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FUNCTIONAL MORPHOLOGY OF THE HORSESHOE SPOT IN THE FRUSTULE OF *PLANOTHIDIUM* SPECIES (*BACILLARIOPHYTA*)

Key words: *lensoid, light utilization, virtual modeling, light rays tracing, ecology*

Abstract

Morphology of the horseshoe spot (HHS) with the cavity has been investigated in details in several species of *Planothidium* Round et Bukht. that carry out an attached mode of life. On the base of photomicrographs obtained with light and scanning electron microscopy, the model of the HSS longitudinal section was designed and light rays tracing through it has been implemented. It has been shown that HHS is an optical system that may diffuse or focus light, depending on the refractive index of the matter inside the cavity. For the first time HHS functions are revealed in a living diatom organism: diffusion of light over the chloroplast side turned to the substrate. The vertical part of inner convexity generates on the attached valve the *heating arc* that provides a diatom cell with additional heating energy. Thus, a new mechanism of light utilization was detected in the diatom species widely distributed in benthic ecosystems. A new term, *lensoid*, and terminology for its morphological description are proposed.

Introduction

Diatoms, unicellular algae, are among major components of aquatic ecosystems. They accumulate 20—25 % of the world net primary production (Treguer et al., 1995) and play a significant role in biogeochemical processes (Vernadskiy, 1923; Streett-Perrott et al., 2008). The siliceous frustule covering a diatom cell greatly contributed to evolutional success of the diatoms; however, its functions are still poorly investigated (Bukhtiyarova, 2009).

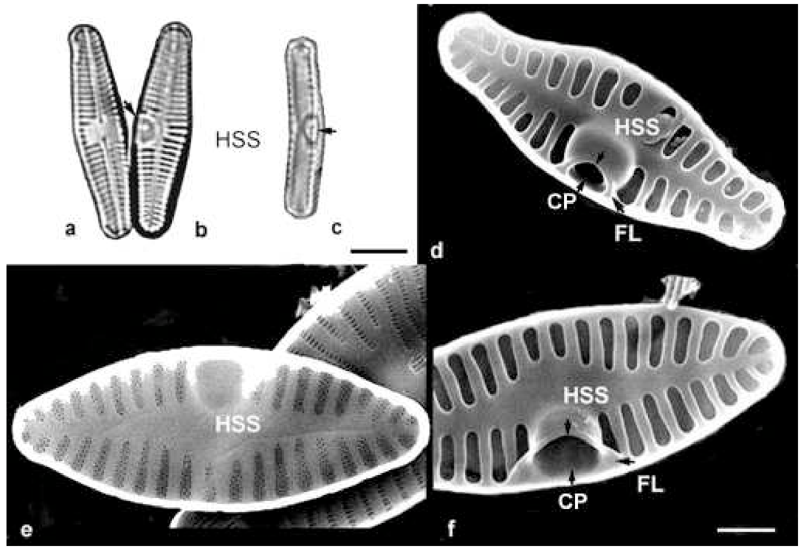
Studies of the diatom frustule (DF) interaction with light have revealed various physical effects. Experiments with freshwater *Melosira variance* C. Agardh and marine *Coscinodiscus wailesii* Gran et Angst have shown that DF plays the role of an *optical filter* that absorbs light mainly in the blue wavelength region (Yamanaka et al., 2008), transmitting approximately 80 % for red, 30 % for green, and 20 % for blue light diapasons (Noyes, 2008). It has been found that regular geometry of pores on DF of *C. wailesii* acts as a *lensless optical system* to focus incoming light into a spot of a few microns, which dimension and spectral properties are strongly wavelength-dependent (De Stefano et al., 2007; De Tommasi et al., 2010). Pore arrangement on DF of *Coscinodiscus granii* Gough has properties of a *photonic crystal* (Fuhrmann et al., 2004).

Many species of the genus *Planothidium* Round et Bukht. have on their frustule a conspicuous semicircular structure that in light microscope (LM) resembles a horseshoe (Fig. 1, *b*) and is called the *horseshoe spot* (HSS). Morphological variability of HSS was observed using scanning electron microscopy (SEM) (Round, Bukhtiyarova, 1996; Maidana, 2000; Van de Vijer et al., 2003; Potapova, 2010, and others); however, its functions were unknown (Morales, 2006). Our study was performed on HSS with the cavity inside the frustule. Available data on its morphology have suggested that it consists of natural lens-like elements (LLE). The aim of the present study was to test our hypothesis about HHS optical nature and to define its functions in a living diatom organism.

Objects and Methods

Diatom samples were collected from submerged higher aquatic plants in the Dnipro (Dnieper) River and in the Holosiivsky Pond (Kiev, Ukraine) and processed using standard methods (Topachevsky, Oksiyuk, 1960). Photomicrographs were recorded from a Zeiss Primo Star light microscope with T100 Science Lab 10 MP digital camera, and with a scanning electron microscope JEOL JSM-6060 LA. The horseshoe spot planar optical model was designed on the basis of LM and SEM micrographs of *Planothidium* species. It corresponds to the longitudinal section via the HSS centre and consists of four lenses. Dimensions and radii of the lenses were calculated from the micrograph of *Planothidium frequentissimum* (Lange-Bert.) Lange-Bert. (Potapova, 2010) using CorelDRAW vector editor. The light rays tracing model was generated with OptoCad computer program (Schilling) on condition that water was inside of and around HSS. The obtained image was combined with scheme drawing of the longitudinal section via living *Planothidium* species attached to the substrate. Refractive indexes (RI) used in calculations: diatom silicon — 1.48, cell cytoplasm — 1.34, lipids — 1.47 (Aas, 1996), cell membrane — 1.50 (Meyer, 1979), mix of gases — 1.00, and fresh water — 1.33.

Fig. 1. Horseshoe spot (HSS) on the rapheless valve of *Planothidium* species. *P. lanceolatum*, raphe (*a*) and rapheless (*b*) valves of the same frustule; *Planothidium* sp., view from the lateral frustule side (*c*); *Planothidium* sp. 1, inside valve surface (*d*); *Planothidium* sp. 2, outside (*e*) and inside valve surface (*f*). FL, flange, arrow; CP, cavity port, pair of opposite arrows. Scale bars: *a—c* = 5 µm, LM; *d—f* = 2 µm, SEM



Results and Discussion

A significant character of *Planothidium* species is *frustule heterovalvity* — different morphology of both valves in the same frustule (Bukhtiyarova, 2006). In particularly, the raphe valve (RV) has the *raphe*, two long through slits, lacking in a rapheless valve (RLV). The horseshoe spot is always disposed on RLV along the transapical axis and displaced asymmetrically to one valve side (Fig. 1, *b—f*).

Observations of HSS with SEM have revealed that in some species a second convexity on the inner RLV surface is adjoined to the outer one, and they form a cavity with a lateral port (CP) (Fig. 1, *d*, *f*, CP pair of opposite arrows). Both HSS convexities are smooth, without any kind of perforations, and consist of homogenous silica. Morphology of HSS corresponds to an optical system formed by its inner (Fig. 2, ISL — inner siliceous lens) and outer (Fig. 2, OSL — outer siliceous lens) siliceous convexities, a lens-shaped cavity between them (Fig. 2, CL — cavity lens) on RLV and the convex imperforated part of RV under HSS (Fig. 2, *a*, RVSL — siliceous lens on raphe valve).

The light tracing via HSS virtual model has shown that at different conditions such optical system may diffuse or focus sunlight (Fig. 2). These processes are

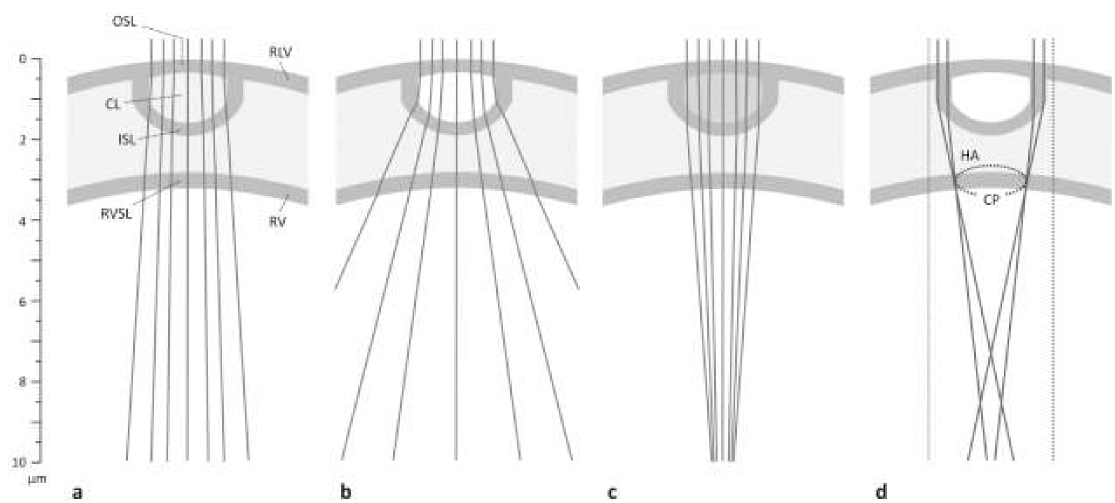


Fig. 2. Light rays tracing via an optical model of a horseshoe spot (HSS) in *Planothidium* species. Influence of different filling matters in the cavity between siliceous convexities on the light rays tracing: cytoplasm slightly diffuses (*a*), gas mix strongly diffuses (*b*), lipids focuse (*c*) light rays. Focusing of light rays that pass via vertical parts of HSS (*d*); the same rays tracing via frustule by HSS (*d*, dotted lines). RV, raphe valve; RLV, rapheless valve; OSL, outer siliceous lens; ISL, inner siliceous lens; CL, lens-shaped cavity; RVSL, raphe valve siliceous lens; HA, heating arc; CP, cavity port

determined by refractive index of filling matter (fm) in the cavity between inner and outer siliceous convexities (Fig. 2, *a*). Cell cytoplasm, produced by cell lipids or mix of carbonic gas with oxygen, potentially can be present inside the cavity; therefore the light rays tracing through these matters was tested on HSS model. The matter with interfacial RI_{fm}=1.40 does not change the light rays trajectory. Filling matter with lower RI_{fm} diffuses light (Fig. 2, *b*), with higher RI_{fm} value — focuses light (Fig. 2, *c*). Intensity of diffusion also depends on the RI_{fm} value: the lower is the RI_{fm} value, the more intense diffusion (Fig. 2, *a*, *b*). After proceeding through the vertical parts of ISL, the light is focused on RV (Fig. 2, *d*) that is additional evidence of HSS optical properties. The light rays that avoid HSS and pass via imperforated frustule parts do not decline from the initial trajectory (Fig. 2, *d*, dotted rays). Thus, it was confirmed that HSS is an optical system with lens-like elements.

Illumination of a chloroplast. Further, we have tried to elucidate LLE functions in a diatom unicellular organism. Species of *Planothidium* often dominate in attached benthic assemblages (Wojtal, 2013). They can be attached to various solid substrates: macroalgae, higher plants, stones and sand, reinforced constructions, plastic etc. The raphe valve is always attached to substrate and the rapheless valve is turned towards the light source.

In a living diatom, the chloroplast is tightly pressed to RLV around LLE; however, it does not overlay them (Fig. 3, *a*). The *flanges* on ISL free edge (Fig. 1, *d*, *f*, FL, arrow) together with the lateral frustule side prevent cytoplasm from entering inside the cavity between two siliceous lenses. It is therefore most likely that the filling matter inside the cavity is carbonic gas (CO_2) and oxygen (O_2). The most verisimilar light rays tracing via LLE corresponds to Fig. 2, *b,* that means that LLE disperse light in living *Planothidium* cells. This pattern of light rays tracing via LLE is combined with a scheme drawing of an apical section via whole diatom cell with protoplast (Fig. 3, *c*). The light rays, passed through LLE, reflect from RV, attached to the opaque substrate, and turned to substrate chloroplast side accepts reflected light (Fig. 3, *c*). Our model has allowed to detect the evolution trend of frustule development towards improvement of chloroplast illumination. However, we understand that real light transmission via LLE is much more complex because areola areas (Fig. 3, *b*, Ar) reflect light in other manner than the costae (Fig. 3, *b*, Cs) and smooth imperforated RV parts. Cell membrane (Fig. 3, *b*, CM), adjacent to the inner frustule surface, slightly influences on light rays tracing via the model due to its minor thickness.

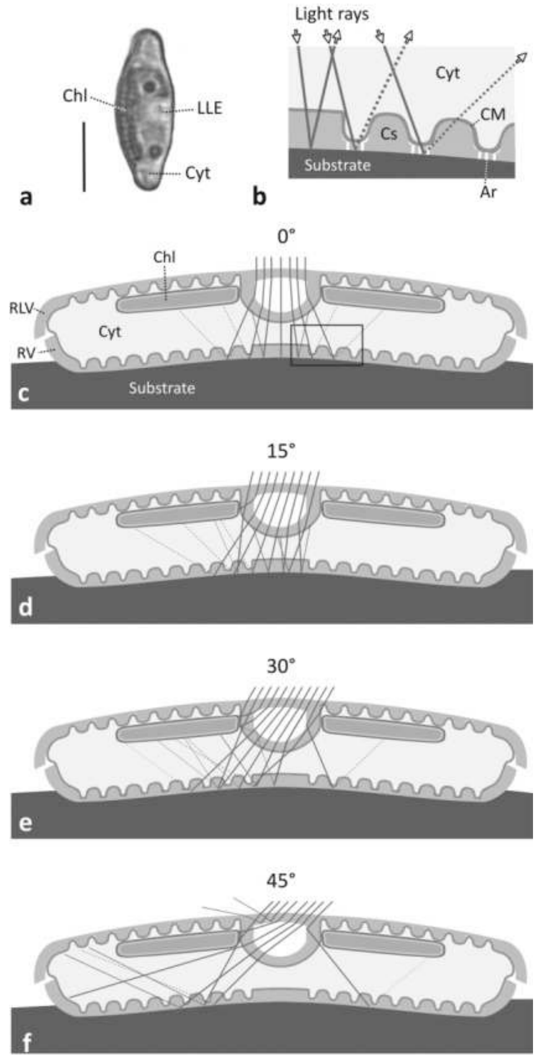


Fig. 3. Light rays tracing via lens-like elements (LLE) in living cell of *Planothidium* species. Living cell of *Planothidium* sp. (*a*): Chl, chloroplast; Cyt, cytoplasm. Enlarged fragment of fig. 3, *c* (*b*): RV, raphe valve; RLV, rapheless valve; Ar, section through areolae; Cs, costa; CM, cell membrane. Influence on light rays tracing of the angles of light incidence, 0º (*c*), 15º (*d*), 30º (*e*), 45º (*f*). Scale bar = 10 μm, LM

The field of light dispersion formed by LLE depends on the angle of rays incidence. The more the angle of incidence, the larger the field of light dispersion covers the chloroplast (Fig. 3, *c—d*). The angle of sunlight incidence varies during a year season and day time; therefore, LLE illuminate different parts of a chloroplast for a day time. When illumination is insufficient, LLE compensate lack of light (Fig. 3, *f*). For instance, better chloroplast illumination can be observed when substrate is situated vertically or at different angles regarding to the bottom of a water body.

Heating effect of LLE. Vertical walls of ISL focus light on RV on which the *heating arc* (HA) is formed (Fig. 2, *d*, HA). Based on our calculations, the HA diameter of *P. frequentissimum* was about 1.8 μm (67 % of LLE bases diameter), its width is equal to LLE thickness and corresponded to about 0.4 μm. We have presumed that such adaptation to supplying the protoplast with heat energy gives the diatom species advantages in cold seasons and in climate conditions with low temperatures. This suggestion is confirmed by frequent records of *Planothidium s*pecies with LLE from corresponding habitats: *P. frequentissimum, P. oestrupii* (Cleve-Euler) Round et Bukht., were found in Chukotka, a northeastern region of Russia (Kharitonov and Genkal, 2012); *P. fragilarioides* (Petersen) Round et Bukht. — in Chukotka (Kharitonov and Genkal, 2012), Victoria Island, Canadian Arctic Archipelago, Canada (Van de Vijer et al., 2003).

We still do not know other effects that LLE cause in a living diatom; however, these peculiar patterns of light utilization in a single-cell organism are amazing for human imagination.

Terminology. The proposed new terms for description of diatom frustule morphology take into account HHS optical properties and functions. They are defined on earlier suggested universal basis that includes the following concepts. *Basic element of the diatom frustule* (*db-element*) is a morphologically detached, homogeneous part of the frustule that possesses special physical-chemical features and provides primary basis for the frustule hierarchical construction. *Morph of the diatom frustule* (*df-morph*) is a compound structural unit of the frustule that is constructed of db-elements or/and structural units of lower orders, performs particular functions in a diatom organism, and has its own evolutionary history. Size scale is included in the definitions since physical properties of any material depend on dimensions of its structural particles (Bukhtiyarova, 2009).

Lensoid (*лінзоїд* in Ukr., *линзоид* in Rus.) — unique micro df-morph of the first order that consists of lens-like elements, additional db-elements, and serves for utilization of light energy (Fig. 1, *b—f*).

Lens-like element (*лінзовидний елемент* in Ukr., *линзовидный элемент* in Rus.) — unique micro db-element in form of smooth imperforated siliceous convexity (Fig. 2, *a*, OSL, ISL).

Cavity lens (*лінзовидна порожнина* in Ukr., *линзовидная полость* in Rus.) — unique micro db-element in form of lens-like hollow in lensoid (Fig. 2, *a*, CL).

Cavity port (*вхід лінзовидної порожнини* in Ukr., *вход линзовидной полости* in Rus.) — entrance to the cavity lens (Fig. 1, *d*, *f*, 2d, CP).

Flange (*фланець* in Ukr., *фланец* in Rus.) — unique micro (nano) db-element in form of a smooth imperforated siliceous plate around the free edge of a lens-like element (Fig. 1, *d*, *f*, FL).

Heating arc (*теплова дуга* in Ukr., *тепловая дуга* in Rus.) — arc-like area on the valve of a diatom frustule where the lensoid focuses light rays (Fig. 2, *d*, HA).

Conclusion

For the first time, physical properties of the horseshoe spot on a diatom frustule were substantiated as an optical system with lens-like elements. In a living diatom, it disperses sunlight over the chloroplast side turned to the substrate and forms on the valve a heating arc supplying a diatom cell with additional heat energy. A new term, *lensoid*, reflects optical properties of this df-morph and its functions in diatom unicellular organisms. The new mechanism of light utilization is revealed that is realized by lens-like elements on the diatom frustule. Attached diatoms play a significant role in benthic ecosystems; therefore, the understanding of their adaptation to insufficient illumination and low temperature conditions are the key issues in aquatic ecology and evolution of benthic organisms.

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Recommended for publication by P.M. Tsarenko   Submitted 12.09.2013

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ФУНКЦІОНАЛЬНА МОРФОЛОГІЯ ПІДКОВОПОДІБНОЇ ПЛЯМИ В ПАНЦИРІ ВИДІВ *PLANOTHIDIUM* (*BACILLARIOPHYTA*)

Детально досліджено морфологію підковоподібної плями (ПП) із внутрішньою порожниною в панцирі видів роду *Planothidium* F.E. Round et Bukht., що ведуть прикріплений спосіб життя. На основі мікрофотографій, отриманих за допомогою світлового та електронного скануючого мікроскопів, розроблено модель поздовжнього перетину ПП і здійснено трассировку променів світла крізь неї. Показано, що ця морфологічна структура панцира є оптичною системою, яка залежно від коефіцієнта заломлювання матеріалу, що заповнює порожнину, може розсіювати або фокусувати світло. Вперше відкрито функції ПП у живому організмі діатомової водорості: розсіювання світла з боку хлоропласта, що звернений до субстрату. Крім того, вертикальна частина внутрішньої опуклості фокусує світло на прикріпленій до субстрату стулці й утворює *теплову дугу,* яка забезпечує клітину додатковою тепловою енергією. Таким чином, відкрито новий механізм утилізації світла у видів, широко розповсюджених у бентосних екосистемах. Запропоновано новий термін, *лінзоїд,* і супровідну до його морфологічного опису термінологію.

К л ю ч о в і с л о в а: *лінзоїд, утилізація світла, віртуальне моделювання, трассировка променів світла, екологія.*

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ФУНКЦИОНАЛЬНАЯ МОРФОЛОГИЯ ПОДКОВООБРАЗНОГО ПЯТНА В ПАНЦИРЕ ВИДОВ *PLANOTHIDIUM* (*BACILLARIOPHYTA*)

Детально исследована морфология подковообразного пятна (ПП) с внутренней полостью в панцире у видов рода *Planothidium* F.E. Round et Bukht., ведущих прикрепленный образ жизни. На основе микрофотографий, полученных с помощью светового и электронного сканирующего микроскопов, разработана модель продольного сечения ПП и проведена трассировка лучей света через нее. Показано, что данная морфологическая структура панциря представляет собой оптическую систему, которая в зависимости от коэффициента преломления материала, заполняющего полость, может рассеивать или фокусировать свет. Впервые выявлены функции ПП в живом организме диатомовой водоросли: рассеивание света над стороной хлоропласта, обращенной к субстрату. Кроме того, вертикальная часть внутренней выпуклости, фокусируя свет на прикрепленной к субстрату створке, образует *тепловую дугу,* обеспечивающую клетку дополнительной тепловой энергией. Таким образом, открыт новый механизм утилизации света у видов, широко распространенных в бентосных экосистемах. Предложены новый термин, *линзоид,* и сопутствующая его морфологическому описанию терминология.

К л ю ч е в ы е с л о в а: *линзоид, утилизация света, виртуальное моделирование, трассировка лучей света, экология.*