

Exploring the diversity of brominated alkaloids naturally released by the sponge *Aplysina cavernicola*



Morgane MAUDUIT¹, Stéphane GREFF¹, Thierry PEREZ¹, Charlotte SIMMLER¹

¹ Institut Méditerranéen de Biodiversité et d'Écologie Marine et Continentale, UMR 7263

Endoume Marine Station, Rue de la batterie des lions, 13007 Marseille (e-mail: morgane.mauduit@imbe.fr)

Context

- Aplysina* species are marine sponges known to produce **spiro-isoxazoline bromotyrosine** alkaloids (e.g. aerothionin).
- It has been reported that the Caribbean sponge *A. fistularis* releases aerothionin in seawater with/without intentional stress.¹
- Such released compounds, herein called **exo-metabolites (EM)**, could possibly exert their biological effects as marine chemical cues.²



The present study aims at determining (1) whether the Mediterranean sponge *Aplysina cavernicola* (Vacelet, 1959) naturally releases brominated alkaloids other than aerothionin, (2) what are their identity and, (3) in what proportions are they released.

General Experimental Workflow



1 Untargeted Metabolomic Comparison

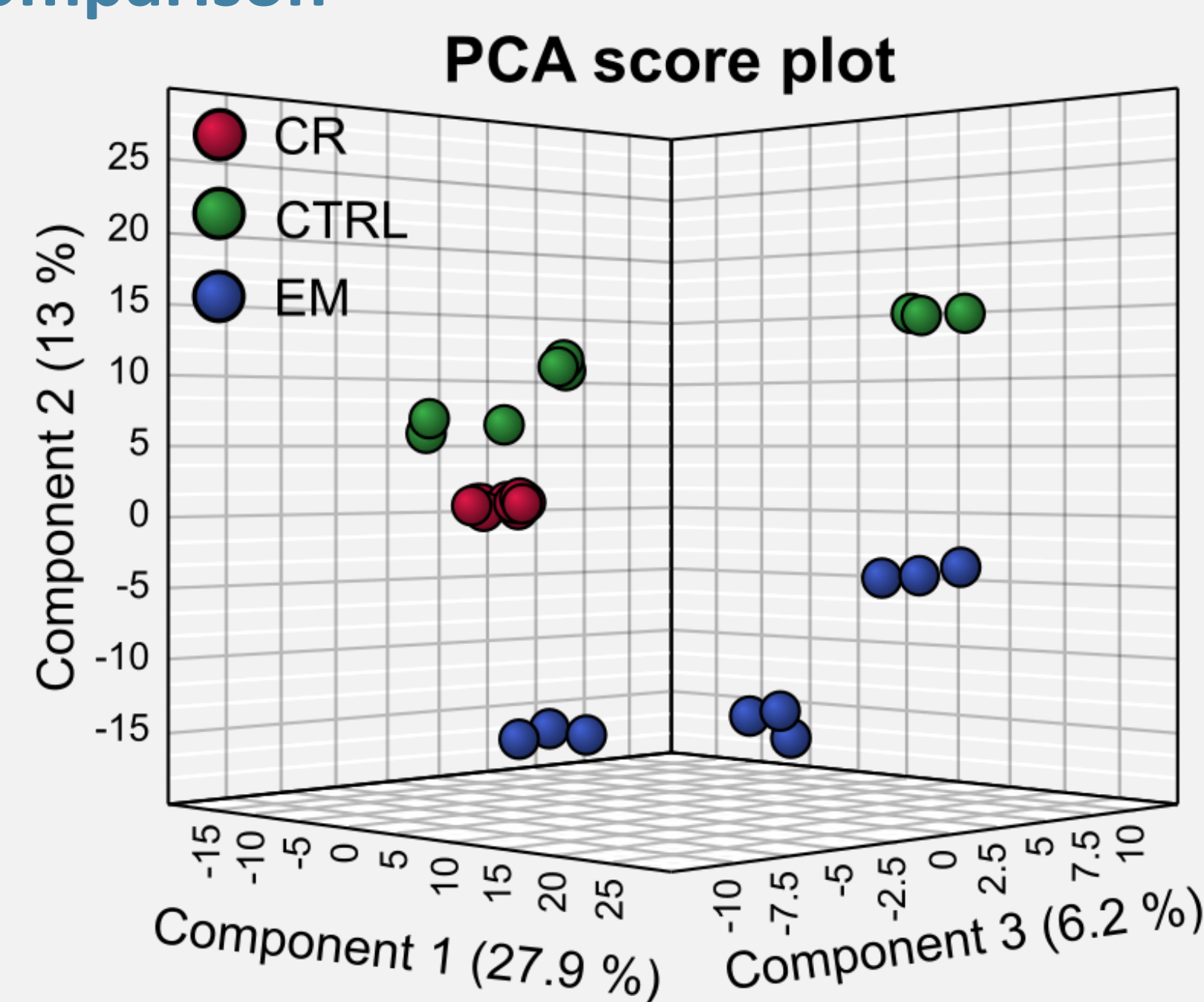
CTRL: Aquarium capture setting without sponges

Mass recovered

EM: 50.4 mg

CTRL: 17.4 mg

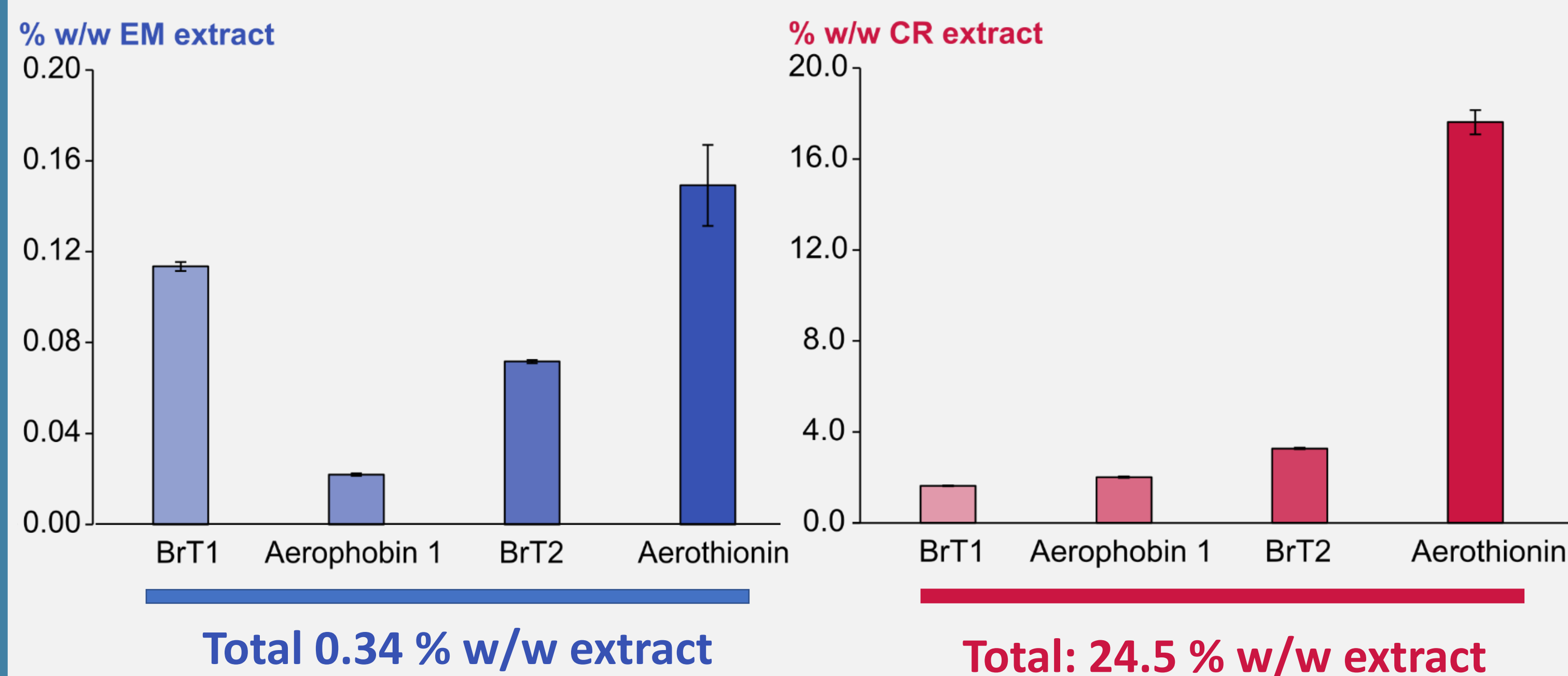
CR: 13.1 % w/w of sponge



- The EM extracts were found to be statistically different from the CTRL and from the CR.
- The amount of collected EM corresponds collectively to a concentration of 1 ppm in the aquarium (traces).

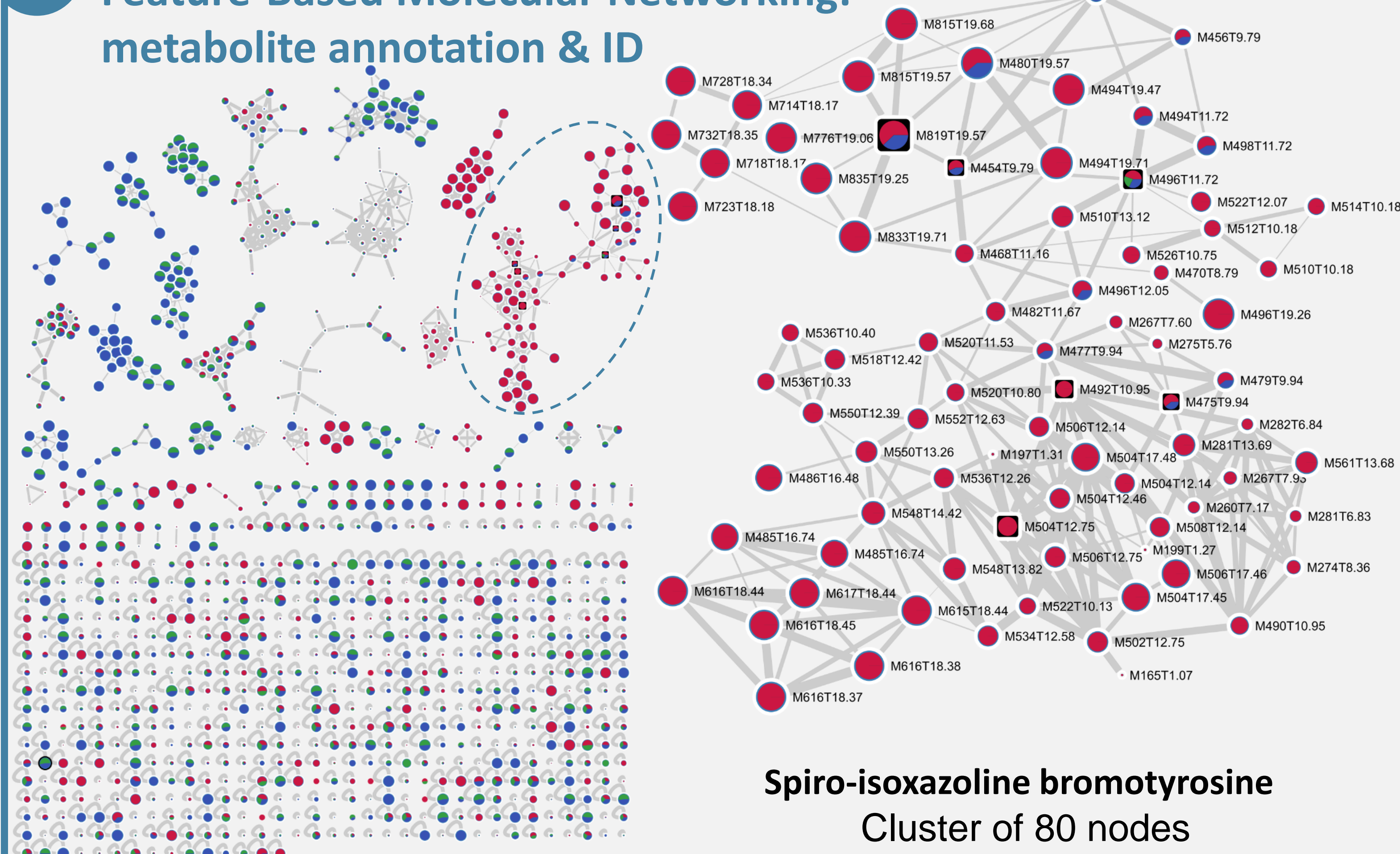
3 Quantification

The 4 brominated metabolites were isolated and used as calibration standards for comparative quantitative MS-based analysis.



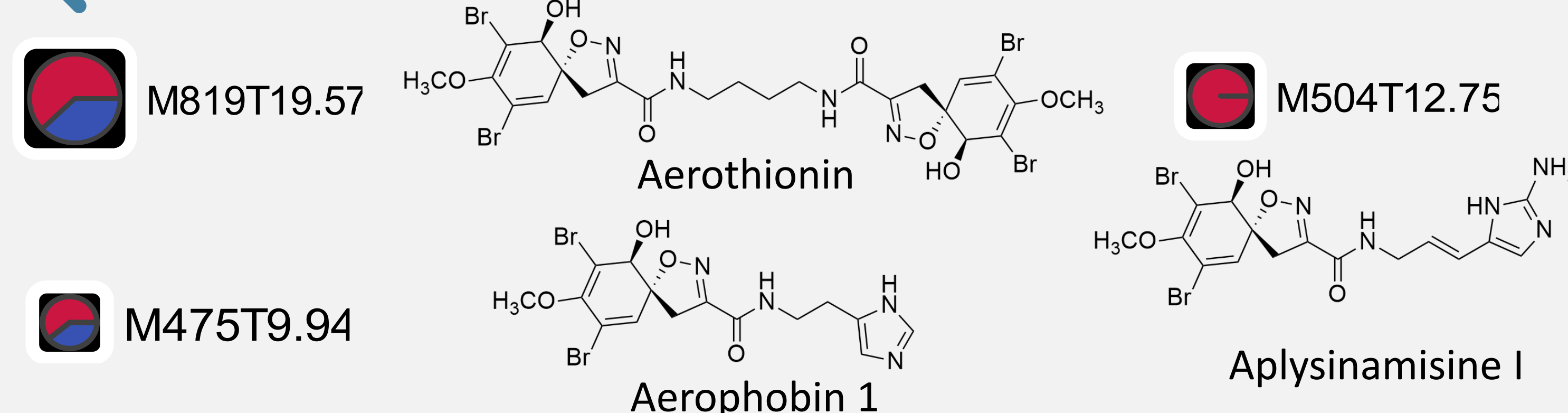
- The proportion of bromotyrosine alkaloids are different in **EM extract** than in the **sponge crude extract (CR)**.
- Aerothionin remains the major metabolite in both cases.
- Collectively, the identified bromotyrosine alkaloids released in seawater are ~100 times more diluted compared to their concentration in the CR.

2 Feature-Based Molecular Networking: metabolite annotation & ID



Spiro-isoxazoline bromotyrosine
Cluster of 80 nodes

Isolation & structure elucidation by NMR and MS²



- Up to now, a total of 4 brominated metabolites including aerothionin were identified as being released by *A. cavernicola*.
- Other major bromotyrosine alkaloids such as Aplysinamisine I were not recovered as EM.

Conclusions

- Aerothionin together with other bromotyrosine alkaloids were found to be naturally released in the sea water by *A. cavernicola* in aquarium.
- Those metabolites were found to be released in trace quantities.
- Their relative proportions were found to be different from those characterizing the sponge crude extract.

→ Such results will help to develop experiments evaluating the roles of sponge exo-metabolites in ecosystem functioning.

References:

¹ Walker, R. P. et al. Mar Biol 88, 27-32 (1985), ² Santonja, M., et al. Mar Biol 165, 121 (2018)



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