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Vitellogenesis in *Archigetes sieboldi* Leuckart, 1878 (Cestoda, Caryophyllidea, Caryophyllaeidae), an intestinal parasite of carp (*Cyprinus carpio* L.)

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Summary. Vitellogenesis in the caryophyllidean tapeworm *Archigetes sieboldi* Leuckart, 1878, from carp *Cyprinus carpio* L. in Slovakia, has been examined using transmission electron microscopy and cytochemical staining with periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP) for glycogen. Vitelline follicles extend in two lateral bands in the medullary parenchyma along both sides of the monozoic body. They are surrounded by an external basal lamina and contain vitellocytes and an interstitial tissue. The general pattern of vitellogenesis is essentially like that of other caryophyllideans. It involves four stages: immature, early maturing, advanced maturing cells and mature vitellocytes. During vitellogenesis, a continuous increase in cell volume is accompanied by an extensive development of cell components engaged in shell globule formation, e.g. granular endoplasmic reticulum and Golgi. Shell globule clusters are membrane-bound. Nuclear and nucleolar transformation are associated with formation and storage of large amounts of intranuclear glycogen, a very specific feature of the Caryophyllidea. For the first time, (a) additional vitelline material in *Archigetes* is represented by lamellar bodies and (b) lipid droplets are described in the mature vitellocytes from vitelline follicles and vitelloduct of the Caryophyllidea. Our results indicate that there may be a double origin of lamellar bodies: either from the endoplasmic reticulum or through transformation of shell globule/shell globule clusters. Lamellar body clusters and some single lamellar bodies appear to have a membrane. Other ultrastructural features of vitellogenesis and/or vitellocyte in *A. sieboldi* from its vertebrate (fish) and invertebrate (oligochaete) hosts are briefly compared and contrasted with those in other caryophyllideans and/or Neodermata.

Key words: Caryophyllidea, *Archigetes sieboldi*, vitellogenesis, Ultrastructure

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Introduction

The monozoic caryophyllidean tapeworm *Archigetes sieboldi* Leuckart, 1878 has been the subject of extensive observations and discussions in conjuction with the origin and evolution of tapeworms (Mackiewicz, 1972, 1981, 1982, 2003). This cestode can have either the more common two-host life cycle characteristic of the Caryophyllidea, or a monoxenic one in an invertebrate, a tubificid annelid. Although the phylogenetic position of *Archigetes* has recently been analysed using molecular systematics (Olson et al., 2008), there are still questions regarding the evolutionary significance of this enigmatic tapeworm.

Ultrastructural studies have described the fine structure of various organ systems of progenetic *Archigetes sieboldi* from the oligochaete, including some comments on the vitelline system (Poddubnaya, 2003; Poddubnaya et al., 2003). However, there are no comparative data of vitellocytes of *Archigetes* species from both its vertebrate (fish) and invertebrate (oligochaete) hosts. Investigations on vitellogenesis in six caryophyllidean species, from the vertebrate hosts only, and belonging to the Caryophyllaeidae and Lytocestidae, have revealed variations in the composition and fine structure of vitelline material

(Ortner-Schönbach, 1913; Mackiewicz, 1968; Swiderski and Mackiewicz, 1976; Świderski et al., 2004a,b, 2009; Bruňanská et al., 2009).

The purpose of the present study is, therefore, to determine ultrastructural features of vitellogenesis in *A. sieboldi*, an intestinal parasite of carp (*Cyprinus carpio L.*) from Slovakia. Particular attention is paid to characteristics of the mature vitellocytes as related to comparative embryogenesis in tapeworms, e.g. presence/absence and origin of lamellar bodies. Such knowledge will not only provide developmental data currently lacking for *A. sieboldi* but also better define variation in vitellogenesis in the basal group of cestodes.

Materials and methods

Four adult specimens of *Archigetes sieboldi* were obtained in 2008 from the intestine of *Cyprinus carpio* from the Tisa River, Slovakia. Worms were immediately fixed in 3% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) for 10 hours at 4°C, postfixed in 2% OsO_4 in 0.1M cacodylate buffer for 2 hours at 4°C, dehydrated in a graded series of ethanol and embedded in Spurr epoxy resin. Semithin sections were cut with glass knives on an ultramicrotom LKB Bromma 8880, stained with methylene blue and examined under a light microscope for localization of vitellaria. Ultrathin sections were cut with a diamond knife on 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 operating at 80 kV.

The periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP) technique of Thiéry (1967) was applied in order to determine the specific cytochemical localization of glycogen.

Given the differing terminology and homologies with respect to the nature and function of vitelline cell inclusions having a lamellar structure (Młocicki et al., 2011), the general term "lamellar bodies" is used to denote any inclusion with that morphology. Clearly, more data is needed before the various forms or types of *lamellar bodies* in trematodes and cestodes can be distinguised from each other and named and their functions determined.

Results

Vitellaria of *A. sieboldi* extend in two lateral bands in the medullary parenchyma along both sides of the monozoic body. They are interrupted in the ovary region and continue posteriorly as postovarian vitelline follicles. Numerous oval or lobate follicles varying in size (Fig. 1) are interconnected by vitelline ductlets. The two larger lateral and one transverse vitelline ducts connect the pre- and postovarian vitellaria to the oötype.

Vitelline follicles contain vitelline cells, at various stages of development, and interstitial tissue (Fig. 2). The nucleus of the interstitium occurs at the periphery of the follicle (Fig. 3). Its nucleoplasm contains 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 granular cytoplasm of the interstitial tissue contains a few mitochondria with a dense matrix. The follicles are surrounded by an external basal lamina (Fig. 3).

Four different stages (I-IV) can be recognized during cytogenesis of the vitellocytes (Fig. 1): (I) immature cells; (II) cells at early stage of maturation; (III) cells at advanced stage of maturation; and (IV) mature vitelline cells.

Immature vitellocytes (I) (Figs. 2, 4, 5 inset)

Immature vitelline cells occur predominantly at the periphery of the vitelline follicles (Figs. 2, 4). These undifferentiated cells with a high nucleo-cytoplasmic ratio have a more or less polyhedral shape (approximately 5 μm in diameter). The large nucleus shows irregular clumps of dense chromatin scattered in the nucleoplasm (Fig. 4). The nucleolus was not found in the early immature vitelline cells. It is present in the late immature vitellocytes which show the first signs of secretory activity in their cytoplasm (Fig. 5 inset).

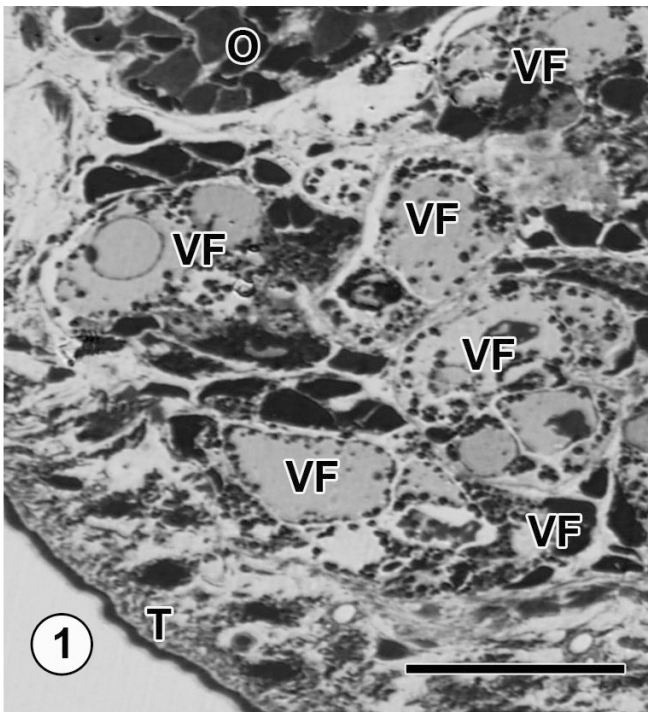

Fig. 1. General view of the vitellarium of *Archigetes sieboldi* in semithin transverse section. O: peripheral region of the ovary, T: tegument, VF: vitelline follicle. Bar: 50 μm.

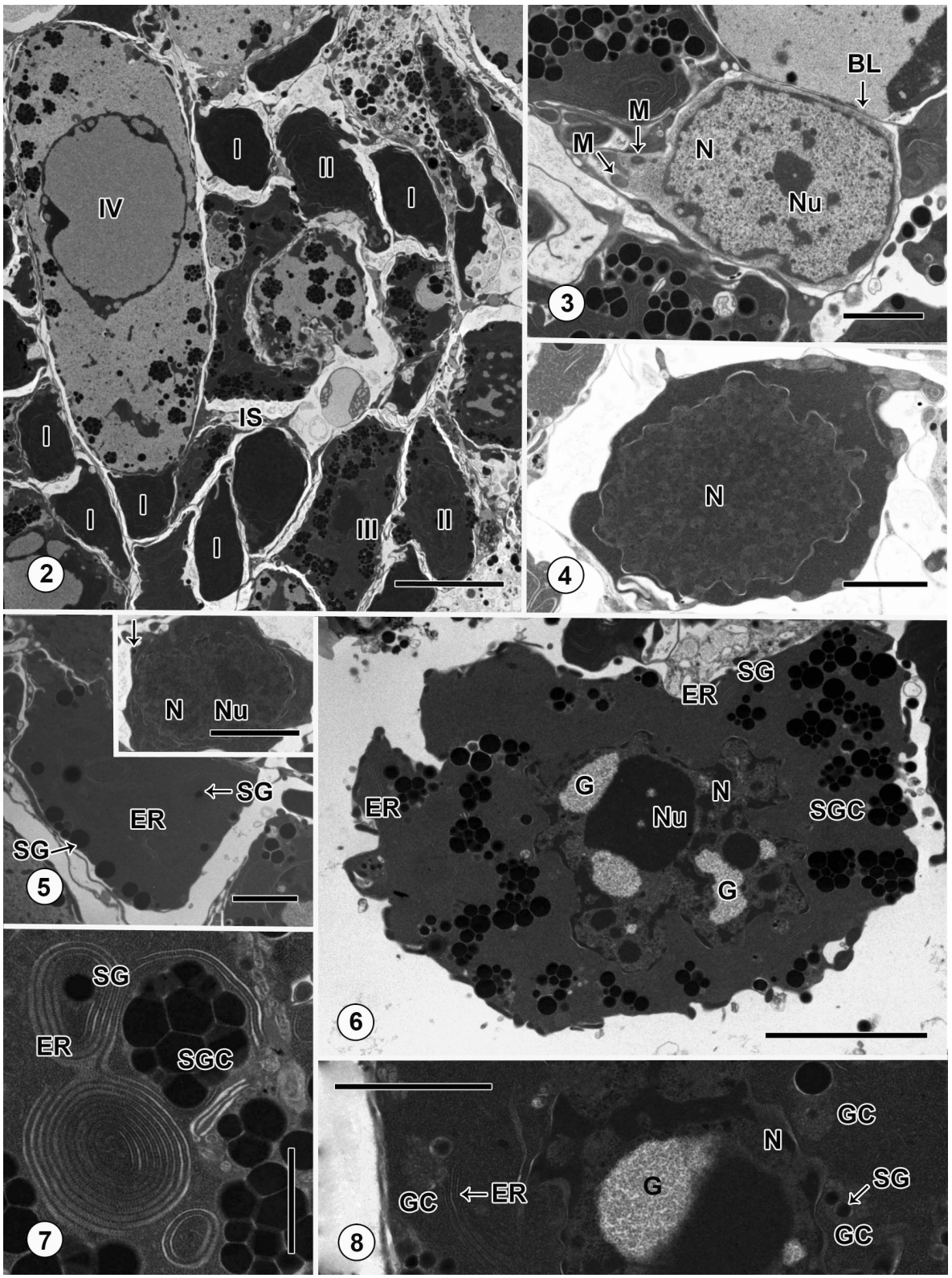

Fig. 2. Tangential section through a vitelline follicle of *Archigetes sieboldi* illustrating vitellocytes at various stages of development. I: immature vitellocytes, II: early maturing vitellocytes, III: advanced maturing vitellocytes, IV: mature vitellocytes, IS: interstitial tissue. Bar: 10 μm. **Fig. 3.** The nucleus (N) of interstitial tissue cell at the periphery of the vitelline follicle. BL: basal lamina, Nu: nucleolus, M: mitochondria. Bar: 2 μm. **Fig. 4.** Detail of an immature vitellocyte (I). N: nucleus. Bar: 2 μm. **Fig. 5.** Vitelline cytoplasm of the early maturing vitellocyte (II) showing various stages of electron-dense shell globule formation (SG). Bar: 2.5μm. Inset: Note nucleolus (Nu) in the nucleus (N) of a late immature vitellocyte (I), and formation of a small electron-dense vesicle (arrow). Bar: 5 μm. **Fig. 6.** Advanced maturing vitellocyte (III). ER: endoplasmic reticulum, G: intranuclear glycogen, SG: shell globule, SGC: shell globule clusters. N: nucleus. Bar: 5 μm. **Fig. 7.** Parallel concentric rows of endoplasmic reticulum (ER) with a shell globule (SG) or shell globule clusters (SGC) at their centre. Bar: 1 μm. **Fig. 8.** Golgi (GC) are in close association with endoplasmic reticulum (ER) in the maturing vitellocytes. Note electron-dense shell globules (SG) within GC. G: intranuclear glycogen, N: nucleus. Bar: 2 μm.

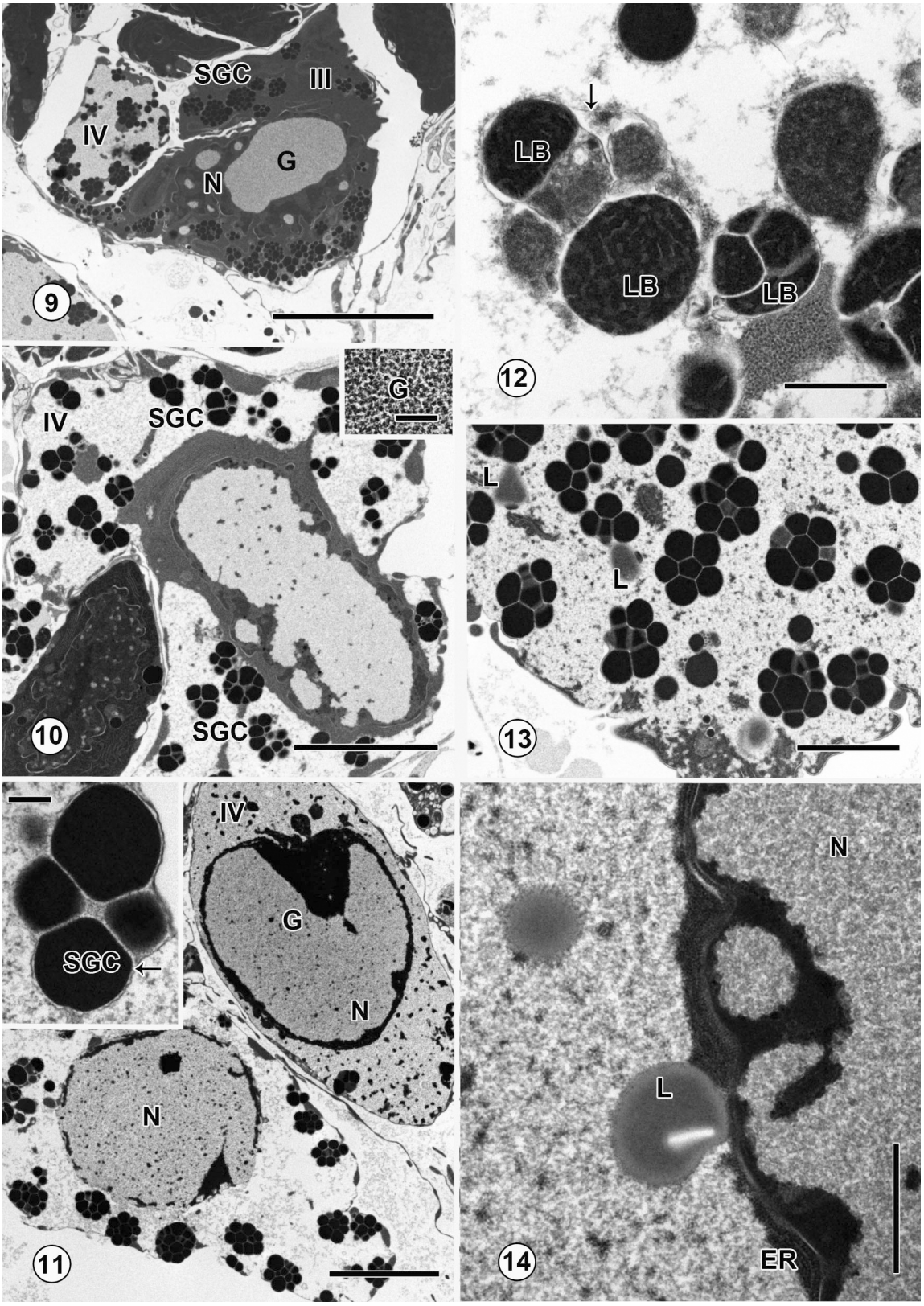

Fig. 9. The amount of intranuclear glycogen (G) increases with maturing of vitellocytes (III). Note that the cytoplasm is still electron-dense and contains predominantly shell globule clusters of various size (SGC). N: nucleus. Bar: 10 μm. **Fig. 10.** Cytoplasm of the mature vitellocytes (IV) becomes more electron-lucent. Remnants of an electron-dense cytoplasm are concentrated in the perinuclear region and at the periphery of the cell. SGC - shell globule clusters. Bar: 5 μm. Inset: Glycogen (G) in the nucleus of mature vitellocytes as demonstrated by Thiéry method. Bar: 0.5 μm. **Fig. 11.** Nucleus (N) of mature vitellocytes (IV) is packed with glycogen (G) and it is lined with thin electron-dense perinuclear region. Bar: 5μm. Inset: Note membrane-bound (arrow) shell globule cluster (SGC) in the mature vitellocyte. Bar: 0.25 μm. **Fig. 12.** Gradual transformation of shell globule/shell globule clusters into lamellar body/ lamellar body clusters (LB). Arrow: outer membrane of the lamellar body cluster. Bar: 1 μm. **Fig. 13.** Cytoplasm of the mature vitellocytes contains lipid droplets occasionally (L). Bar: 2 μm. **Fig. 14.** Lipid droplets (L) in the perinuclear region of a mature vitellocyte. Possible electron-lucent region in lipid droplet. ER: endoplasmic reticulum in the perinuclear region, N: nucleus. Bar: 1 μm.

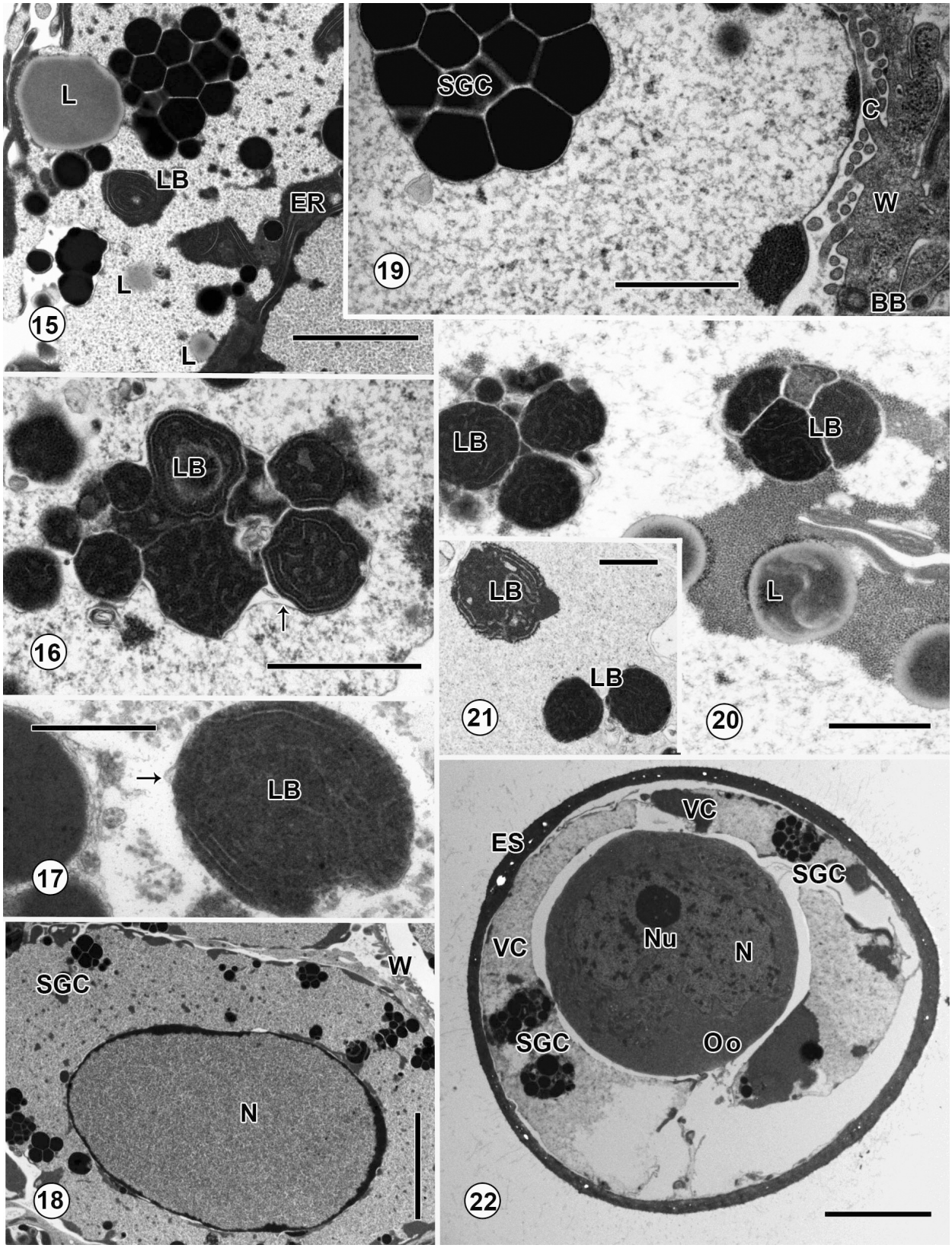

Fig. 15. Formation of the lamellar body (LB) is closely associated with perinuclear cytoplasm containing endoplasmic reticulum (ER). L: lipid droplets. Bar: 2 μm. **Fig. 16.** Detail of the lamellar bodies (LB) within the cytoplasm of the mature vitellocytes. Note outer membrane (arrow) surrounding LB. Bar: 1 μm. **Fig. 17.** Lamellar body (LB) from a mature vitelline follicle. Note outer membrane (arrow). After application of Thiéry method. Bar: 500 nm. **Fig. 18.** Vitellocyte in the vitelloduct. N: nucleus of vitellocyte, SGC: shell globule clusters, W: the wall of the vitelloduct. Bar: 5 μm. **Fig. 19.** Detail of the membrane-bound shell globule clusters (SGC) and the vitelloduct wall (W). BB: basal body of the vitelloduct cilium, C: cilium. Bar: 1 μm. **Fig. 20.** The lamellar bodies (LB) within the cytoplasm of the mature vitellocytes in the vitelloduct. Bar: 1 μm. **Fig. 21.** The lamellar bodies (LB) within the cytoplasm of the vitellocytes in the intrauterine egg. Bar: 1 μm. **Fig. 22.** Some of the vitellocytes (VC) within intrauterine egg still contain complete shell globule clusters (SGC). ES: eggshell, Oo: fertilised oocyte, N: nucleus of the oocyte, Nu: nucleolus of the oocyte. Bar: 5 μm.

Early maturing vitellocytes (II) (Figs. 2, 5)

Early maturation is characterised by formation of electron-dense vesicles predominantly in the perinuclear cytoplasm. At this stage, secretory activity is associated with the endoplasmic reticulum (Fig. 5) which consists of short rows of membranes and well developed long parallel cisternae. Individual secretory globules of various size and electron-density are in the cytoplasm of the early maturing vitellocytes. They represent the earliest stage of shell globule formation.

Advanced maturing vitellocytes (III) (Figs. 2, 6, 7, 8, 9)

The maturation of vitellocytes is accompanied by an increase in cell size (about three times), a decrease in the nucleo-cytoplasmic ratio, nuclear and nucleolar transformation, and extensive formation of numerous shell globules and shell globule clusters in the cytoplasm (Figs. 2, 6).

The large lobate nucleus occupies a central region of the cell. Its nucleoplasm includes numerous clumps of condensed chromatin and glycogen particles, the latter forming nuclear glycogen islands of various size. Due to the apparent disintegration, the nucleoplasm within the glycogen islands becomes electron-lucent (Figs. 6, 8). The nucleus contains a large heterogenous nucleolus (Fig. 6). The nucleolus is composed of electron-dense and electron-lucent areas. The latter are in fact projections of nucleoplasm, penetrating into the nucleolus at its periphery.

Secretory activity rapidly increases in the advanced maturing vitellocytes due to the extensive development of the granular endoplasmic reticulum and Golgi, both participating in production of shell globules and shell globule clusters (Figs. 6, 7, 8).

The endoplasmic reticulum is composed of long parallel narrow membranes forming cisternae or concentric whorls, often surrounding the shell globules (Figs. 6, 7). Components of endoplasmic reticulum are often in close association with the Golgi (Figs. 6, 8). Golgi vesicles have different sizes and electron-density (Fig. 8). The smallest ones are electron-lucent and their electron-density continuously increases with the enlargement of vesicles. Minute secretory vesicles fuse and form secretory globules, which join together to form the shell globule clusters. At this stage of development, the cytoplasmic matrix is of moderate electron-density (Fig. 9).

Mature vitellocytes (IV) (Figs. 10-17)

Mature vitellocytes have an oval shape and measure about 15 μm in diameter (Figs. 2, 10). Their nuclei are tightly packed with glycogen particles, as evidenced by the Thiéry method (Fig. 10 inset). The periphery of the nucleus and the cell are bordered by electron-dense cytoplasm (Fig. 10). An electron-lucent amorphous part of the vitelline cytoplasm is predominant in the mature vitellocytes (Fig. 11). It contains either single shell globules or membrane-bound shell globule clusters (Fig. 11 inset) with electron-dense globules of various sizes that are embedded into an electron-lucent matrix. Some of the shell globule/shell globule clusters undergo transformation resulting in the formation of lamellar body/lamellar body clusters (Fig. 12). At the same time, single lipid droplets have been observed occasionally in the mature vitellocytes (Figs. 13-15). In addition, there are single lamellar bodies of moderate electron-density, comprising dense spiral or concentric configuration and measuring approximately 0.3-0.4 μm (Figs. 15, 16). The occurrence of these lamellar bodies coincides with the breakdown of granular endoplasmic reticulum predominantly on the perinuclear region of mature vitellocytes (Fig. 15). The result of the Thiéry test indicates the absence of glycogen/polysaccharides within lamellar bodies (Fig. 17).

Vitellocytes within the vitelloduct (Figs. 18-20)

Mature vitellocytes from the vitelloductlets and lateral vitelloducts do not differ from those within the vitelline follicles (Fig. 18). The wall of the vitelloduct is built by flat epithelium lined with cilia (Fig. 19). Vitellocytes passing through the vitelloduct contain nucleus, single shell globules, membrane-bound shell globule clusters (Fig. 19), intranuclear and cytoplasmic glycogen, lipid droplets and lamellar bodies/lamellar body clusters (Fig. 20).

Vitellocytes within the intrauterine eggs (Figs. 21, 22)

Remnants of the vitelline cells are present in the polylecithal eggs from the proximal uterus (Fig. 22). These vitellocytes surround a fertilized oocyte and still contain some shell globule clusters, glycogen particles and single lamellar bodies (Fig. 21). The electron-dense eggshell is about 0.6 μm in thickness.

Discussion

Vitellogenesis in the Caryophyllidea

The caryophyllaeid cestode *Archigetes sieboldi* is unique among Eucestoda in the palearctic in having two types of life cycle: the common two-host, oligochaete-fish cycle, and the less common monoxenic one in oligochaetes (Poddubnaya et al., 2003). Earlier studies of vitellogenesis in this species have been from cestodes from oligochaetes, or experimentally infected fish (Poddubnaya, 2003; Poddubnaya et al., 2003), however, there are no data on vitellogenesis from this species naturally parasitizing carp. This parasite is very rare from fishes in Czech and Slovak Republics, with only one record of *A. sieboldi* from carp in South Bohemia (Moravec, 1986).

Our work on *A. sieboldi* from naturally infected carp reveals that vitellogenesis has the same basic pattern as

described from other caryophyllidean cestodes from oligochaetes, or experimentally infected fish (Mackiewicz, 1968; Świderski and Mackiewicz, 1976; Świderski et al., 2004a,b, 2009). Unlike other caryophyllideans, however, mature vitellocytes of *A. sieboldi* contain four, not two types of vitelline inclusions: shell globules/shell globule clusters, glycogen, lamellar bodies/lamellar body clusters, and lipid. The latter two cytoplasmic inclusions have not been previously reported in *A. sieboldi*. Three types of vitelline material (shell globules/shell globule clusters, "lamellar" granules {= lamellar bodies, present paper}, glycogen) have been found in the triploid *A. huronensis* (Bruňanská et al., 2009), whereas other caryophyllids posses only two types (shell globule clusters and glycogen) (Mackiewicz, 1968; Świderski and Mackiewicz, 1976; Świderski et al., 2004a,b). The significance of these differences remains obscure. To what extent the frequency or presence of products of vitellogenesis may vary or be influenced by factors such as age of worm, for example, is not known. It remains to be seen whether they represent genuine differences of fact, or reflect differing states of development, which may be resolved with restudy of more extensive sampling of all stages of vitellogenesis.

Lamellar bodies in vitellocytes of the neoophoran Platyhelminthes

The present study has revealed the presence of lamellar bodies in the mature vitellocytes within vitelline follicles, vitelloducts and in intrauterine eggs of *A. sieboldi*. Lamellar inclusions in parasitic flatworms have been classified either as a kind of membrane-bound shell globules or as cytosegresomes that take part in focal degradation of the cell (Młocicki et al., 2011). These structures have been classified with various terminology and have been extensively reviewed in the latter contribution. In Digenea, lamellar granules of *Fasciola hepatica* are classified as labyrinthine shell globules (Björkman and Thorsell 1963), yolk bodies/globules (Irwin and Threadgold, 1970; Stitt and Fairweather, 1996), heterolysosomal bodies (Threadgold, 1982) or membrane-bound glycan vesicles (Schmidt, 1998). Structures with spiral or concentric lamellar configuration have been described in the vitellocytes of Monogenea (Halton et al., 1974).

Within the Cestoda, lamellar bodies are found in mature vitelline cells of the two spathebothriideans, *Cyathocephalus truncatus* (Bruňanská et al., 2005) and *Didymobothrium rudolphi* (Poddubnaya et al., 2006). In the Caryophyllidea, lamellar structures (= lamellar bodies, present paper) have also been found in mature vitellocytes of vitelline follicles of *Atractolytocestus huronensis* (Bruňanská et al., 2009), *Khawia sinensis* (Bruňanská et al., 2010), *A. sieboldi* (present study), and vitellocytes within intrauterine eggs of *Wenyonia virilis* (Młocicki et al., 2011), *K. sinensis* (Bruňanská et al., 2012), or *A. sieboldi* (present study). In contrast, the lamellar, single, non-membrane bound inclusions in *W. virilis* are described as GER bodies, being formed in eggs in the proximal part of the uterus, and they were not observed in mature vitellocytes within the vitelline follicle (Świderski et al., 2009). Lamellar structures are said to represent remnants of granular endoplasmic reticulum and may participate in synthesis of glycoproteins. It should be noted that unlike vitellogenesis in *A. sieboldi*, which culminates in a nucleus with massive amounts of glycogen in a well-defined inclusion, the so called "nuclear vacuole" (Mackiewicz, 1968) so characteristic of over 50 other caryophyllideans, the nucleus in *W. virilis* apparently does not ever assume the configuration beyond stage II, as described here for *A. sieboldi*.

The present study appears to indicate that in *A. sieboldi*, cytoplasmic inclusions with a lamellar appearance may be formed in two ways in the mature vitellocytes: a) from gradual transformation of shell globule/shell globule clusters (Figs. 11, 15, 19), and b) from the breakdown of granular endoplasmic reticulum (ER) (Fig. 14). It should be noted, however, that the ER-derived GER-bodies in eggs of *W. virilis* are without a membrane (Młocicki et al., 2011). In contrast, the single lamellar bodies from *A. sieboldi* vitelline folicles appear to have a membrane. Our data, therefore, indicate that the way the lamellar bodies are formed is far from clear. Single lamellar bodies, though they appear to arise directly either through transformation of single shell globules or from the breakdown of ER in our study, appear not to be the same. As far as we are aware, ER has never been reported in shell globules. Whether or not ER-like lamellar body clusters can develop from shell globule clusters and be involved in glycoprotein synthesis with ER by the processes discussed recently (Młocicki et al., 2011) remains a subject for further research. To be sure, there is little question that some lamellar bodies are indeed formed from ER, as in the egg, and are not membrane-bound, so that the designation GER-bodies (Młocicki et al., 2011) seems appropriate for certain inclusions (in the egg). However, as we have observed, some single lamellar bodies and membrane-bound clusters of lamellar bodies are in mature vitellocytes in conjuction with the transformation of shell globule/shell globule clusters. Therefore, until the origin of all of the lamellar bodies is clarified, the more general and succint terminology of lamellar bodies is used in the present study for all inclusions with a lamellar structure. To what extent the various inclusions may represent shell precursors and enzymes or be involved in protein synthesis has yet to be determined.

Lipids in vitellocytes of the Cestoda

Earlier studies have concluded that the absence of cytoplasmic lipid droplets and the large amount of glycogen are the most characteristic features of vitellocytes in the Caryophyllidea (Świderski and Xylander, 2000). More recently, however, lipid droplets

have been reported from degenerating vitellocytes within intrauterine eggs of caryophyllideans *W. virilis* (Młocicki et al., 2010) and *K. sinensis* (Bruňanská et al., 2012). Though lipid droplets were also described from mature vitellocytes of the triploid *A. huronensis* in 2007 in a brief abstract (Bruňanská et al., 2007), a subsequent detailed study in 2009 (Bruňanská et al., 2009) from the same material, however, could not positively identify lipids. The true status of lipids in the vitellocytes of *A. huronensis*, therefore, has yet to be established. In the present study, we have found scattered lipid droplets in mature vitellocytes of *A. sieboldi*. Vitellocytes of *A. sieboldi* containing lipid droplets, however, did not show distinct features of degeneration. Lipids were not reported in the developing vitellocytes of progenetic *A. sieboldi* (Poddubnaya, 2003), or cestodes from experimentally infected carp (Poddubnaya et al., 2003). The significance of these inconsistent differences of lipids from mature vitellocytes and occurrence in vitellocytes in intrauterine eggs is unclear. It is important to note, however, that both of the earlier studies on *Archigetes* (Poddubnaya, 2003; Poddubnaya et al., 2003) focused on the general ultrastuctural features of the morphology of *A. sieboldi*, with little attention to the specific process of vitellogenesis.

The occurrence of lipids in the vitellocytes is characterized by a great variability in different cestode groups (Świderski and Xylander, 2000). Lipid droplets are described in the vitelline cytoplasm either as electron-lucent units representing saturated fatty acids e.g. in amphilinideans, gyrocotylideans, caryophyllideans (Xylander, 1987, 1988; present study), spathebothriideans (Bruňanská et al., 2005; Poddubnaya et al., 2006), pseudophyllideans (Świderski and Mokhtar, 1974; Levron et al., 2007), tetraphyllideans (Mokhtar-Maamouri and Świderski, 1976), trypanorhynchs (Świderski et al., 2006a,b), diphyllideans (Świderski et al., 2011), and/or proteocephalideans (Bruňanská, 1997, 1999). Electron-dense lipid droplets containing a high level of unsaturated fatty acids were detected in vitellocytes of spathebothriideans (Bruňanská et al., 2005), bothriocephalideans (Korneva, 2001), trypanorhynchs (Świderski et al., 2006a,b, 2007) or diphyllideans (Świderski et al., 2011). From these studies, and a review of vitellogenesis in cestodes (Świderski and Xylander, 2000), it is postulated that lipid droplets in vitelline cells may serve as nutritive reserves for the development of embryos. Glycogen has been cited as the primary energy source for embryo development in the caryophyllideans with the apparent absence of lipid from mature vitellocytes in follicles (Mackiewicz, 1981; Bruňanská et al., 2009; Młocicki et al., 2010). Given this apparent absence, it has been hypothesized that the lipid in degenerating vitellocytes within intrauterine eggs of *Khawia* spp may represent waste metabolic products of early embryos (Bruňanská et al., 2007; Młocicki et al., 2010). Clearly, lipids in the developing vitellocytes of the caryophyllideans is very rare, indicating that its role in embryogenesis may be more variable than previously thought.

Glycogen in vitellocytes of the Caryophyllidea

Glycogen represents a predominant nutrient within vitellocytes of all caryophyllideans (Mackiewicz, 1968; Świderski and Mackiewicz, 1976; Świderski et al., 2004a,b, 2009; Bruňanská et al., 2012). Unlike nuclear inclusions of other cestodes, that of caryophyllideans involves massive amounts of glycogen as a dense, non membrane-bound, coherent mass within the nucleus (Mackiewicz, 1968, 1981; Świderski and Mackiewicz, 1976). Except for *Wenyonia*, in which the nuclear glycogen inclusion appears to be less well-defined (Świderski et al., 2009), that of other caryophylideans is similar to that described here for *A. sieboldi*. Earlier literature referred to this concentration of nuclear glycogen as a "nuclear vacuole" (Mackiewicz, 1968, 1981). The nucleus in *W. virilis* apparently does not ever assume the configuration beyond stage II, as described here for *A. sieboldi*. This unusual, well-defined concentration of nuclear glycogen in vitellocytes of the Caryophyllidea, as well as nuclear inclusions in other groups, may constitute a plesiomorphy of the Cestoda (Poddubnaya et al., 2006).

Conclusions

New data on cytoplasmic inclusions in vitellocytes of *A. sieboldi* indicates that vitellogenesis within the Caryophyllidea is more complex that previously thought. Further investigations into vitellogenesis and fertilization of other caryophyllideans and related species is therefore desirable. Only additional detailed knowledge on ultrastructure and cytochemistry of vitellocytes in the Caryophyllidea, and other lower cestodes, may help us to understand the role of various cytological components during embryonic development. At the same time, the discovery of new characters at the ultrastructural level might be useful in the elucidation of evolutionary interrelationships of the lower tapeworms.

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