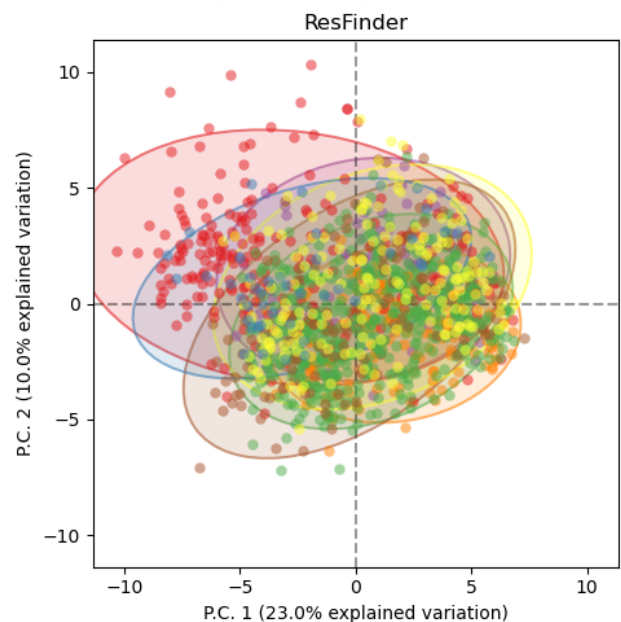
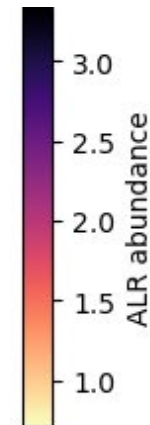
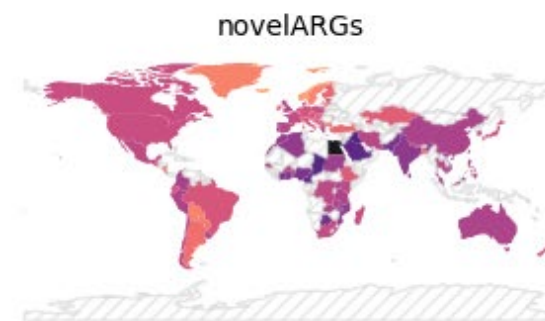
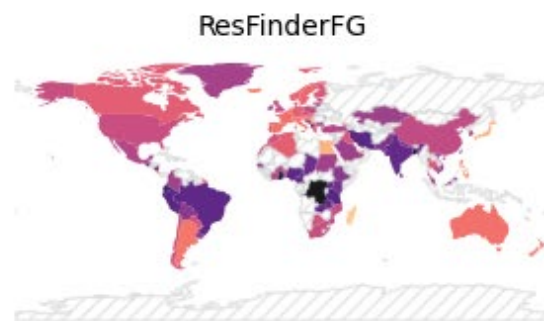
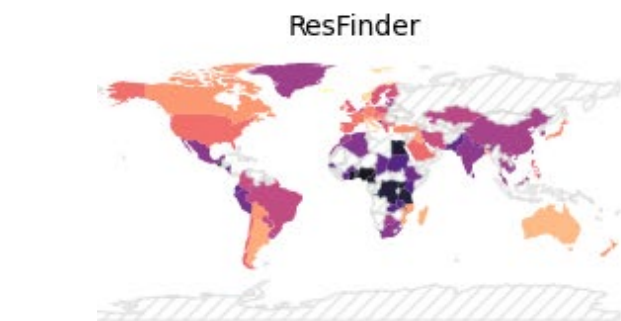
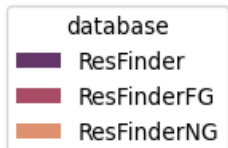
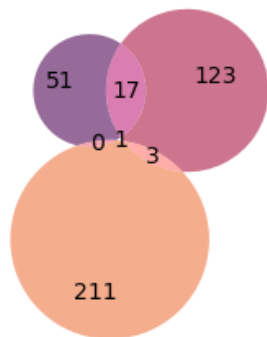


Looking at the resistance classes



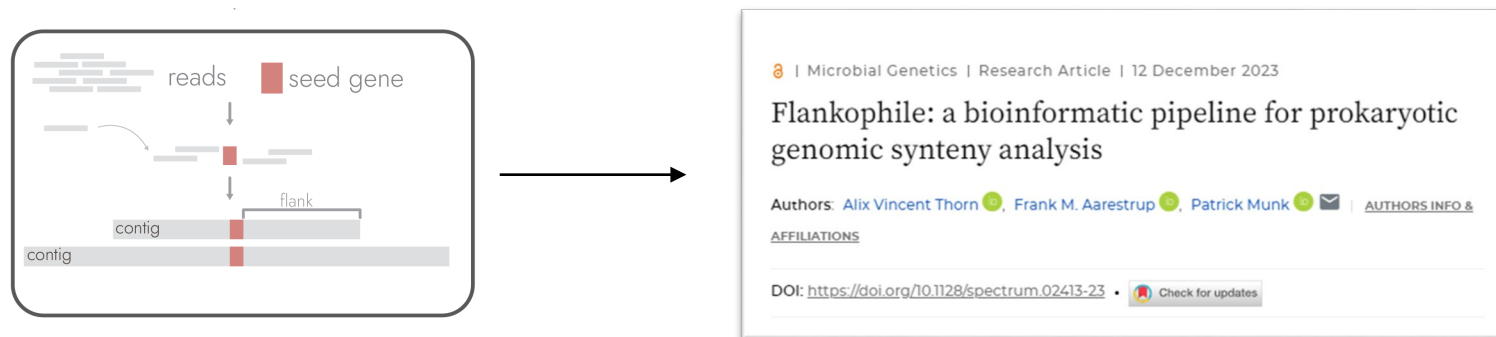
Looking at the resistance classes

Tetracycline



What's next?

- Cluster samples on other metrics: geodesic distance, Ward?
- Include information from assemblies



ResFinder¹

3,131 ARGs
2,117 found
(67.61%)
479 assembled
(15.30%)

ResFinderFG²

3,842 ARGs
3,560 found
(92.66%)
900 assembled
(23.43%)

ResFinderNG³

1,090 ARGs
980 found
(81.65%)
300 assembled
(27.52%)

What's next?

- Investigate other groupings than world regions: geodesic distance?
 - Include information from assemblies
 - Are the functional genes intrinsic or not?
-
- Other ideas? 😊

this is how i finish a presentation:

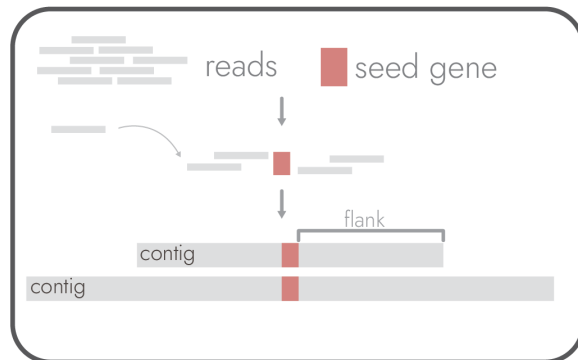


Focus on a subset of PanRes



ResFinder¹

3,131 ARGs
2,117 found
(67.61%)



479 assembled
(15.30%)

ResFinderFG²

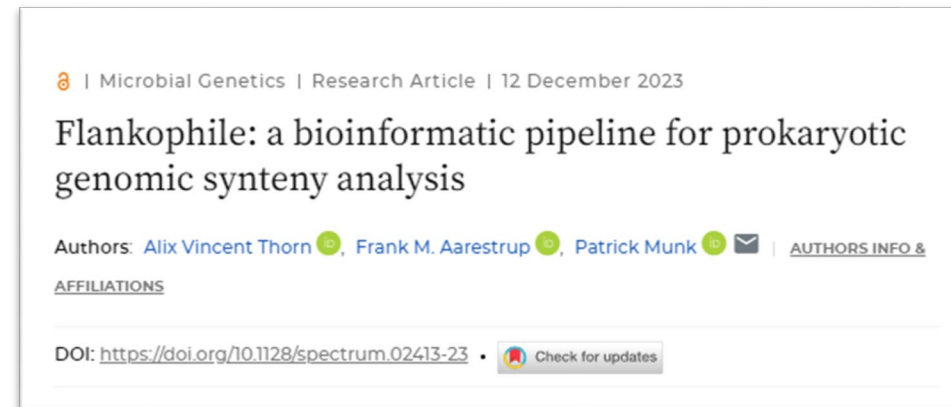
3,842 ARGs
3,560 found
(92.66%)

900 assembled
(23.43%)

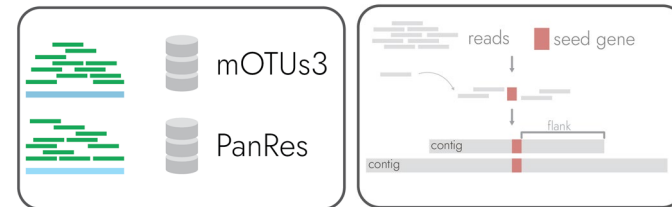
ResFinderNG³

1,090 ARGs
980 found
(81.65%)

300 assembled
(27.52%)

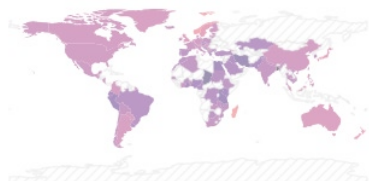


Focus on a subset of PanRes



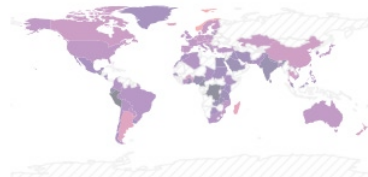
ResFinder¹

3,131 ARGs
479 assembled
(15.30%)



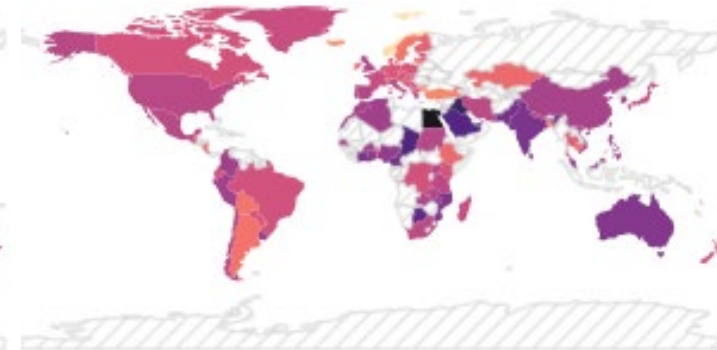
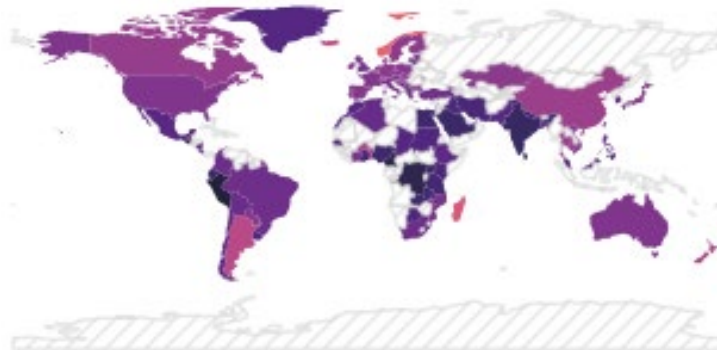
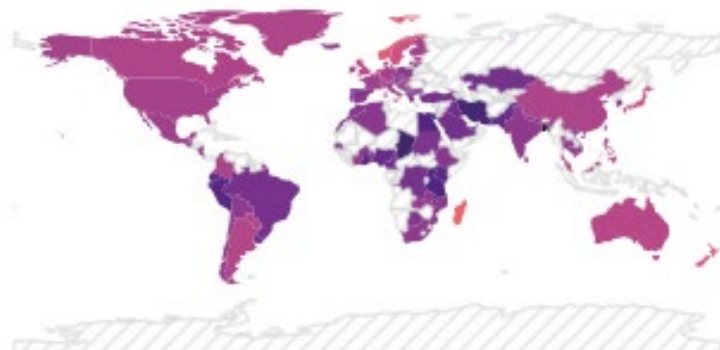
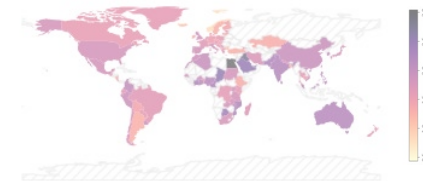
ResFinderFG²

3,842 ARGs
900 assembled
(23.43%)

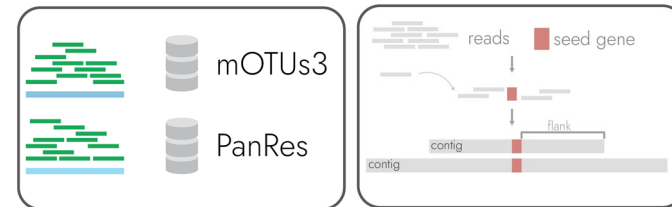


ResFinderNG³

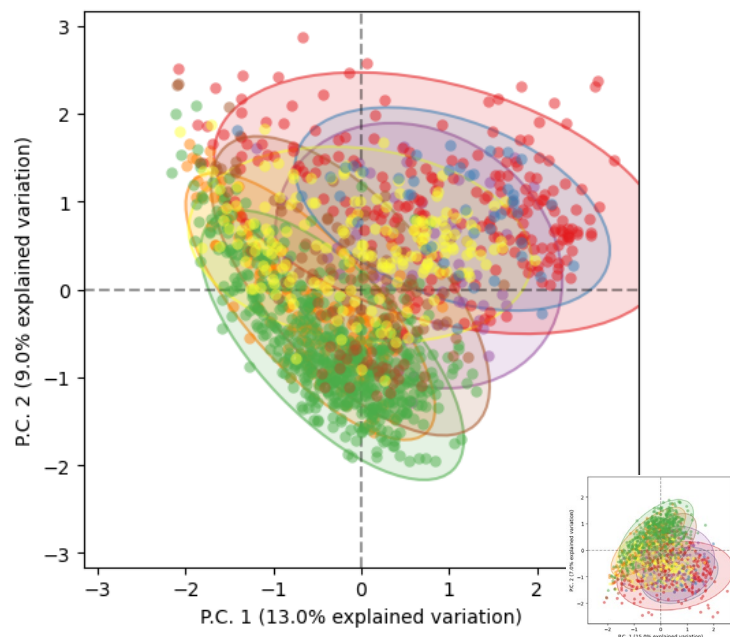
1,090 ARGs
300 assembled
(27.52%)



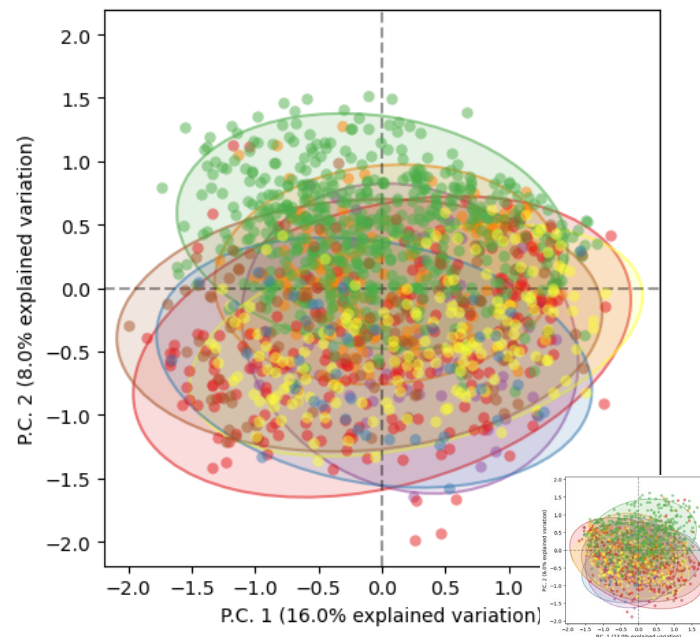
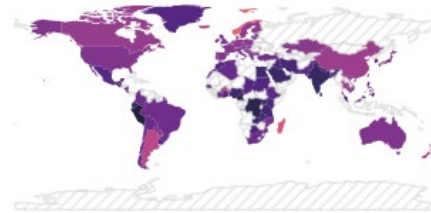
Focus on a subset of PanRes



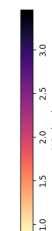
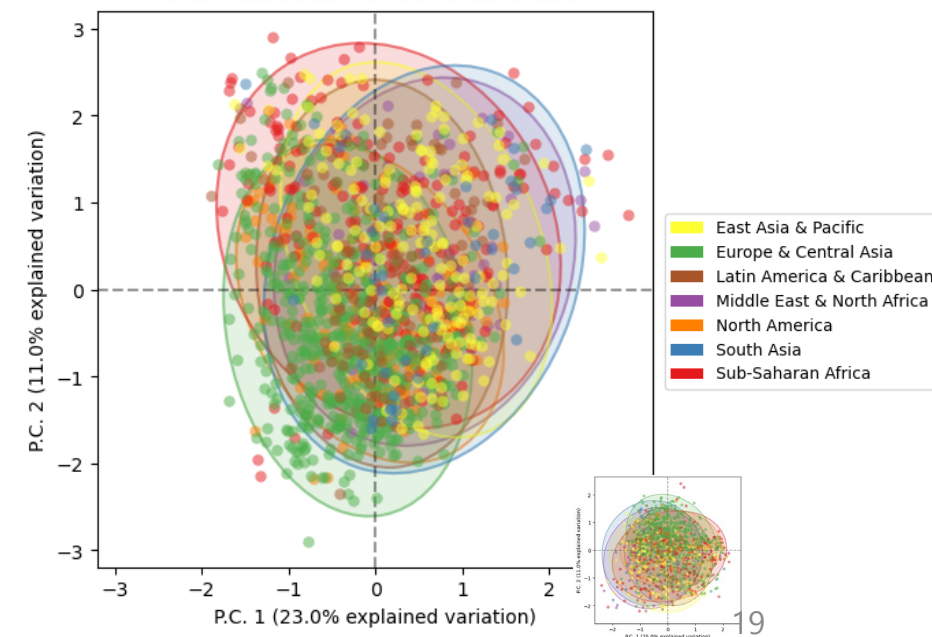
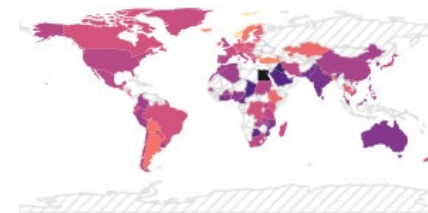
ResFinder¹



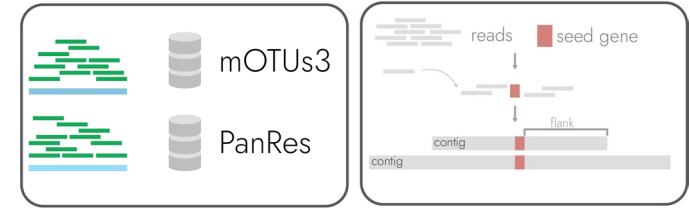
ResFinderFG²



ResFinderNG³



Focus on a subset of PanRes



ResFinder¹

3,131 ARGs
479 assembled
(15.30%)

ResFinderFG²

3,842 ARGs
900 assembled
(23.43%)

ResFinderNG³

1,090 ARGs
300 assembled
(27.52%)