Morphological Peculiarities Statoconia in Statocysts of Terrestrial Pulmonary Snail *Helix Lucorum*

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Vast majority of statoconia in statocysts of *Helix lucorum* are of oval shape and have smooth surface. Each statoconium in its central part has a nucleus, a spherical mass of 1.5 μ in diameter, surrounded by concentric structures. Minority of statoconia are of subcircular, elongated, rectangular, triangular, irregular, and sometimes fanciful shape and are structured around several nuclei or around small statoconia consolidated by shared growth layers. Apart from statoconia, spherical formations lacking mineral composition of 0.3-2.5 μ in diameter were also found in the statocyst cavity. Similar formations were found in vacuoles of sensory cells of statocyst epithelial lining. It is hypothesized that statoconium nuclei and growth layers around them are of different origin: the nuclei are formed by statocyst sensory cells, while the mineral component is a result of activity of supporting cells.

Key Words: *organ of equilibrium; statocyst; statokonia; morphometry*

Statoconia, microscopic biomineral structures forming an inertial mass in the statocyst (organ of equilibrium) of gastropods are formed by growth layers of mineral and organic origin. This is why statoconia, similar to teeth, nails, bones, scale, shells and some other formations in animals are reckoned among the so-called recording structures.

The aim of the study was to reveal morphological parameters and ultrastructure of statoconia from terrestrial pulmonata snail *Helix lucorum*. This species was recently used for investigation of the functional state of statocyst sensory cells (also called statoreceptors and gravity receptors) during the period of readaptation after prolonged exposure to null-gravity at Mir orbital space station. It was also demonstrated that the main mineral element, which provides statoconium heaviness, is calcium carbonate in the form of aragonite crystals [1-6].

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MATERIALS AND METHODS

Experiments were conducted on 76 adult animals *H. lucorum* (*var. taurica Kryn.*) weighing 13.0±0.04 g with shell diameter 35.7±0.3 mm collected from a small area of city park Mziuri in Tbilisi (snails are considered to be adult, if their shell edges near the opening are slightly excurved and form a small thickening, called a lip; in this state the shell loses its ability to grow).

The body of the snail was taken out of the shell and fixed on the preparation table with entomological needles, the body was dissected on the dorsal side along the medial line, the statocysts were cut out from the uncovered subesophageal ganglionar complex under a binocular lens. The statocysts were opened, the statoconia were placed on microscope slides or on a special graphite film and examined under a MIK-MED-2 phase-contrast microscope (LOMO) or under a scanning electron microscope (CamScan) after preliminary gold coating in a sputter, respectively. Morphological parameters of statoconia were estimated using SSIMP purpose-designed software, element composition was determined using ISIS microanaly-

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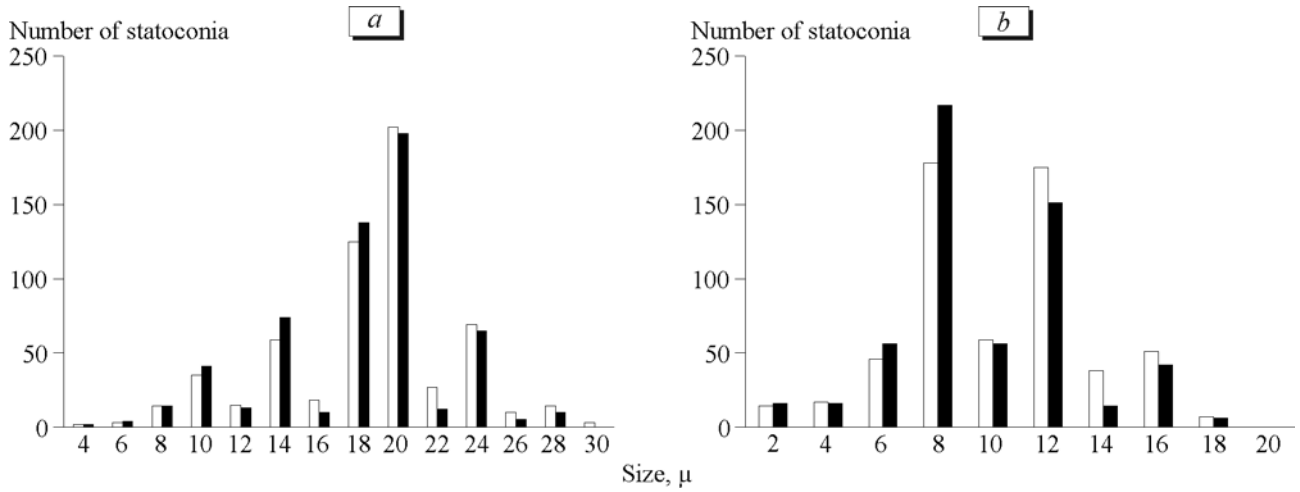

Fig. 1. Length (*a*) and width (*b*) distribution of statoconia from the left and right statocysts of 13-g snails (*n*=9).

sis system equipped with a X-ray detector. Statocysts were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4, and after dehydratation were placed into epon-araldite mixture. Ultrathin section were used for transmission electron microscopy using a Hitachi H-300 microscope; toluidine blue-stained 1.5-μ sections were used for light microscopy. In a series of experiments, statocysts after glutartaldehyde and OsO_4 fixation and dehydaratation were dried at a critical point in the atmosphere of amyl acetate and carbon dioxide, after which they were opened, coated with gold and placed on object tables of scanning electron microscope.

Experimental material underwent computer processing using Statistics 5.1 software.

RESULTS

Statocysts of *Helix lucorum* are paired formations located on the dorsolateral surface of the pedal ganglia of the subesophageal ganglionar complex. Each statocyst is surrounded by a connective tissue membrane containing smooth muscles and collagen fibers. In native state, statocysts of 13-g snails used in our experiments were of spherical shape with external diameter 198.0±0.3 μ on the right and 197.4±0.6 μ on the left, and internal diameter of about 180 μ.

The statocyst cavity contained a large number (on the right 587.1±17.7, on the left 579.6±19.3) of highly morphologically dissimilar statoconia of length and width of 2.5-30.0 and 2-20 μ, respectively, and thickness of 2-7 μ. Statoconia of intermediate size predominated, while the proportion of small and large statoconia scaled by length varied within 15-25% and by width within 10-15% (Fig. 1).

Statoconia are transparent under light microscope; however, a small number (4-27) of dark statoconia and solitary (2-7) partially or fully destroyed statoconia were found. At high magnifications of scanning electron microscope, the surface of statoconia was slightly rough, sometimes small cavitations of 0.1-0.6 μ were found (Fig. 2).

Vast majority of statoconia (504.4±20.1 on the right and 504.2±23.8 on the left) were of oval shape and had flattened surfaces with smoothened edges (Fig. 2). In the central part of these statoconia, a spherical electron-dense formation of 1.5 μ in diameter is located. It is a sort of core or nucleus surrounded by electron-transparent and electron-dense stratified structures. The smallest statoconia (length 3.0-3.5 μ and width 2.0-2.5 μ) have only a single electron-transparent layer seen around the nucleus; the number of layers surrounding the nucleus increased with increasing the statoconium size. Apart from concentric stratified structures, radial striation formed by electron-dense grains is also typical for statoconium structure.

About 15% statoconia contained in one statocyst (82.9±12.3 on the right, 75.4±8.8 on the left) differ

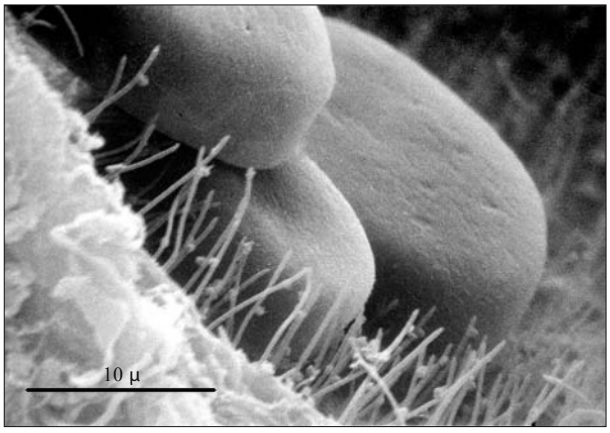

Fig. 2. Opened statocyst. Three statoconia and multiple sensory hairs of receptor cells are seen. Scanning electron microscopy.

from the picture described above. They are subcircular, elongated, rectangular, and triangular, or have irregular or sometimes intricate shape and contain not one but 2 and more (up to 12) nuclei. Generally, statoconia with 2 nuclei predominated, statoconia with multiple nuclei were rare. Another feature of these statoconia consists in the fact that around each nucleus 2-3 or more growth layers are revealed, which, in turn, are surrounded by similar additional layers. Nuclei of some statoconia lack their own stratification. In this case they all are surrounded by common growth layers. There are also formations consisting of several statoconia with intergrown surfaces, which makes their borders quite easily distinguished (Fig. 3).

Major chemical elements constituting the statoconia are calcium, carbon, and oxygen. Acid medium (diluted solutions of hydrochloric acid, sulphuric acid, nitric acid) dissolved the statoconia: initially statoco-

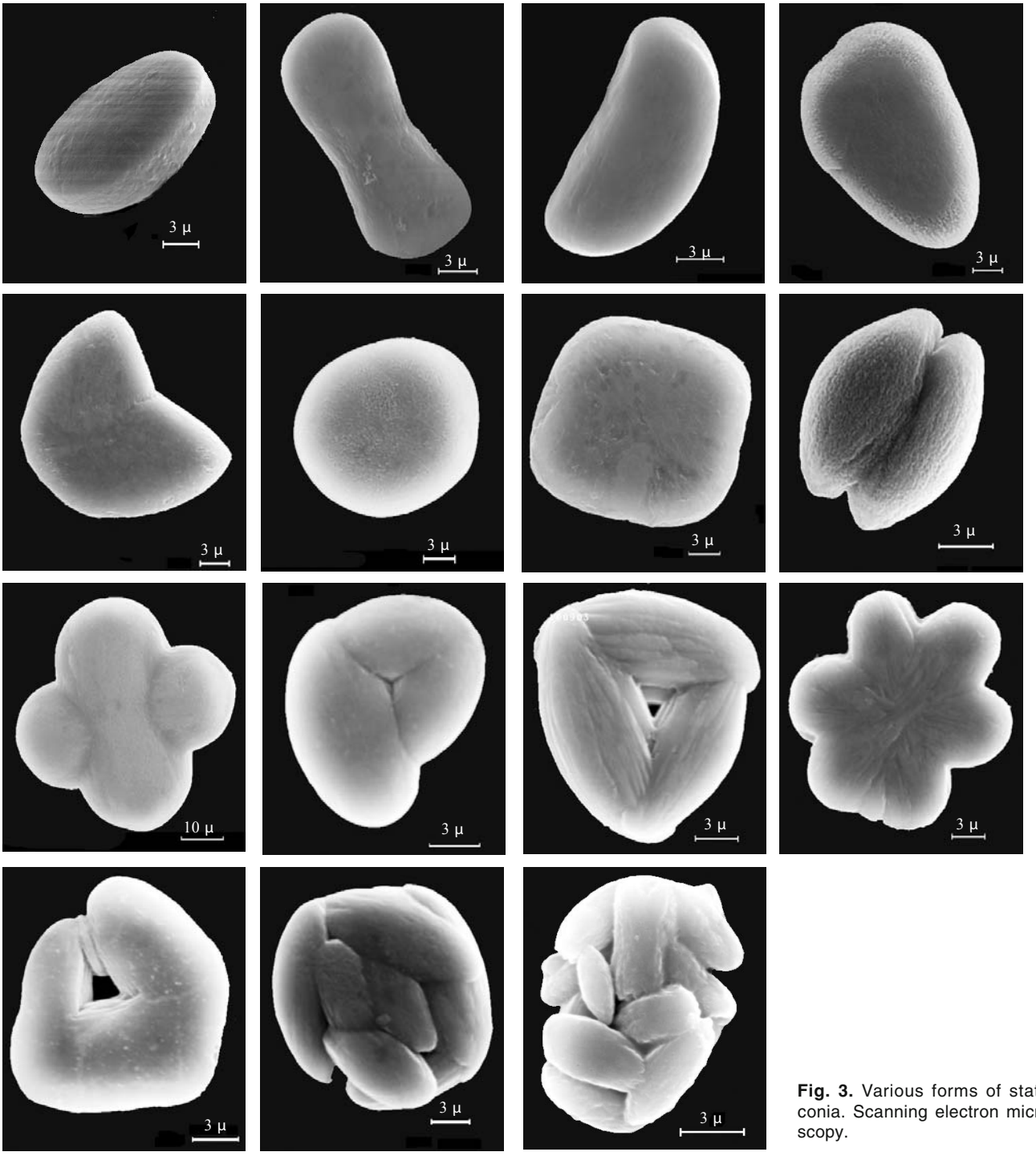

Fig. 3. Various forms of statoconia. Scanning electron microscopy.

nium surface loses transparency and takes a tuberous form; then statoconium diameter gradually decreases and pale rim appears and grow with decreasing the statoconium diameter. Finally, there remains a thin and transparent structure in the form of a film, which partially retains the form of statoconia with stratification, normally typical for them.

Apart from statoconia, statocyst cavity also contains spherical formations 0.3-2.5 μ in diameter (many tens in some cases). They are optically dense under phase contrast microscope and have homogenous structure and sometimes are stuck together in groups of 2, 4, or more. These structures can often be seen under scanning electron microscope between kinocilia on the surface of sensory cells covered with glycocalyx. The smallest of them lack mineral composition and unlike statoconia are not dissolved in acid medium. First signs of calcification are revealed in structures of 2-3 μ in diameter. It should be emphasized that the same structures are present in vacuoles of sensory cells of statocyst equatorial zone. They are usually located on the periphery of vacuoles, in some cases they entirely border their inner surface, on semithin sections they are dark blue-colored with toluidine blue and under transmission electron microscope they have high electron density. In this case, the apical surface of cells exerts into the statocyst cavity. At the same time, these vacuoles quite often can contain only a small number of such structures or can be empty; in this case they are much smaller in size. In this state, the apical surface of sensory cells looks quite smooth.

The obtained results show that the majority of statoconia are of oval shape with flattened surfaces, but not egg-shaped as it was stated before. Such statoconia contain only one nucleus, and this is why we called them "simple" in contrast to "complex" statoconia. The latter are of various shape and are structured around several nuclei or formed by small statoconia integrated by shared growth layers. The structures consisting of several statoconia with intergrown surfaces (which makes their borders easily distinguished) should obviously be considered complex statoconia.

Statoconia are in the state of generation and growth during the whole life of the animal. This is proved by the presence of small and very small statoconia together with large statoconia in the same statocyst in animals with completed growth process. "Growth potential" became clearly apparent under conditions of weightlessness. In experiments, conducted on terrestrial and freshwater snails (*Helix lucorum*, *Ampullaria gigas*, *Biomphalaria glabrata*) exposed at artificial Earth satellites, Mir station and Shuttle, for a period from 14 to 148 days, a significant increase of the total number and size of statoconia was revealed [1-3,9]. On the contrary, under conditions of excessive gravity produced by rotation in the centrifuge, destruction and elimination of the majority of statoconia were observed in statocysts of *Helix lucorum* [4]. Similar conditions led to reduction of statolith dimensions in seashell *Aplysia californica* larvae and total number of statoconia in the same animals after metamorphosis [8,10], as well as reduction of utriculus and sacculus statolith dimensions in Cyclide *Oreochromis mossambicus* larvae [7].

It was demonstrated that statocyst cavity in addition to statoconia contains structures differing from them. They were characterized by the absence of mineralization, resistance to acid medium, small size, and spherical shape. We suggest that these formations are nuclei of future statoconia generated by sensory cells. This hypothesis is supported by the fact that similar structures were revealed in vacuoles and on the surface of sensory cells. Vacuoles filled with these structures empty their content into the statocyst cavity from time to time, after that a new cycle of accumulation and evacuation starts. Meanwhile, the mineral component, which sediments in the form of growth layers around the nuclei, is a product of supporting cells [2].

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