ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2009 Vol. 52, Št. 2: 33–40

Diatoms: Their strange evolution and remarkable properties

Kremenaste alge: Njihov nenavadni razvoj in izjemne lastnosti

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Abstract: We review some new literature on diatoms, with emphasis on genomics, evolution, ecology and biomimetic nanotechnical applications. Diatoms account for a substantial part of the photosynthetic production on this planet, and their genome is a mosaic of contributions from different sources. They occupy very diverse ecological niches, and may have been the first organisms to carry out C4 photosynthesis. Their frustrules (silica enclosures) with their elaborate sculpturing make it possible to follow the occurence of different forms back in time, and the frustrules is also the main reason that they are interesting for biotechnology.

Keywords: C4 photosynthesis, chloroplasts, diatoms, dynamite, endosymbiosis, nanotechnology, omega-3 fatty acid, silica

Izvleček: Prispevek je pregled novih virov o kremenastih algah s povdarkom na genomiki, evoluciji, ekologiji ter biomimetični nanotehnološki aplikaciji. Kremenaste alge prispevajo velik delež k fotosintezni produkciji našega planeta. Njihov genom je mozaik elementov različnega izvora. Zasedajo različne ekološke niše, in verjetno so bile prvi organizmi s C4 način fotosinteze. Njihove frustule (silikatni ovoji) z izdelanimi raznolikimi vzorci omogočajo sledenje različnih oblik v zgodovini in prav frustule so tiste, zaradi katerih so kremenaste alge zanimive za biotehnologe.

Ključne besede: C4 fotosinteza, kloroplasti, kremenaste alge, dinamit, endosimbioza, nanotehologija, omega-3 maščobne kisline, silicij

Introduction

Diatoms are photosynthetic, unicellular organisms. In some species several cells remain attached in colonies, but without any differentiation or division of functions between cells (see Hayakawa *& al.* 1994). Diatoms belong to the so-called heterokonts which, together with oomycetes and others form the stramenopiles. Brown algae are among the most well-known close relatives of diatoms, and both groups have fucoxanthin as an accessory photosynthetic pigment.

Diatoms form one of the most successful groups of organisms on our planet. They are present in most niches of the biosphere where there is, at least from time to time, some water: in seas, lakes and stream water, hot springs (up to 50°C), salty brines up to saturated concentration, dry rock and stone walls, desert surface crusts, in the surface layer of other soils, and as symbionts inside dinoflagellates and foraminifers. Some diatoms harbour cyanobacteria as endosymbionts.

The diatoms of the sea are the most important ones in a global perspective. Marine dinoflagellates produce about 40 percent of the biomass in the sea, and for sea and continents combined they produce about 20 percent of the biomass and the oxygen. Experts do not agree on the number of diatom species. Twenty-five thousand species have been described (Alverson 2008), but some of them have been shown to be different forms of the same species. Certainly there are more than 15 thousand species; Mann & Droop 1996 say 200 thousand, and both Drum & Gordon 2003 and Sterrenburg & *al*. 2007 give a range of one hundred thousand to one million. The upper limit of this interval appears unrealistic. A discussion is going on about how the species concept should be defined for diatoms, since it will be impossible to carry out mating experiments except in a few cases.

The first diatoms probably appeared on land about 280 million years ago, but the oldest unquestionable fossils date from early Cretaceous, 120 million years ago. There is reason to believe in a radiation into different evolutionary lines between 160 and 150 million years ago. The first diatoms were »centric«, i.e. had radial symmetry (Figure 1), and the elongated and bisymmetric »pennate« forms (Figure 2) arose about 125 million years ago. It is thought that diatoms (as well as dinoflagellates) were favoured by the great extinction that marks the end of the Cretaceous, 65.5 million years ago, as this was a catastroph not only for the dinosaurs, but also for coccolithophores and silicoflagellates, competitors of the marine diatoms. Diatoms and dinoflagellates could survive, probably thanks to their ability to form resistant resting cells. During their whole evolution the diatoms have also been favoured by the expanding terrestrial vegetation, which, because its roots and mycorrhiza have been expanding ever

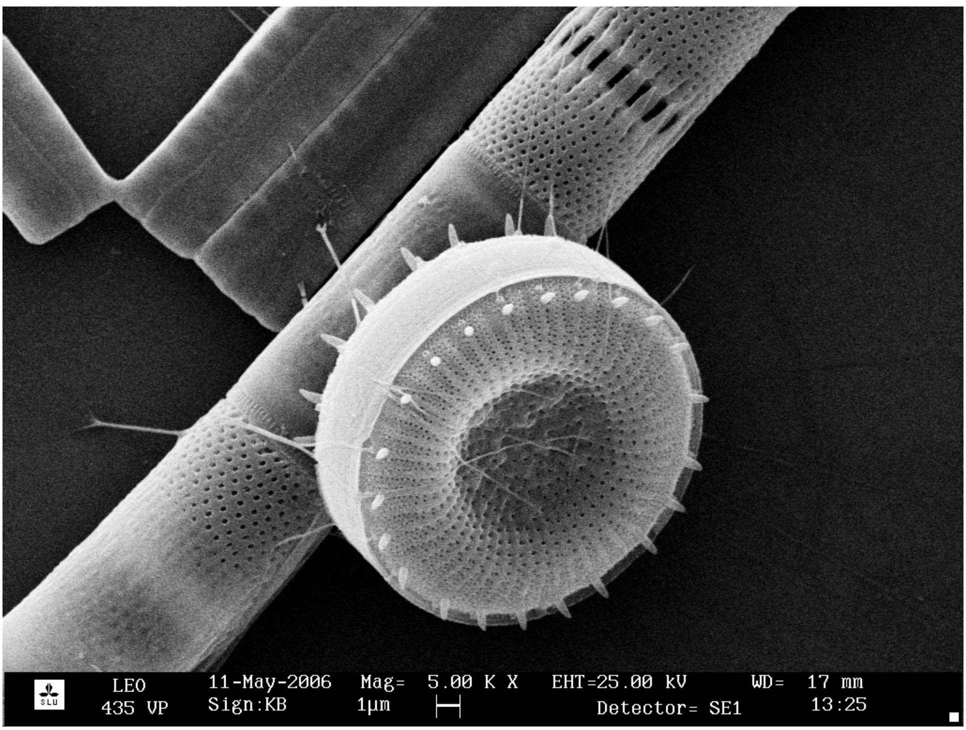

Fig. 1: Centric diatoms, *Cyclostephanus dubius* in the foreground and *Aulacoseira sp.* in the background (scanning electron microscope image by Gertrud Cronberg).

Slika 1: Kremenasti algi iz reda *Centrales*; vrsta *Cyclostephanus dubius* spredaj in predstavnica iz rodu *Aulacoseira sp.* v ozadju (foto: Gertrud Cronberg).

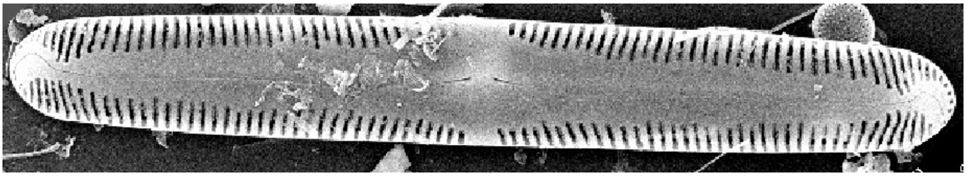

Fig. 2: Pennate diatom (*Pinnularia sp.*) (scanning electron microscope image by Gertrud Cronberg).
Slika 2: Kremenasta alga iz reda *Pennales* (*Pinnularia sp.*) (foto: Gertrud Cronberg).

wider and penetrating ever deeper, has contributed to increased weathering and transport of silicic acid to fresh waters and to the sea.

Peculiar genomes

Genomes have now been studied for both centric and pennate diatoms, and several strange circumstances have recently been brought to light. But let us begin what has been known for several years. Diatom chloroplasts, as most other chloroplasts with the exception of those of green algae and plants, derive from a red alga which has entered into an intimate symbiosis with an originally non-photosynthetic organism. This »secondary endosymbiotic event« is thought to have happened about a billion years ago, the chloroplasts of this and other red algae, in turn, being originally derived from a cyanobacterium (blue-green alga) which entered into »primary endosymbiosis« with a non-photosynthetic organism more than 1.2 billion years ago. But the recent analysis of diatom genes reveal that things are much more complicated than this.

Most of the genes originally present in the »engulfed« blue-green and red algae are not left in the chloroplast of the diatom, but have either disappeared or been transferred to the nucleus. This is because some of them were no longer needed, since similar genes were present in the nucleus of the original, non-photosynthetic organism. The genes which were still needed, for instance those necessary for photosynthesis, had better leave the chloroplast, because this is a dangerous place for DNA, as it has high concentrations of radicals and oxygen. And so they did, by the process of natural selection. Another »reason« to leave the chloroplast was that chloroplast genes cannot benefit from the advantages of sexual reproduction (a tricky question discussed by many, and recently in Nick Lane's wonderful book »Life ascending«). A few genes were left in the chloroplast, because some functions related to photosynthesis require very rapid regulation of gene activity by signalling pathways originating in the photosynthetic apparatus.

The chloroplast origin of these genes that did move to to the nucleus is recognized because these genes code for chloroplast proteins. If all of these genes in a diatom had arrived with the engulfed red alga, they would show greater similarity to genes in red algae (the »red line of evolution«) than with genes in green algae and plants (the »green line of evolution«). This is most often the case. But in quite a few cases the similarity is greater to the »green line of evolution«, for reasons that are not currently understood. One theory is that the diatoms (or diatom ancestors) have first harboured a »green« chloroplast, which at a later time has been exchanged for the present one. Another theory is that »horizontal gene transfer« has taken place by infection by viruses or bacteria. The sea is teeming with viruses, and several of them are known to harbour photosynthesis genes, although in the known cases these genes are from cyanobacteria (e.g., Mann & *al.* 2003; Lindell & *al.* 2005; Hellweger 2009; Sharon & *al.* 2009). Horizontal gene transfer would have been a reasonable explanation if it had only been a few genes. But according to Moustafa & *al.* 2009 more than 1,700 »green« genes have been transferred, which is 17% of the whole complement of a little more than 10,000 genes. This high transfer frequency is so surprising that not all workers in the field have wholeheartedly accepted it (Dagan & Martin 2009).

But not only photosynthesis genes have been added by side-steps in the evolution of diatoms. In both centric and pennate diatoms Bowler & *al.* 2008 have found hundreds of genes from various prokaryotic organisms: cyanobacteria, various proteobacteria, and archaea. The more they are investigated, the more the diatoms appear as heaps of disconnected twigs from the great tree of evolution. Animals, fungi, and some microorganisms have, based on their molecular biology, been grouped together under the heading Opisthokonta, a rather thick branch on the tree of evolution. Scala et al. (2002) found as many genes in the diatom *Phaeodactylum tricornutum* being closely related to opisthokont genes as being related to genes in higher plants.

In contrast to chloroplasts of plants and green algae, which are surrounded by two membranes, there are four membranes around the chloroplasts of diatoms. This is understandable, as these chloroplasts have originated by two successive endosymbiotic events. Between the membranes one can in some cases find a »nucleomorph«, the remains of the nucleus of the engulfed red alga. The nucleomorph contains very few genes, but those that exist are typical chloroplast genes.

Motility, carbon assimilation, reproduction, and technical applications

One might think that diatoms, enclosed in glass jars as they are, would not be able to move actively. But for a long time people have studied how pennate diatoms can creep over a substratum, and now it is known that many centric diatoms possess this ability, too (Sato & Medlin 2006). Some diatoms leave a mucus track behind, as snails do, and it is thought that their movement is somehow connected to this slime exudation. The mucus exits through a slit called a raphe in the middle of one or both halves of the frustrule (silica enclosure). The slime is set in motion by microfibrils which inside the cell are connected to filaments of actin and myosin, the same kinds of protein molecules that we have in our muscles (Bertrand 2008). This view is, however, partly based on speculation, and slime trails are not to be seen after individuals of all species of motile diatoms. The movements are regulated by, among other things, various light-perceiving systems. Cells with a raphe can move with up to 25 $\mu m\ s^{-1}$, but some species without a raphe can also move, albeit at a slower speed, about 1 $\mu m\ s^{-1}$.

One might also imagine that it would be difficult for the armor-enclosed diatoms to take up carbon dioxide or bicarbonate ions for their photosynthesis, but they are, in fact, very efficient in doing this. The pretty perforations in the frustrules contribute to this, and some species are able to carry out both C3-photosynthesis with incorporation of carbon dioxide into 3-phosphonolglycerate, and C4-photosynthesis with incorporation of bicarbonate into oxaloacetate (review by Roberts & *al.* 2007). Contributing to the efficient uptake of inorganic carbon is also an exudation of extracellular carbonic anhydrase, enabling a rapid interconversion between carbon dioxide and bicarbonate. Diatoms are unusual in that they have a carbonic anhydrase which can use cadmium instead of zinc without decrease in activity (Strasdeit 2001; Xu & *al.* 2008), which is very useful as zinc is depleted from the ocean surface. Cadmium can often replace zink in enzymes, but usually with a large decrease or complete loss of activity.

The two halves of a diatom are of unequal size and form a box with lid (Figure 3). When cells divide, each daugher cell gets one half, and then forms a new half inside the existing one. Therefore one daughter cell will be of the same size as the original cell, while the other one will be smaller. With repeated divisions smaller and smaller cells will be created, and when a size of about one third of the original one has been reached meiosis takes place and sex cells (gametes) with half the number of chromosomes result. They can be of equal size, or half of them can be smaller and act as sperm cells, depending on the systematic position of the species. After fusion of gametes the resulting cell grows to the size we started the story with, and only then is a continuous silica enclosure produced again.

Diatoms have a long story of technical uses. The most well-known one from a Swedish perspective is as component in Alfred Nobel's dynamite, a mixture of diatom frustrules and glyceryl trinitrate (»nitroglycerine«). Diatom frustrules have also been used in toothpaste, but this use is declining since they are so hard that they damage the enamel of the teeth. By their ability to form patterns in silica (silicon dioxide) the diatoms have attracted interest in the field of nanotechnology (Bozarth & *al.* 2009). One is more interested in understanding

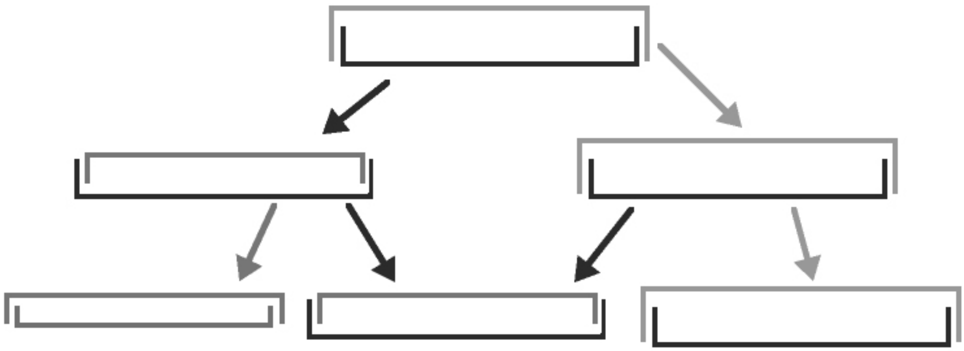

Fig. 3: The diagram shows repeated divisions of a diatom. The shades of the arrows indicate which of the two frustrule halves is transmitted to the daughter cell. The cells on the right maintain the original size, while those to the left become smaller and smaller for each division. When they have reached about one third of the original size, sex cells are produced and the silica cover discarded.

Slika 3: Digram prikazuje ponavljajoče se delitve kremenastih alg. Barve puščic nakazujejo kateri dve polovicise preneseta na hčerinsko celico. Celice na desni strani ohranjajo originalno velikost, medtem ko tiste na levi postajajo po vsaki delitvi manjše.

how diatoms manage to do this, and copy their methods, than to use diatoms as such (Drum & Gordon 2003; Gordon & *al*. 2009; Noyes & *al.* 2008). Nevertheless the frustrule of a diatom has been used instead of a more conventional lens for focusing a 100 µm wide laser beam to a spot of 10 µm (De Stefano & *al.* 2007; Figure 4).

Diatom frustrules can be regarded as photonic crystals, periodic structures with special properties with regard to light propagation (Fuhrmann & *al.* 2004).

The sculpturing of diatom frustrules is such that they combine mechanical strength with low weight (the density of the silica in the frustrules is ca. 2, i.e. about twice that of the surrounding water). The strength affords protection against predators, while the low weight is important in particular for planktonic species to avoid sedimentation out of the photic zone. The mechanical properties have been studied by Hamm & *al.* 2003.

For most diatoms silicon is an essential element, but an exception is afforded by *Phaeodactylum tricornutum* (Brzezinski & *al.* 1990). The silicon is in most cases taken up as $Si(OH)_4$ (Del Amo & Brzezinski 1999), but *Phaeodactylum tricornutum* absorbs it as the anion $SiO(OH)_3^-$. Uptake takes place only during two phases of the cell cycle, namely at the end of G1 and throughout G2 (Brzezinski 1992). The silicon concentration (≈ 1 m*M*) in the Archaean sea was orders of magnitude greater than that of the contemporary (Konhauser & *al.* 2007), and due to volcanic eruptions during the Triassic the sea probably became saturated with silicic acid during the Jurassic. This is likely to have favoured diatoms, and their flourishing in the sea eventually again decreased the concentration to such an extent that many sponges with silica skeletons died out (Maldonado & *al*. 1999). Silicon is now frequently limiting for diatom growth (e.g., Shipe & *al*. 2007). A large drop in silica availability seems to have taken place in the late Eocene, 35 Ma ago, in connection with opening of the Southern Ocean, increased stratification, and increased abundance of diatoms. This is reflected in the decreased silification at this time of radiolarians (Lazarus & *al*. 2009), which may have less efficient acquisition of silicate than diatoms do. Still, modern marine diatoms generally have less silica in their frustrules than freshwater diatoms do, reflecting the generally higher silicic acid content in freshwater (≈ 100 µ*M*) as compared to surface seawater (≈ 10 µ*M*) (Alverson 2007). It is estimated that the silica input to the ocean is 6.1±2.0 Tmol/year and the sedimentation 7.1±1.8 Tmol/year. Biogenic production is 240±40 Tmol/year, of which only a small fraction ends up as sediment. The residence time in surface waters is 400 years (Tréguer & al. 1995).

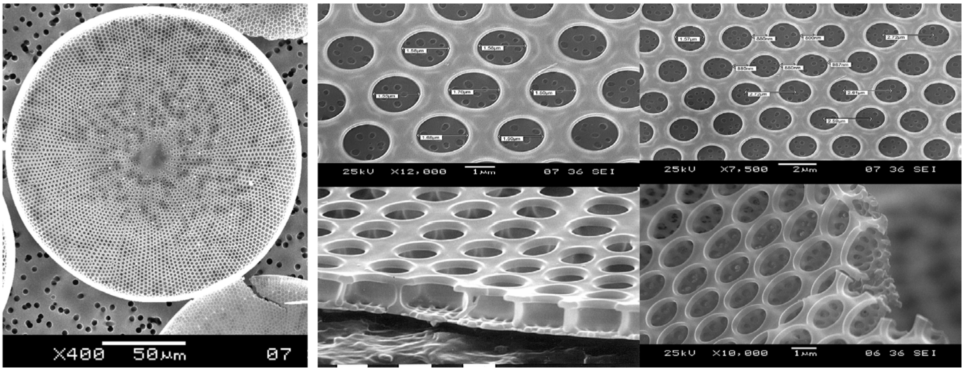

Fig. 4: Whole cell of the diatom *Coscinodiscus walesii*, used to focus laser light, and details of the regular perforations in the frustrule that cause the diffractive bending of the light. The data on width of the perforations indicated in some places are printed in a font too small to be read here, but range 1.5 to 1.7 µm, and the distances between them 800-987 nm. From De Stefano L., I. Rea, I. Rendina, M. De Stefano & L. Moretti 2007: Lensless light focusing with the centric marine diatom *Coscinodiscus walesii*. Optics Express **15**: 18082–18088.

Slika 4: Celotna celica kremenaste alge vrste *Coscinodiscus walesii*, uporabljena za fokusiranje laserskega žarka, in detajli luknjic v frustuli, ki povzročijo difrakcijo svetlobe. Podatki o širini luknjic, ki so na nekaterih mestih označeni so premajhni, da bi jih lahko prebrali. So v razponu od 1.5 do 1.7 µm, razdalje med njimi pa so od 800 do 987 nm. From De Stefano L., I. Rea, I. Rendina, M. De Stefano & L. Moretti 2007: Lensless light focusing with the centric marine diatom *Coscinodiscus walesii*. Optics Express **15**: 18082–18088.

Diatoms are important also for their contents of omega-6 and omega-3 fatty acids, which reach us indirectly via fish in our diet. Both are essential for us, since they are needed for our brain and some other organs, and we cannot make them ourselves. The requirement of omega-6 acids is not very great, and too much inhibits the uptake in the brain cells of the omega-3 acids (Novak *et al*. 2008). Work is now in progress to circumvent the fish route and get the acids more directly into human diet, and at the same time to optimize the omega-6/omega-3 ratio. The fats of diatoms have also been considered as biofuels for motor vehicles.

Acknowledgement

The authors stand in great debt to Professor Helen Ghiradella for her improvements of our original manuscript.

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