

PANGENOME ANALYSIS OF MARINE NITRIFIERS SUGGESTS DIFFERENCES IN VITAMIN B₁₂ METABOLISM

Kiera Sullivan, Barbara Bayer, Alyson Santoro - UC Santa Barbara Department of Evolution, Ecology, and Marine Biology

WHAT'S AN NOB?

Nitrification, the oxidation of ammonia (NH₄⁺) to nitrate (NO₃⁻) with nitrite (NO₂⁻) as an intermediate, plays an important role in the global nitrogen cycle. Chemolithoautotrophic nitrite-oxidizing bacteria (NOB) in the dark ocean perform the second step of this process, oxidizing nitrite through a reaction that provides them with the energy for carbon dioxide (CO₂) fixation¹.

Members of the phylum Nitrospinae comprise the majority of marine NOB, yet relatively little is known about them due to the lack of available cultured strains. Uncultured Nitrospinae are the dominant type of NOB from the water column to deep ocean sediments, but the metabolic potential encoded in their genomes has remained largely unexplored.

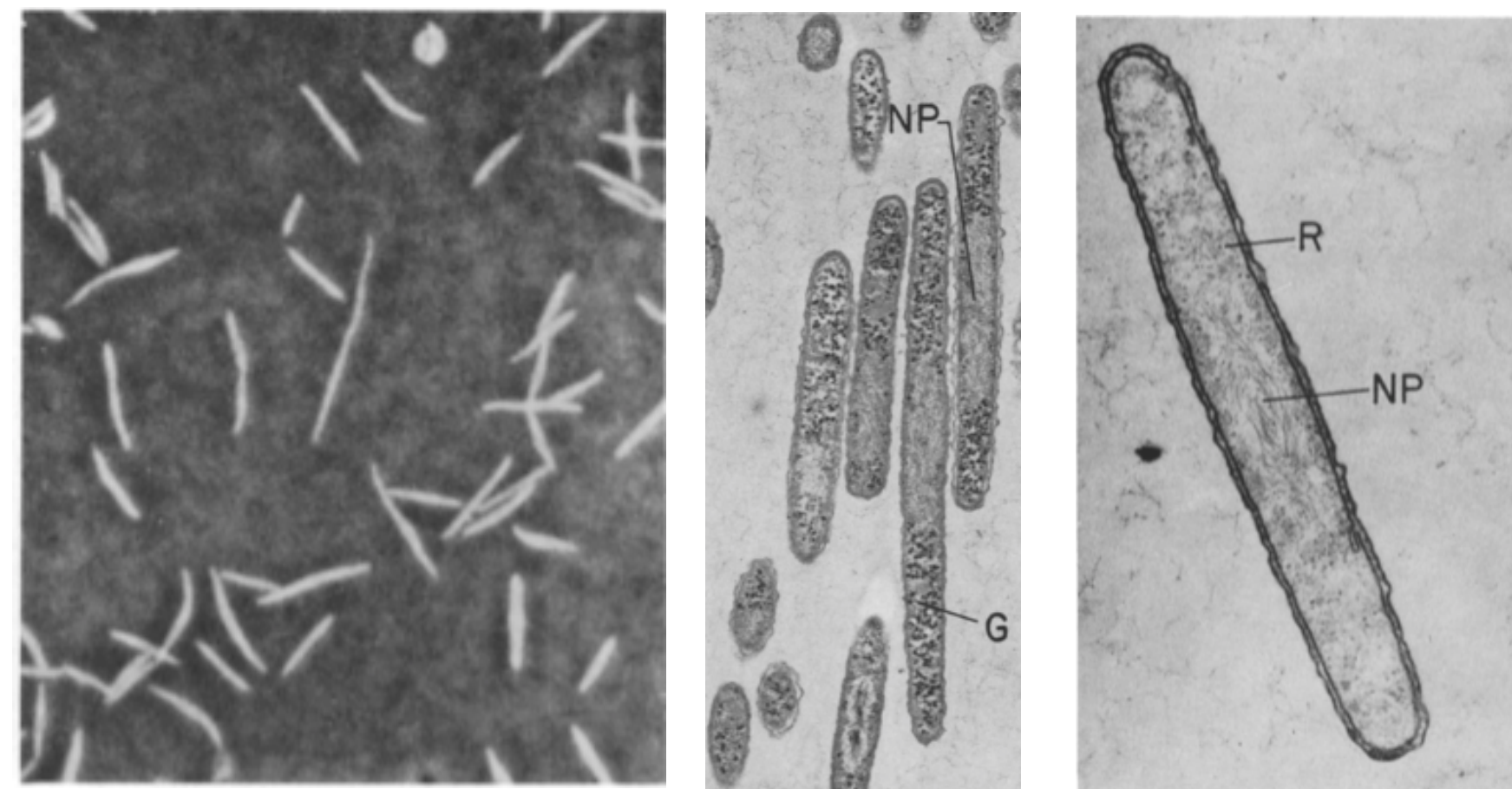


Figure 1. Electron microscopy images of Nitrospina gracilis. Originally sourced from Watson and Waterbury (1971), these images show the overall shape of Nitrospina gracilis with labeled nucleoplasm (NP), glycogen deposits (G), and ribosomes (R)². *N. gracilis* is the only available pure culture of a marine NOB.

WHAT'S A PANGENOME?

A pangenome is the sum of all genes from a set of organisms. Visually, it is represented by a diagram that shows the differential presence and absence of genes across multiple genomes. Each ring in the diagram represents the genetic contents of a single genome. Darker colors represent areas in the genome where a gene is present, while light colors indicate the absence of a gene.

The large cluster of genes that are present across most of these genomes is called the “core region,” and genes that fall into this region are called “core genes.” Many of the genes in the core region are housekeeping genes shared by multiple types of bacteria, while others may only be specific to the phylum Nitrospinae.



Figure 2. Clade 1 vs. Clade 2 Pangenome

Pangenome of the two primary Nitrospinae clades was created using the Anvi'o Pangenomics Workflow. Genomes were sourced from the NCBI and JGI. Gene functions were also determined using Anvi'o³. Vitamin B₁₂ synthesis genes were manually identified and marked on pangenome.

WHAT DID WE FIND?

Phylogenetic analyses of Nitrospinae genomes revealed the distinction of three clades of Nitrospinae. Clade 1 (blue) and Clade 2 (green) are the primary clades of these nitrite oxidizers and appear to be only distantly related to the only known cultured representative of the phylum, Nitrospinae gracilis⁴. Due to their high abundance in the ocean, Clades 1 and 2 were analyzed for differential presence of genes that may impact the type of environments they can live in, with a particular emphasis being placed on the ability to synthesize vitamin B₁₂. Genes predicted to encode enzymes that participate in the vitamin B₁₂ synthesis pathway were detected primarily in Clade 2 genomes, indicating that members of this clade of Nitrospinae may be able to synthesize their own vitamin B₁₂ while members of Clade 1 likely cannot.

WHY DO WE CARE ABOUT VITAMIN B₁₂?

Vitamin B₁₂ is an important cofactor whose de novo synthesis is carried out only by certain bacteria and archaea. However, organisms that cannot produce this compound commonly require it for basic metabolic functions, like the synthesis of the amino acid methionine and other important biological molecules. It has previously been suggested that Nitrospinae rely on other prokaryotes as a source of vitamin B₁₂, but this analysis suggests that certain representatives of Clade 2 may be able to synthesize this nutrient on their own.

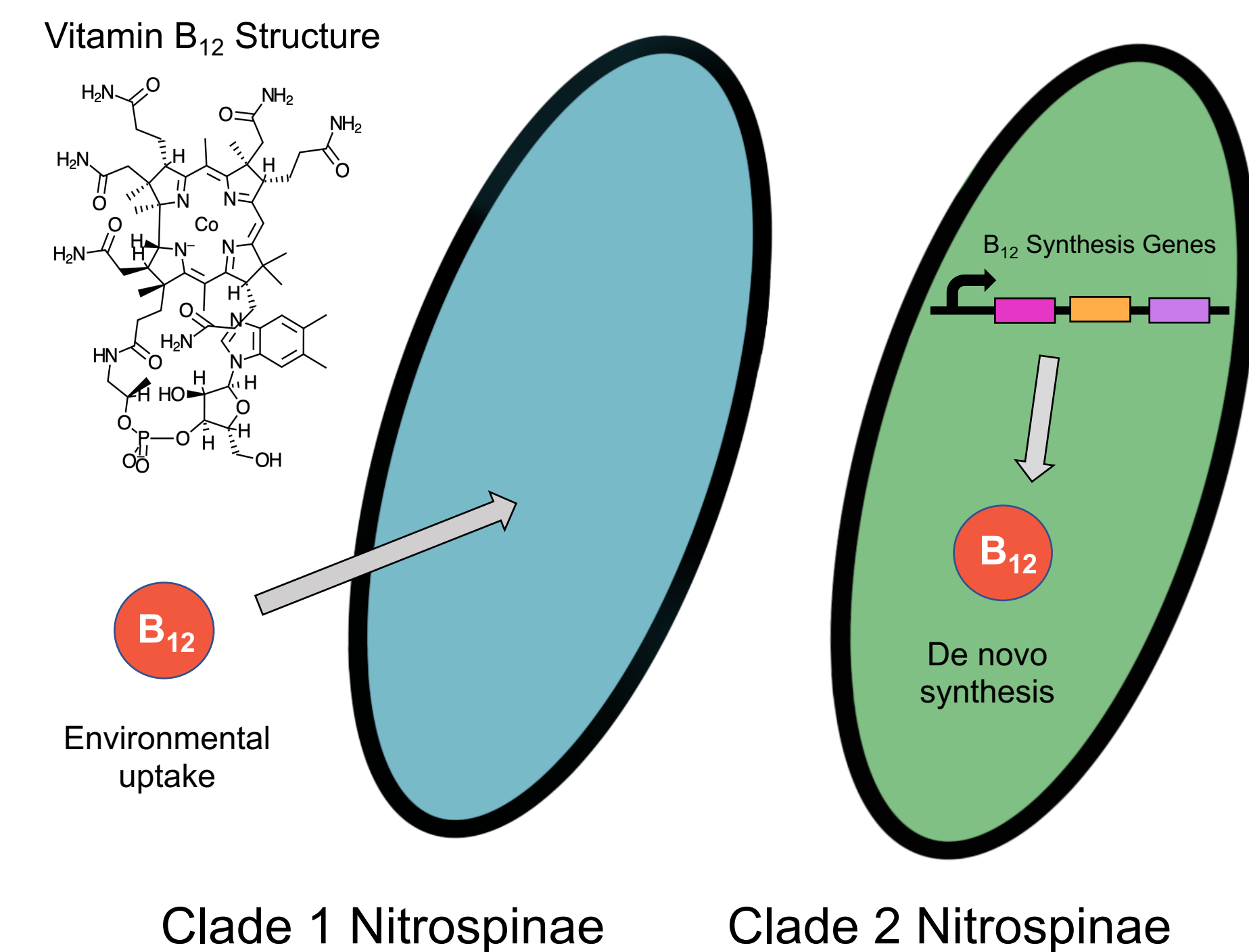


Figure 3. Vitamin B₁₂ dependencies.

Depiction of vitamin B₁₂ uptake in Clade 1 and de novo vitamin B₁₂ synthesis in Clade 2 Nitrospinae bacteria.

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