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Ecologically Guided Searches for New Riboswitch Functions

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Abstract

Riboswitches are structured RNA elements that regulate gene expression by directly binding ligands. Bioinformatics approaches have greatly accelerated the discovery of candidate riboswitches by searching for conserved RNA structures. However, discovering the ligand that binds to a conserved element remains challenging. Traditional approach search for clues to the binding partners of riboswitches by analyzing the function of downstream genes. This approach has proven highly successful, but poses challenges in poorly annotated genomes, such as from metagenomic samples, and may miss important environmentally delivered ligands. Here we propose to use ecological interactions to search for riboswitch functions. For our model system we will use herbivores which feed on sagebrush, highly defended by toxic chemicals known as plant secondary metabolites (PSM). We hypothesize that the microbial communities from these gut environments will contain riboswitches responding to the plant chemicals. Our collaborative research will produce shotgun metagenomics data from the fecal samples collected from animals ingesting sagebrush, bioinformatics searches for candidate riboswitch variants in the samples, and biochemical and biophysical validation of riboswitches using plant extracted chemicals. If successful, the research will produce new antibiotic candidates and targets, and will advance our understanding of how microbial communities contribute to the plant-herbivore arms race that drives biodiversity across the planet.



Ecologically Guided Searches for New Riboswitch Functions

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Background

- Riboswitches are structured RNA elements that regulate gene expression by directly binding ligands¹.
- Bioinformatics approaches have greatly accelerated the discovery of candidate riboswitches by searching for conserved RNA structures².
- Discovering the ligand that binds to a conserved element remains challenging.
- Traditional approaches search for clues to the binding partners of riboswitches by analyzing the function of downstream genes.
- This approach has proven highly successful³, but poses challenges in poorly annotated genomes, such as from metagenomic samples, and may miss important environmentally delivered ligands.

Methods

Extraction: Whole leaves were extracted for sesquiterpene lactones using chloroform; leaves ground in liquid nitrogen were extracted with acidified methanol/water for polyphenols. Extracts were filtered and stored in amber vials at -20°C.

HPLC separation of polyphenolic compounds: Reverse phase high performance liquid chromatography (HPLC) with UV diode array detection was used to separate, quantify, and identify polyphenolic compounds in sagebrush. Extracts were fractionated over a C18 column with an acidified water:acetonitrile or water:methanol gradient.

TLC separation of sesquiterpene lactones: Silica gel thin layer chromatography (TLC) and staining with H₂SO₄ was used to extract, separate, and identify sesquiterpene lactones.

Mass spectrometry: Identity of chemical components of sagebrush extracts were established with tandem liquid chromatography/mass spectrometry (LCMS) retention times for [M+H]⁺ parent ion masses (University of Eastern Finland), and with extracted ion chromatographs (EIC), and MS/MS fragmentation patterns (Boise State University Biomolecular Research Center.) Data were compared to reference standards and also compared to entries in the MassBank of North America (MoNA, <http://mona.fiehnlab.ucdavis.edu>).

Metagenomics: The cecal metagenomes of three sage-grouse were collected during late fall and early winter when the birds feed exclusively on sagebrush, as confirmed by crop content⁴. Total cecal DNA was isolated using a MoBio PowerFecal DNA extraction kit and sheared using a Covaris Sonicator. Metagenomic shotgun libraries were made using an Illumina TruSeq DNA preparation kit and sequenced on an Illumina HiSeq2000 platform.

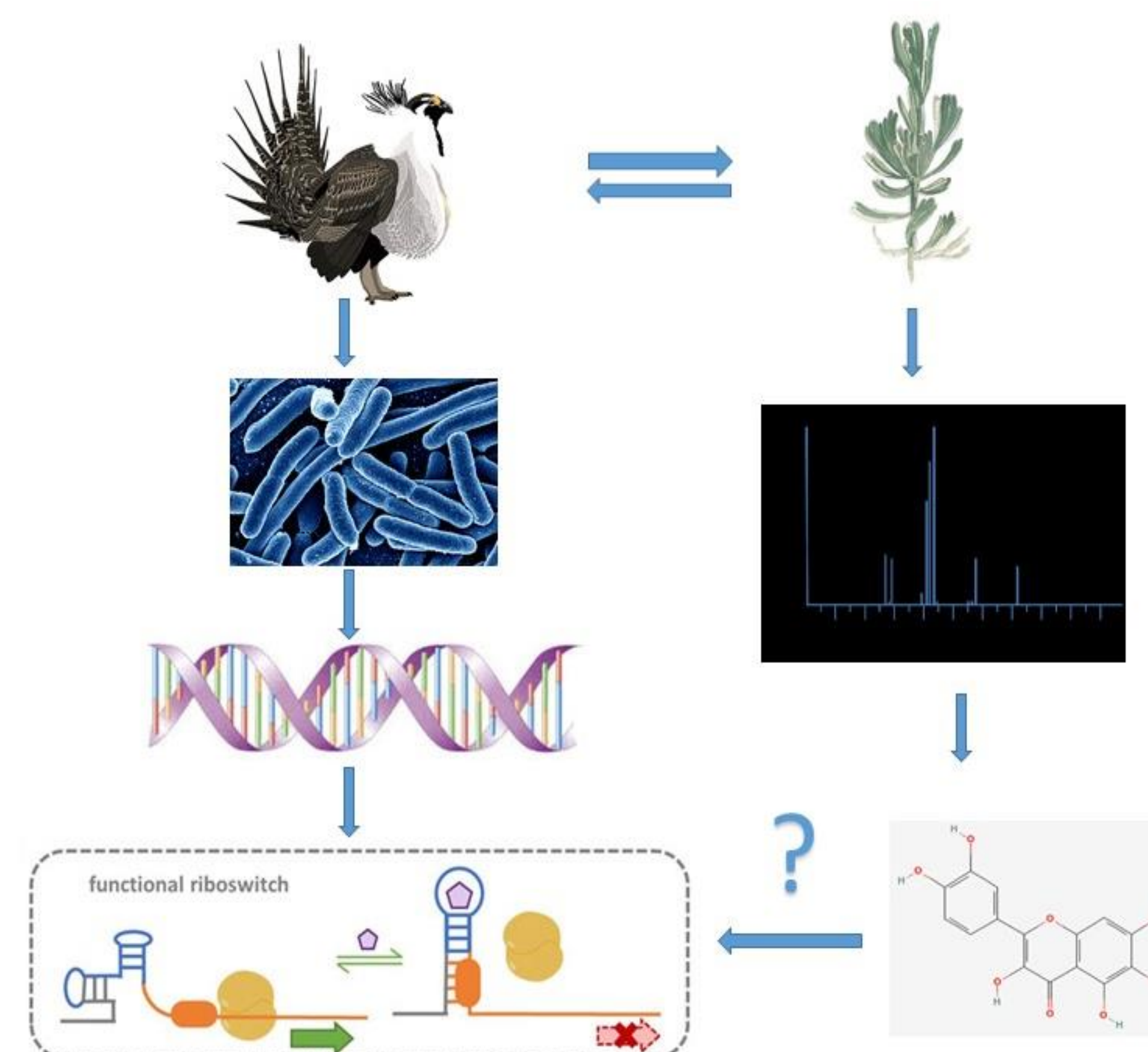
Riboswitch identification: HiSeq sequences were assembled into contigs using Velvet (<https://www.ebi.ac.uk/~zerbino/velvet/>). Covariance models of riboswitches of interest were downloaded from Rfam (rfam.xfam.org) and aligned to the contigs using Infernal (<http://eddylab.org/infernal/>) to identify candidate riboswitches within the microbiome.

Future Goals

- Improve chemical isolations from sagebrush.
- Bioinformatics:
 - Identify sequence variation in riboswitches and downstream genes
 - New candidate riboswitch discovery. Cmfinder (<http://bio.cs.washington.edu>) will be used to find new RNA motifs which may function in binding the identified plant chemicals in sagebrush.
- Riboswitch characterization:
 - Biophysical approaches to determine ligand interactions → in-line probing, reporter gene assays, Kinexa/SPR

Objective

Use ecological interactions to guide the search for interactions between plant chemicals and riboswitches. For our model system we will use Sage Grouse (*Centrocercus urophasianus*) which feed on sagebrush (*Artemisia spp.*) known to be highly defended by toxic chemicals known as plant secondary metabolites (PSMs). We hypothesize that the microbial communities from these gut environments will contain interactions between riboswitches and PSMs that contribute to plant herbivore coevolution.



Mass Spectrometry Results

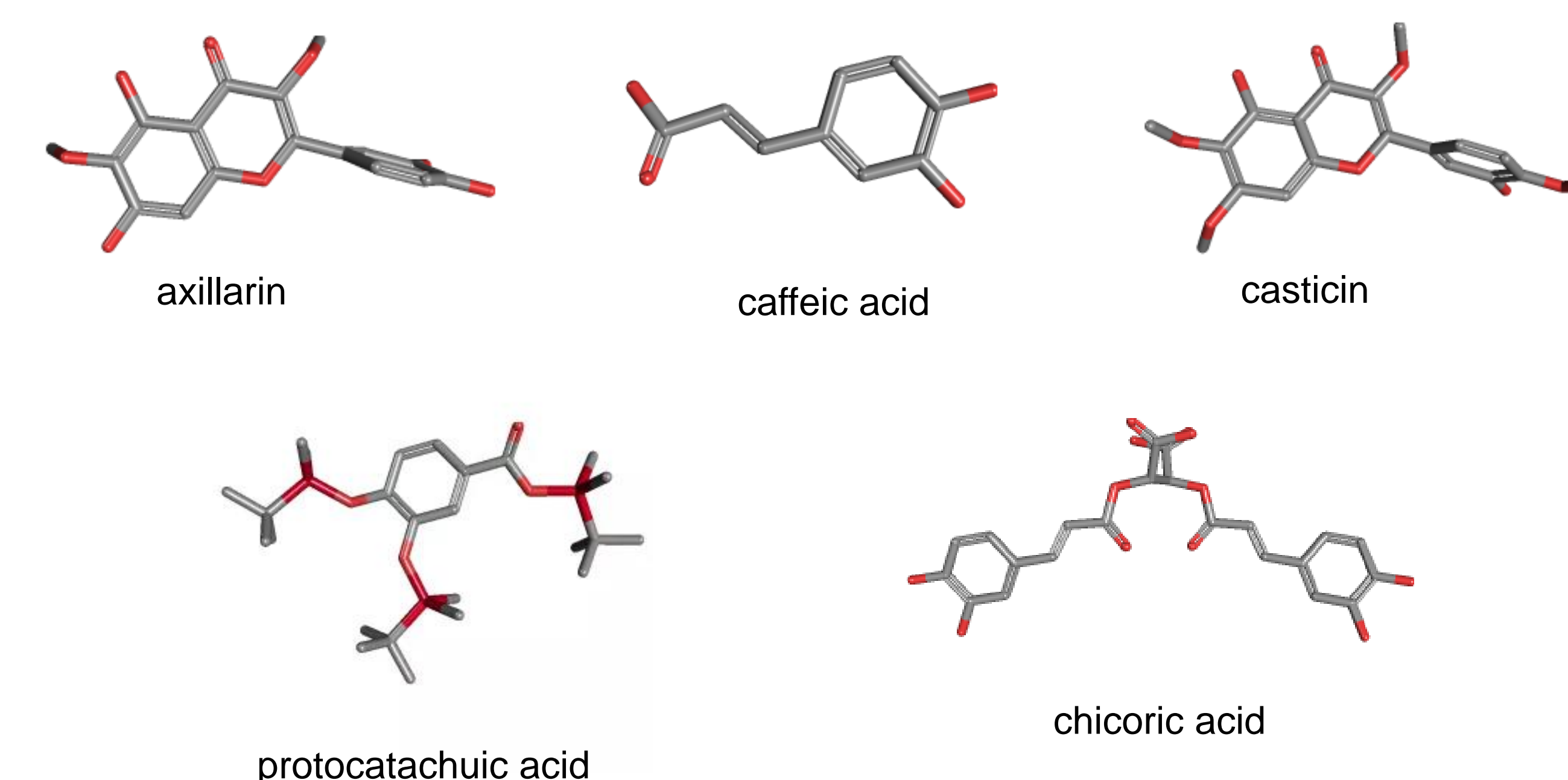
Polyphenols identified:

apigenin-7-glucoside, axillarin, caffeic acid, casticin, chicoric acid, chlorogenic acid, esculetin, esculin, 7-hydroxy coumarin, isoscapoletin, luteolin, kaempferol, methyl axillarin, protocatechuic acid, quercetin, rutin, scopoletin, and umbelliferone.

Sesquiterpene lactones identified:

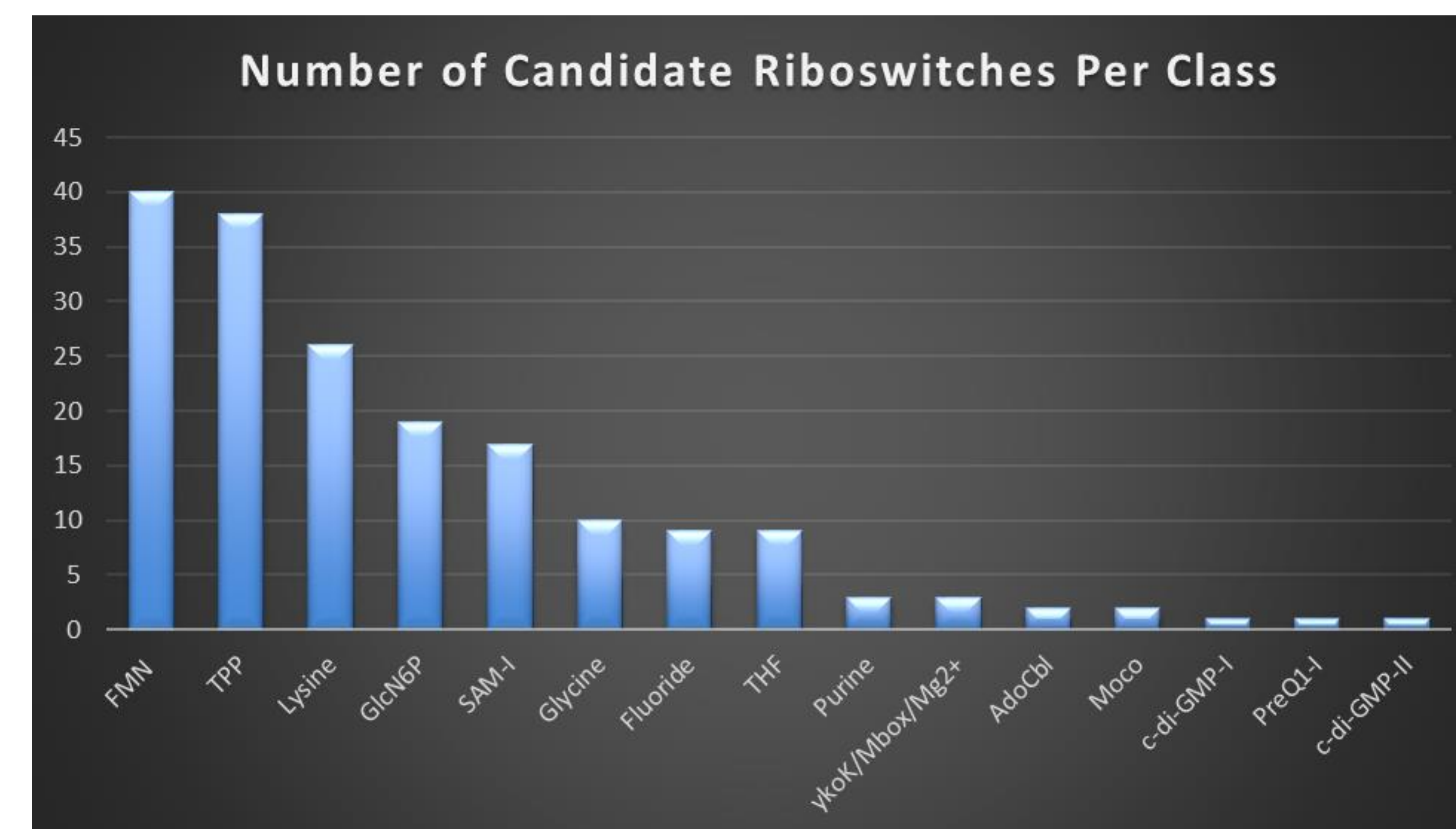
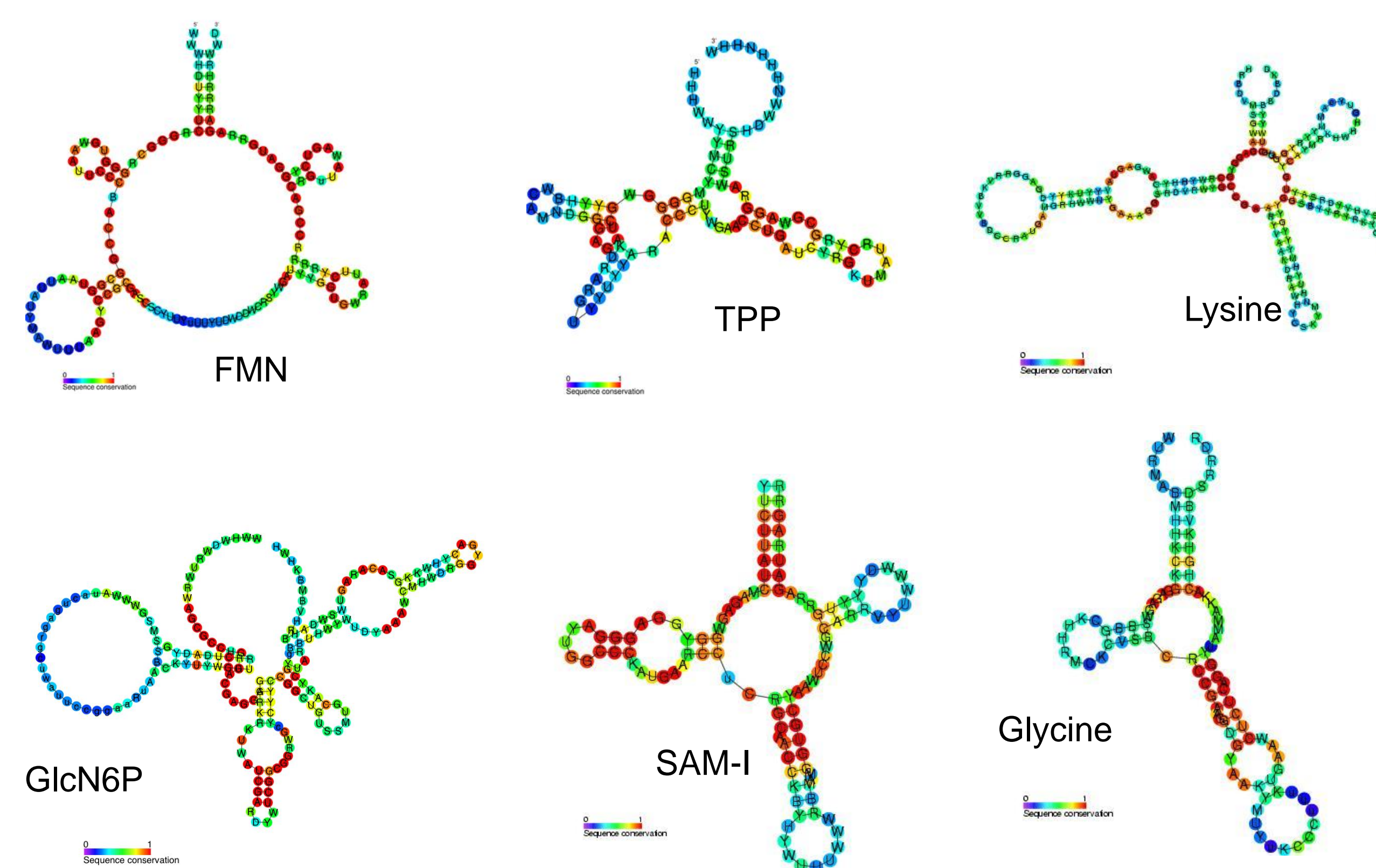
cumambrin-A, matricarin, and deactylmatricarin.

Those below are previously unreported in sagebrush.



Riboswitch Identification

Riboswitch identification: Candidate riboswitches were identified in Infernal and sequences were submitted to Rfam (<http://rfam.xfam.org>) for secondary structure analysis



References

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