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SPECIFIC CHARACTERISTICS OF CHITIN EXOSKELETON OF LEAF WEEVIL *PHYLLOBIUS MACULICORNIS* (GERMAR, 1824)

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Biological systems such as butterflies and beetles have developed highly elaborate photonic crystals to create their striking coloration. The vigorous development of nanoscience and nanotechnology, together with the fast convergence of physics and materials science with biology, has renewed interest in naturally occurring photonic nanoarchitectures. Until now most photonic nanostructures are observed in exotic species, however our investigations with widely occurring weevil *Phyllobius maculicornis* (Germar, 1824) shows that structures of this type occurs in nature more frequently, and it is possibly quite widespread phenomenon.

Key words: Structural color, photonic nanoarchitectures, weevil, *Phyllobius maculicornis*.

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INTRODUCTION

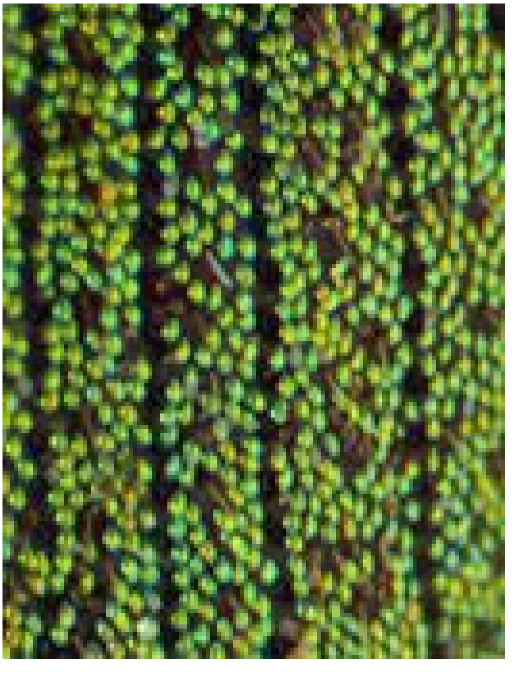
Structural colours in animal world are subject of investigation of many scientists in whole world and for several years, such famous outstanding researchers as Hooke (Hooke 1961), Newton (Newton 1952) and Lord Rayleigh (Rayleigh 1923) have studied these phenomena.

Nevertheless at the last years interest on this issue is raised due to new discoveries of several nanostructures and such structures as photonics crystals (Yablonovitch 1987) in biological material (Biró et al. 2003), therefore there are numerous new publications in this field nowadays. Photonic crystals (PhCs), or photonic band gap (PBG) materials, introduced to the physics and materials science communities only twenty years ago by Yablonovitch (Yablonovitch 1987) and John (John 1987), have been present in the living world for millions of years. Structural (or physical) colouration is due to nanometre-sized structures with periodically changing refractive indices, causing coherent light scattering. If the structures causing the physical colours are regular with a periodicity in the order of the wavelength of visible light, the materials are referred to as photonic crystals (Joannopoulos 2008). One-dimensional photonic crystals consist of parallel thin film layers of alternating high and low refractive index materials, i.e. the well-known multi-layers. They create the metallic and polarized reflections of, for example, the skin of cephalopods (Mäthger & Hanlon 2007) and fishes (Mäthger et al. 2003), the elytra of jewel beetles (Stavenga et al. 2011, Hariyama et al. 2005, Mason 1927, Durrer & Villiger 1972), scarabs (Sharma et al. 2009, Brady & Cummings 2010), and the breast feathers of birds of paradise (Stavenga et al. 2011). Two-dimensional photonic crystals, that is, structures with periodicity in two dimensions, underlie the coloration of peacock feathers (Zi et al. 2003, Loyau et al. 2007). Three-dimensional photonic crystals have been found in the scales of many weevils and beetles (Wilts et al. 2012, Parker et al. 2003, Wilts et al. 2012, Galusha et al. 2008, Welch et al. 2007), but also in butterflies (Michielsen & Stavenga 2008, Poladian et al. 2009, Saranathan et al. 2010, Saba et al. 2011, Shawkey et al. 2009). Quasi-ordered three-dimensional photonic crystal structures, which are periodic in all three dimensions, although imperfect, have been identified in bird feathers (Dong et al. 2010) and in the scales of some coleopterans (Morrone 2002).

Recent research shows that species of Curculionidae family can possess structures of photonic crystals, which generate specific colour effects in reflected light (Simonis & Vigneron 2011).

However till now most investigations on photonic crystals of weevils are made with exotic species (*Entimus imperialis, Pachyrrhynchus congestus, Lamprocyphus augustus*) mainly from tropic regions. This research is done with weevil species *Phyllobius maculicornis* (Fig. 1) widespread in

a)



b)

Fig. 1. Leaf weevil *Phyllobius maculicornis* a) and scales b).

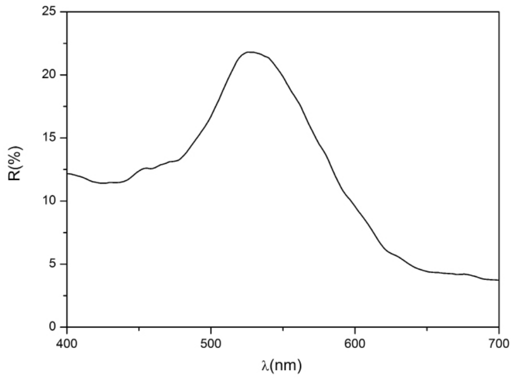


Fig. 2. Reflected spectrum of leaf weevil *Phyllobius maculicornis*

Europe, which can be found feeding on the foliage of various plants.
The coloration of this weevil comes from a covering of dense scales, which are easily rubbed off. Through age, adults can become devoid of virtually all green scaling and generally appear black or very dark brown (especially on the legs).

Experimental methods

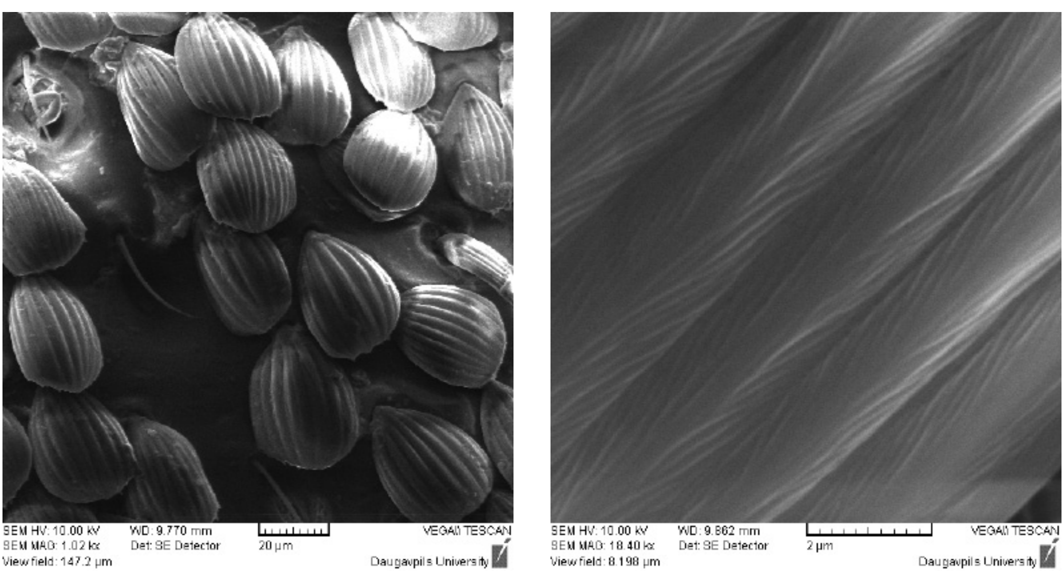
Dried specimens of *Phyllobius maculicornis* (Germar, 1824) were obtained from the collection of the Institute of Systematic Biology (Daugavpils University, Latvia).

The research of material structure was realized by Electron scanning microscope Vega II LMU (Tescan), samples were coated by 10 nm Ag coating. Spectroscopic investigations were done by Microspectrophotometer MSP500 (Angstrom Sun Technologies). Reflected spectra were obtained from elytral scales of beetles with well-expressed reflective characteristics.

RESULTS

Reflected spectra obtained during research are represented in Fig. 2. It is evident that the reflectivity reaches a maximum value at around 525 nm which corresponds to green light. Reflected light observed is with metallic shine and slight effect of iridescence.

This weakly expressed iridescence effect is based on surface structure of weevil's elytral scales (Fig. 3. a) – structure which corresponds to diffraction grid with approximate period of 2 micrometres. There is well-expressed second level (secondary) structure: nanostructural riffling - approximately



a) b)

Fig. 3. Surface scales a), and structure of surface scales b) of leaf weevil *Phyllobius maculicornis*

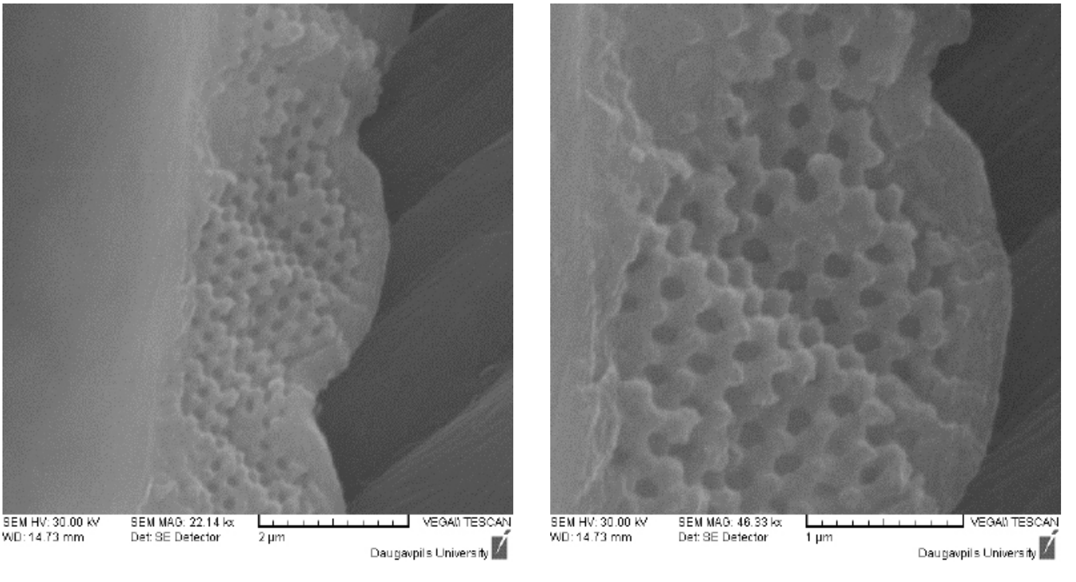
100 nm in width, some micrometres in length with period of approximately 200 nm (Fig. 3. b). The hypothetical biological importance of such secondary structures is maintenance of surface cleanliness, because waxy nanostructures can ensure self-cleaning property of surface – so-called "lotus effect" (Furstner et al. 2005). Nano-structuring of surface increases its hydrophobic characteristics as well (Byuna et al. 2009). There is number of cases when primary function of micro- and nanostructural formations in insects is friction-reducing or water-repelling (Seago et al. 2009). In our case it is impossible to estimate if primary function of these structures is self-cleaning and hydrophobic features, or it is reflection of light, without additional investigations.

Nevertheless it is clear that quasi-ordered scattering (Seago et al. 2009) determined by internal structural architecture of scale-like structures is responsible about dominant color of reflected light – 525 nm. Structural nanoarchitecture can be seen in Figure 4 (a) and (b) – lattice with period about 250 nm.

The structure of lattice differs from all previously revealed cases (Simonis et al. 2011, Rassart et al. 2009) where lattice was composed of spherules in specific arrangement. The architecture of structure is more similar to structures observed by Victoria Welch etc. (Welch et al. 2007). Still, research (Simonis et al. 2011) offers the model, which allows predicting basic frequencies of reflected light depending on dimensions of structural elements. It is likely to generalize this model to other types of structures, because conclusions of this model correspond to results of our research as well.

CONCLUSIONS

The structure with nanostructures built of chitin, occurring in the beetle's individuals representing the species *Phyllobius maculicornis* were investigated by electron microscopy and reflectance spectroscopy. The multiple structures which are composed from micro- and nanostructures were observed on the surface of scale as well as on three-dimensional nanostructural lattice inside the scale. We believe that interaction of these three structures altogether determines the ultimate colouring of the beetle.



a) b)

Fig. 4. Internal structural architecture of surface scale of leaf weevil *Phyllobius maculicornis*, magnification 22,14 kx -a), and magnification 46,33 kx -b).

Until now most photonic nanostructures are observed in exotic species, however our research shows that structures of this type occurs in nature more frequently, and it is possibly quite widespread phenomenon.

Structures observed are interesting from point of view of biological systematics, because it is possible to employ them as identification feature of species. Research of the nanostructures is valuable for multiple uses in bionics, for example, samples for material scientists for finding new nanoarchitectures, iridescent lattices can be worthwhile for development of holograms with reproduction of natural structures and iridescence effects.

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