

A divergent bacterium lives in association with bacterivorous protists in the ocean

Marine protists abound, but are challenging to study, and their interactions with other microbes in nature remain largely unknown. We captured wild predatory protists (choanoflagellates) and discovered a divergent, obligately co-associated bacterial group that lives by extracting resources from these predators.

This is a summary of:

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The mission

Cell-to-cell interactions are fundamental to many evolutionary and ecological processes. The activities and specific interactions of ocean protists (unicellular eukaryotes) with viruses, prokaryotes and other microbial eukaryotes are largely unknown, despite the key position of protists in marine food webs¹. Thus, the explicit roles of protists are rarely incorporated into global carbon cycle and climate models or other major microbial oceanography conceptual frameworks. We aimed to reduce this knowledge gap by developing and implementing methods for recovering protists in their native state (that is, maintaining their physical associations with other entities) while circumventing biases inherent to mainstream approaches.

The discovery

The two most commonly used methods for examining ocean microbial communities are ill-suited to exploring natural physical interactions among microbes. Filtering seawater and sequencing all the DNA retained on the filter enables one to determine microbial community composition, but cell-to-cell interactions are lost. Similarly, cultures of seawater samples typically lead to major shifts in the microbial community because natural conditions cannot be simulated and, therefore, unique lineages are often eliminated owing to overgrowth by protistan and prokaryotic 'weeds' (species that thrive under laboratory conditions). Moreover, for many non-photosynthetic, predatory protists, identifying their natural prey or other crucial biological factors required for growth has been so challenging that most protistan predators remain uncultured¹.

By pursuing approaches that circumvent culturing and keep delicate protists and interacting partners together (including fluorescence-activated cell sorting (FACS) at sea, staining and sequencing methods), our team has gained unprecedented insights into protistan ecology and evolution, and the giant viruses of marine protists^{2–4}. Here, we single-cell sorted using FACS and analysed predatory protists from the Pacific Ocean together with any physically co-associated microbes. We discovered a clade of divergent gamma-proteobacteria that belongs to the enigmatic bacterial lineage UBA7916, which was first identified in 'omics' data⁵ and comprises purely marine, uncultivated taxa (Fig. 1).

We named the new bacterium Comchoano, as it co-associated with choanoflagellates, unicellular relatives of animals that thrive as predators of other microbes in aquatic ecosystems. Comchoano has hallmarks of an obligately host-dependent lifestyle, including signatures of possibly parasitic or pathogenic

behaviour. For example, it lacks amino acid synthases, pathways for vitamin B synthesis and steps in glycolysis. It also presents a secretion system that is used by several known pathogenic bacteria to inject effector proteins into their animal hosts. The choanoflagellate host in which we detected Comchoano, *Bicostaminor*, is found in many ocean environments, but both *B. minor* and Comchoano remain uncultured. With complete Comchoano genomes in hand, the field is now positioned to unravel key features of their co-association, and by extension co-associations of the broader UBA7916 lineage – whose members may remain uncultured because a host-associated lifestyle has never before been considered.

The implications

Our discovery reshapes how we think about the flow of energy and carbon in marine food webs. Predatory protists are considered a key link between small algae and bacteria and higher trophic levels – moving material up the food chain while also stimulating free-living prokaryotes with 'sloppy feeding' (the release of dissolved and particulate organic matter). Comchoano's residence with a predatory protist recasts the traditional view, whereby rather than being digested by the choanoflagellate, the bacterium consumes resources from the predator, redirecting energy flow.

Depending on environmental conditions, resource use by Comchoano could manifest as acquisition of host material that has little impact on the choanoflagellate host, or steer towards more detrimental parasitism or pathogenicity. Quantifying these effects, and the usages of a secretion system that is rare in cultivated marine bacteria, will require considerable research.

The discovery of Comchoano, a relative of the animal pathogen *Coxiella burnetii*, emphasizes that culturing 'unculturable' microbes requires the consideration of interconnectedness – a factor that is increasingly appreciated in microbiota or holobiont studies. It also highlights aspects of choanoflagellate biology in the single-cell state, providing a mechanism for enhanced local concentrations of bacterial compounds that can trigger host transition to multicellularity. Our study provides a springboard for investigating the biology of the widespread UBA7916 lineage – exposing direct dependencies on eukaryotes and unexpected biogeochemical influences. Moreover, the marine protists themselves were not known to have co-associates, revealing dimensions of their ecology as yet unknown.

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EXPERT OPINION

This paper is a fantastic example of technology-enabled discovery: the novel association was discovered by FACS and sequencing *B. minor* from open ocean water samples. The proposed associate, called

Comchoano, is most similar to the human pathogen *C. burnetii*, and evidence for the association was discovered in samples from across different ocean basins.” Samantha Joye, University of Georgia, Athens, GA, USA.

FIGURE

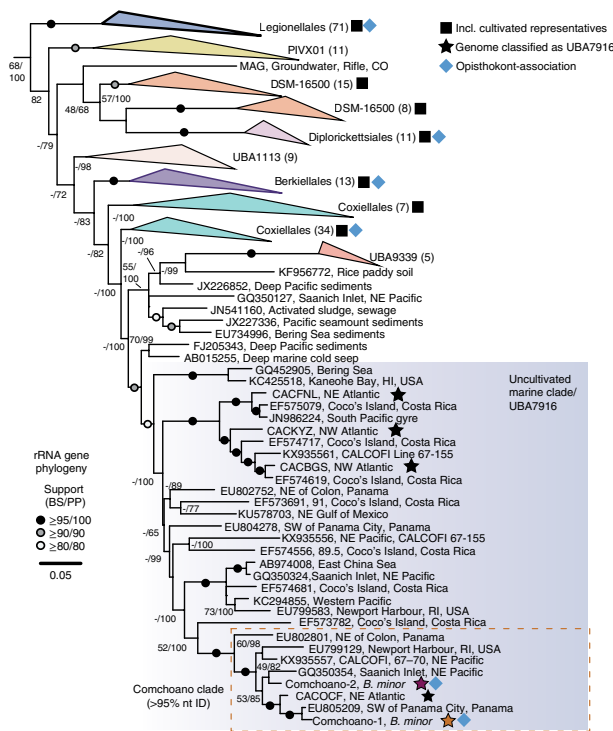


Fig. 1 | The Comchoano bacterial lineage. Phylogenetic relationships from a 16S rRNA gene maximum likelihood reconstruction. Stars indicate bacteria from metagenome-assembled genomes (MAGs) (black); partial MAGs obtained from other studies) and complete Comchoano genomes (colours) assembled from single-cell sorted protists (the choanoflagellate *B. minor*). Note that both choanoflagellates and animals belong to the Opisthokont supergroup. BS, bootstrap support; PP, posterior probability; 0.05, nucleotide (nt) evolutionary distance. © 2022, Needham, D. M. et al., CC BY 4.0.

BEHIND THE PAPER

In 2011, four of us from different research backgrounds (Patrick Keeling, Thomas Richards, Alyson Santoro and A.Z.W.) agreed that innovation was urgently needed to describe uncultivated marine protists and to identify and characterize co-associations involving these cells. Protists were often being overlooked in the microbial ‘omics’ world owing to challenges working with them. With funding friendly to ‘high-risk’ research in place by 2013, we began to unveil a host of interactions and evolutionary information on long-mysterious

uncultivated protists. The supportive, empathetic environment created by the team was important, especially given experimental complications — and several of us having young children. At each Eureka moment, unique constructive criticism(s) advanced analyses and ecological and evolutionary integration. We were determined to capture cells in their native state, even during seasons that were ‘rough’ (when sea states were uncooperative) but particularly important for ocean productivity and overall carbon cycling. **A.Z.W.**

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This article implements genome-based phylogenetics to identify and classify multiple unique uncultivated lineages of bacteria.

FROM THE EDITOR

From the human gut to unicellular plankton, the myriad symbiotic interactions between a host organism and its microbiota are essential. But what if the host is a bacterivorous predator that could also eat its own microbiota? The microbial association revealed by this work stands out because it reverses the traditionally accepted flow of resources in microbial predator–prey relationships, showing that a reimagining of marine food web structure might be necessary.” Kyle Frischkorn, Senior Editor, Nature Microbiology.