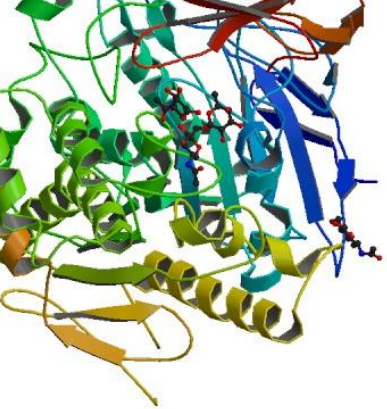

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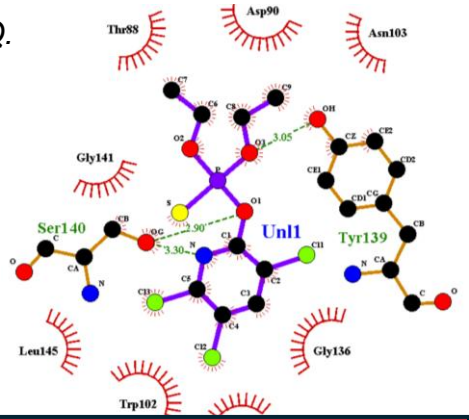
Data-Driven Mechanistic Forecasting of Species-Specific Chemical Sensitivity Using Docking-Derived Molecular Features

Rama Krishnan [1]*, David Spurgeon [2], Stephen Short [2], Bruno Campos [3], Claudia Rivetti [3], Claire Peart [3], Peter Kille [1]

[1] *School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff, CF10 3AX.*
[2] *UK Centre for Ecology and Hydrology, Benson Ln, Maclean Building, Crowmarsh Gifford, Wallingford OX10 8BB.*
[3] *Safety, Environmental and Regulatory Sciences (SERS), Unilever, Colworth Science Park, Sharnbrook MK44 1LQ.*



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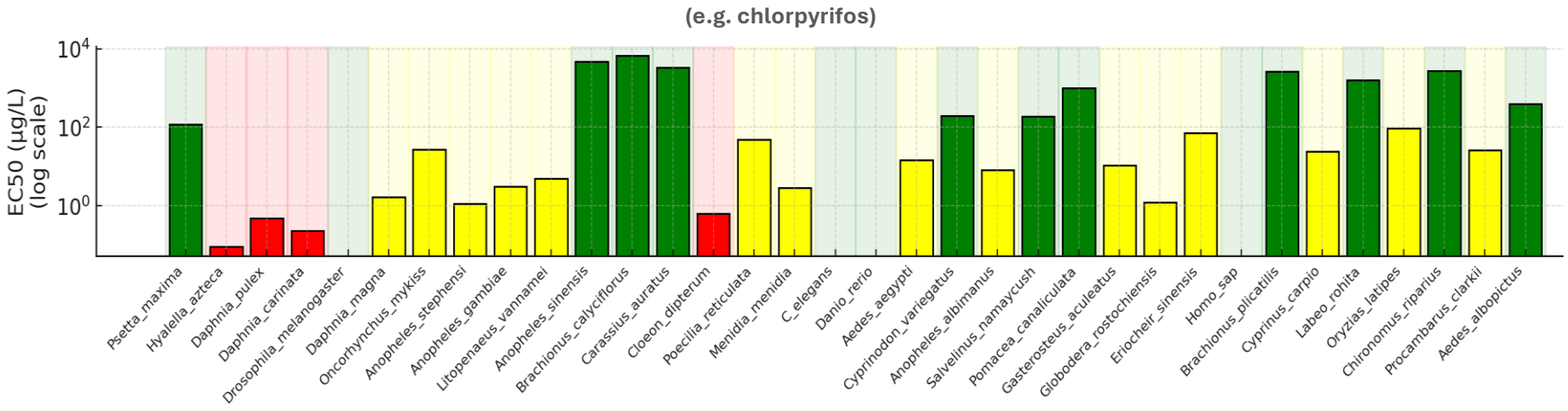
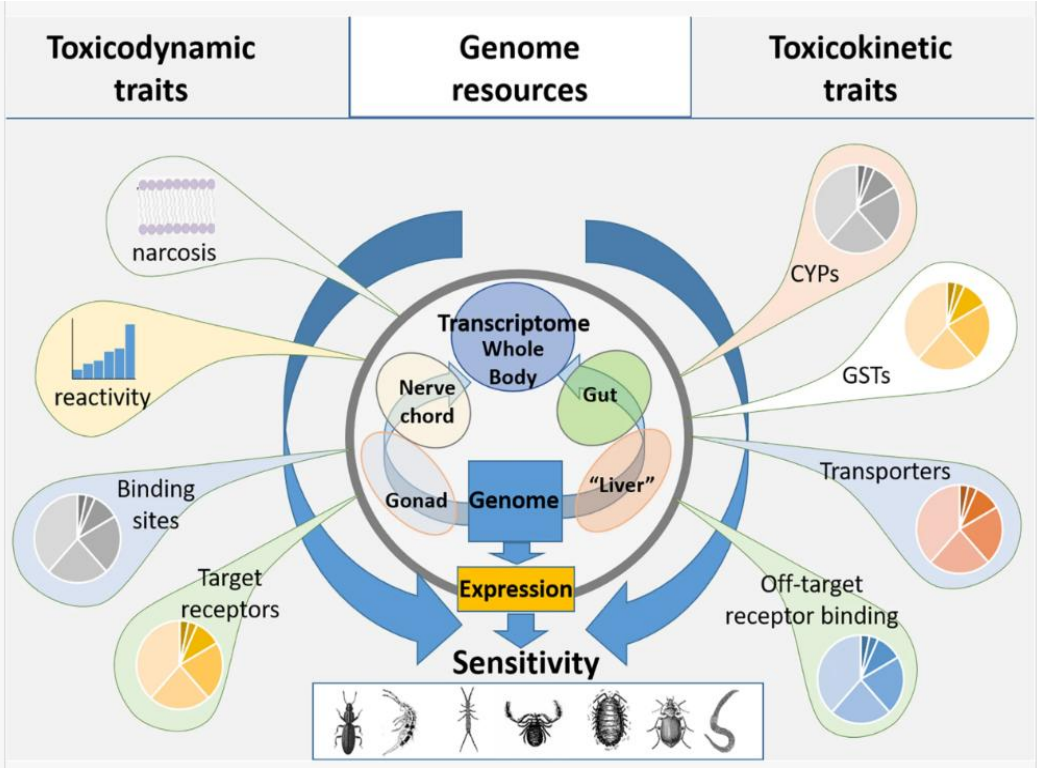
➢ Species are not equally sensitive/susceptible to toxicants: making risk assessment difficult



➢ Revealing the knowledge required to make informed predictions



Spurgeon D, Lahive E, Robinson A, Short S and Kille P (2020) Species Sensitivity to Toxic Substances: Evolution, Ecology and Applications. Front. Environ. Sci. 8:588380. doi: 10.3389/fenvs.2020.588380

Omic approaches to modelling toxicodynamic traits: i.e. receptors and known gene pathways.

Level 1 tools that already exist and are being continually improved.

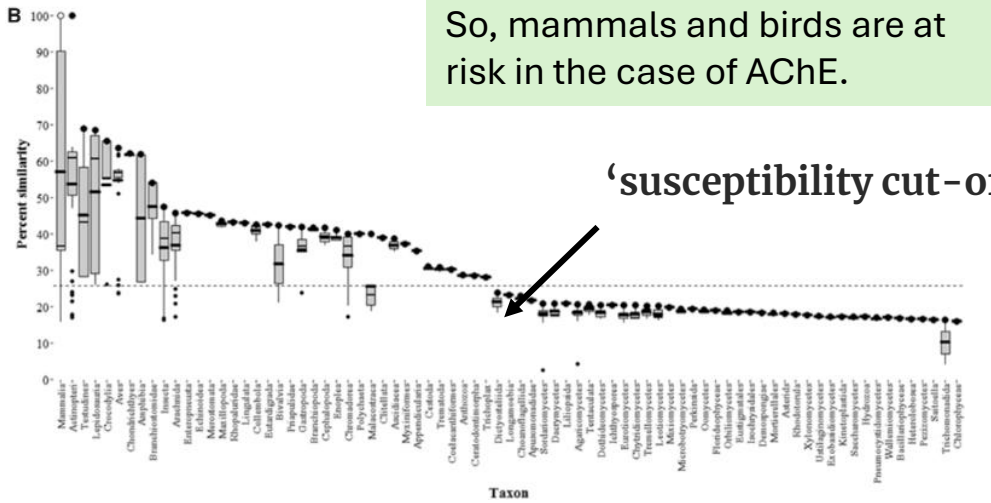
JOURNAL ARTICLE

In Silico Site-Directed Mutagenesis Informs Species-Specific Predictions of Chemical Susceptibility Derived From the Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) Tool FREE

Jon A Doering, Sehan Lee, Kurt Kristiansen, Linn Evenseth, Mace G Barron, Ingebrigt Sylte, Carlie A LaLone ✉

Toxicological Sciences, Volume 166, Issue 1, November 2018, Pages 131–145, https://doi.org/10.1093/toxsci/kfy186

Published: 27 July 2018



So, mammals and birds are at risk in the case of AChE.

'susceptibility cut-off'

Tools that cross level 1

and level 2

Environmental Toxicology and Chemistry

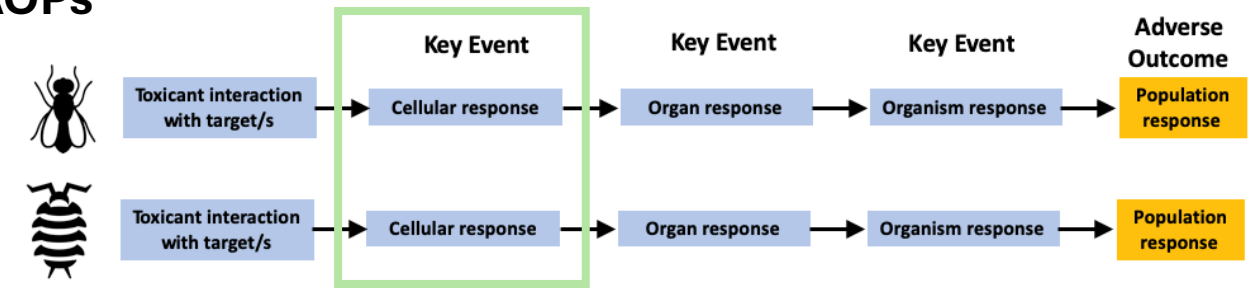
Hazard/Risk Assessment | Open Access

Genes-to-Pathways Species Conservation Analysis: Enabling the Exploration of Conservation of Biological Pathways and Processes Across Species

Claudia Rivetti ✉, Jade Houghton, Danilo Basili, Geoff Hodges, Bruno Campos

First published: 01 March 2023 | https://doi.org/10.1002/etc.5600

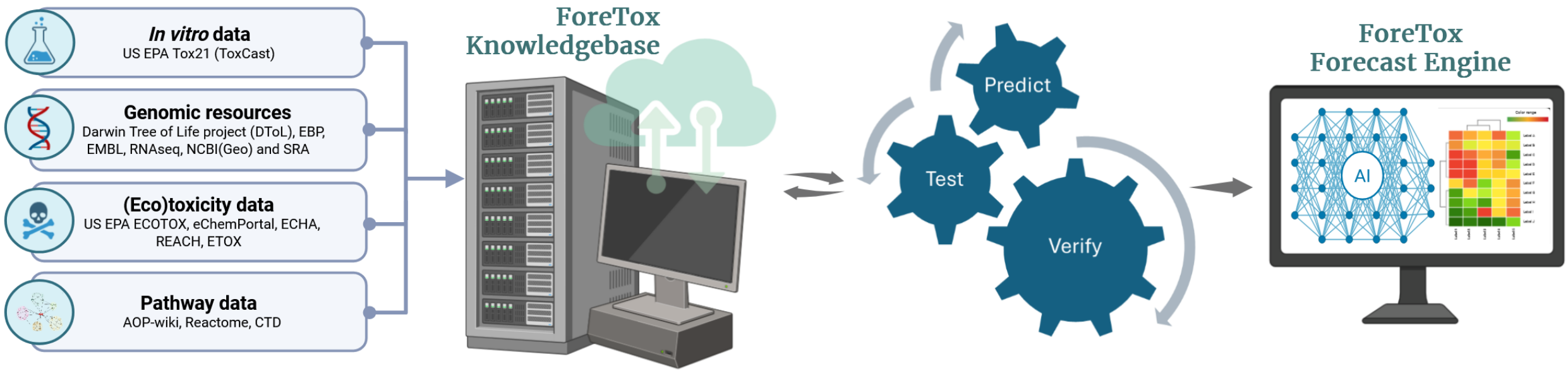
Cross-species comparison of molecular components of AOPs



Omics based predictions – The scalable holy grail!

Leverage publicly available genomic and toxicological data from tested and/or untested species and compare the pertinent molecular differences.

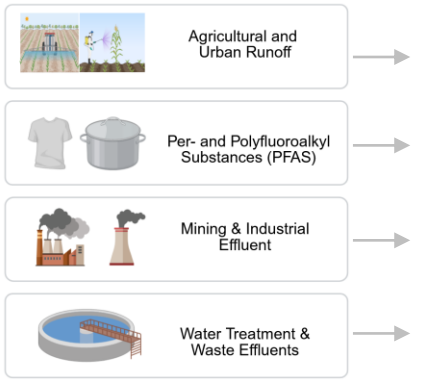
Score the differences and predict sensitivity and mixture responses.

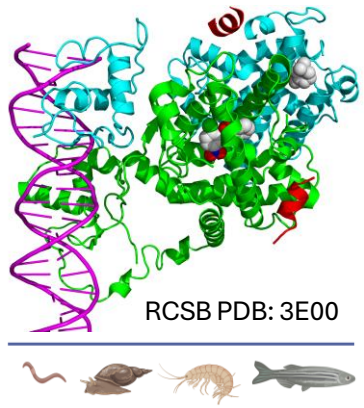


ForeTox Case Study: Ecdysone receptor (EcR) Agonism
Species Sensitivity and Vulnerability Predictions

The binding of natural ligands (ecdysteroids) to EcR is critical for regulating moulting and metamorphosis in insects and crustaceans.

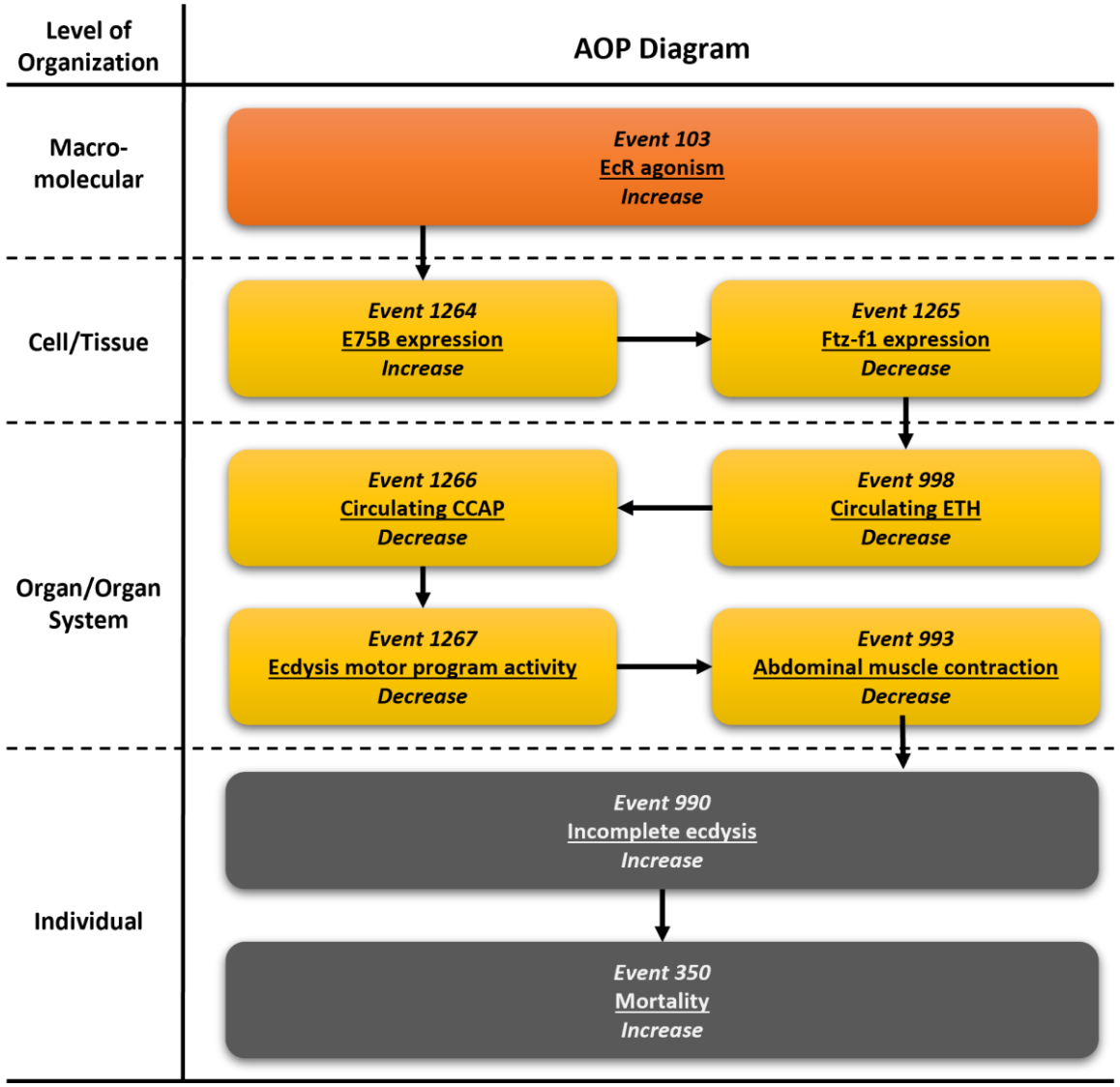
Molecular Initiating Event (MIE)
Binding of exogenous ligands to the **EcR/USP Heterodimer**, initiating a premature transcriptional cascade.





Apical Adverse Outcome

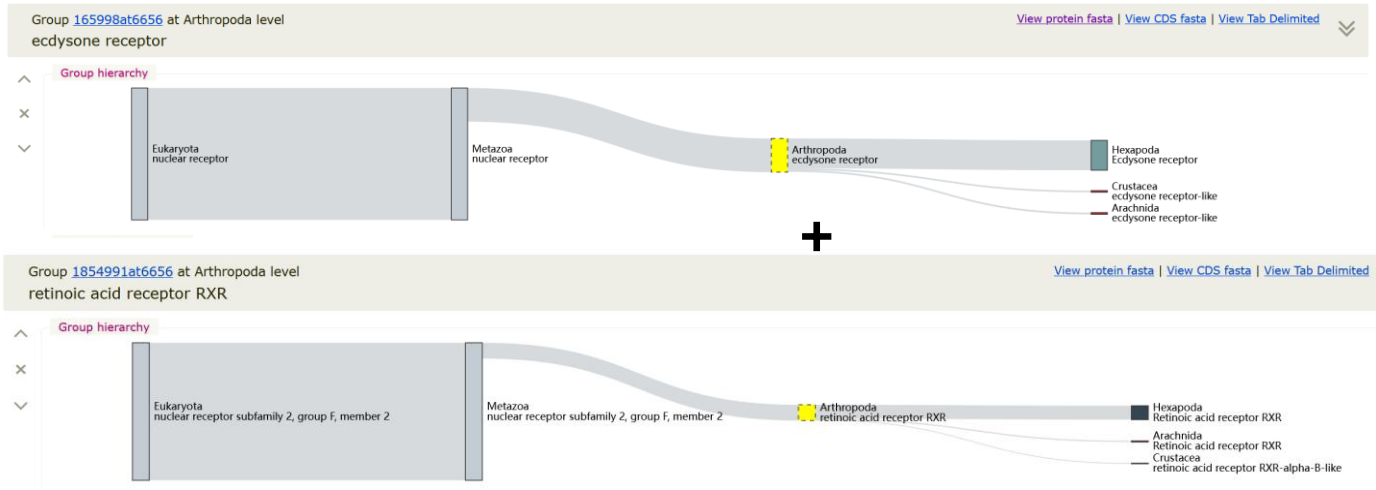
Population Decline resulting from widespread developmental arrest and mortality during ecdysis.



(https://aopwiki.org) AOP: 04: Ecdysone receptor agonism leading to mortality via suppression of Ftz-f1

➢ EcR\USP heterodimer ortholog retrieval

OrthoDB

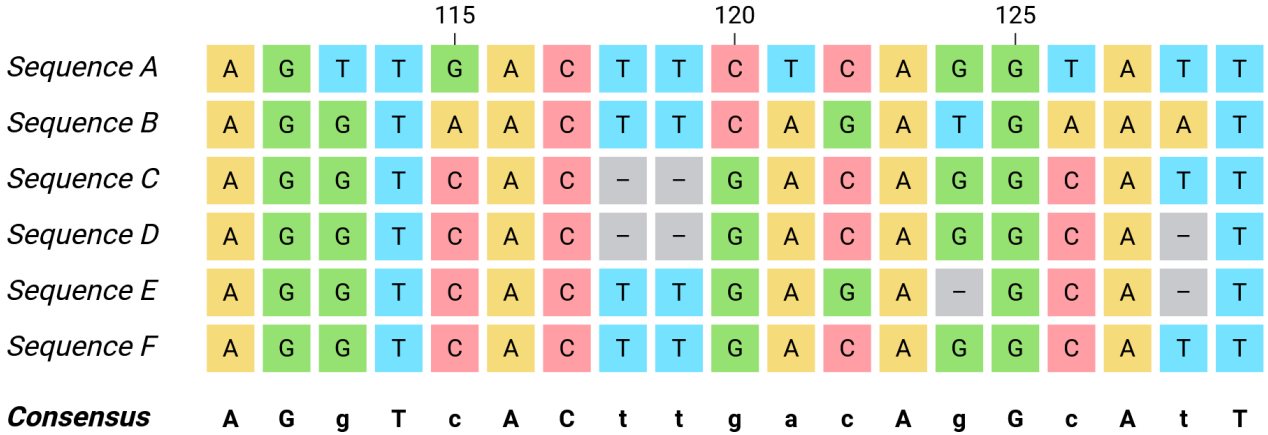


EcR – 1264 genes in 1067 Sp.

USP/RXR – 1226 genes in 1057 Sp.

➢ Candidate pair selection

Multiple Sequence Alignment



HMMER – Domain/Sequence Completeness

Family					Domain e-values	
Accession	Clan	Description	Start	End	Independent	Conditional
PF00104		Ligand-binding domain of nuclear hormone receptor	14	201	6.9e-28	2.9e-32

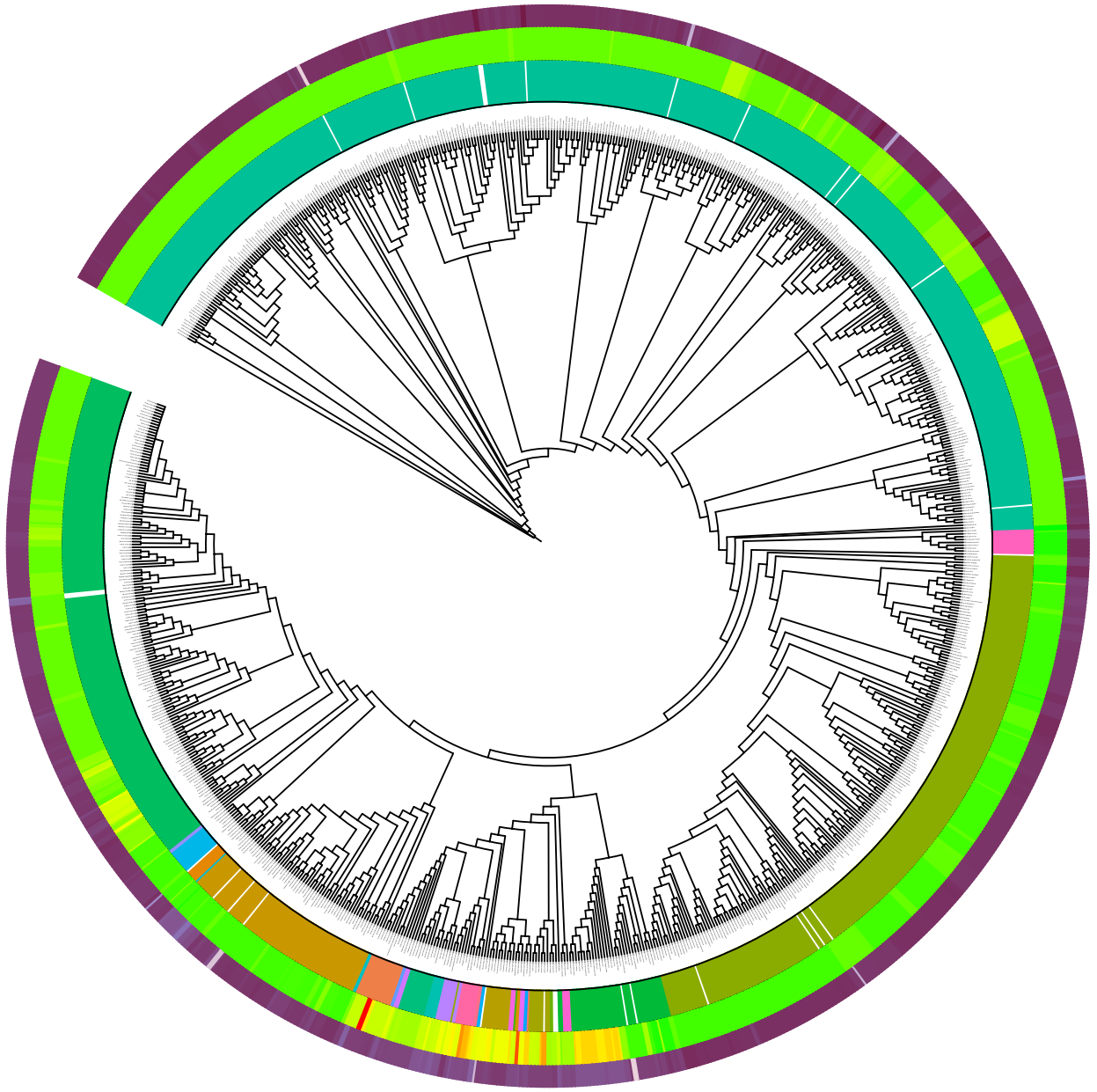
```
                 .........*.........*.........*.........*.........*.........*.........*.........*
Target     7 klrkagkeelcglwekdlllvagWlkyfpeFqeLpledqmalLksfwlewlrlekgarsaklrrkkllgekllllsddda 86
               ++   + +++e+    ++l++e+ k +p+F eL  edq+aLlk++ +e+++l++a+++   +++ i++++   ++
Query     19 DISDYKFRHITEITILTVQLIVEFSKRLPGFDELLREDQIALLKACSSEVMMLRMARKYDVQSDSIIFANNQPYTR---- 94
PP           566678999*********************************************************96555....

                 .........*.........*.........*.........*.........*.........*.........*.........*
Target    87 ddkeveddsswesKyskeqlkifepfidslfdelvkplieLnpddvElayllaqllfqyagkl..lqgeileivekLqek 164
                   d  ++  + ++++ +l          ++++++  +++++ E+a+l a+++f   ++r  l +    +vek+qe+
Query     95 ------DSYNVAGMGETIEDLL---------HFCRQMYAMKVNNAEYALLTAIVIF---SERpnLVEG--WKVEKIQEI 153
PP           ......4557888888888888.........**********************9...555555444..59*******

                 .........*.........*.........*.........*.......
Target   165 laneLhdYyvnklklpnysgRlakllkilnslrkisrerrgklelak 211
             + ++L+ Y+ n+ ++p+    +akll++l++lr++ ++ +e  +  k
Query    154 YLEALKAYVDNR-RRPKSGTIFAKLLSVLTELRTLGNQNSEMCFSLK 199
PP           *********655.7888777*********************9988776
```

ECR-USP paired cladogram

Species: 858 | Total ortholog isoforms: 1203



Order (Inner ring)

- Amphipoda
- Araneae
- Blattodea
- Calanoida
- Coleoptera
- Decapoda
- Diplostraca
- Diptera
- Entomobryomorpha
- Harpacticoida
- Hemiptera
- Hymenoptera
- Ixodida
- Lepidoptera
- Mesostigmata
- Neuroptera
- Odonata
- Orthoptera
- Pantopoda
- Pedunculata
- Pseudoscorpiones
- Psocodea
- Sarcoptiformes
- Scorpiones
- Sessilia
- Siphonostomatoida
- Thysanoptera
- Trichoptera
- Trombidiformes
- Xiphosurida

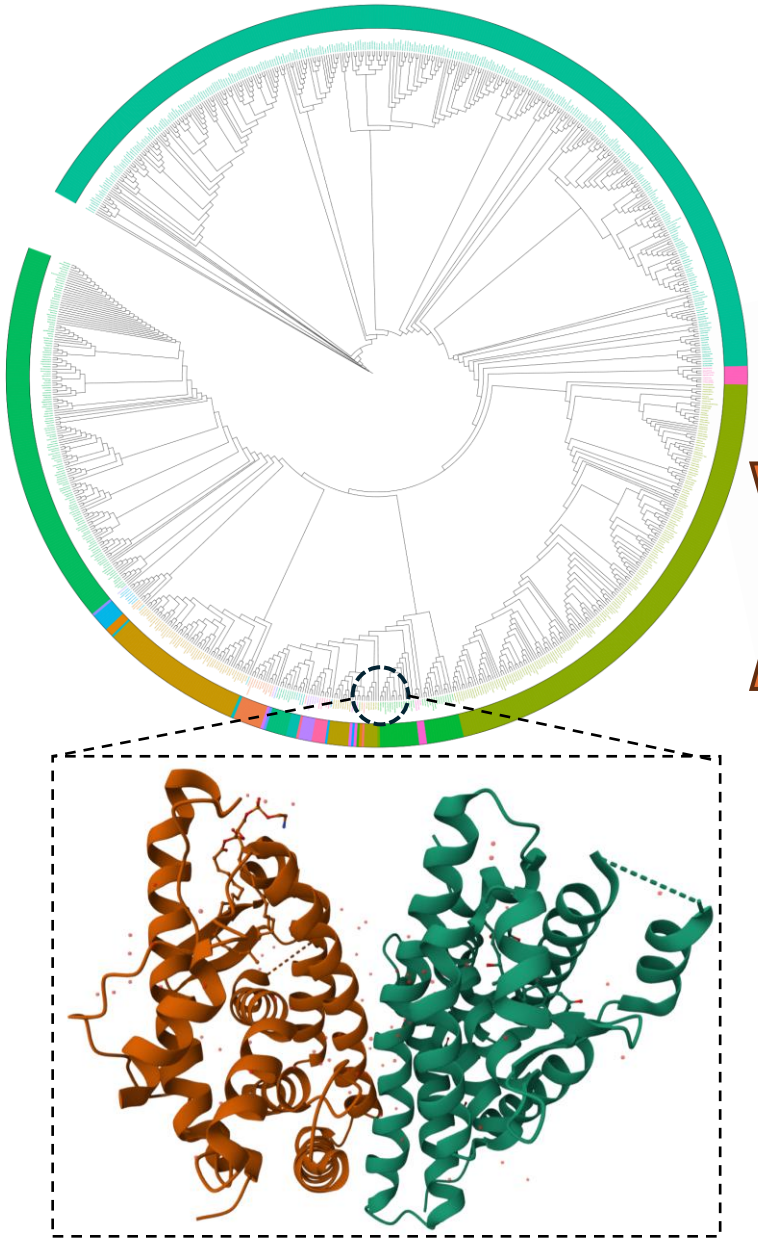
EcR 20E-LBD conservation (middle ring)

- 0.375
- 0.438
- 0.5
- 0.563
- 0.625
- 0.688
- 0.75
- 0.813
- 0.875
- 0.938
- 1

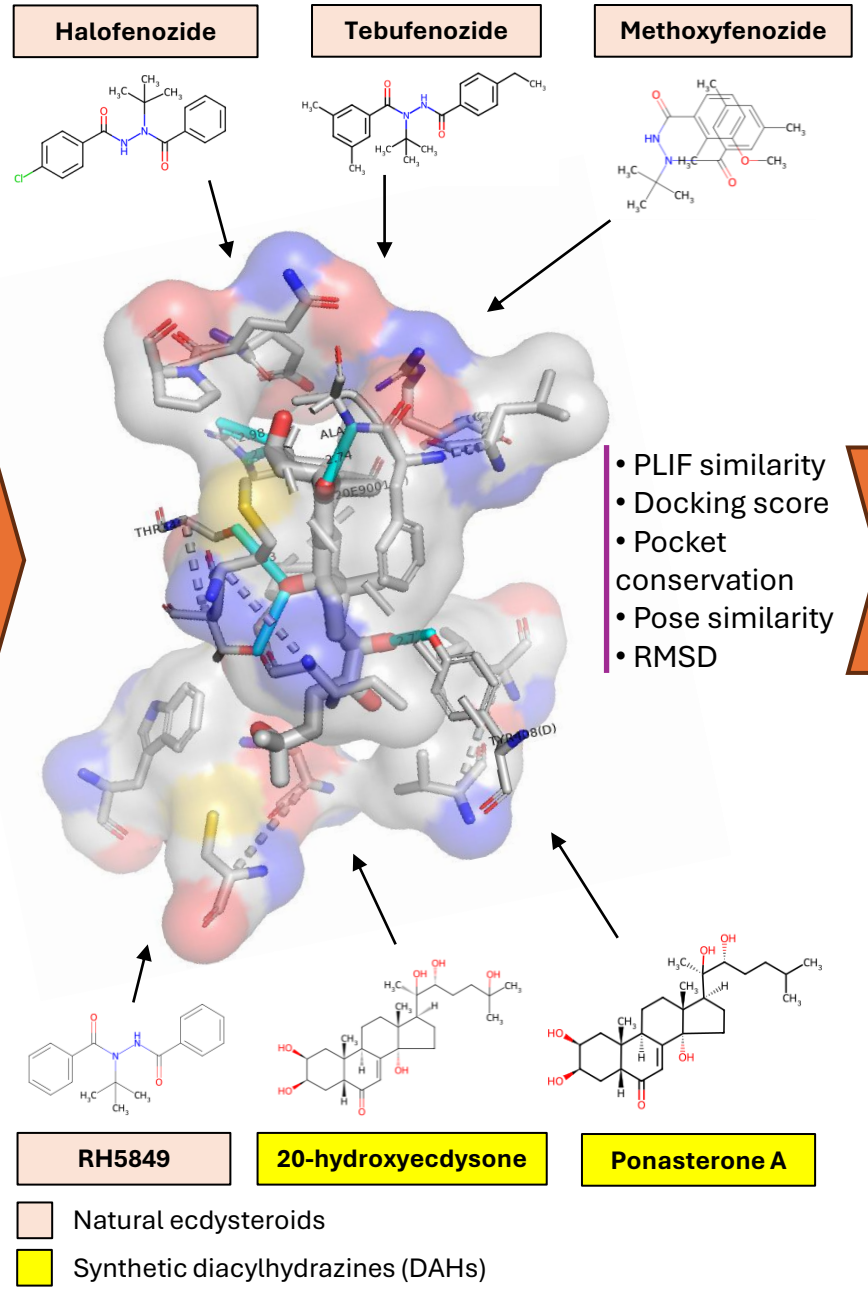
USP conservation (outer ring)

- 0
- 0.5
- 1
- 1.5
- 2
- 2.5
- 3
- 3.5
- 4
- 4.5
- 5

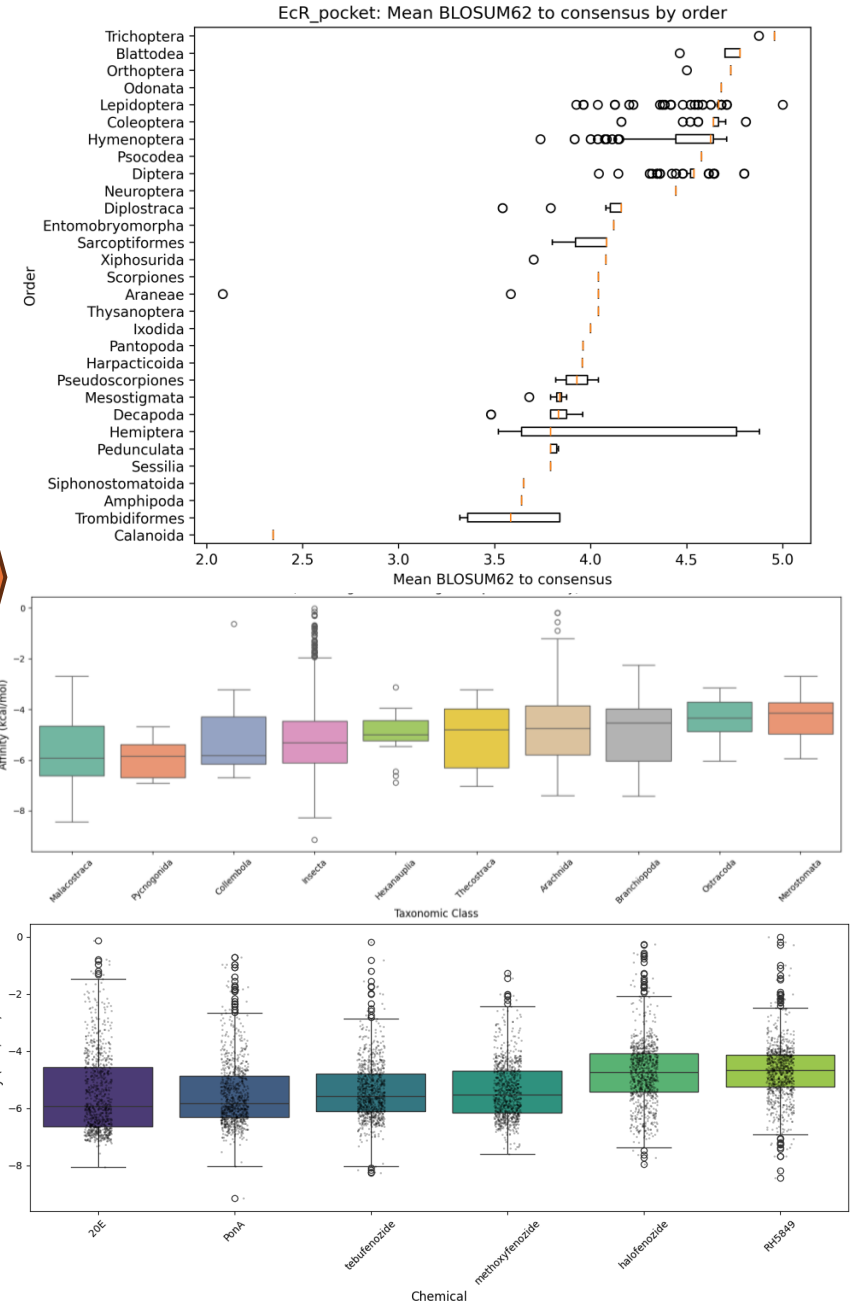
Protein Ortholog Extraction + Protein Folding



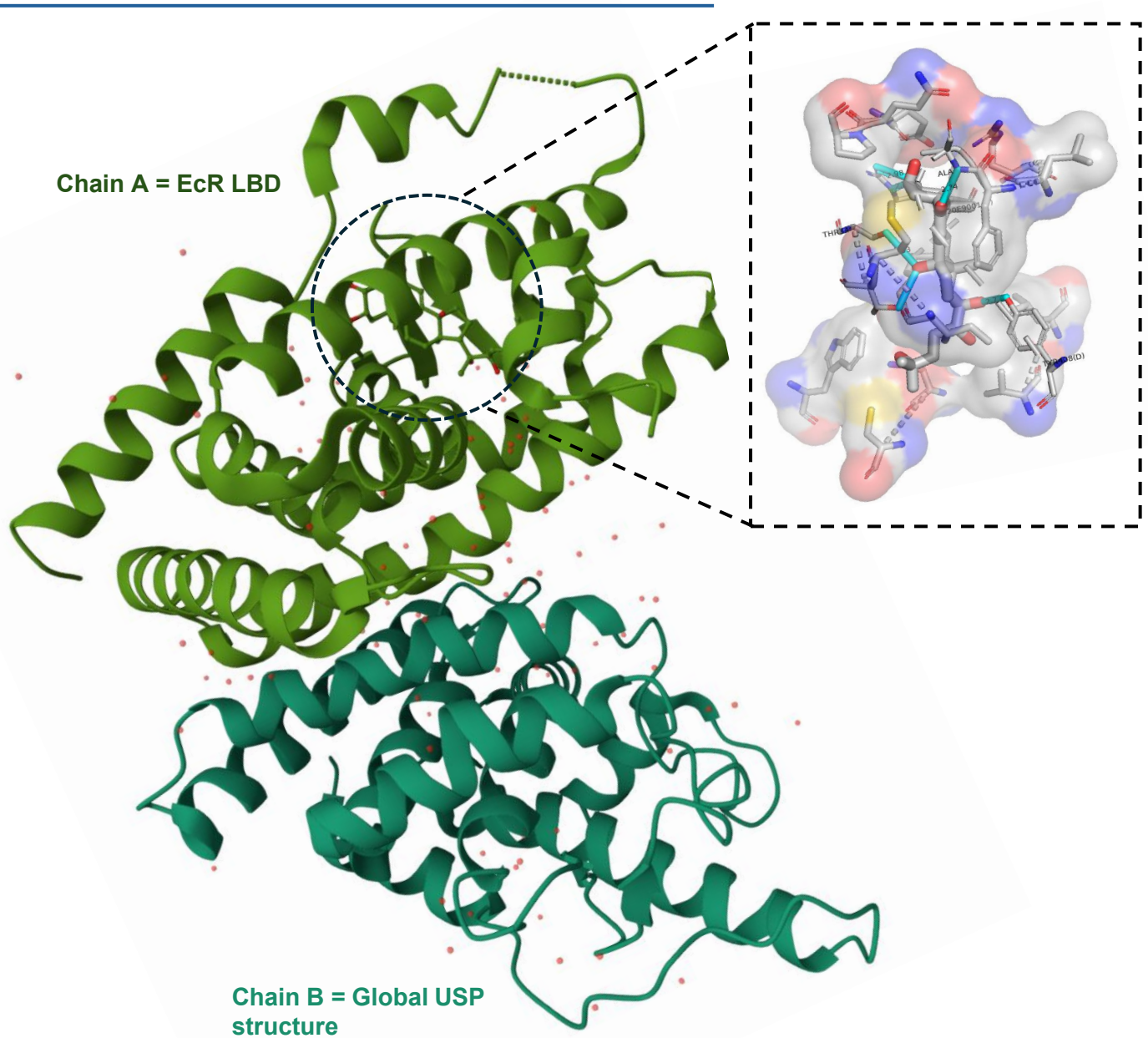
Molecular Docking

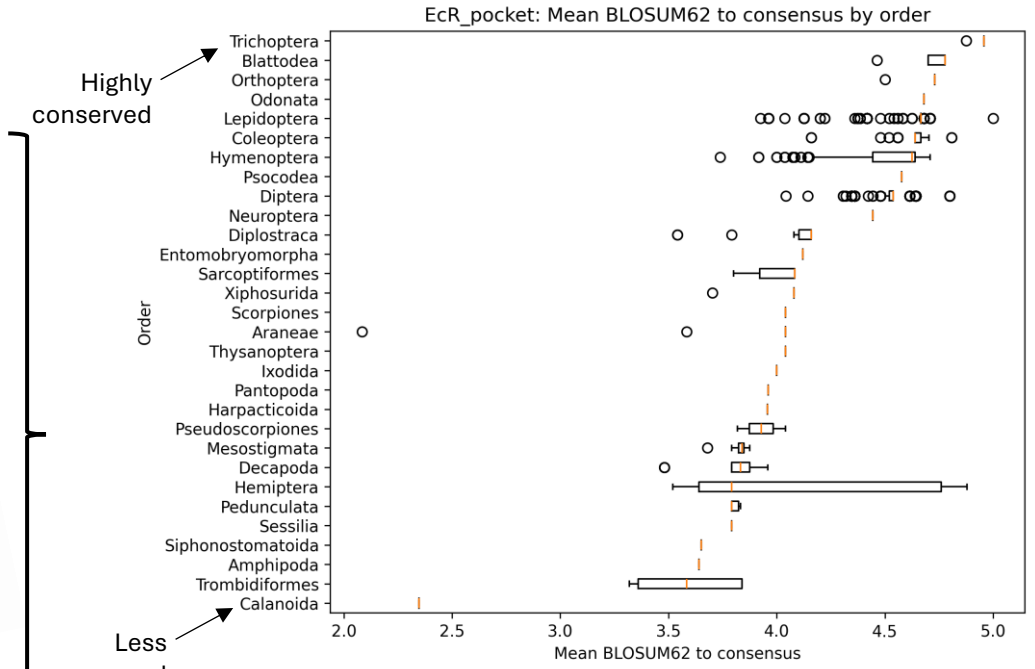


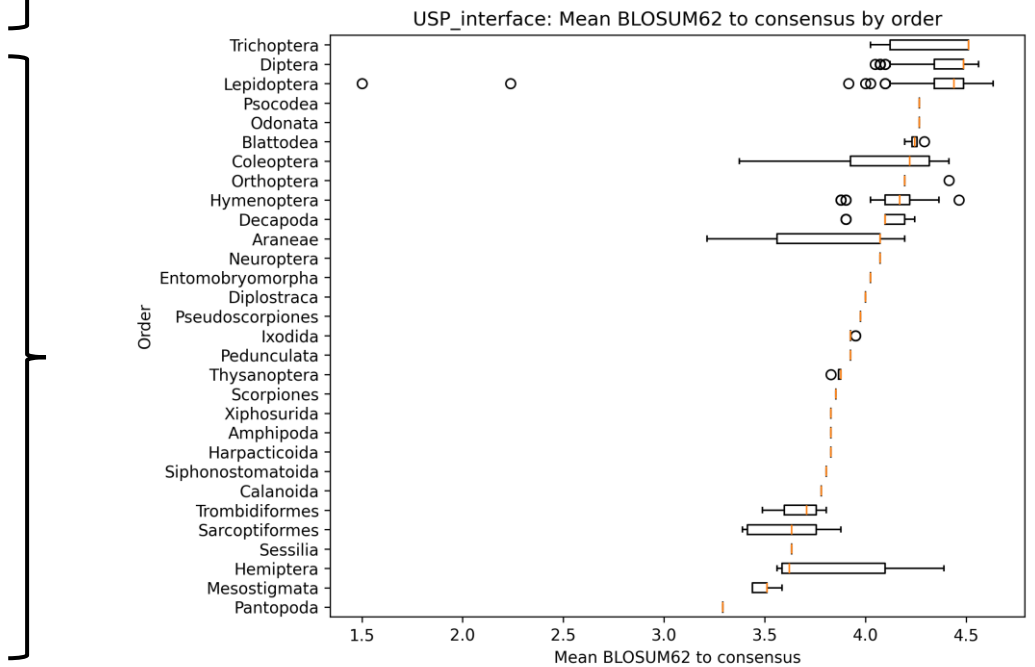
Species Sensitivity Distributions (SSDs) Modelling



Ligand binding domain (LBD) residue conservation across arthropod families – sequence level susceptibilities

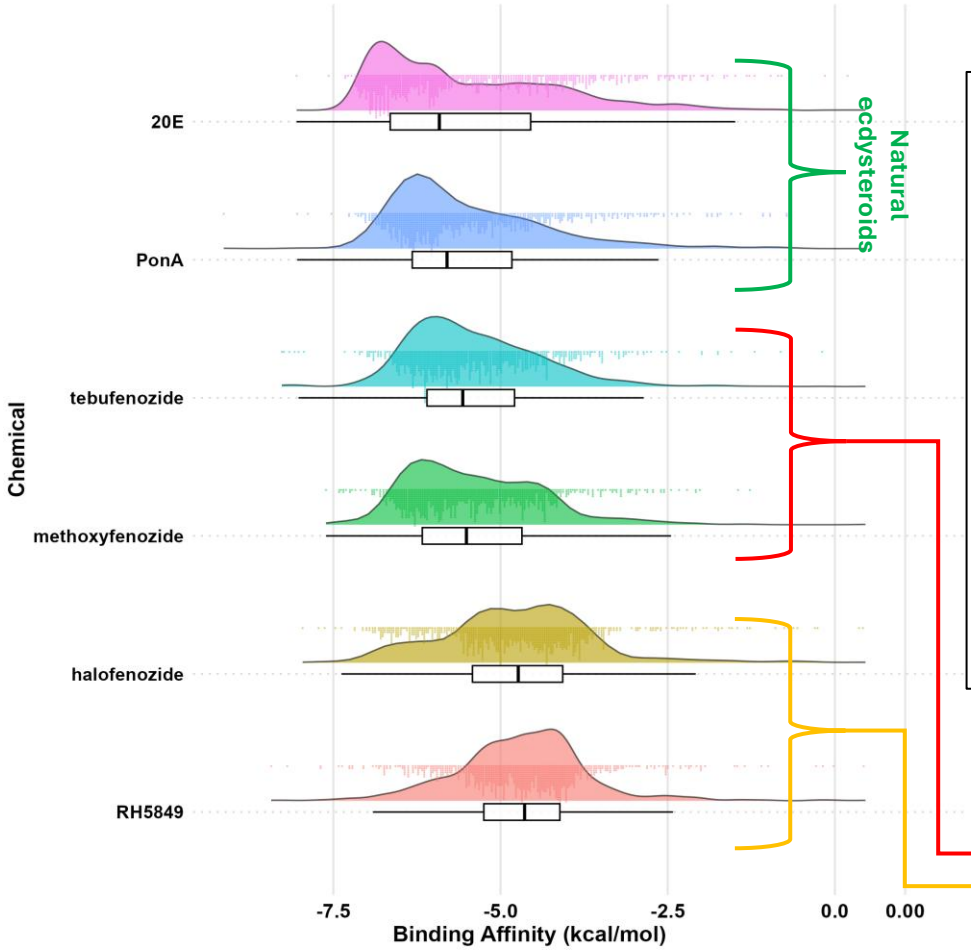




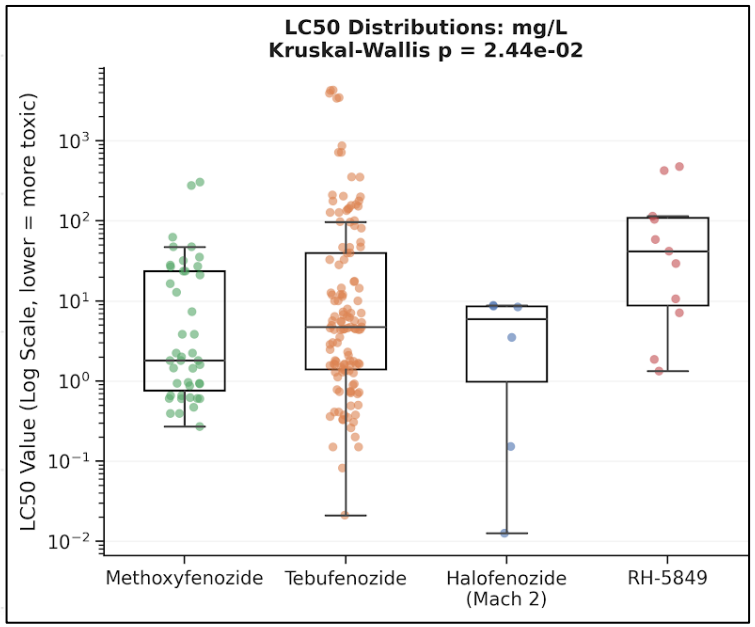


ForeTox Binding Affinities: Comparing to experimentally measured data

(A) Binding Affinity by Chemical (kcal/mol)



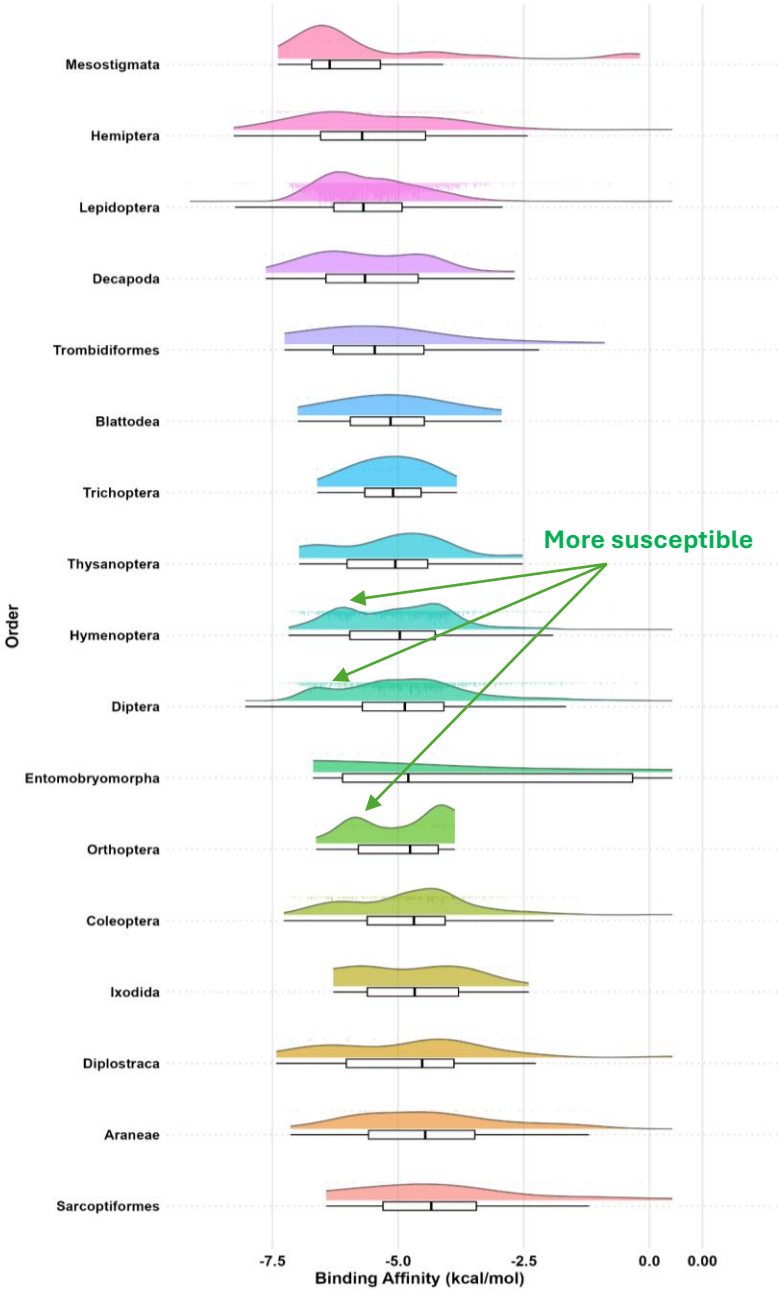
(B) Acute toxicity (LC50) distributions of ecdysone agonists from ECOTOX-derived exposure route.



More potent

Less potent

(C) Binding affinities by taxonomic order (kcal/mol)



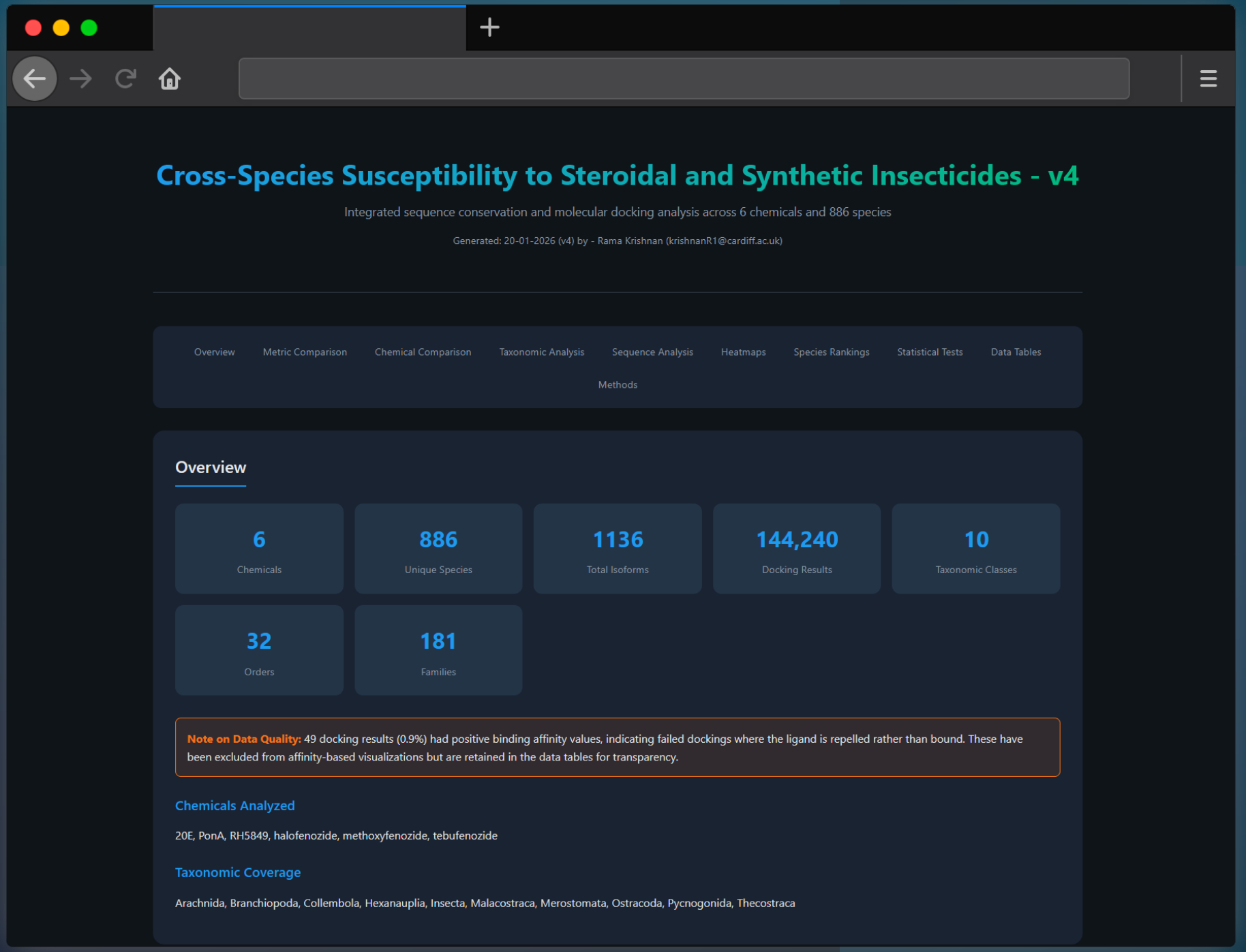
Interactive Dashboard Integration

Environmental Risk Assessment: Assess susceptibilities across 800+ species to identify species requiring attention.

Evaluate Docking: Analyse docking pose scores and binding affinities across 6 chemical libraries.

Investigate Orthologous Divergence: Query species-specific structural variability and binding pocket orthology.

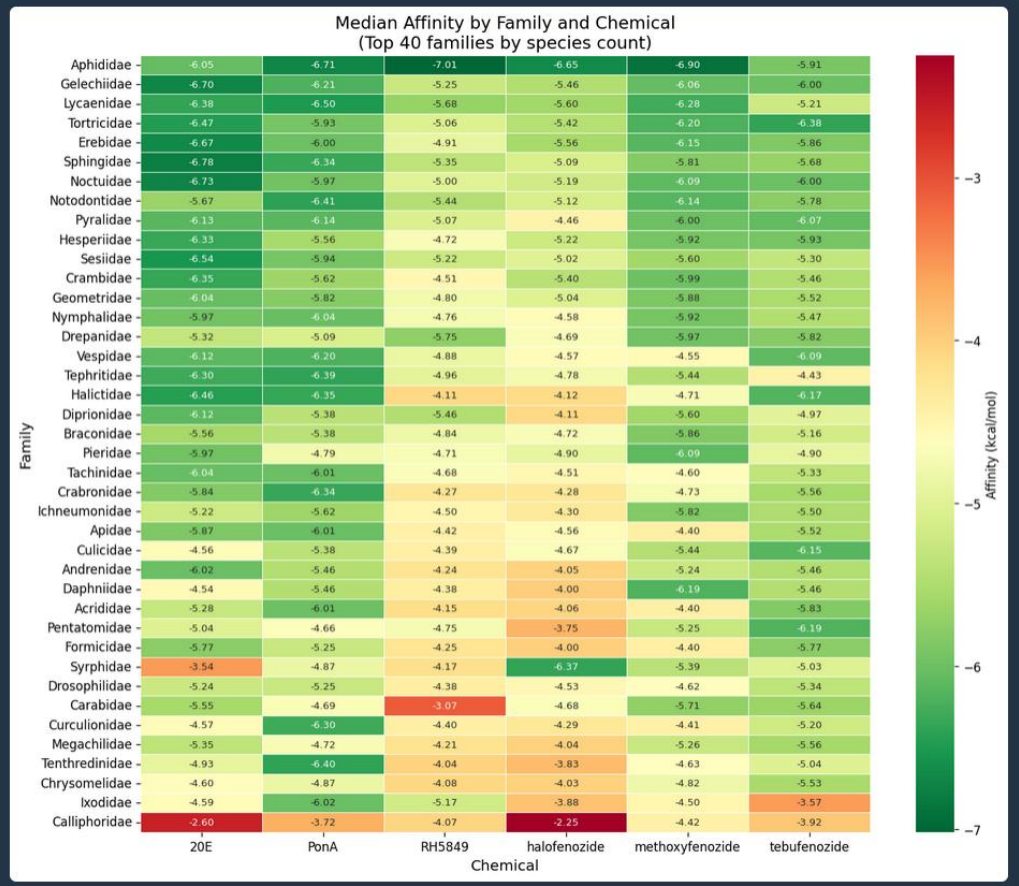
Rank Species Susceptibility: Compare predicted chemical potency through integrated sequence conservation and docking scoring metrics.



Family-level binding affinities

Family-Level Binding Patterns

CNN Pose Score | Binding Affinity



Median binding affinity by family and chemical (top families by species count).

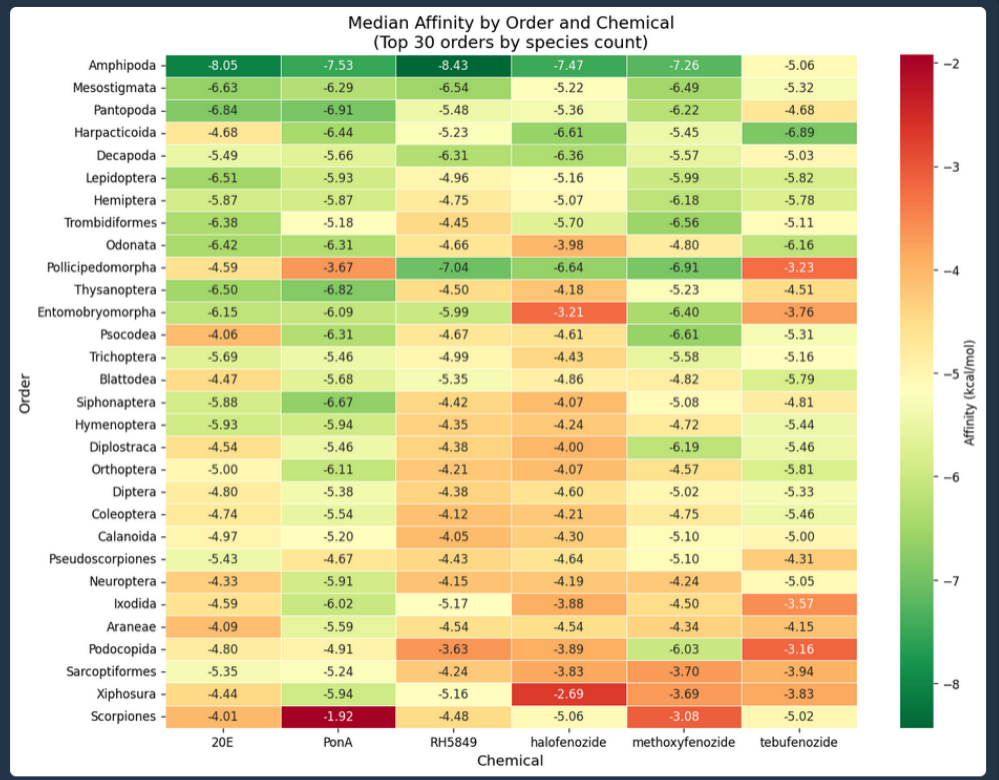
PNG

Order-level binding affinities

Binding Heatmaps

Order-Level Binding Patterns

CNN Pose Score | Binding Affinity



Median binding affinity by order and chemical. Green = stronger binding (more negative).

PNG

Family-Level Binding Patterns

CNN Pose Score | Binding Affinity

Example chemical:

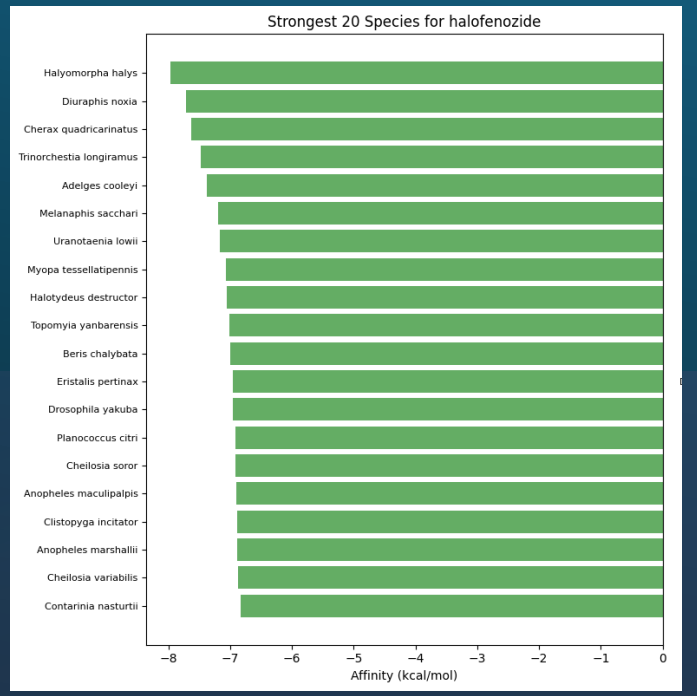
Halofenozide

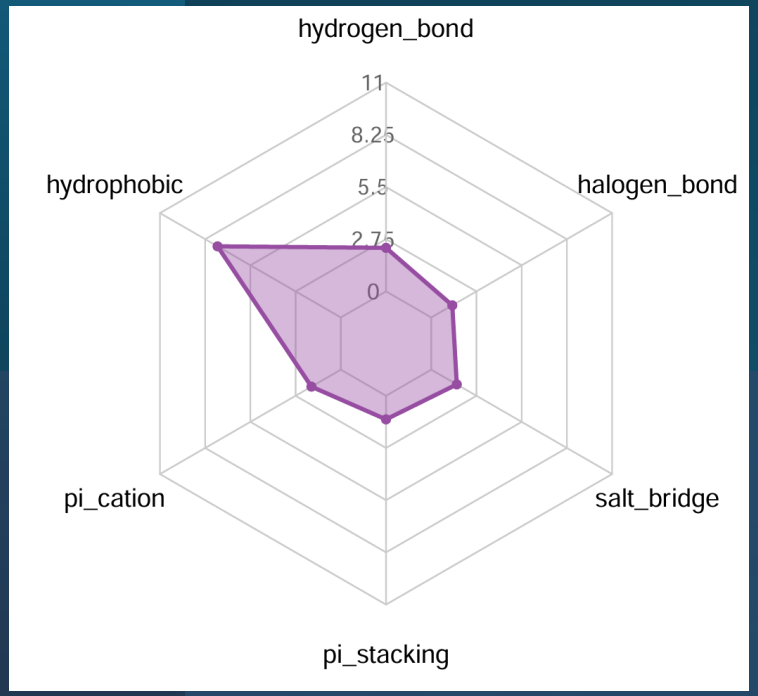
Potency Gradient: Affinities range from **-8.0 kcal/mol** (*H. halys*), defining a clear susceptibility spectrum.

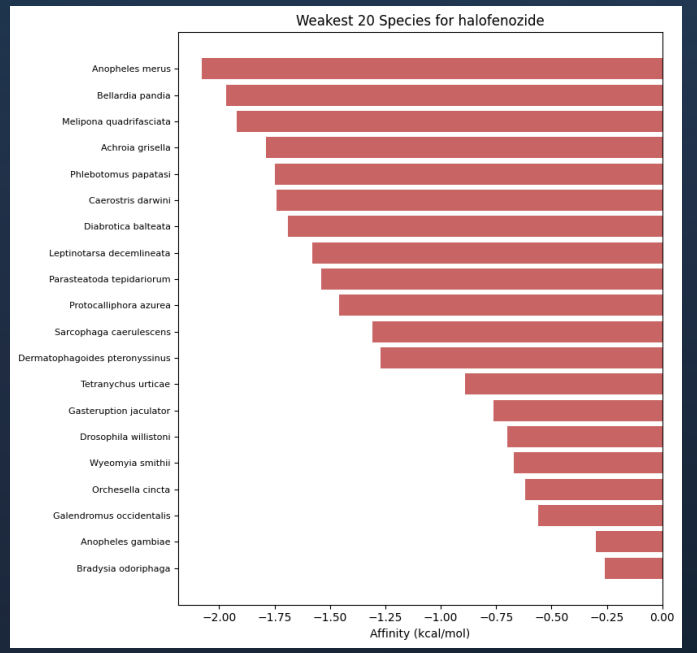
Hydrophobic Dominance: Radar plots confirm binding is primarily driven by **hydrophobic forces,** with unique **halogen bonding**, yet minimal polar or ionic bonds.

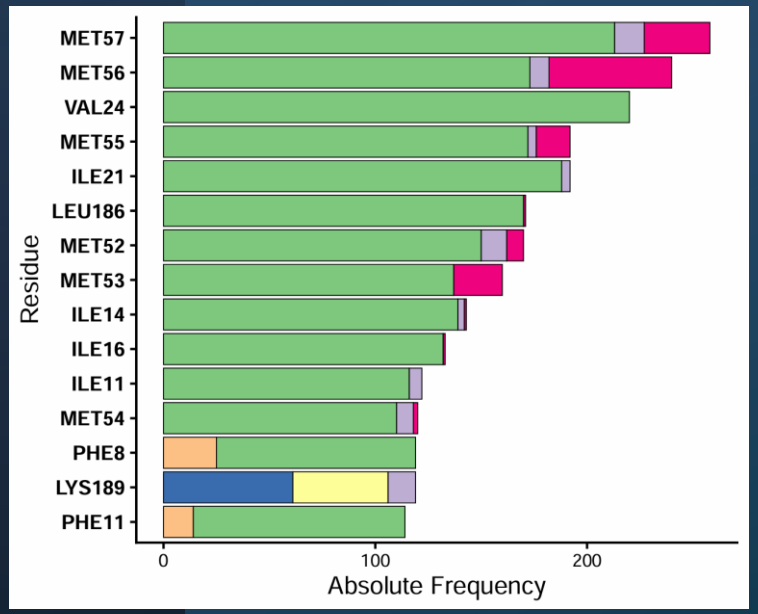
Key Residue Hotspots: MET57, MET56, and VAL24 are identified as the most frequent and critical interaction sites.

Methionine Dependency: High contact frequency across the **MET52-57 cluster** as primary residues for ligand stabilisation.

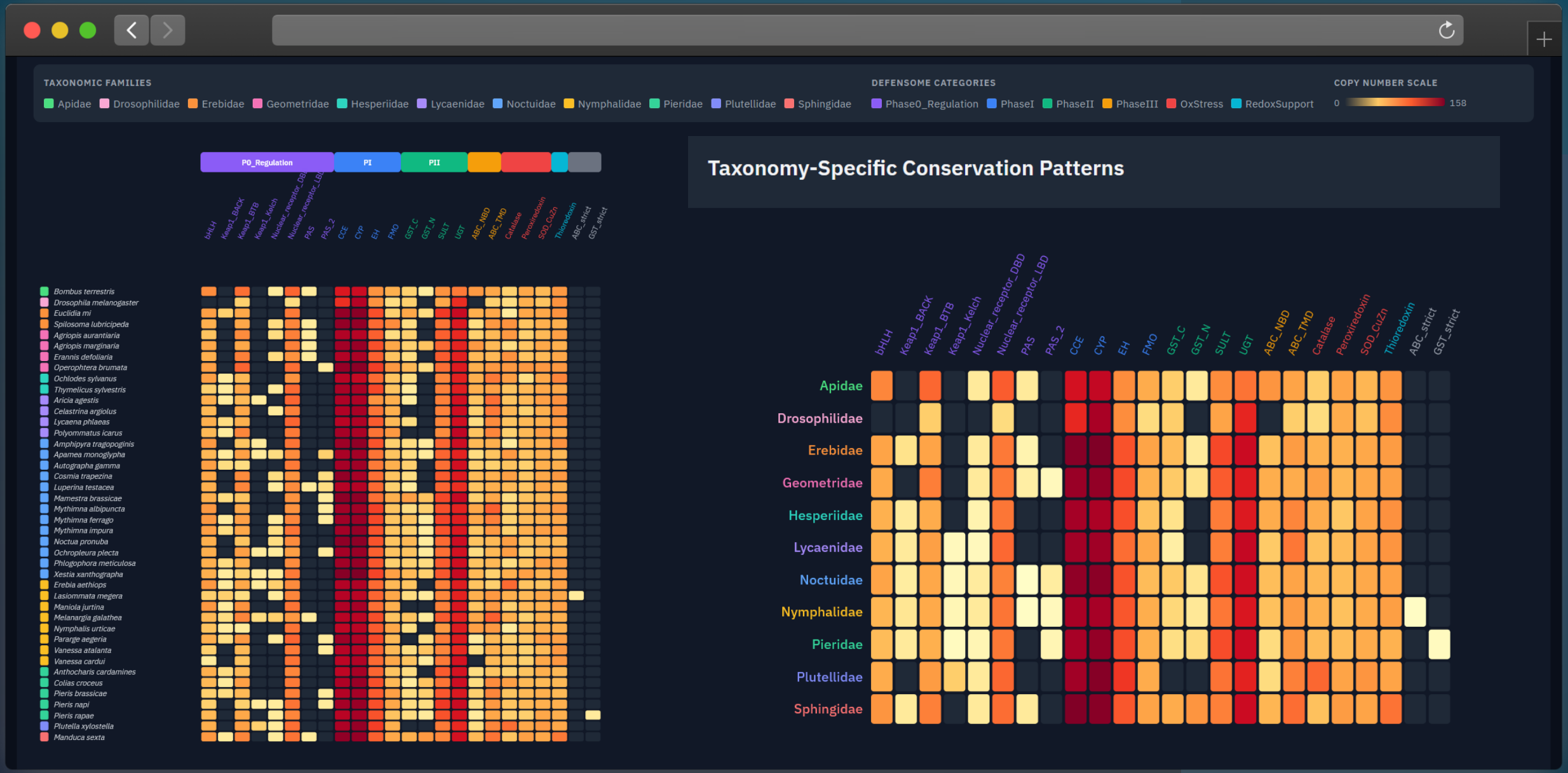


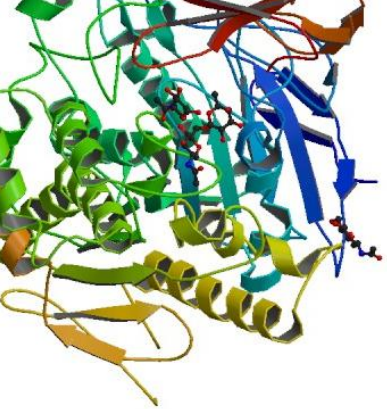






Modelling Toxicokinetic (TK) traits





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Thank you!

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UKRI Biotechnology and Biological Sciences Research Council