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CHORION STRUCTURE OF DIAPAUSE AND SUBITANEOUS EGGS OF FOUR DIAPTOMID COPEPODS (CALANOIDA, DIAPTOMIDAE): SEM OBSERVATIONS

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Chorion Structure of Diapause and Subitaneous Eggs of Four Diaptomid Copepods (Calanoida, Diaptomidae): SEM Observations. Samchyshyna L., Santer B. — Scanning electron microscopic examination of diapause eggs of freshwater Calanoida *Hemidiaptomus amblyodon* Marenzeller*, Eudiaptomus vulgaris* (Schmeil)*, E. graciloides* (Lilljeborg) and subitaneous one s of *E. gracilis* (Sars) is described. The chorion surface ornamentation and number of coated layers are revealed. Diapause eggs have a thick (0.6–4.8 μ), at least two-layered chorion with patterned surface, whereas subitaneous eggs have a thin (0.13–0.25 μ), single-layered chorion with smooth surface. The mean number of eggs in egg sac, diameter of eggs and thickness of chorion were measured. The significance of the egg chorion thickness for diaptomid species inhabited temporary and/or permanent water-bodies is discussed.

Key words: diapause and subitaneous eggs, chorion structure, SEM, Diaptomidae.

Структура хориона диапаузирующих и субитанных яиц четырех видов диаптомидных копепод (Calanoida, Diaptomidae): СЭМ-исследования. Самчишина Л., Сантер Б. — Сканирующая электронная микроскопия яиц пресноводных каланоид *Hemidiaptomus amblyodon* Marenzeller*, Eudiaptomus vulgaris* (Schmeil)*, E. graciloides* (Lilljeborg) и *E. gracilis* (Sars) дала возможность показать особенности покровов и количество оболочек у диапаузирующих и субитанных яиц этих видов. Диапаузирующие яйца имеют толстую (0,6–4,8 мкм), двухслойную оболочку с орнаментом на поверхностном слое, в то время как субитанные яйца имеют тонкую (0,13–0,25 мкм), однослойную оболочку с гладким поверхностным слоем. Среднее количество яиц в яйцевом мешке, диаметр яиц и толщина хориона указаны. Обсуждается значение толщины оболочки яиц в связи с обитанием диаптомид во временных и постоянных водоемах.

Ключевые слова: диапаузирующие и субитанные яйца, структура яйцевой оболочки, СЭМ, Diaptomidae.

Introduction

The majority of freshwater calanoid copepods in temperate and boreal latitudes survive unfavourable environmental conditions in diapause egg stage (Dahms, 1995, etc.). Morphological differences between diapause and subitaneous eggs were studied mostly in marine calanoid copepods (Belmonte, 1997; Castro-Longoria, 2001; Santella, Ianora, 1990, 1992; Ianora, Santella, 1991; Toda, Hirose, 1991; etc.). Eggs of these two types are showed striking structural differences. In addition, some pontellids have a third egg type, which is morphologically similar to diapause ones, however hatched within 2 to 3 days (Santella, Ianora, 1990). Moreover, the external morphology of subitaneous eggs of some Acartiidae species shows even intraspecific variation, and it was proposed to use this as a diagnostic feature (Belmonte, Puce, 1994; Belmonte, 1998).

Morphological peculiarities of eggs in freshwater calanoid copepods were studied much less. Diapause eggs of freshwater calanoid copepods for the first time were described more than hundred years ago by Wolf (1905). He showed that *Diaptomus coeruleus Fisch*. (now *Eudiaptomus transylvanicus* (Dad.)) has two types of eggs: ones with a thick layered chitinous shell, like those in *D. castor* Jur., and others with a thin chitinous shell. It has been revealed by the author that eggs of the first type can withstand freezing. This caused Wolf to call them wintering eggs (“Wintereier”). The presence of a resistant external shell over a thin internal one in diapause eggs of calanoids was confirmed later (Rylov, 1930). Ekman (1904) reported for diapause eggs of *Eudiaptomus graciloides* (Lill.) a single-layered shell, which is twice as thick as that one in subitaneous eggs. The differences were also noted in the coloring of these two egg types in diaptomids (Cooley, 1971). Presence of space between chorion and vitelline membrane in the diaptomid subitaneous eggs differ them from diapause eggs, there it is absent (Lohner et al., 1990). Morphological study of diapause and subitaneous eggs with the use of transmission electronic microscopy (TEM) in freshwater calanoid *Boeckella triarticulata* Thomson showed that diapause eggs of this species have a thick three-layered chorion, whereas the subitaneous eggs have a thin single-layered chorion (Couch et al., 2001); the distinct difference in physiology of these two types of eggs was assumed by the authors. In *Sinodiaptomus indicus* Kiefer diapause eggs have a highly complex, four-layered chorion; in turn, subitaneous eggs have a three-layered chorion and there is a marked variation in the surface ornamentation of those two types of eggs (Dharani, Altaff, 2004).

Mentioned studies conform a significant variety of egg structure in freshwater calanoid copepods, however, lack of egg morphology data for most taxa of Calanoida put obstacles in understanding of role of observed structures. Indeed, sinking down to the lake bottom, diapause eggs are exposed to other environmental factors than subitaneous eggs which develop in the water column in a few days. Among bottom factors affecting diapause eggs are toxic gases (e. g. hydrogen sulphide), oxygen deficiency (hypoxia), the desiccation or freezing (in shallow water-bodies), *etc.* Furthermore, the duration of exposure of diapause eggs to the stress factors could be rather long (months, years, decades) that can lead to depression of embryo development and decreasing of egg viability. Thus, diapause eggs have to be strong enough to resist the power of stress factor and duration of its influence. As diapause eggs differ from subitaneous by their ecological and functional role, they attract the interest to investigate their morphological features. Present study is focused on observation of the egg surface and structure of chorion in four species of the family Diaptomidae, the representatives of fresh continental waters which have less stable environmental parameters compared to marine ecosystems.

Material and methods

Material was collected using a plankton net with 0.25 m^2 opening and 250 μ mesh size at the next sites: *H. amblyodon* in the spring puddle at the ploughing up field, Zhytomyr, Ukraine (14.05.2001); *E. vulgaris* in the shallow pool, *ibid.* (25.08.2001); *E. graciloides* in the lake Selentersee, Holstein, Germany (14.11.2001); *E. gracilis* in the lake Pluβsee, *ibid.* (31.10.2001). Diapause eggs (which haven't hatched by incubation) were examined in the first three species. Subitaneous eggs were studied in *E. gracilis*, species spawning only this one egg type (Santer et al., 2000). Specimens and eggs selected for SEM were prepared by method described in Hernandez-Chavarria and Schaper (2000) and observed under scanning electronic microscopy LEO Elektronenemikroskopie, Zeiss Leica (SEM CO nanolab 7). The mean number of eggs in the egg sac, diameter of eggs and thickness of chorion were measured.

Results

The egg sac membrane of *H. amblyodon* is thick and optically dense (fig. 1, *a*). It fit not close to the eggs (e. g., like in *E. graciloides*). The mean number of eggs in the sac is 96 ± 23 (n = 22). Egg (fig. 1, *b*) diameter is 113.3–145 μ (n = 7). The thickness of chorion varies from 3.2 up to 4.8 μ (n = 9). Chorion (fig. 1, *c*) has at least two well expressed layers, the internal one is very thick (as far as can be observed under the SEM). This layer has visible microfibrils, probably of chitinous nature (fig. 1, *d*). Short richly interlaced folds are on the chorion surface (fig. 1, *e*). The dotted character of egg surface became visible only at the higher magnification (fig. 1, *d*).

Membrane covering the egg sac of *E. vulgaris* is also quite thick and fit more closely to eggs following their shapes (fig. 2, *a*). Average number of eggs per sac is 16 ± 4 (n = 20). Egg (fig. 2, *b*) diameter is 82.5–97.9 μ (n = 8). Thickness of the chorion is 1.4–1.7 μ (n = 5) (fig. 2, *c*) and it is quite dense optically. The chorion sculpture is rough (fig. 2, *d*). Higher magnification has shown the dotted character of egg surface, as in *H. amblyodon* (fig. 2, *e*).

The egg sac of *E. graciloides* is covered by relatively thin membrane closely fitting to the eggs (fig. 3, *a*). Number of eggs in egg sac is 4 ± 2 (n = 20). The diameter of eggs is 87.5–106.8 μ (n = 6) (fig. 3, *b*). The chorion has thickness 0.6–1.1 μ (n = 5) with two distinct layers (fig. 3, *c*), the internal one is approximately 3 times as thick as the external. The surface of eggs is smooth (fig. 3, *d*), however, at high magnification it reminds that in *E. vulgaris*, while waves are smaller and sparse (fig. 3, *e*).

The diameter of subitaneous eggs of *E. gracilis* is 123.4–131.7 μ (n = 6). There are 9 ± 3 (n = 20) eggs in the egg sac. Eggs are covered by extremely gentle, thin, single-layered chorion (fig. 4, *a*) not more than 0.25 μ of thickness (0.13–0.25 μ, n = 5). These

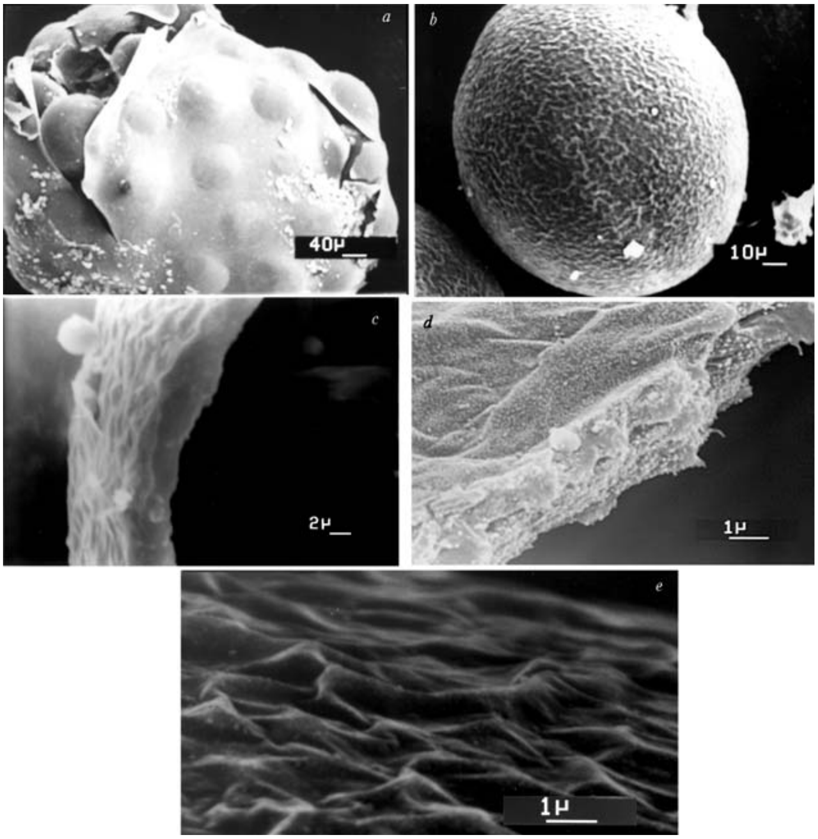

Fig. 1. *Hemidiaptomus amblyodon,* diapause egg: *a* — egg sac; *b* — egg; *c*, *d* — section of chorion; *e* — surface of chorion.

Рис. 1. *Hemidiaptomus amblyodon,* диапаузирующее яйцо: *a* — яйцевой мешок; *b* — яйцо; *c*, *d* — срез хориона; *e* — поверхность хориона.

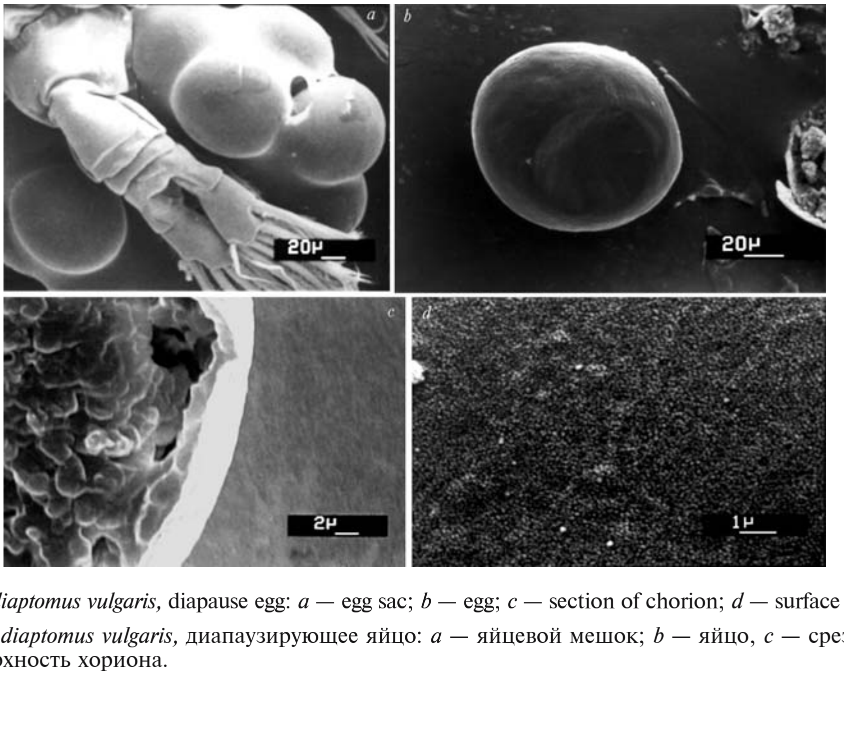

Fig. 2. *Eudiaptomus vulgaris,* diapause egg: *a* — egg sac; *b* — egg; *c* — section of chorion; *d* — surface of chorion.

Рис. 2. *Eudiaptomus vulgaris,* диапаузирующее яйцо: *a* — яйцевой мешок; *b* — яйцо, *c* — срез хориона, *d* — поверхность хориона.

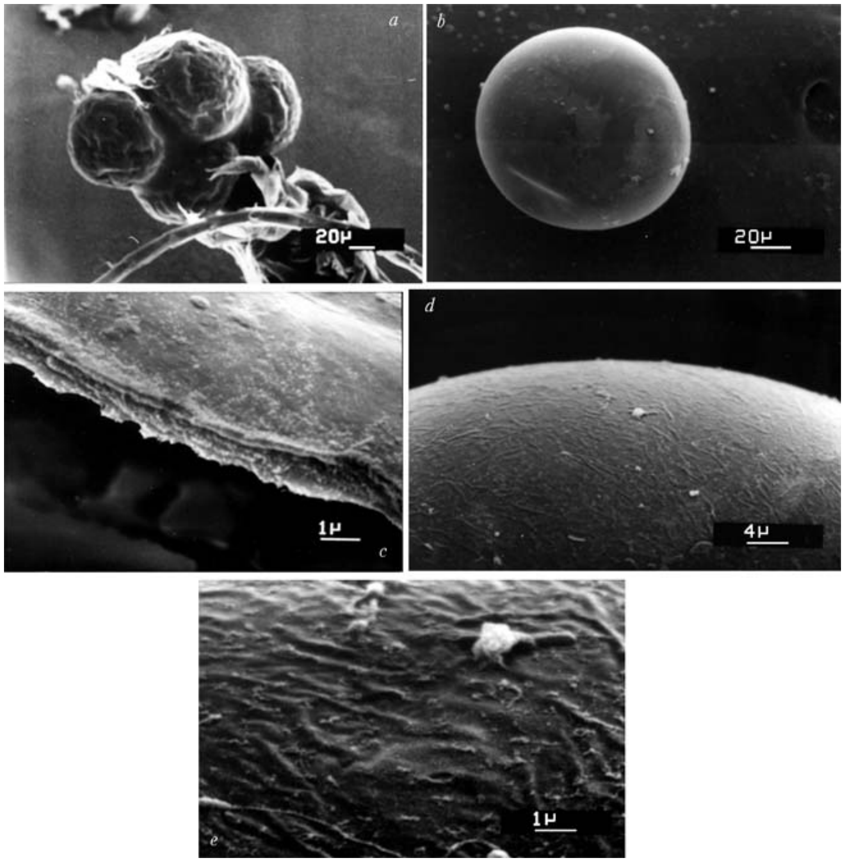

Fig. 3. *Eudiaptomus graciloides,* diapause egg: *a* — egg sac; *b* — egg; *c* — section of chorion; *d*, *e* — surface of chorion.

Рис. 3. *Eudiaptomus graciloides,* диапаузирующее яйцо: *a* — яйцевой мешок; *b* — яйцо, *c* — срез хориона, *d*, *e* — поверхность хориона.

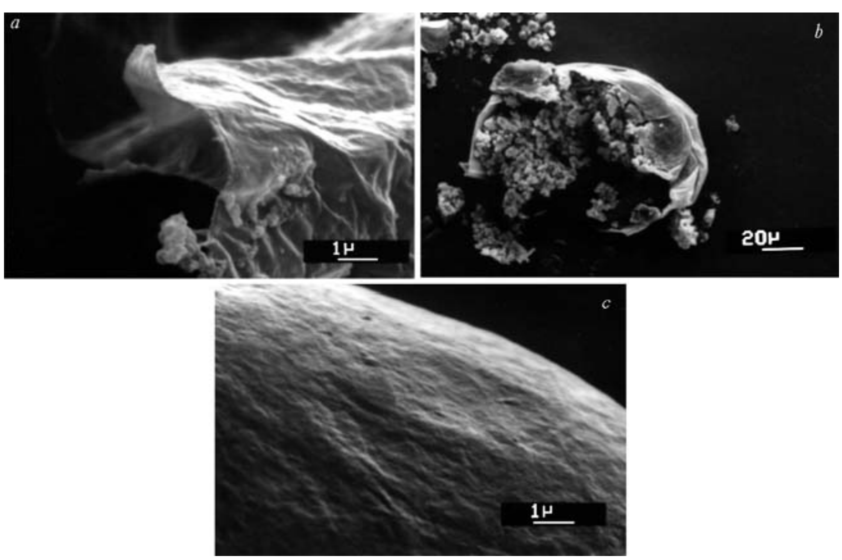

Fig. 4. *Eudiaptomus gracilis,* subitaneous egg: *a* — chorion; *b* — destroyed egg; *c* — surface of egg.

Рис. 4. *Eudiaptomus gracilis,* субитанное яйцо: *a* — хорион; *b* — разрушенное яйцо; *c* — поверхность хориона.

eggs were extraordinarily fragile. They simply have been broken up into pieces by attempts to make a section (fig. 4, *b*). The egg surface is smooth even at high magnification (fig. 4, *c*).

Discussion

The egg chorion sculpture of observed diaptomid copepods varies from smooth (*E. gracilis*) up to strongly folded (*H. amblyodon*). The typical astatic dweller, monocyclic species *H. amblyodon* spawns only diapause eggs. It inhabits shallow spring puddles, which are filled up for a short time (up to three months) by the snow melted water. Hence, for the nine months the species exists in the dormant stage as a diapause egg. After drying up of pool in May, diapause eggs are remained lying on a ground and the rest of the year they are exposed to different stress factors (desiccation, freezing etc.). Hence, two main functions of the egg chorion are to protect the embryo from the losses of moisture and from physical damage. Solid and thick chorion (fig. 1, *c*) serves to keep viability of *H. amblyodon* eggs during long period of desiccation. Eggs of the species can be spread by birds, moved by wind, etc. Sharp folds on a surface of chorion might better protect eggs from mechanical blows in contrast to smooth eggs. Folded and rough sculpture of the outermost chorion layer, we might suppose, serve as a bumper. The structure and presence of proteinaceous components of N-acetylglucosamine in egg chorion of *Hemidiaptomus* species indicate that it is similar to the cuticle of other arthropods, particularly copepods (Bouligand, 1965). Cuticle, composed by chitin, proteins, lipids and waxes (Beklemishev, 1964) plays the important role in terrestrial arthropods, protecting them from evaporation of water out their body. As more thick the cuticle as more reliably the body is protected from the losses of moisture. Egg chorion of diaptomids, inhabited non-stable inland waters, performs the similar function. The literary data show that the chorion thickness in *Hemidiaptomus* species can vary from 2.8 up to 9.4 μ (Petkovski, 1983). The chorion thickness of eggs in Ukrainian population of *H. amblyodon* is 3.2–4.8 μ ($n = 7$). Obviously, such thickness of chorion allows the diapause eggs to survive a long period of desiccation.

The chorion thickness of diapause eggs of investigated *Eudiaptomus* species is different. In *E. vulgaris*, the dweller of shallow, sometimes temporary, reservoirs, the eggs have a thicker chorion than in *E. graciloides* (1.4–1.7 μ to 0.6–1.1 μ). The latter species prefers deep-water reservoirs. Egg chorion of *E. graciloides* serves not only for protection of the embryo from low winter temperatures etc. Diapause eggs of *E. graciloides* can be easily spread by wind that allows species quickly populates new water reservoirs (Nauwerck, 1980). However, the bigger thickness of chorion as well as the higher number of eggs in egg sac (16 ± 4 in *E. vulgaris* to 4 ± 2 in *E. graciloides*) are the adaptive strategies owing to that *E. vulgaris* can survive in temporal waters more successfully than *E. graciloides*. In addition, the same egg clutch of diaptomids may contain both subitaneous and diapause eggs in it (Makrushin, 1987; Watras, 1980; Hairston, Olds, 1984; Couch et al., 2001 etc.). Such morphological and ecological peculiarities are adaptations of species to exist in temporary water-reservoirs.

The nature of dotted chorion surface (fig. 2, *d*) in eggs of *E. vulgaris* and *H. amblyodon* (fig. 1, *d*) remains unknown and invites further hystological investigations. These dots might be, as we guess, the micropores for gaseous metabolism or structures that can function like sensors for detection of environmental changes.

The diapause is absent in the life cycle of the overwintering species *E. gracilis* (Santer et al., 2000). Subitaneous eggs of this species we compare with diapause eggs of ecologically similar congener *E. graciloides*. The chorion of subitaneous eggs of *E. gracilis* is at average 4.5 (2.4–8.7) times thinner than in diapause eggs of *E. graciloides*. It is clear that resistance of subitaneous eggs, which are kept for a few days in the egg sac of female from the spawning till hatching, is lower than resistance of diapause eggs. Subitaneous eggs, probably, are not capable to pass alive through the strong stress factors (e. g. desiccation). However, at the same time previous studies shown that a small percent of subitaneous eggs of *E. gracilis* can pass undigested through the gut of fish (Bartholme et al., 2005). Probably, the cuticle-like nature of egg chorion can protect an embryo from digestive ferments of the predator (by functional analogy with tegument of helminthes).

Variability of chorion thickness in eggs of the freshwater calanoid copepods in relation with ecology of species was discussed by Champeau (1970). He showed that the monocyclic species, inhabitants of temporary waters, spawn eggs with the chorion thickness > 1.5 μ. In contrast, species from permanent waters have eggs with the chorion thickness < 1.5 μ. The author established that this rule can be applied even on the population level. Thus, different populations of *E. vulgaris* from temporary and permanent lakes have eggs with chorion thickness of 1.1–2.3 and 1–1.2 μ, respectively (Champeau, 1970). Between four observed diaptomid species, *H. amblyodon* and *E. vulgaris* have chorion thickness > 1.5 μ, whereas diapause eggs of *E. graciloides* and subitaneous eggs of *E. gracilis* have chorion thickness < 1.5 μ. This completely confirms the revealed by Champeau natural phenomenon, since the first two species inhabit mostly shallow temporary waters, while the second two, *E. graciloides* and *E. gracilis*, often are found in permanent lakes.

Knowledge of the egg morphology is still incomplete, especially in understanding of the processes of termination of diapause (by reaction on environmental changes) and level of sensitivity (and resistance) to surrounding factors, climate change.

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