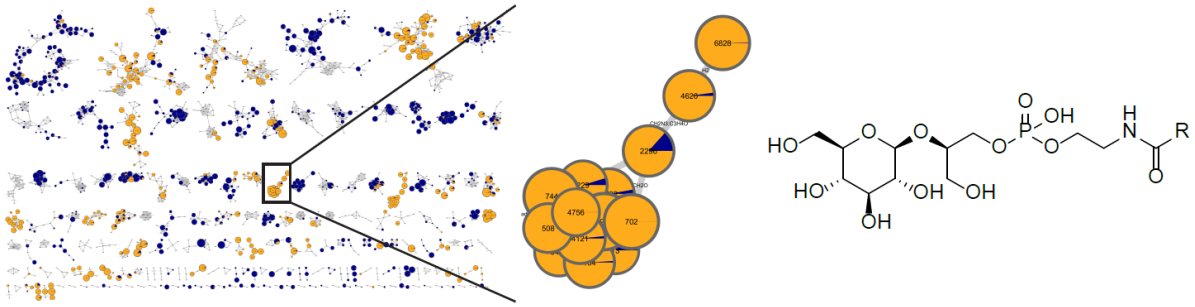
Combining comparative metabolomics and molecular networking to study secondary metabolism of *Caenorhabditis elegans* and *briggsae*

Marie-Désirée Schlemper-Scheidt, Stephan H. von Reuss

Institute of Chemistry, Université de Neuchâtel, Avenue de Bellevaux 51, Neuchâtel, Switzerland

Secondary metabolism in nematodes is characterized by complex modular metabolites that incorporate building blocks from diverse primary biosynthetic pathways [1]. In order to characterize the chemical space of nematode secondary metabolism we employed comparative metabolomics and molecular networking using the hermaphroditic model organism *Caenorhabditis elegans* and the satellite model organism *Caenorhabditis briggsae* [2].
Using GNPS the combined ESI-(–)-MS/MS data from exo- and endometabolome extracts of well-fed and starved cultures were pooled to generate a molecular network of *Caenorhabditis* metabolites. Here, we report the identification of two clusters, which demonstrates the utility of molecular network analysis for the characterization of nematode secondary metabolism. As a proof of concept, we initially focused on the cluster representing ascarosides. Ascarosides represent highly conserved signaling molecules in nematodes, which regulate a wide range of inter- and intraorganismal interactions [1, 3]. Comparative metabolomics using the reference strains *C. elegans* N2 and *C. briggsae* AF16 further enabled us to characterize ascaroside production under different growth conditions.



In addition, a second prominent cluster was identified as amphiphilic *N*-acylethanolamine derivatives, which are highly abundant in *C. elegans* and predominantly excreted into the exometabolome under well-fed conditions. The underlying lipophilic *N*-acylethanolamine building blocks (NAEs) represent a conserved class of signaling molecules in plants, animals and microorganisms [4]. In the nematode *C. elegans* some NAEs have previously been identified, and shown to be retained in the endometabolome [5]. Using a combination of large-scale cultivation, RP-C18 chromatography and NMR spectroscopy a variety of 2-(β-glucosyl)-glyceryl *N*-acyl phosphoethanolamines were unambiguously identified. Their amphiphilic properties, enrichment in the exometabolome, and potential cleavage to known signaling molecules suggests potential roles in intraorganismal interactions.
Taken together, our results demonstrate the utility of comparative metabolomics and molecular networking to explore structural diversity, species-specificity, and growth condition dependency of nematode secondary metabolism.

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[2] M. A. Félix, F. Duveau *et al.*, *BMC Biol.*, **2012**, *10,* 59.
[3] S. H. von Reuss, *Chimia*, **2018**, *72*, 297-303.
[4] E. B. Blancaflor *et al.*, *The Plant Journal*, **2014**, *79*, 568-583.
[5] M. Lehtonen *et al.*, *Chemistry&Biodiversity*, **2008**, *5*, 2431-2441.