

Structure of the ovaries and oogenesis in *Cixius nervosus* (Cixiidae), *Javesella pellucida* and *Conomelus anceps* (Delphacidae) (Insecta, Hemiptera, Fulgoromorpha)

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Abstract

Ovary organization in representatives of two families of Fulgoromorpha, Cixiidae (*Cixius nervosus*) and Delphacidae (*Javesella pellucida* and *Conomelus anceps*), was examined by light and transmission electron microscopy. Ovaries of studied fulgoromorphans consist of telotrophic ovarioles. From apex to base individual ovarioles have four well defined regions: a terminal filament, tropharium (trophic chamber), vitellarium and pedicel (ovariolar stalk). Tropharia are not differentiated into distinct zones and consist of syncytial lobes containing multiple trophocyte nuclei embedded in a common cytoplasm. Lobes are radially arranged around a branched, cell-free trophic core. Early previtellogenic (arrested) oocytes and prefollicular cells are located at the base of the tropharium. The vitellarium houses linearly arranged developing oocytes each of which is connected to the trophic core by a broad nutritive cord. Each oocyte is surrounded by a single layer of follicular cells that become binucleate at the beginning of vitellogenesis.

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1. Introduction

Hemipterans have traditionally been subdivided into two groups – Homoptera and Heteroptera (Latreille, 1810 after Campbell et al., 1995). The Homoptera consisted of the Sternorrhyncha (Aphidinea, Coccinea, Aleyrodinea and Psyllinea) and Auchenorrhyncha (Cicadomorpha and Fulgoromorpha). The monophyletic origin of Sternorrhyncha is well documented and they are regarded as sister to the remaining Hemiptera (Fig. 1) (Hennig, 1981; Campbell et al., 1995; Sorensen et al., 1995; von Dohlen and Moran, 1995). In contrast, the monophyly of Auchenorrhyncha is still under discussion (Hennig, 1981; Campbell et al., 1995; von Dohlen and Moran, 1995; Sorensen et al., 1995; Yoshizawa and Saigusa, 2001). Recent molecular data suggest that Auchenorrhyncha represent a paraphyletic group (Campbell et al., 1995; von Dohlen

and Moran, 1995; Sorensen et al., 1995). Moreover, some morphological and molecular characters indicate that Fulgoromorpha are more closely related to Heteroptera than to Cicadomorpha (Bourgoin, 1993; Campbell et al., 1995; Sorensen et al., 1995; von Dohlen and Moran, 1995; Bourgoin et al., 1997). However, more recently, Yoshizawa and Saigusa (2001) based on the forewing base structure supported the monophyly of Auchenorrhyncha.

Insect ovaries consist of a varying number of tubes termed ovarioles (e.g. 2 in curculionids, about 300 in some scale insects) (Büning, 1979; Szklarzewicz, 1998). A typical ovariole is divided from apex to base into a terminal filament, germarium, vitellarium and ovariolar stalk (pedicel). The terminal filaments combine to form a suspensory ligament joining the ovary to the body wall or to lobes of the fat body. The germ cells divide mitotically in the germarium to produce oocytes in panoistic ovarioles or oocytes and trophocytes (nurse cells) in meroistic ovarioles. Trophocytes do not transform into functional gametes and instead they are engaged into production of

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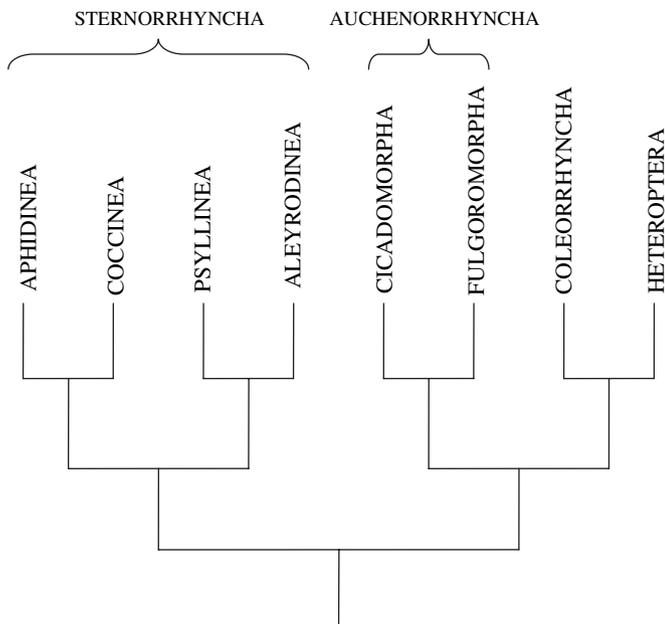


Fig. 1. Cladogram of hemipteran taxa based on morphological characters assuming monophyletic origin of Auchenorrhyncha (according to data presented in Hennig, 1981).

different types of RNAs, proteins and various organelles. In insects two categories of the merostic ovarioles are distinguished: polytrophic and telotrophic (Gross, 1903). In polytrophic ovarioles each oocyte possesses its own group of trophocytes and is connected to them by intercellular bridges. In telotrophic ovarioles trophocytes remain in the trophic chamber and are connected to oocytes in the vitellarium by long nutritive cords.

In all types of ovarioles the vitellaria comprise linearly arranged oocytes (or groups: oocyte + trophocytes in polytrophic ovarioles) surrounded by follicular cells. While passing down the vitellarium, oocytes accumulate or synthesize RNAs during previtellogenesis, accumulate yolk during vitellogenesis and become covered by two egg envelopes (the vitelline envelope and chorion) during choriogenesis.

Ovarioles of hemipterans are telotrophic. Extensive studies of ovaries of aphids (Aphidinea), scale insects (Coccinea), whiteflies (Aleyrodinea), psyllids (Psyllinea), cicadas and leafhoppers (Cicadomorpha), and true bugs (Heteroptera) have revealed these organs to vary in the structure of their tropharia (Büning, 1985, 1994; Książkiewicz-Kapralaska, 1985, 1991; Huebner, 1981; Biliński et al., 1990; Simiczyjew et al., 1998; Szklarzewicz, 1998; Štys et al., 1998; Szklarzewicz et al., 2000). In aphids, scale insects, whiteflies, cicadas, leafhoppers and in basal heteropterans, mononucleate trophocytes join the trophic core individually, whereas in advanced heteropterans the trophocytes fuse to form cytoplasmic lobes. The apices of tropharia in adult cicadas, leafhoppers and some heteropterans contain mitotically active germ cells (trophocyte precursors), whereas in remaining hemipterans germ cells do not divide. In spite of these differences, hemipteran ovaries share synapomorphic characters strongly supporting the monophyly of this clade: in each cluster more than one oocyte

develops (1); the centre of the tropharium is cell-free, i.e. a trophic core occurs (2); and in the trophic core and nutritive cords numerous microtubules are present (3).

Since there are no data on ovary structure in fulgoromorphans and because numerous studies have demonstrated that ovary organization is useful in phylogenetic analysis (Štys and Biliński, 1990; Büning, 1998), we hope our investigations provide useful information about ovary anagenesis in hemipterans.

2. Material and methods

Adult females of *Cixius nervosus* (Linnaeus, 1758) were collected in July in Ojców (south Poland). Specimens of *Javesella pellucida* (Fabricius, 1794) and *Conomelus anceps* (Germar, 1821) were collected in July near Nowy Targ (south Poland). Ovaries from 5 females of each species were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature, rinsed in 0.1 M phosphate buffer (pH 7.4) with addition of sucrose and postfixed in 1% osmium tetroxide in the same buffer. After dehydration in a graded series of ethanols and acetone, the material was embedded in epoxy resin Epox 812 (Fullam Inc., Latham, N.Y., USA). Semithin sections (0.7 µm thick) were stained with 1% methylene blue in 1% borax and photographed in a Jenalumar (Zeiss Jena) microscope. Ultrathin sections (90 nm thick) were cut on a Reichert-Jung Ultracut E microtome, contrasted with uranyl acetate and lead citrate and examined in a JEM 100 SX EM at 60 kV.

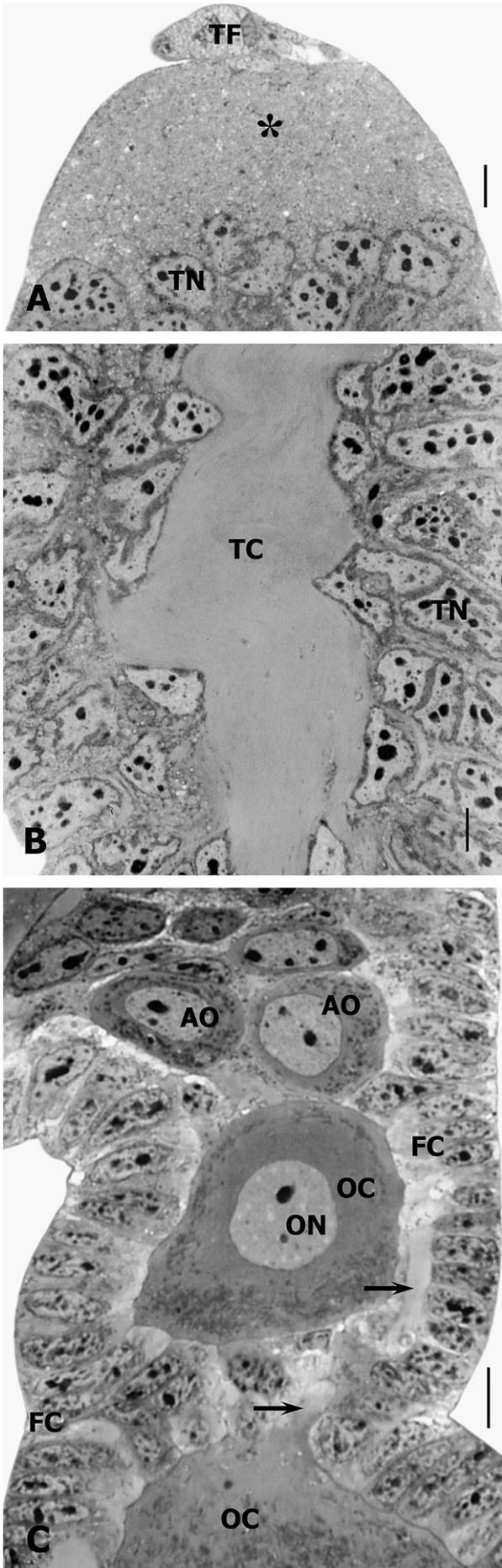
3. Results

3.1. Gross architecture of the ovary

Each ovary of *Cixius nervosus*, *Javesella pellucida* and *Conomelus anceps* contains over a dozen telotrophic ovarioles. Each ovariole is subdivided from apex to base into: a fine terminal filament, tropharium (trophic chamber), vitellarium and ovariole stalk (pedicel) that opens into the calyx of a lateral oviduct. The terminal filament is not separated from the tropharium by a transverse septum. All trophocytes and oocytes in each ovariole are interconnected and belong to the same cluster. The vitellarium in mature female comprises 4 or 5 linearly arranged oocytes that are surrounded by a one-layered follicular epithelium.

3.2. The tropharium

Within each long, club-shaped tropharium are two well defined regions: an anterior region comprising syncytial lobes housing 3–5 trophocyte nuclei embedded in a common cytoplasm (Figs. 2A,B, 3A, 4A and 5A) and a posterior region containing prefollicular cells and early previtellogenic (arrested) oocytes (Figs. 2C, 3C and 4B). The apex of the tropharium in females of *Cixius nervosus* is devoid of trophocyte nuclei and is tightly packed with endosymbiotic microorganisms (Figs. 2A and 3B). Cytoplasmic lobes are radially arranged around a central, branched trophic core (Figs. 2B, 4A and 5A). The latter is extended peripherally into cytoplasmic lobes

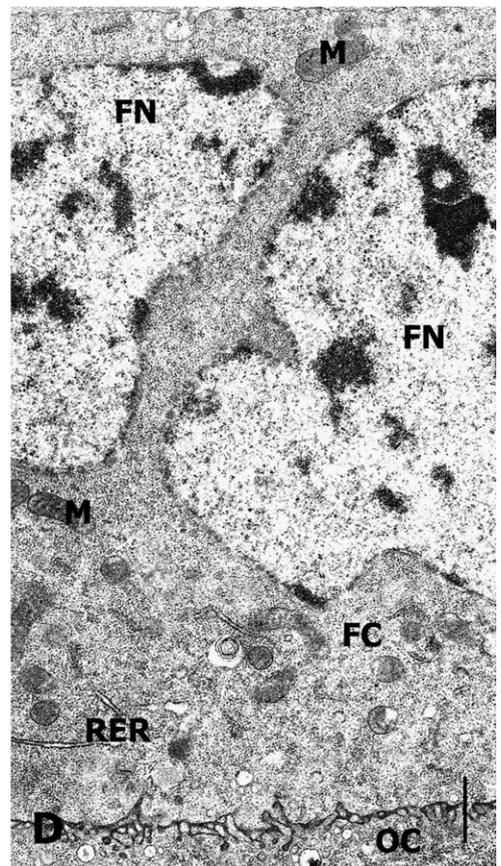
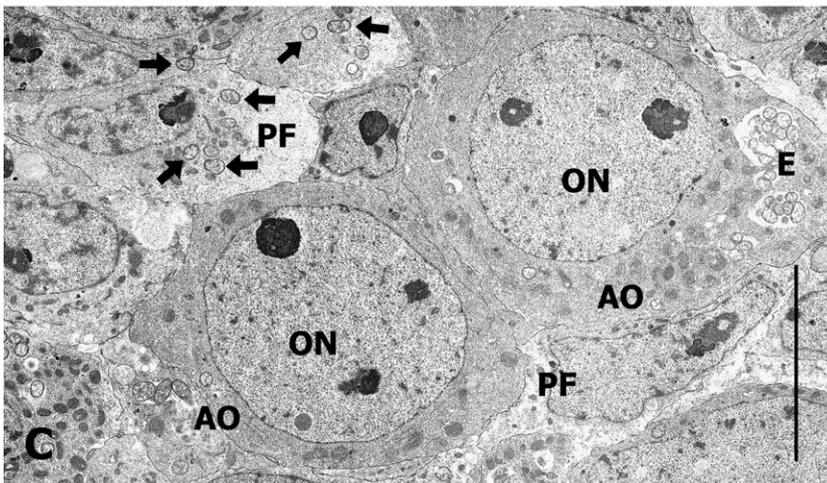
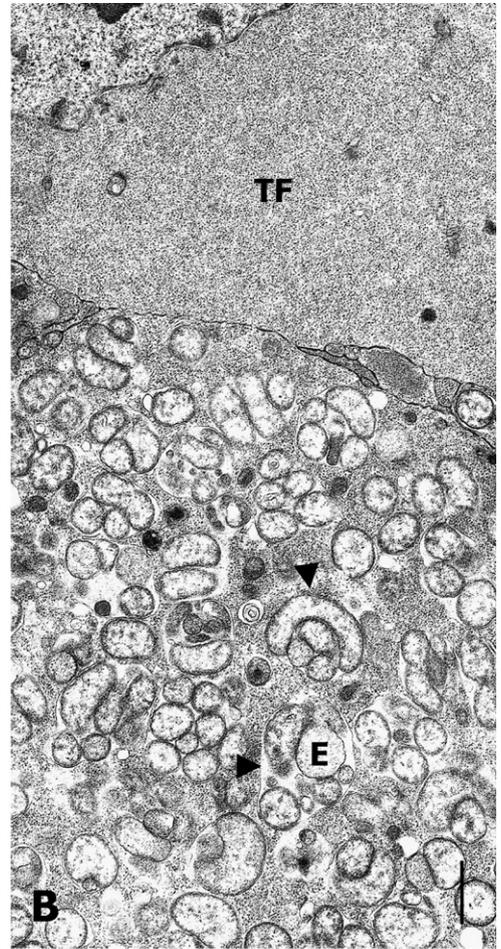
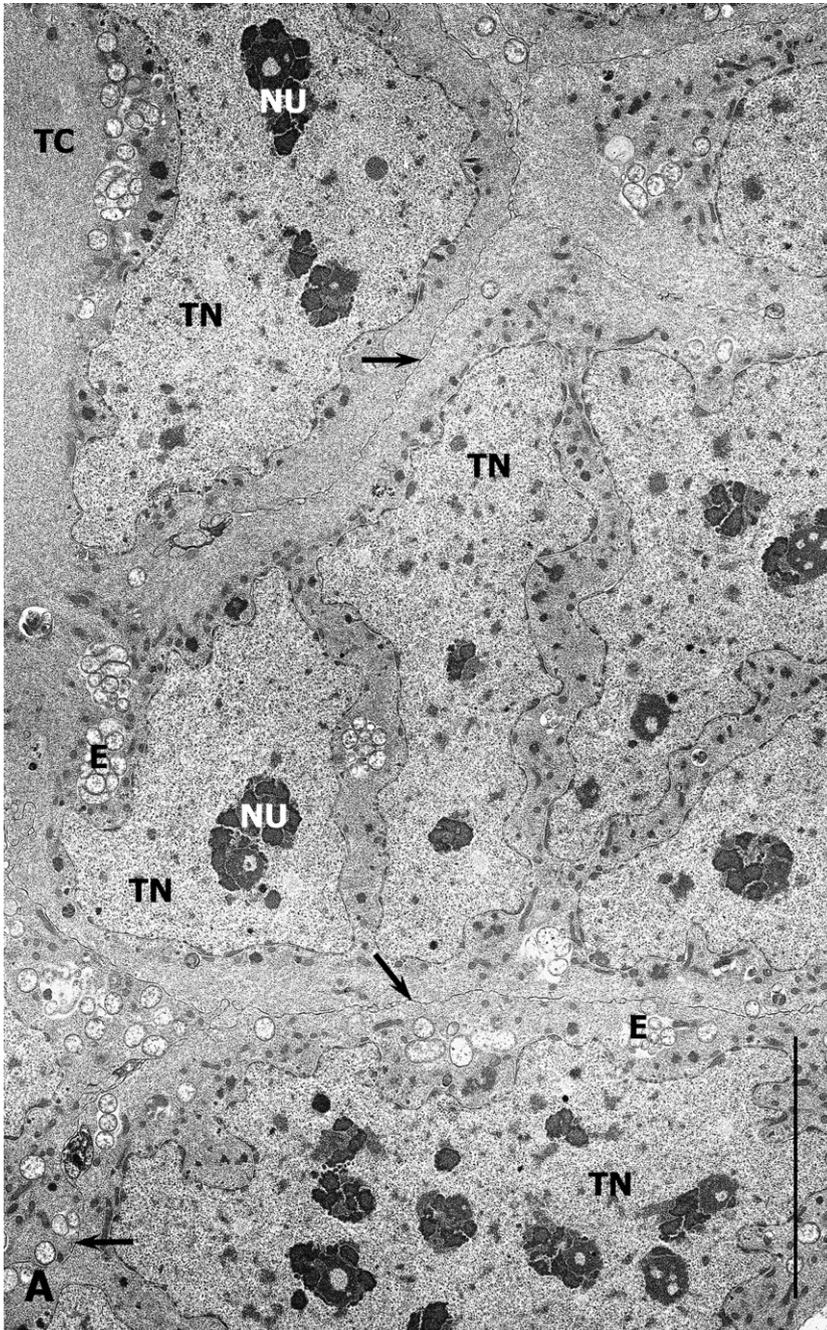


(Figs. 2B, 3A, 4A and 5A) and proximally is continuous with arrested and developing oocytes (Figs. 2C, 3C and 4B). The cytoplasmic lobes are joined to the core *via* broad cytoplasmic strands (Figs. 2B, 4A and 5A) and to the oocytes by nutritive cords (Figs. 2C and 4B). Both trophic core and nutritive cords are tightly packed with longitudinally disposed microtubules (not shown). The latter are accompanied by ribosomes and mitochondria (not shown). In the trophic core and nutritive cords of *Cixius nervosus*, numerous endosymbiotic bacteria are present (Fig. 3A). The analysis of serial semithin sections has shown that tropharia contain about 200 trophocyte nuclei. In all investigated species, trophocyte nuclei are large and mitotically inactive (Figs. 2A,B, 3A, 4A and 5A,C). The trophocyte nuclei of *Conomelus anceps* ramify (Fig. 5A,C), while those of *Javesella pellucida* (Fig. 4A) and *Cixius nervosus* (Figs. 2A,B and 3A) are only slightly extended. In *Conomelus* ovarioles, the nuclei closest to the trophic core extend broad, short extensions towards the trophic core (Fig. 5D). In all examined species, the trophocyte nuclei contain dispersed chromatin and about 10 prominent, irregular nucleoli (Figs. 2A,B, 3A, 4A and 5A,C). In the perinuclear cytoplasm, aggregations of electron-dense “nuage” material occur (Fig. 5D). The remaining cytoplasm is filled with ribosomes and mitochondria (Figs. 3A and 5C). Trophocyte cytoplasm of *Cixius nervosus* contains numerous, rod-shaped, endosymbiotic bacteria (Fig. 3A,B). Usually, accumulations of 2–7 micro-organisms surrounded by a common, perisymbiotic membrane were observed (Fig. 3B). The posterior region of the tropharium is occupied by small prefollicular cells (Fig. 3C) and 6–10 arrested oocytes (Figs. 2C, 3C and 4B). Arrested oocytes have little cytoplasm (Fig. 2C) filled with ribosomes, mitochondria and rod-shaped endosymbiotic bacteria (Fig. 3C). Oocyte nuclei (germinal vesicles) are large, spherical, and contain decondensed chromatin (Figs. 2C and 3C).

3.3. The vitellarium

The vitellaria of all examined species house 3 or 4 linearly arranged oocytes at consecutive stages of oogenesis: previtellogenesis, vitellogenesis and choriogenesis. Developing oocytes are surrounded by a monolayer of follicular cells (Figs. 2C, 3D, 5B and 6A) that during previtellogenesis are columnar and mononucleate (Fig. 2C). At the onset of vitellogenesis, these cells become binucleate with their nuclei arranged one above the other in the long axis of the cell (Fig. 5B). As vitellogenesis progresses, the follicular cells change their shape from columnar (Fig. 5B) to squamous (Fig. 6A). Simultaneously, their nuclei shift next to one another (Figs. 3D and 5A). During

Fig. 2. (A–C). *Cixius nervosus* (Cixiidae). Sagittal section through tropharium. (A) and (B). Anterior region. Note the apex of tropharium devoid of trophocyte nuclei (asterisk), the syncytial lobes containing multiple trophocyte nuclei (TN) and the centrally located trophic core (TC). (C) Posterior region. Note arrested oocytes (AO) and the previtellogenic oocyte (OC) surrounded by a single layer of follicular cells (FC). Arrows indicate a nutritive cord connecting the oocyte with the trophic core. Oocyte nucleus (ON), terminal filament (TF). (A–C) Methylene blue, bar = 10 μ m.



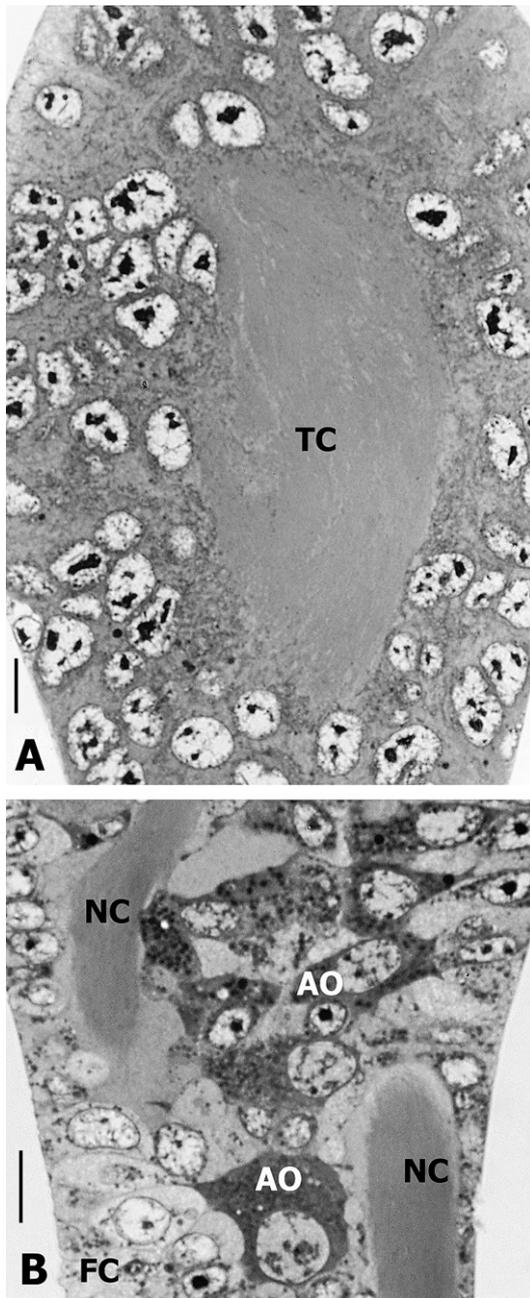


Fig. 4. (A), (B). *Javesella pellucida* (Delphacidae). Longitudinal section through tropharium. (A) Anterior region. Note centrally located trophic core (TC) and syncytial lobes. (B) Posterior region. Note arrested oocytes (AO), nutritive cords (NC) and follicular cells. (A) and (B) Methylene blue, bar = 10 μ m.

choriogenesis, numerous mitochondria, cisternae of rough endoplasmic reticulum and Golgi complexes appear in the cytoplasm of the follicular cells (Fig. 3D). Oocytes have spherical, centrally located nuclei at the beginning of oocyte

growth (Fig. 2C). At the onset of vitellogenesis, the oolemma of each oocyte starts to form microvilli (Fig. 3D). Concurrently, in the peripheral ooplasm numerous endocytotic vesicles appear (Fig. 3D). As vitellogenesis continues, the oocyte nucleus moves into the peripheral ooplasm (Fig. 5B). In females of *Javesella pellucida* and *Conomelus anceps*, the posterior ends of ovarioles are invaded by endosymbiotic, yeast-like microorganisms (Fig. 6A,B). These endosymbionts transverse the pedicel and enter the perivitelline space (between oocyte and follicular epithelium) (Fig. 6A). They then are located in a deep depression of the oocyte and form a characteristic “symbiont ball” (Fig. 6B).

4. Discussion

4.1. Morphology of the ovary

Results of numerous studies of hemipteran ovaries (see introduction) have revealed that they differ markedly in tropharium organization. Contrary to those of examined auchenorrhynchans (Książkiewicz-Kapralska, 1985, 1991; Lupa et al., 1999), the tropharia of adult fulgoromorphans lack a morphological gradient of trophocytes from apex to base. Since a zone of mitotically active trophocyte precursors seems to be absent in adult tropharia, the number of trophocyte nuclei per ovariole does not increase any more. The tropharia of examined fulgoromorphans have lobes containing multiple trophocyte nuclei embedded in a common cytoplasm. Both nuclei and cytoplasm are typical of insect trophocytes: the nuclei are large and lobed, the nucleoli are numerous and massive, perinuclear cytoplasm accumulates electron-dense “nuage” material, and the cytoplasm is filled with numerous ribosomes. Such ultrastructure correlates with the synthetic activity of these cells: in producing of mRNA and rRNA. Like those of other hemipterans, the trophic core and nutritive cords of fulgoromorphans are filled with densely packed microtubules involved in transporting macromolecules, organelles and endosymbiotic microorganisms from the trophic chamber into individual, developing oocytes in the vitellarium (Stebbins, 1986). Although development of the hemipteran trophic chamber has been thoroughly examined (Huebner and Anderson, 1972a; Choi and Nagl, 1976; Lutz and Huebner, 1980, 1981), the mode of formation of syncytial lobes remains unclear. According to Lutz and Huebner (1981), cytoplasmic lobes arise through fusion of trophocyte membranes, whereas Choi and Nagl (1976) postulated that syncytialization of tropharia results from amitotic division of trophocyte nuclei.

Fig. 3. (A–D). *Cixius nervosus* (Cixiidae). (A) Detail of tropharium. Note trophocyte nuclei (TN) in a common cytoplasm. Arrows indicate membranes of the syncytial lobes. (B) Detail of terminal filament (TF) and apex of tropharium devoid of trophocyte nuclei and filled with endosymbiotic bacteria (E). Arrowheads indicate perisymbiotic membrane covering groups of endosymbionts. (C) Posterior region of tropharium containing arrested oocytes (AO) and prefollicular cells (PF). Short arrows indicate endosymbiotic bacteria in the cytoplasm of prefollicular cells. (D) Detail of a binucleate follicular cell (FC). Nucleus of follicular cell (FN), mitochondrion (M), trophocyte nucleolus (NU), oocyte (OC), oocyte nucleus (ON), cisternae of rough endoplasmic reticulum (RER), trophic core (TC). (A–D) TEM, (A) and (C), bar = 10 μ m, (B) and (D), bar = 1 μ m.

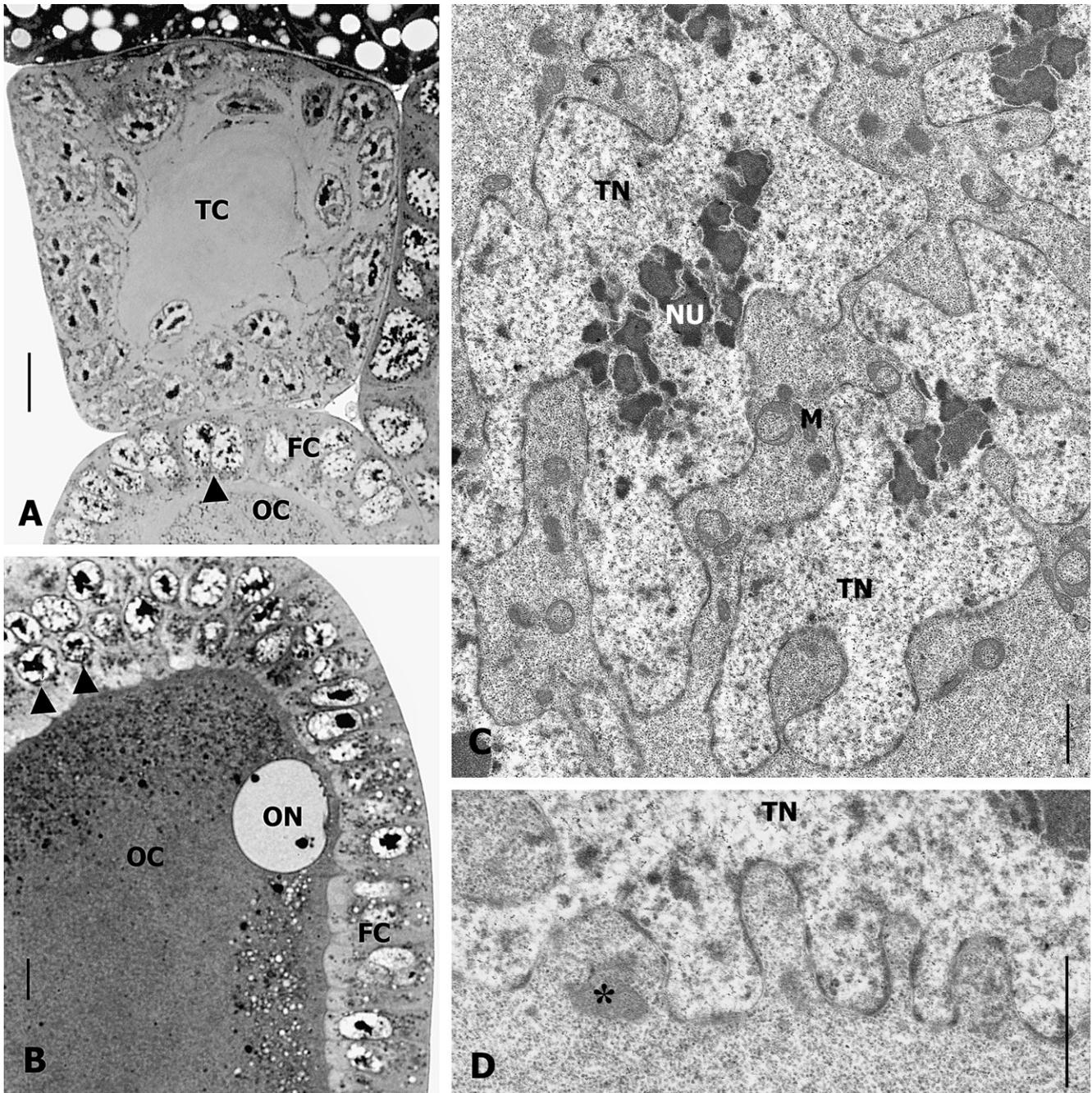


Fig. 5. (A–D). *Conomelus anceps* (Delphacidae). (A) Cross section through tropharium. Note the centrally located trophic core (TC) and syncytial lobes. Arrowhead indicates a binucleate follicular cell. (B) Longitudinal section through early vitellogenic oocyte (OC). Note binucleate follicular cells (arrowheads) and oocyte nucleus (ON). (C) Detail of tropharium. (D) Detail of nucleus and perinuclear cytoplasm of trophocyte. Note broad, short extensions of the nucleus directed towards the trophic core and electron-dense “nuage” material (asterisk). Follicular cells (FC), trophocyte nucleus (TN), trophocyte nucleolus (NU), mitochondria (M). (A) and (B) Methylene blue, bar = 10 μm . (C) and (D) TEM, bar = 1 μm .

Like those in other insects (Kaulenas, 1992; Büning, 1994), developing oocytes of examined fulgoromorphans are surrounded by a single layer of follicular cells that at the beginning of vitellogenesis become binucleate. Binucleate follicular cells have not been reported in non-heteropteran hemipterans, but are common in heteropterans (Huebner and Anderson, 1972b; Simiczjzew, 1999). It is suggested that presence of binucleate follicular cells is connected with their basic function,

i.e. synthesis of precursors of egg envelopes. In most insects, follicular cells possess single, polyploid nuclei with a folded nuclear envelope.

In ovarioles of examined fulgoromorphans, numerous endosymbiotic microorganisms are observed. Presence of endosymbionts is correlated with restricted diet of host insects (for detailed information see Buchner, 1965; Douglas, 1989; Moran and Baumann, 2000; Baumann, 2005). Since

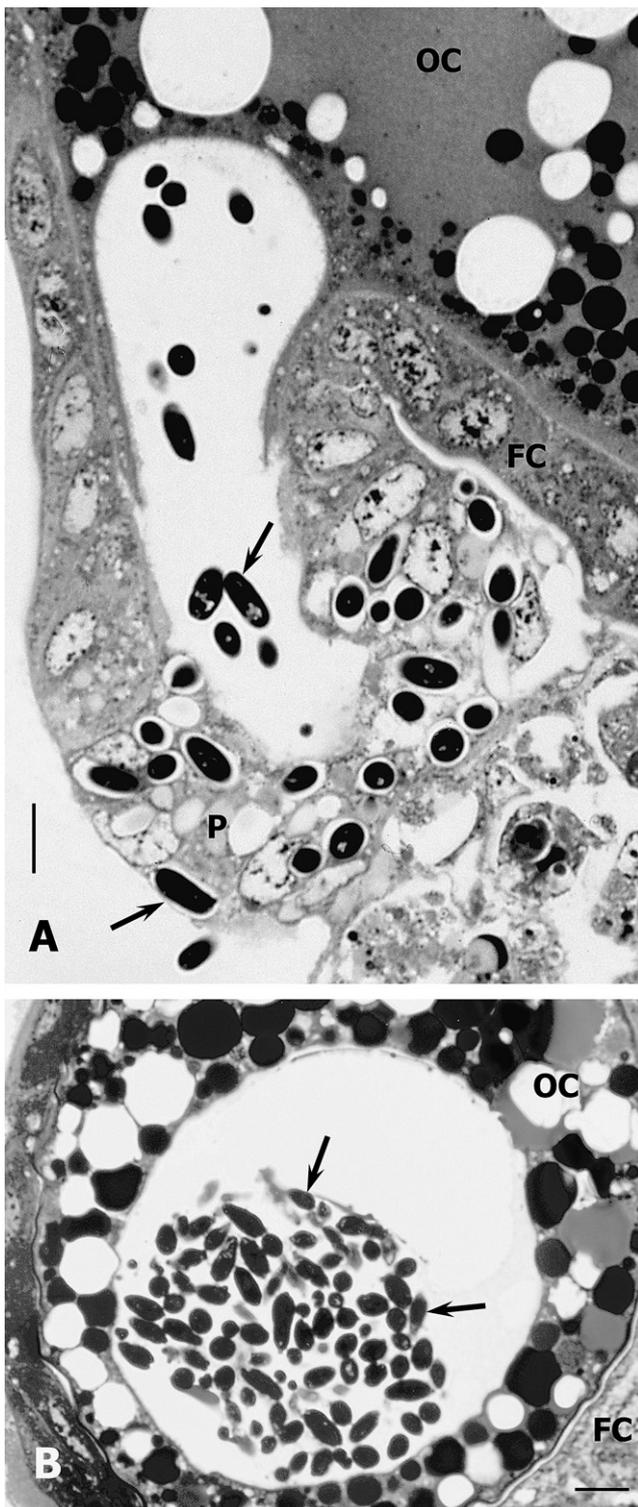


Fig. 6. (A) *Conomelus anceps* (Delphacidae). Sagittal section through pedicel (P) of an ovariole. Yeast-like microorganisms (arrows) invade the ovariole. (B) *Javesella pellucida* (Delphacidae). Cross section through posterior part of the ovariole. A large group of yeast-like endosymbionts ("symbiont ball") is located within a deep depression of a vitellogenic oocyte (OC). Follicular cells (FC). (A) and (B) Methylene blue, bar = 10 µm.

fulgoromorphans, like most hemipterans, consume phloem sap deficient in certain essential amino acids, their endosymbionts are responsible for synthesis of missing but required substances. Such microorganisms are transmitted transovarially from one generation to the next (Żelazowska and Biliński, 1999; Cheng and Hou, 2001; Szklarzewicz and Moskal, 2001; Szklarzewicz et al., 2006). Our ultrastructural studies confirm Buchner's (1965) observations that fulgoromorphans have a diversity of endosymbionts. In ovarioles of *Cixius nervosus* we found endosymbiotic bacteria in the apex of the tropharium, in trophocytes and oocytes and in somatic, prefollicular cells. The occurrence of huge masses of endosymbionts in the apex of the tropharium of *Cixius nervosus* is of special interest. Since such a phenomenon has never been observed in other hemipterans, its biological significance remains unclear. An enormous number of these bacteria indicates that they play a crucial role for the host insect. It may be also speculated that these microorganisms invade the ovaries before the germ line cells differentiate into oocytes and trophocytes. In the apical part of the tropharium they intensively multiply. Then they migrate via the trophocyte cytoplasm, trophic core and nutritive cord into the oocyte. In contrast, yeasts in *Javesella pellucida* and *Conomelus anceps* appear to infect the posterior end of ovarioles by transferring through pedicellar cells. Transovarially transmitted yeasts have also been observed in several species of other delphacids (Noda et al., 1995; Cheng and Hou, 2001; Xet-Mull et al., 2004); however, their distribution within the body of the host, ultrastructure, reproduction and the mode of transmission have only been described in detail in brown planthopper, *Nilaparvata lugens* (Cheng and Hou, 1996, 2001). Our observations show that European delphacids, *Javesella pellucida* and *Conomelus anceps*, developed similar symbiotic system as the Asiatic delphacid, *Nilaparvata lugens*. Recent examinations of 18S rDNA of yeasts inhabiting Asian and American delphacids revealed their high similarity (over 98%) (Xet-Mull et al., 2004). Thus, yeasts may be typical of delphacids (1); and endosymbiosis of yeasts in delphacids is ancestral (2).

4.2. Phylogenetic conclusions

Hemipteran ovaries vary in the organization of their tropharia and this character is thus useful for phylogenetic consideration (Biliński et al., 1990; Büning, 1994, 1998; Simiczyjew et al., 1998; Szklarzewicz, 1998; Štys et al., 1998). Our studies show the tropharia of the investigated species of Cixidae and Delphacidae to be syncytial. This character has never been reported for Auchenorrhyncha (Książkiewicz-Kaprańska, 1985, 1991; Lupa et al., 1999), but is characteristic for females of advanced Heteroptera (Reduviidae, Aradidae, Coreidae, Pyrrhocoridae, Lygaeidae, Pentatomidae) (Simiczyjew et al., 1998). Tropharia in basal heteropterans, however, consist of individual trophocytes (Biliński et al., 1990; Štys et al., 1998). Büning (1998), Simiczyjew et al. (1998), Štys et al. (1998), and Koteja et al. (2003) postulated that the tropharium of the hemipteran ancestor contained individual trophocytes not undergoing

mitosis. Simiczjew and co-workers (1998) regarded syncytialization of tropharia of advanced hemipterans as a synapomorphy for members of this lineage. Thus, we postulate that the syncytial tropharia of fulgoromorphans represent a homoplasy, i.e. that they evolved independently and convergently to those of advanced Heteroptera. Therefore, based on the morphology of hemipteran ovaries, it is suggested that Auchenorrhyncha are monophyletic (Fig. 1).

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