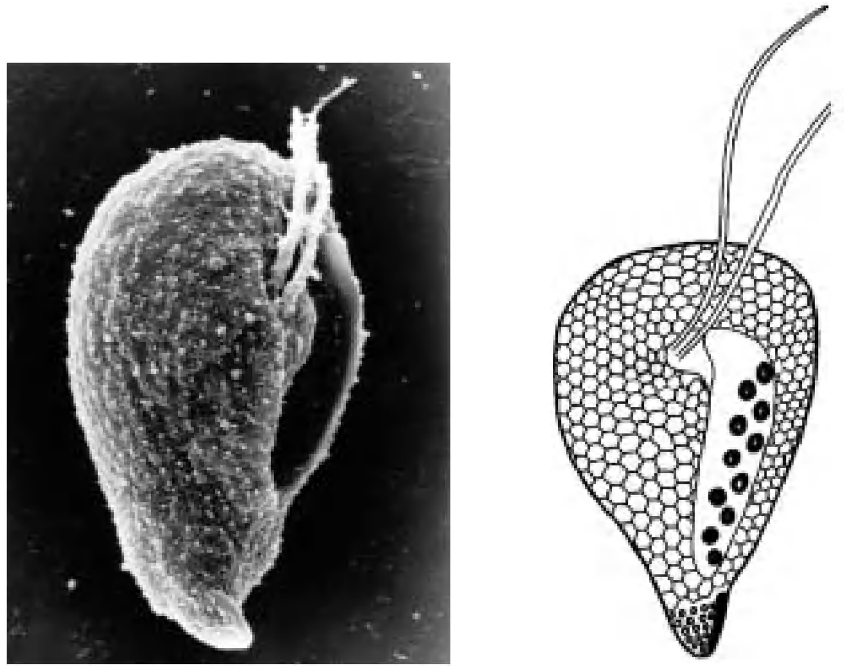
New Species

Cryptomonad flagellates

By Gianfranco Novarino

FREE-LIVING FLAGELLATE PROTISTS (flagellates for short) are arguably the most abundant and widespread eukaryotic microorganisms in the biosphere. As a group, flagellates are highly polyphyletic – they are defined simply by the possession of one or more flagella used for locomotion or feeding, or both. This "flagellate condition" has been a huge evolutionary success as shown by the presence of flagellate cells in the great majority of protistan, protozoological and algal phyla, as well as in many multicellular organisms – including ourselves.



Plagioselmis species inedita Novarino 2005 from the Mediterranean Sea.

Marine planktonic flagellates, especially the nanoplankton-sized (1-20μm) fraction, contribute significantly to marine primary production or carbon consumption, or both, and therefore play pivotal and intricate roles in the functioning of the marine ecosystem. Their diversity is far from having been described at both morphological and molecular levels. Improving our knowledge of nanoplankton diversity is a scientific imperative if we are to address such questions as the relationships between morphological, molecular, physio-ecological and geographical diversity, the occurrence of cryptic speciation, and ultimately the very nature and mechanisms of speciation and evolution in these organisms. These and other questions are being addressed in MarBEF within the MARPLAN Responsive Mode Project.

Cryptomonad flagellates, which are very abundant and widespread in the sea, are a moderately species-rich group (200 known species, possibly 300 to 1,200 species in total) on morphological grounds. Undescribed morphospecies of the genera *Cryptochloris*, *Hemiselmis* and *Plagioselmis* [see box] were found recently during ongoing investigations of nanoplankton diversity in the western Mediterranean. Despite being new, they have not been formally described, which may appear surprising but is becoming quite commonplace when dealing with protists. Previously undescribed species found in chemically-fixed water samples are often christened informally as species ineditae until they can be isolated in pure laboratory culture for further analysis, especially molecular sequencing.

All species ineditae of cryptomonads were characterised using field-emission scanning electron microscopy. This revealed several ultrastructural features of taxonomic significance, especially the architecture of the cell surface (periplast) and the features of the vestibular region of the cell from which the flagella arise. *Plagioselmis* species inedita is particularly interesting owing to the superficial similarity to other cryptomonads – especially *Teleaulax* Hill – in terms of cell shape and pigmentation. It is a pear-shaped flagellate, slightly compressed along the perlateral axis, 6-9 x 4-5.5 x 3-4 μm in size, with equal or slightly unequal flagella inserted to the right-hand side of a broad ventral furrow reaching the median part of the cell. Its periplast allows it to be identified as a true member of its genus. Although the hexagonal plates on the cell body are very small (0.15-0.25μm) and barely perceptible, there is a non-plated periplast with a very short ventral band on the pointed cell posterior, as expected for a true member of *Plagioselmis*.

The Mediterranean species inedita brings the count of *Plagioselmis* morphospecies to five. The relationships between morphological, molecular, physio-ecological and geographical diversity within this genus are still unknown but these new discoveries add to the ongoing debate on the applicability of general biogeographical concepts to the protists.

The full names of these genera are *Cryptochloris* Schiller non Bentham non Shortridge & Carter, *Hemiselmis* Parke and *Plagioselmis* Butcher ex Novarino Lucas & Morrall.

Cryptomonad nomenclature is "ambiregnal" because it falls under the dual jurisdiction of the Botanical and Zoological Codes. The long string of names constituting the authority reflects the ambiregnal history of the generic name.

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Genetic Diversity

Macrophytes as models

The emerging role of genetic diversity for ecosystem functioning

By Thorsten B.H. Reusch

DURING THE PAST decade, manipulative experiments have provided compelling evidence of the importance of biological diversity for ecosystem functioning and stability. Diversity at the species and functional-group level begets productivity, resistance and resilience in the face of disturbance and stability with respect to temporal fluctuations (overview in Loreau *et al*, 2002).

However, the emerging consensus is still incomplete. The majority of these studies have been performed in grassland communities or experimental micro- and mesocosms, while marine systems are clearly understudied. Moreover, a number of aquatic communities seem not to fall under the 'species diversity is good' consensus. What could be more uniform than endless meadows of reed, saltmarshes or seagrasses that are locally dominated by a canopy of a single species? Yet these macrophyte-based communities represent some of the most productive and stable shallow-water and estuarine ecosystems. Seagrasses in particular, a phylogenetically diverse group of 60 or so marine angiosperms, often form meadows that consist of only a few or even one species. Their low taxonomic diversity is in striking contrast to their ecological importance (Duarte, 2002). As foundation or ecosystem engineering species, seagrasses allow a rich invertebrate and fish community to thrive on otherwise barren sedimentary coastlines. Moreover, seagrass beds fix nutrients, stabilise the sediment and prevent coastal erosion. It has been estimated that the ecosystem services provided by macrophyte stands are among the highest for all ecosystems compared (Costanza *et al*, 1997).

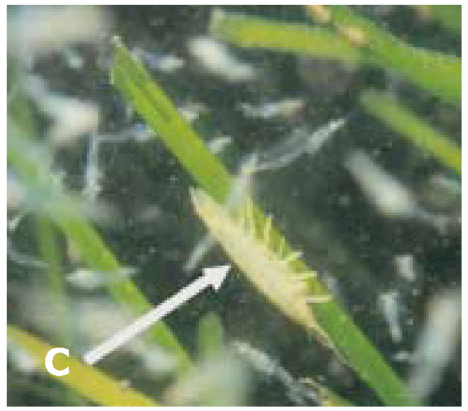
The apparent uniformity of a seagrass canopy disappears when viewed through the eyes of molecular genetics. Modern molecular markers, in particular DNA microsatellites (see Box 1), tell a completely different tale, revealing hidden genotypic diversity resulting from the complex pattern of clonal reproduction of these plants (Fig 1, page 16). In many aquatic plants, including seagrasses, genetically identical shoots of a genotype proliferate in space and form clones of various sizes and spatial arrangement. The so-called 'genets' (clones) may comprise several thousands of morphological individuals, or 'ramets' that may be hundreds of years old.

Given striking differences in clonal diversity between seagrass populations on one hand, and the experimental evidence on the importance of species diversity on the other, marine ecologists have begun to address the role of genetic diversity for ecosystem functioning. Recent experiments using the widespread seagrass species *Zostera marina* (eelgrass) indicate that genotypic (or clonal) diversity may replace the function of species diversity in ecosystems with few or a single foundation species. In an experiment in California by Hughes and Stachowicz (2004), mixtures of 1, 2, 4 and 8 eelgrass genotypes were planted into the intertidal zone of Bodega Bay. Diverse treatments colonised the area more rapidly, and recovered significantly faster, than monoculture plots after nearly destructive grazing by migrating Brant Geese *(Branta bernicla)*. This effect, however, disappeared a few months after disturbance. In the southwestern Baltic (Germany), Reusch and colleagues combined six genotypes into diversity treatments of 1, 3 and 6 genotypes (Reusch *et al*, 2005). Their experiment coincided with a period of extreme water temperatures caused by the unparalleled heatwave that hit Europe in 2003 (Schär *et al*, 2004). These conditions can be viewed as a precursor for the predicted global warming in the coming decades. Water temperatures attained 25°C in the southwestern Baltic, leading to mortality among cold-temperate plant and animal species, including eelgrass. Similar to the response in the Californian experiment, the more diverse assemblages of seagrass genotypes showed faster recovery after the summer mortality. Moreover, there were more shoots, biomass and associated invertebrates in diverse plots at the end of the experiment. These findings should provide an additional motivation for conservation and restoration biology to protect and maintain genetic resources in addition to the diversity of species and functional groups.

Since Reusch and co-workers also transplanted all genotypes tested in mixtures as monocultures, the underlying mechanism of the positive biodiversity effect could be assessed (Reusch *et al*, 2005). Surprisingly, the enhancement of recovery and biomass at the end of the experiment were not due to a few dominant genotypes that happened to be the most resistant given the extraordinary conditions during the 2003 heatwave (the

Edge of an eelgrass *(Zostera marina)* bed in the southwestern Baltic on a sandy sediment in 3m depth.

Green crab *(Carcinus maenas)* feeding on juvenile blue mussels settling on eelgrass blades.



Mysiid shrimps (background) and isopod (*Idothea balthica,* white arrow) grazing on epiphytes of eelgrass leaves during a night dive.

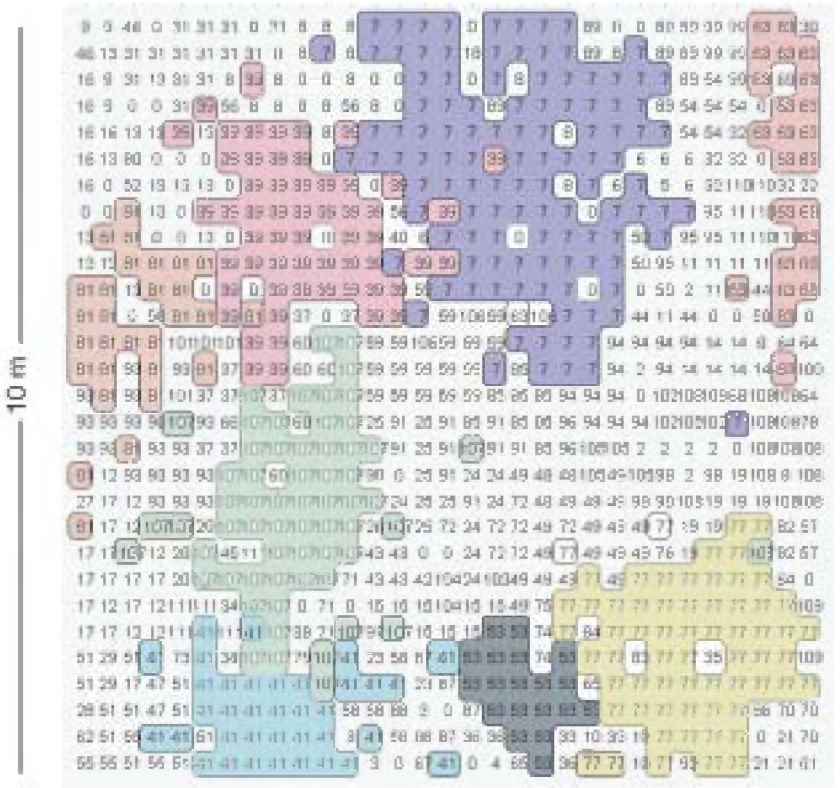


Figure 1: High-resolution clonal map obtained through non-destructive genotyping of eelgrass shoots at 31x31 grid intersections (distance 33.3cm). Genotyping was done based on the length polymorphism displayed by microsatellite markers specifically designed for eelgrass. The eight largest clones are marked by different colours.

'selection' or 'sampling' effect, see Box 2). On the contrary, the best eelgrass genotype in monoculture performed badly in mixtures, whereas all 'weak' performers in monoculture were enhanced in their performance in diverse treatments. The latter finding supports a true biodiversity effect, or complementarity, to be responsible for the enhanced performance of diverse treatments (see Box 2).

Many questions remain open. Firstly, experiments using different macrophyte species are urgently needed to assess the generality of the effects identified in *Zostera marina*. Secondly, does genotypic diversity only play a role under stress or disturbance, as the studies in eelgrass beds suggest? Experiments are underway in which the performance of test populations with low and high genotypic diversity are tested in heat-stressed mesocosms versus non-stressed ones. If genotypic diversity has a positive effect only under stress, this will manifest itself through a 'genetic diversity x temperature' interaction.

As a first step, the above experiments only addressed the level of genotypic (or clonal) diversity as a subset of the total genetic diversity using a clonally reproducing organism as a model. How to define genetic diversity among a collection of unique genotypes, for example in exclusively sexually reproducing organisms, is an open and much-debated question in conservation ecology and population genetics. It is clear that the diversity displayed by neutral genetic markers, the focus of the overwhelming number of studies conducted thus far, is only weakly linked to selectively-relevant genetic diversity. What is needed is a novel generation of genetic markers that measure diversity directly at selectively relevant traits (van Tienderen *et al*, 2002). Probably, an *a priori* decision would have to be made as to which traits are relevant under a given ecological challenge that should be assessed and manipulated.

In conclusion, recent experiments allow for a generalisation of ecological theory, because the effects of genotypic and species diversity on ecosystem functioning appear analogous. The further development of molecular tools will soon allow an integrative experimental approach to address consequences of biological diversity at the genotypic, genetic (*sensu strictu*) and species level.

DNA microsatellites as genetic markers

DNA microsatellites (synonymous to short tandem repeats STR or simple sequence repeats SSR) are sites on the DNA consisting of small motifs (2-6 nucleotides) that are repeated >10 times, for example GAGAGAGAGAGAGAGA GAGA. Due to their rapid stepwise mutation rates, many microsatellites display several length variants within populations. This polymorphism can be utilised to distinguish individuals or to assess relatedness, mating patterns and differentiation between populations.

Decomposition of biodiversity effects into complementarity and selection

Positive biodiversity effects may arise from two different processes. As a statistically-inevitable consequence, diverse communities are expected to comprise the best performing species (or genotype = clone) within their mixture that may dominate the total response at termination. Whether or not such a 'sampling' (or selection) effect is causally related to biodiversity *per se* is controversial. On the other hand, true biodiversity effects arise when the average performance of species is enhanced by the presence of other species (or genotypes), for example through facilitation or niche differentiation. Loreau and Hector (2001) developed a statistical procedure that allows a decomposition of the net biodiversity effect into complementarity and selection effects. Based on appropriate experimental designs, the relative roles of complementarity versus selection can be disentangled provided that all species tested in mixtures are also present as experimental monocultures. The procedure can also be adapted for genotypic instead of species diversity (Reusch *et al*, 2005).

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Functional Diversity

The influence of the lugworm *(Arenicola marina)* on biodiversity and ecosystem functioning in an intertidal mudflat

By James Saunders, Kirstie Dyson David Paterson

Intertidal mudflat on the German island of Sylt, February 2005.

ON THE GERMAN island of Sylt, just south of the Danish North Sea coast, researchers at the Alfred Wiener Institute established an experiment to examine the influence of *Arenicola marina* upon an intertidal mudflat.

Six 20m x 20m exclusion plots were created (three each at high and low tide) by burying a fine mesh 10cm deep into the sediment, preventing *A. marina* from burrowing or establishing the U-shaped tubes in which it lives.

Excluding *A. marina* allowed observations on how its presence influenced the abundance and distribution of other intertidal species and what effect this had on the physical nature and functioning of the system.

The first stage of the experiment was completed in February and March 2005 (supported partly by MarBEF funds) where the sites were studied under winter conditions. Macrofaunal and microphytobenthic communities were sampled from each plot and the adjacent control plots without mesh. Measurements of ecosystem functioning and environmental factors included nutrient production, algal content and photosynthetic production, sediment grain size, water content and sediment stability.

During the field campaign the mudflat was subjected to extremely cold conditions and was regularly covered in ice. With biofilms present on the sediment surface underneath the ice sheets, measurements of primary productivity and biomass were taken.

Macrofauna abundance was greater on the low shore than high shore but biodiversity was low at all sites as it was dominated by the gastropod *Hydrobia ulvae*. The exclusion of *A. marina* resulted in a small increase in biodiversity due to increasing numbers of smaller worms such as the polychaete *Nereis diversicolor*, oligochaetes and *Spionidea* species.

Sediment stability was low at all sites. However, high-tide sites were slightly more stable than low-tide, although this may have been a result of larger sediment particles in these sites. The exclusion of *A. marina* from high- and low-tide sites did not affect the sediment stability.

Analysis of the winter samples is still continuing and a return visit in the summer of 2005 will allow comparisons of winter and summer conditions.

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Production readings under the ice.

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