Reinvestigation of vitellogenesis in *Caryophyllaeus laticeps* (Pallas, 1781) (Cestoda, Caryophyllidea, Caryophyllaeidae), monozoic tapeworm of *Abramis brama* (Pisces, Teleostei)

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Summary

Reinvestigation of vitellogenesis in the caryophyllidean cestode *Caryophyllaeus laticeps* (Pallas, 1781) has been performed using light microscope (LM) and transmission electron microscopy (TEM), and cytochemical staining with periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP) for glycogen. Vitellogenesis is generally similar to that reported in the past, however, some new observations were made. The present study reveals the first evidence of: (i) interstitial tissue in the vitelline follicles, (ii) lipid droplets in maturing and mature vitellocytes from vitelline follicles, and (iii) lamellar bodies in vitellocytes from the vitellogonad in *C. laticeps*. Projections of interstitial tissue surround each vitellocyte and the follicle periphery. The perinuclear cytoplasm of the interstitial tissue contains granular endoplasmic reticulum and vesicles of various size and density. Cytoplasmic osmiophobic lipid droplets and lamellar bodies, previously believed to be absent in most caryophyllid cestodes, are readily apparent in vitellocytes of *C. laticeps*. The origin and presumed function of these inclusions are discussed. On the other hand, the formation and storage of massive amounts of glycogen in the nucleus and large amounts in the cytoplasm of mature vitelline cells are similar to the condition found in other caryophyllids. Results are compared and contrasted with previous studies on vitellogenesis in other monopleuroid cestodes (Amphilinidea and Gyrocotylidae) as well as polypleuroid cestodes (Spathelbothriidae) and the remaining strobilated Eucestoda.

Keywords: Caryophyllidea; *Caryophyllaeus laticeps*; vitellogenesis; vitellocyte; ultrastructure

Introduction

Ultrastructural characters of the vitellarium might be useful

for a better understanding of the relationships between different taxa within the neoophoran Platyhelminthes (Gremigni & Falleni, 1998) including Cestoda (Świderski & Mackiewicz, 1976; Świderski & Xylander, 2000; Bruňanská *et al.*, 2005; Poddubnaya *et al.*, 2006, 2012; Levron *et al.*, 2007). The structure and composition of eggshell-forming globules in vitellocytes have been considered to be especially phylogenetically informative (Gremigni & Falleni, 1991; Falleni *et al.*, 2001).

To date, limited data are available on vitellogenesis in the Caryophyllidea, one of the basal groups of the Eucestoda. Out of about 150 species of four currently recognised families (Mackiewicz, 1994; Oros *et al.*, 2008) seven members belonging to the two families (Caryophyllaeidae, Lytocestidae) were studied, namely *Archigetes sieboldi* (by Poddubnaya *et al.*, 2003; Bruňanská *et al.*, 2012b), *Caryophyllaeus laticeps* (Pallas, 1781) (by Ortner-Schönbach, 1913; Mackiewicz, 1968; Świderski *et al.*, 2004a; Bruňanská *et al.* 2010), *Caryophyllaeides fennica* (by Mackiewicz, 1968), *Glavidacris catostomi* (by Świderski & Mackiewicz, 1976), *Wenyonia virilis* (by Świderski *et al.*, 2009), *Atractolytocestus huronensis* (by Bruňanská *et al.*, 2009), *Khawia armeniaca* (by Świderski *et al.*, 2004b) and *Monobothrioides chalmersius* (by Arafa 2012). As far as *C. laticeps* is concerned, the first histological and histochemical investigation on vitellogenesis was based on light microscopy (Mackiewicz, 1968), and supplemented by ultrastructural data in two brief abstracts (Świderski *et al.*, 2004a, Bruňanská *et al.*, 2010). The latter reported lipid droplets, never found previously in vitellocytes of the Caryophyllidea.

The purpose of the present paper is to give detailed information on the ultrastructure of vitellaria, developing vitellocytes and vitellogonad of *C. laticeps*, the oldest fish tapeworm parasitizing a wide spectrum of cypriniform fishes in the Palaearctic region (Scholz *et al.*, 2011). These data

may extend our current knowledge on reproductive biology and evolutionary history of the Caryophyllidea.

Materials and methods

Adult specimens of the caryophyllidean cestode *Caryophyllaeus laticeps* (Pallas, 1781) were collected from the intestine of bream, *Abramis brama* (Pisces: Cyprinidae), from Rybinsk Reservoir, Yaroslavl Province, Russia. The worms were cut into small pieces, fixed immediately in ice cold 3 % glutaraldehyde in sodium cacodylate buffer (pH 7.4) for 3 – 8 h, followed by 3 changes of cacodylate buffer, and postfixed in 1 % OsO₄ for 2h. Material was dehydrated in a graded ethanol series, and embedded in Araldite. Ultrathin sections (60 – 70 nm) were cut using a Leica Ultracut UCT ultramicrotome, placed on copper grids and double-stained with uranyl acetate and lead citrate. The grids were examined in a JEOL 1010 transmission electron microscope operated at 80 kV.

The periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP) technique of Thiéry (1967) was applied to determine specific cytochemical localisation of glycogen at the ultrastructural level.

Results

Vitellaria of *C. laticeps* are situated in lateral and median bands in the medullary parenchyma of a non-segmented (monozoic) strobila (Fig. 1A; Mackiewicz, 1968), where they occur in preovarian and postovarian positions. Vitellaria consist of numerous oval or lobate follicles (Figs. 1, 2) of various sizes. They are interconnected by small collecting ducts, two larger lateral ducts that connect and form a major transverse vitelline duct that connects the vitellarium to the ootype. Each vitelline follicle is composed of vitelline cells at various stages of development in a matrix of interstitial tissue (Fig. 3).

Interstitial tissue

Interstitial tissue extends between vitellocytes within the vitellarium (Figs. 3, 4) and surrounds the follicles periphery. The nuclei of the interstitium occur either between vitellocytes (Fig. 3), or at the periphery of the vitelline follicle (Fig. 4). They contain an electron-dense nucleolus and scattered clumps of heterochromatin. The periphery of the nucleus is bordered by a thin layer of electron-dense chromatin. The perinuclear cytoplasm of the interstitial tissue contains granular endoplasmic reticulum and vesicles of various size and density (Fig. 4).

Vitellocytes

Four stages (I – IV) can be recognized during differentiation of the vitellocytes: (I) immature, (II) early maturation, (III) advanced maturation, and (IV) mature (Fig. 3).

Immature (I)

Immature vitelline cells are localised at the follicular pe-

riphery. They have a large nucleus with distinct nucleolus and show a high nucleo-cytoplasmic ratio (Fig. 5). Irregular clumps of dense chromatin occur in the nucleoplasm. Several small mitochondria and more abundant ribosomes are present in the cytoplasm.

Early maturation (II)

The most characteristic feature of the vitellocytes at this stage (Fig. 2) is the small electron-dense vesicles that are the earliest form of shell globules (Fig. 6). Formation of electron-dense vesicles is associated with Golgi complexes and endoplasmic reticulum (Fig. 7). The endoplasmic reticulum consists of short rows of smooth membranes and long parallel cisternae.

Advanced maturation (III)

The maturation of cells is characterized by an increase in size, decrease of the nucleo-cytoplasmic ratio, development of endoplasmic reticulum and Golgi complexes, formation of shell globule clusters and glycogen, and nuclear and nucleolar transformation (Fig. 3). Extensive cisternae of endoplasmic reticulum and Golgi complexes continue in producing single shell globules (Fig. 8) and shell globule clusters (Fig. 9). Minute secretory vesicles fuse and form secretory globules, which join together to form shell globule clusters. Initially, electron-dense shell globules are loosely packed in electron-lucent matrix of shell globule clusters (SGCa, Figs. 10, 11). Later, shell globule clusters are built of tightly packed globules (SGCb, Figs. 12 – 19, 21, 22).

The shape of the nucleus changes from oval (Fig. 8) to lobate (Figs. 10, 11). It occupies a central region of the cell. The nucleoplasm includes numerous clumps of condensed chromatin (Fig. 11). In more advanced stages of vitellogenesis, glycogen particles stained with PA-TSC-SP form islands of various sizes in the nucleus (Figs. 12, 13). Moderately electron-dense cytoplasm may contain electron-lucent amorphous parts with scattered glycogen granules (Figs. 11 – 13). Occasionally, single lipid droplets have been observed in developing vitellocytes (Fig. 14).

Mature (IV)

The oval mature vitellocyte measures about 25 µm in diameter. Its nucleus and cytoplasm are filled with glycogen (Fig. 15, 16). Nucleolus and chromatin material are displaced towards the nuclear periphery (Fig. 15). Amorphous vitelline cytoplasm is filled with shell globules and shell globule clusters. The later are represented by tightly packed electron-dense globules within matrix of moderate electron density (SGCb, Figs. 15, 16).

In addition to the vitelline material described above, single osmiophobic lipid droplets have been observed occasionally in mature vitellocytes (Figs. 17 – 19). They are localised either in the perinuclear cytoplasm (Fig. 17), at the periphery of the cell (Fig. 18), or irregularly scattered in the cytoplasm (Fig. 19).

Figs. 1 – 7. Overview of the vitelline follicles in *C. laticeps*. Details of immature vitelline cells (I) and vitellocytes at early stage of maturation (II).
 (1) General view of vitellarium of *Caryophyllaeus laticeps* in semithin transverse section. T – tegument, VF – vitelline follicles. Bar = 100 μ m;
 (2) Vitelline follicle in semithin transverse section. VC – vitellocyte. Bar = 100 μ m; (3) Tangential section through a vitelline follicle of *C. laticeps* showing vitellocytes at various stages of development. I – immature vitellocytes, II – early maturing vitellocytes, III – advanced maturing vitellocytes, IV – mature vitellocytes, IS – interstitial tissue, N – nucleus of the IS. Bar = 10 μ m; (4) Nucleus (N) of an interstitial tissue at the periphery of vitelline follicle. arrows – electron-dense vesicles, ER – endoplasmic reticulum, Nu – nucleolus, VC – vitelline cell. Bar = 2 μ m;
 (5) Immature vitellocyte (I). N – nucleus, Nu – nucleolus, M – mitochondrion. Bar = 2 μ m; (6) Early maturing vitellocyte (II), and formation of small electron-dense vesicles (arrows). N – nucleus, Nu – nucleolus. Bar = 3.3 μ m; (7) Vitelline cytoplasm of early maturing vitellocyte (II) showing endoplasmic reticulum (ER) and Golgi (Go) with a small electron-dense vesicles (arrows). Bar = 0.7 μ m.

Figs. 8 – 15. Vitelline cells at advanced stage of maturation (III).

(8) Advanced maturing vitelloocyte (III) and various stages of electron-dense shell globule formation (SG). ER – endoplasmic reticulum, N – nucleus, Nu – nucleolus. Bar = 2 μ m; (9) Detail of the periphery of the advanced maturing vitelloocyte (III) showing formation of shell globule clusters (SGC). ER – endoplasmic reticulum. Bar = 2 μ m; (10) Shell globule clusters with electron-lucent matrix and loosely packed shell globules (SGCa) in advanced maturing vitelloocyte (III). ER – endoplasmic reticulum, N – nucleus. Bar = 2 μ m; (11) Cytoplasm of advanced maturing vitelloocyte (III) contains electron-lucent area filled with dense granules of glycogen (G). Hch – heterochromatin, N – nucleus, SGCa – shell globule clusters with electron-lucent matrix and loosely packed shell globules. Bar = 6 μ m; (12) Intracellular glycogen (G) in maturing vitelloocytes (III). N – nucleus, Nu – nucleolus, SGCb – shell globule clusters composed of tightly packed shell globules embedded within matrix of moderate electron density. Bar = 6 μ m; (13) Visualisation of glycogen (G) in maturing vitelloocytes (III) by Thiéry method. N – nucleus, SGCb – shell globule clusters. Bar = 1 μ m; (14) Lipid droplet (L) in perinuclear region of a maturing vitelloocyte (III). N – nucleus. Bar = 6 μ m; (15) The amount of intracellular and intracytoplasmic glycogen (G) increases with maturing of vitelloocytes (III). N – nucleus, Nu – nucleolus, SGCb – shell globule clusters. Bar = 3 μ m.

Vitellocytes within the vitellogen

Mature vitellocytes from the vitellogens are not identical to those of the vitelline follicles (Fig. 20). In addition to a nucleus, tightly packed shell globule clusters (SGCb), glycogen and lipid droplets (very rarely), the cytoplasm of mature vitellocytes from the vitellogen (Fig. 22, inset) has additional single bodies with dense spiral or concentric lamellar configuration. These lamellar bodies, absent from vitellocytes within the follicle, measure approximately 0.8 μm . The wall of the vitellogen is built by flat epithelium (Figs. 20, 23) lined with cilia of 9+2 configuration (Fig. 23). The axonemes of the cilia are attached with their basal bodies.

volves a continuous increase in cell size, an extensive development of cell components engaged in shell globule production, and formation of the so-called “nuclear vacuole”, a very specific feature in the Caryophyllidea. Significant differences, however, exist with regard to the presence/absence of the interstitial tissue in the vitelline follicles, and the occurrence of vitelline inclusions, namely lipids, and lamellar bodies.

Interstitial tissue has not been described previously in *C. laticeps*. This component is present in vitelline follicles of some caryophyllidean tapeworms (see Świderski & Mackiewicz, 1976; Bruňanská *et al.*, 2009; Świderski *et*

Figs. 16 – 19. Mature vitelline cells (IV) from the vitelline follicles.

(16) Cytoplasm and nucleus (N) of mature vitellocytes (IV) are packed with glycogen (G). Remnants of an electron-dense cytoplasm are concentrated in the perinuclear region and at periphery of the cell. N – nucleus, SGCb – shell globule clusters. Bar = 5 μm ; (17) Lipid droplets (L) in perinuclear region of a mature vitellocyte. SGCb – shell globule cluster. Bar = 2 μm ; (18) Detail of lipid droplets (L) and shell globule cluster (SGCb) at periphery of the mature vitellocyte (IV). G – glycogen. Bar = 1 μm ; (19) Scattered lipid droplets (L) in cytoplasm of the mature vitellocyte (IV). G – glycogen, SGCb – tightly packed shell globule cluster. Bar = 2 μm .

Discussion

The general pattern of vitellocyte differentiation and ultrastructure in *C. laticeps* resembles that previously reported for this species and/or other caryophyllideans (Mackiewicz, 1968; Świderski & Mackiewicz, 1976; Świderski *et al.*, 2004a, 2004b, 2009). Vitellogenesis in-

al., 2009; Bruňanská *et al.*, 2012b; Arafa 2012; present study). Interstitial tissue has been proposed to be involved in selection and distribution of nutrients transported from the parenchyma toward the vitellocytes of some cestodes (Xylander, 1988; Bruňanská *et al.*, 2009), and monogeneans (Halton *et al.*, 1974). In addition to the caryophyllideans, interstitial tissue has been observed also in spatheboth-

Figs. 20 – 23. Mature vitelline cells (IV) from vitellogenic duct and ultrastructure of the wall of the vitellogenic duct. (20) Vitellocytes in vitellogenic duct. N – nucleus of vitellocyte, NW – nucleus of epithelial wall of the vitellogenic duct, SGCb – shell globule clusters, W – wall of the vitellogenic duct. Bar = 10 μ m; (21) Detail of membrane-bound shell globule clusters (SGCb) in vitellocytes from the vitellogenic duct. W – the wall of vitellogenic duct. Bar = 2 μ m; (22) The lamellar bodies (LB) within cytoplasm of the mature vitellocytes in the vitellogenic duct. SGCb – shell globule clusters, W – wall of vitellogenic duct with traces of cilia. Bar = 2 μ m. Inset: detail of lamellar body (LB). Bar = 0.55 μ m; (23) Detail of the epithelial wall (W) of vitellogenic duct. BB – basal body of cilium, C – cilium. Bar = 0.5 μ m.

riideans (Bruňanská *et al.*, 2005; Poddubnaya *et al.*, 2006), bothriocephalideans (Korneva, 2001; Levron *et al.*, 2007; Świderski *et al.*, 2011a), trypanorhynch (Świderski *et al.*, 2006b; 2012), nipotaeniideans (Korneva, 2002), and proteocephalideans (Korneva & Davydov, 2001). Interstitial cells may also occur in some trypanorhynch (Świderski *et al.*, 2006a, 2007, 2012).

In the present study, mature vitellocytes of *C. laticeps* contain four types of vitelline material (shell globule/ shell globule clusters, glycogen, lipids, lamellar bodies), thus resembling *A. sieboldi* (Bruňanská *et al.*, 2012b). In contrast, only three types of vitelline inclusions (shell globules/shell globule clusters, glycogen, lipid droplets) were previously described in *C. laticeps* in a brief abstract (Bruňanská *et al.*, 2010). Three different types of vitelline material (shell globules/shell globule clusters, glycogen, “lamellar” granules [= lamellar bodies, present paper]) have been found in the triploid *A. huronensis* (Bruňanská *et al.*, 2009). Two types of vitelline material (shell globule clusters and glycogen) have been reported in the light microscope study on the histology/histochemistry of vitellogenesis of *C. laticeps* and *Caryophyllaeides fennica* by Mackiewicz (1968), in a brief abstract of an ultrastructural study of *C. laticeps* by Świderski *et al.* (2004a), and in

ultrastructural studies of some caryophyllideans (Świderski & Mackiewicz, 1976; Świderski *et al.*, 2004b).

It should be noted that the ultrastructural data above are from species in two families: Caryophyllaeidae and Lytocestidae. Recent studies of vitellogenesis from *Monobothrioides chalmersii* of the family Lytocestidae by Arafat (2012), however, have reported five kinds of vitelline cell inclusions: shell globule clusters, glycogen, lipid droplets, translucent vesicles and electron-dense vesicles. The significance of these differences within and between families is unclear and may be elucidated only with more extensive sampling of all stages of vitellogenesis in caryophyllidean cestodes from all of the four families.

Shell globule clusters in the maturing vitellocytes of *C. laticeps* are primarily of two forms that represent early and later stages of cluster development. Similar finding has been described in the caryophyllidean *A. huronensis* (Bruňanská *et al.*, 2009). The first form (loosely packed electron-dense shell globules situated in electron-lucent matrix) occurs in the initial stages of shell globule cluster formation of *C. laticeps*. This form of shell globule cluster appears to be characteristic for the mature vitellocytes of most primitive tapeworms (Xylander, 1987, 1988), some caryophyllideans (Poddubnaya *et al.*, 2003; Świderski *et*

al., 2004b), spathebothriideans (Bruňanská *et al.*, 2005; Poddubnaya *et al.*, 2006) and/or pseudophyllideans (Świderski & Mokhtar, 1974; Korneva, 2001; Levron *et al.*, 2007). The second form (tightly packed shell globules embedded in matrix of moderate electron density) is found in caryophyllideans (Świderski & Mackiewicz, 1976; Świderski *et al.*, 2004a; Bruňanská *et al.*, 2012b; present study), and spathebothriideans (Bruňanská *et al.*, 2005; Poddubnaya *et al.*, 2006).

The lack of cytoplasmic lipid droplets and the large amount of glycogen have been considered to be the most characteristic features of vitellocytes in the Caryophyllidea (Świderski & Xylander, 2000). However, recent investigations have revealed the presence of lipid droplets in the mature vitellocytes of some caryophyllideans, e.g. *A. sieboldi* (Bruňanská *et al.*, 2012b), *C. laticeps* (Bruňanská *et al.*, 2010; present study) and *Monobothrioides chalmersius* (Arafa, 2012). In addition, lipid droplets were also described from mature vitellocytes of the triploid *A. huronensis* in a brief abstract (Bruňanská *et al.*, 2007), in degenerating vitellocytes within intrauterine eggs of caryophyllideans *Wenyonia virilis* (Młocicki *et al.*, 2010) and *Khawia sinensis* (Bruňanská *et al.*, 2012a). Lipid droplets have been detected in the maturing vitellocytes (stage III) of *C. laticeps* (present study) and in the mature vitellocytes (stage IV) of *A. sieboldi* (Bruňanská *et al.*, 2012b) and *Monobothrioides chalmersius* (Arafa, 2012). They are osmiophobic and electron-lucent, a feature being indicative of saturated lipids (Świderski & Xylander, 2000). The presence of saturated lipid droplets has been also described in amphilinideans, gyrocotylideans (Xylander, 1987; 1988), spathebothriideans (Bruňanská *et al.*, 2005; Poddubnaya *et al.*, 2006), bothriocephalideans (Świderski & Mokhtar, 1974; Korneva, 2001; Levron *et al.*, 2007), tetracyphallideans (Mokhtar-Maamouri & Świderski, 1976), trypanorhynchids (Świderski *et al.*, 2006a, b, 2007, 2012), diphyllideans (Świderski *et al.*, 2011b), and/or proteocephalideans (Bruňanská, 1997, 1999). Given that lipids in the developing vitellocytes of caryophyllidans have been detected only very rarely, it is possible that their role in embryogenesis may be more variable than previously supposed (Bruňanská *et al.*, 2012b).

Lamellar bodies were found for the first time in vitelline cells from the vitellogonad in *C. laticeps*. These single dense components with spiral or concentric lamellar configuration have been detected previously in the mature vitellocytes from vitelline follicles of *A. huronensis* (Bruňanská *et al.*, 2009), *K. sinensis* (Bruňanská *et al.*, 2010), *A. sieboldi* (Bruňanská *et al.*, 2012b), and vitellocytes within intrauterine eggs of *W. virilis* (Młocicki *et al.*, 2011), *K. sinensis* (Bruňanská *et al.*, 2012a), or *A. sieboldi* (Bruňanská *et al.*, 2012b). This latter study indicates that lamellar bodies may be formed either during gradual transformation of shell globule/shell globule clusters or from the breakdown of granular endoplasmic reticulum in the mature vitellocytes. The current study on *C. laticeps* supports a close relation between lamellar bodies and the breakdown of granular endoplasmic reticulum. Lamellar

bodies have been proposed to be involved in glycoprotein synthesis and/or the formation of foci of cytoplasmic degradation (lamellar bodies = “GER-bodies” of Młocicki *et al.*, 2011). On the other hand, according to Poddubnaya *et al.* (2006) ‘concentric bodies’ (= lamellar bodies, present paper) in the spathebothriidean cestode *Didymobothrium rudolphii* represent a phase in vitelline globule transformation during the process of egg shell formation.

Ultrastructural characters of the vitellocytes in the Caryophyllidea, as exemplified by the mass of glycogen in the nucleus, for example, are closely related to the nature of their life-history (Mackiewicz, 1981). The occurrence of lipid droplets and lamellar bodies in the vitellocytes of *C. laticeps* (present study), and *A. sieboldi* (Bruňanská *et al.*, 2012b) indicates that vitellogenesis within the Caryophyllidea is more complex than previously thought. Further investigations into vitellogenesis and fertilisation of other caryophyllideans and related cestodes are needed to provide additional ultrastructural data for elucidation of the reproductive biology and evolutionary history of the lower tapeworms.

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