

# Uncovering picoplankton biodiversity by sequencing of environmental rRNA genes

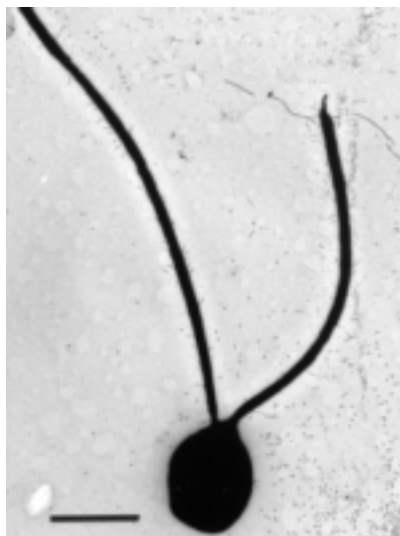
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*Picoplankton, small oceanic organisms that play a major role in the world's primary photosynthetic production, are not well characterized. This article describes the sequencing of marker genes, performed by QIAGEN Genomics Sequencing Services, as an efficient tool to examine the biodiversity of picoplankton.*

Picoplankton



**Figure 1** *Pseudoscurfieldia marina*, one species of eukaryotic marine picoplankton. The bar represents 1  $\mu$ m.

Phytoplankton, microscopic unicellular algae found in the ocean, dominate the world's photosynthetic primary production. However, large areas of the open oceans are oligotrophic, i.e., lacking in nutrients, such as nitrogen, phosphorus, or iron, that are essential for algal growth. A morphological adaptation of the phytoplankton in such waters is a reduction in size, with the corresponding increase in the cell surface-to-mass ratio permitting easier uptake of nutrients. Indeed, in many oceanic regions small phytoplankton, 3  $\mu$ m or less in size and called picoplankton (Figure 1), contribute up to 80% of the total chlorophyll (1, 2).

Despite their obvious importance, picoplankton have not been extensively studied until recently. Most picoplankton species are difficult to culture and, due to their small size, often lack characteristic features that can be distinguished by light microscopy. Molecular techniques such as the sequencing of marker genes or FISH (fluorescence in situ hybridization) can overcome these problems and help in the determination of picoplankton biodiversity. Recently published data based on molecular studies confirmed the presence of new eukaryotic groups in the picoplankton (3, 4, 5).

The aim of the European Union PICODIV project† is to analyze the biodiversity and abundance of picoplankton at various sites throughout the year. For these studies, molecular approaches are combined with "classical" methods such as light/electron microscopy, culturing, and pigment analysis. Here we

describe the large-scale sequencing of environmental rRNA genes by QIAGEN Genomics Sequencing Services as an efficient way to examine the biodiversity of picoplankton.

## Materials and methods

Samples were collected on a monthly or bimonthly basis at three sites in European coastal waters (Roscoff, France; Barcelona/Blanes Bay, Spain; and Helgoland, Germany). 5–10 liters of seawater was filtered through 3  $\mu$ m filters, and this filtrate was then passed through 0.2  $\mu$ m filters.

Environmental DNA was isolated from the 0.2  $\mu$ m filters, and complete 18S and 16S rRNA genes were amplified by PCR using universal primers. PCR products were purified by agarose gel electrophoresis or using the QIAquick® PCR Purification Kit. Purified PCR products were cloned into a TA vector, generating at least 500 clones per sample. Clones were assessed by restriction fragment length polymorphism (RFLP) analysis of either reamplified inserts or of plasmid DNA isolated using QIAprep® columns. Unique clones (as judged by RFLP analysis) were sequenced by QIAGEN Genomics Sequencing Services using an internal and flanking primers.

Sequences provided by QIAGEN Genomics Sequencing Services were compared to each other (to sort out redundant clones) and to 18S/16S rRNA gene databanks (RDP, Genbank). Such comparisons allow a rough classification of sequences at the class or

† [www.sb-roscoff.fr/Phyto/PICODIV/index.html](http://www.sb-roscoff.fr/Phyto/PICODIV/index.html)

genus level and the identification of known species. Sequences were then placed into a universal tree using the ARB program ([www.arb-home.de](http://www.arb-home.de)).

**Results**

Eukaryotic clone libraries were established and sequenced for 2 sites (Roscoff and Helgoland) over a full year. In total, about 1000 clones were partially sequenced, resulting in approximately 500 different sequences. The most predominant algal groups detected were Prasinophyceae, Bolidophyceae, Cryptophyta, Chrysophyceae, and Chlorarachniophyta. The first two groups were present in all libraries. Sequences for many heterotrophic eukaryotes (e.g., Alveolates, Stramenopiles, and Ciliates) were also recovered (Figure 2). The majority of sequences analyzed were previously unknown, potentially indicating the existence of new species of picoplankton.

**Conclusions**

- ◆ Large-scale sequencing of environmental rRNA gene clones is an efficient tool for uncovering the biodiversity of marine

picoplankton. The picoplankton community in coastal waters contains an unexpected degree of biodiversity, with most algal classes represented in this group of organisms. Most of the picoplankton species still await description.

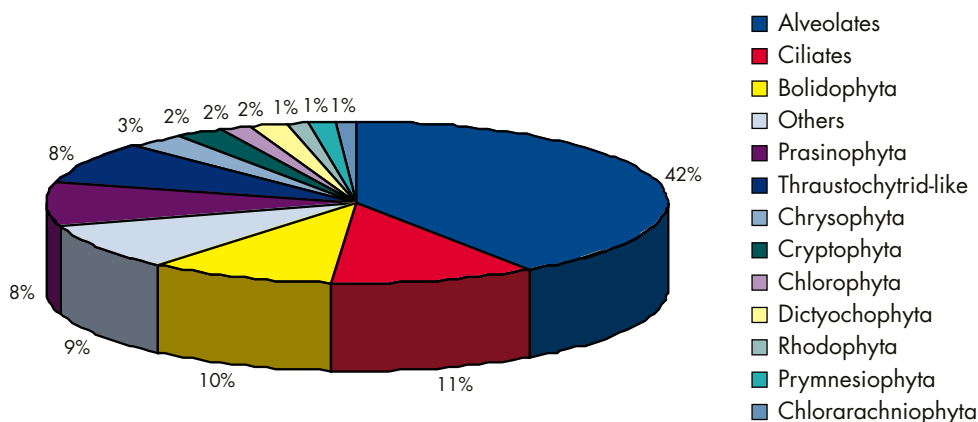
- ◆ Subcontracting the time-consuming sequencing efforts to QIAGEN Genomics Sequencing Services proved to be very cost-effective since it allowed the rapid and accurate acquisition of a large number of sequences.

**Acknowledgements**

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**Diversity of Picoplankton Phyla Found at the Helgoland Site**



**Figure 2** Algal groups identified by sequencing of rRNA genes from picoplankton collected from the Helgoland site throughout 2000.

For information on QIAGEN Genomics Sequencing Services, please call QIAGEN Technical Services.

**References**

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