

Ultrastructure and development during meiosis and the tetrad period of sporogenesis in the leptosporangiate fern *Alsophila setosa* (Cyathea-ceae) compared with corresponding stages in *Psilotum nudum* (Psilotaceae)

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Abstract

The pre-meiotic, meiotic and tetrad stages of development in microsporangia of *Alsophila setosa* were studied with particular emphasis on the early establishment of patterning in the microspore wall and the subsequent development of the sporoderm. The data obtained were compared with corresponding ontogenetic stages of *Psilotum nudum*. Tapetal behaviour was also examined. During the tetrad period, only one layer, a thin undulating sheet, appeared alongside the plasma membrane of the tetraspores, and this was evidently formed on a pre-patterned structure – a fibrillar layer, corresponding to a kind of primexine matrix. The early free microspores had a wavy plasma membrane with a parallel, sinusoidal, thin initial sporoderm layer. The proximal apertural fold was observed to be an extended outgrowth of this initial spore envelope. Sporoderm ontogeny during the tetrad period in *Alsophila* and *Psilotum* show some common points, but also fundamental differences, mainly in the relative timing of events: in *Alsophila* the end of the tetrad period is the starting point for exospore development, whereas in *Psilotum* the exospore is already complete at this stage. Considerable differences were also observed in the tapetum of the two species.

Keywords: *spore wall and tapetum development, ultrastructure, pre-meiosis, meiosis, sporoderm pattern formation, self-assembly, micelles, Alsophila setosa, Psilotum nudum*

Ultrastructural ontogenetic studies have always been a powerful tool for understanding the processes involved in sporoderm development and also for the correct interpretation of mature spore or microspore walls, which at maturity are ‘silent’ or closed structures from which ontogeny cannot be deduced. In spite of the series of a number of important and informative studies on spore wall formation (Lugardon, 1966, 1968, 1969, 1973, 1979, 1990; Pettitt, 1971, 1976, 1977; Sheffield & Bell, 1979, Bell, 1981; Brown & Lemmon, 1982a, 1982b, 1996, 2001a, 2001b, 2009; Sheffield et al., 1983; Parkinson, 1987, 1995; Uehara & Kurita, 1989a, 1989b, 1991; Uehara et al., 1991; Parkinson & Pacini, 1995), the development of pteridophyte spores has received far less attention than corresponding events in seed plants. There have also been

fewer attempts to bring pteridophyte spore development into a phylogenetic context (e.g. Blackmore et al., 2000) or to interpret the basic organisation and mechanisms of spore or microspore formation (Wellman, 2004; Blackmore, 2007; Blackmore et al., 2007).

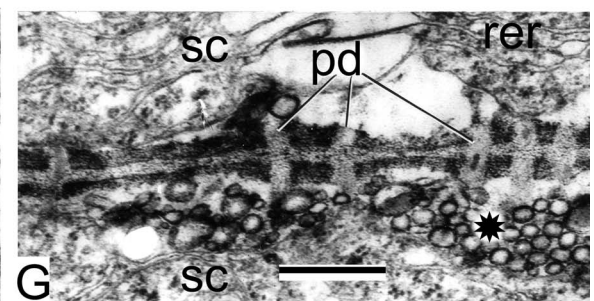
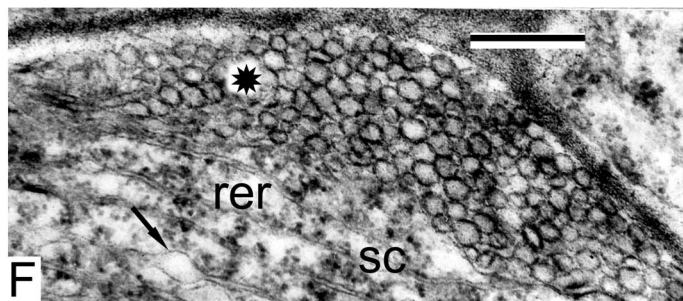
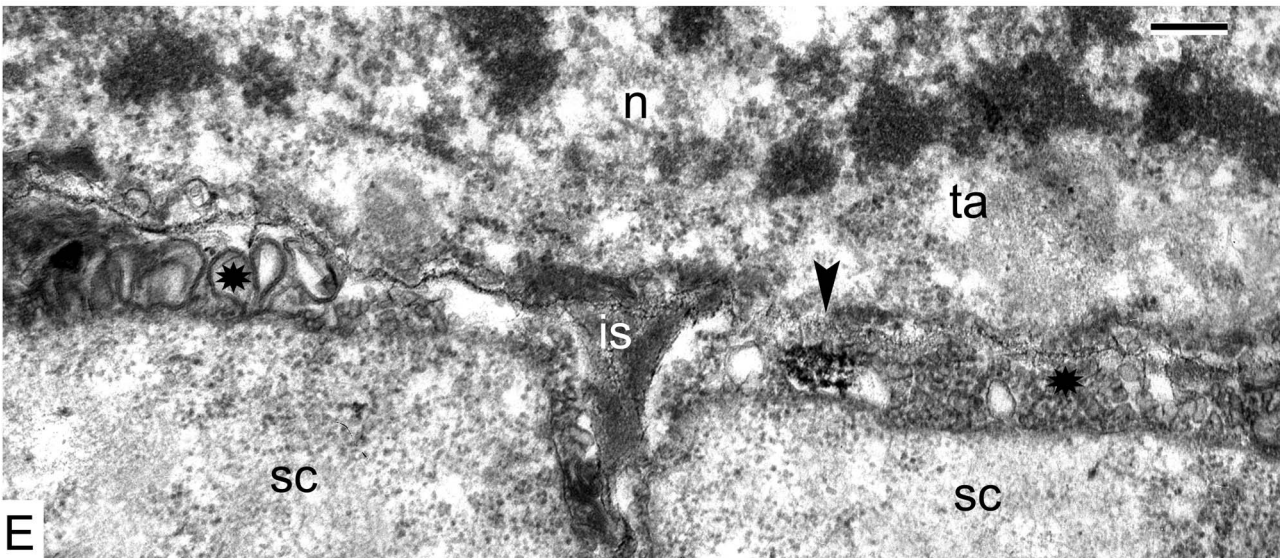
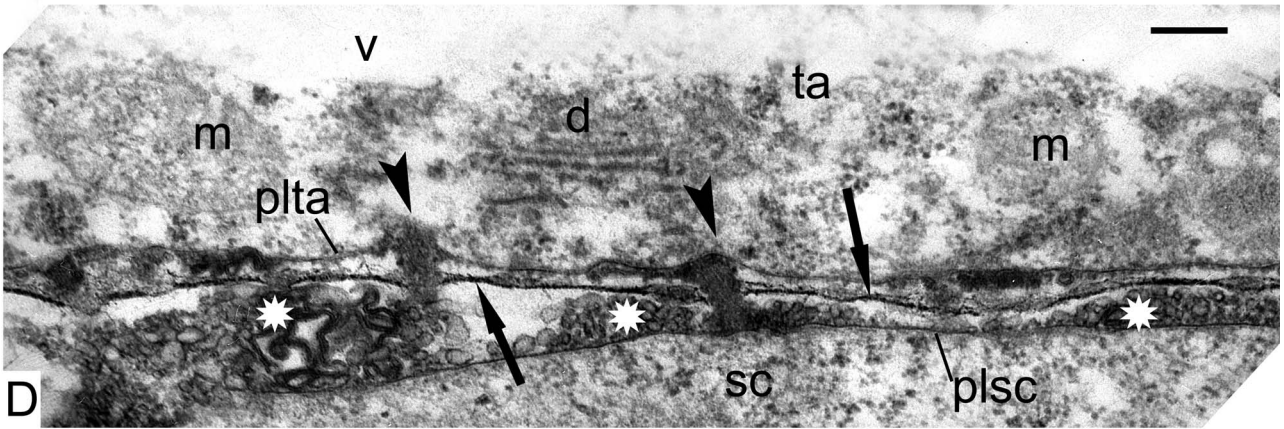
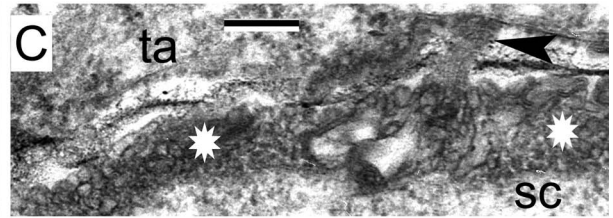
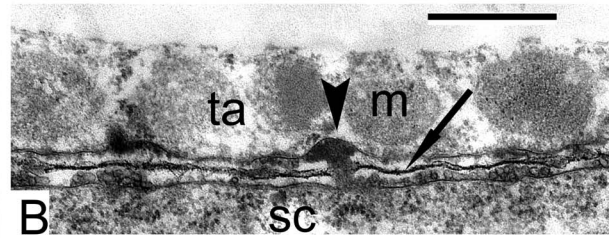
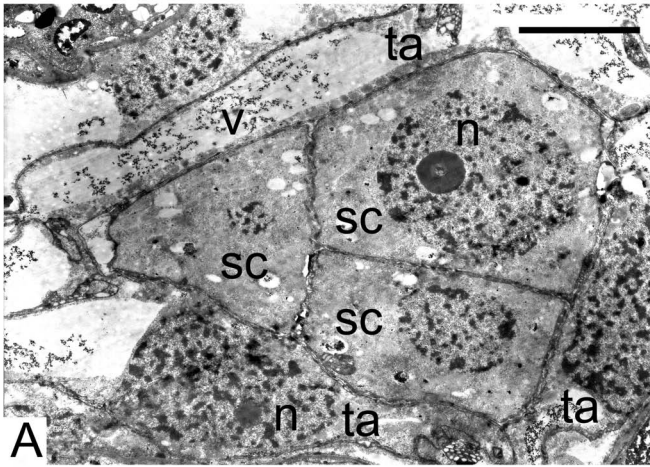
No previous ontogenetic studies have been undertaken on representatives of Cyatheaaceae, including *Alsophila*, the focus of the present study. Gastony (1974) reported some embryological data including the sporangium and spore morphology of Cyatheaaceae: he reported the chromosome number $n=69$ in *Alsophila* and observed that there were four spore mother cells (SMCs) per sporangium and, correspondingly, 16 spores in a sporangium. The form of the sporangium is elongate with a rather narrow loculus. Spore morphology of the

This article is dedicated to the memory of John Rowley, my dear teacher and friend (N.G.).

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Cyatheaceae, including *Alsophila*, was also studied by Gastony and Tryon (1976). Later, mature spores of several genera of the Cyatheaceae (including *Alsophila*) were described and illustrated with scanning electron microscopy (SEM) and transmission electron microscopy (TEM) by Tryon and Lugardon (1991). The mature sporoderm of an endangered homosporous, leptosporangiate Argentinian species, *Alsophila setosa* Kaulf., was studied with SEM and TEM by Marquez et al. (2009) as well. The authors demonstrated some differences between the number of perispore layers they recognised and those defined by Tryon and Lugardon (1991).

Our aim in this study was to trace at the ultrastructural level of the developmental events in *Alsophila setosa* from sporogenous cells through spore mother cell and meiosis stages, and, in particular, to pay special attention to the sporoderm development during the tetrad period. We also wanted to compare the ontogenetic events in the leptosporangiate fern *Alsophila setosa* (Cyatheaceae) and in *Psilotum nudum* (L.) P.Beauv. Psilotaceae, with the two genera *Psilotum* and *Tmesipteris*, had for many years been regarded as the most primitive living vascular plants (Takhtajan, 1978; Parenti, 1980; Bremer, 1985), although Bierhorst (1977) had argued that *Psilotum* was, in fact, a morphologically reduced fern. Later, Stevenson and Loconte (1996) pointed out that *Psilotum* should be placed as second branch within the true ferns (see Blackmore et al., 2000, and references cited therein). Analyses based on molecular sequence evidence strongly support the conclusion that *Psilotum* is a morphologically reduced fern (Qiu & Palmer, 1999; Schuettpelz & Pryer, 2007). In comparing *Alsophila* and *Psilotum*, our intention is to add to our understanding of early sporogenesis

in pteridophytes, and also to find similarities with gymnosperms and angiosperms.

Materials and methods

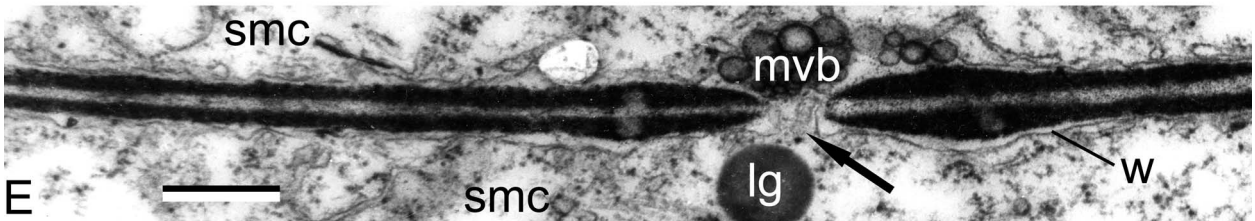
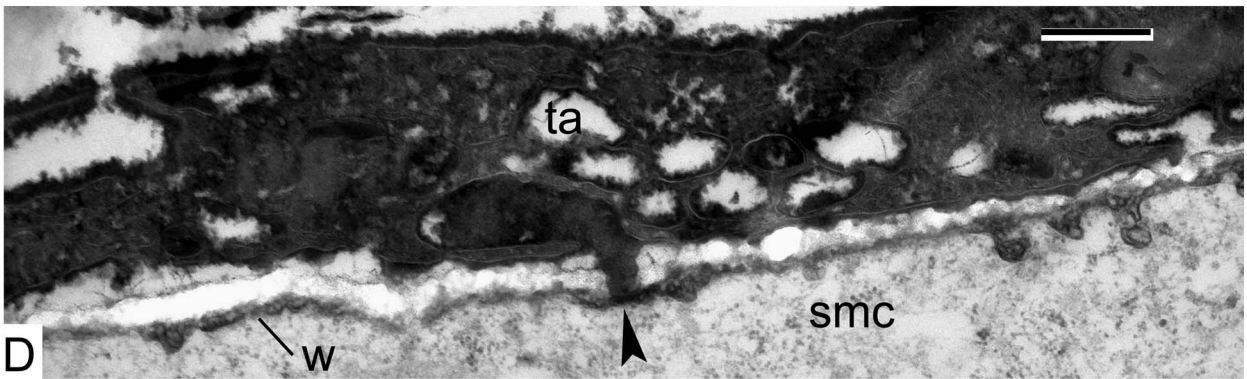
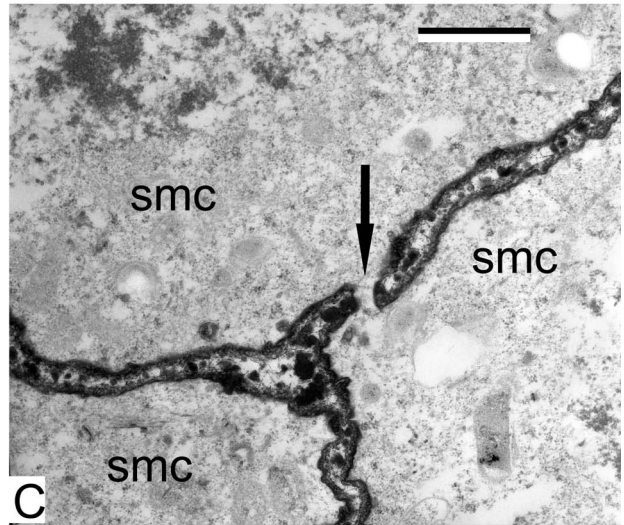
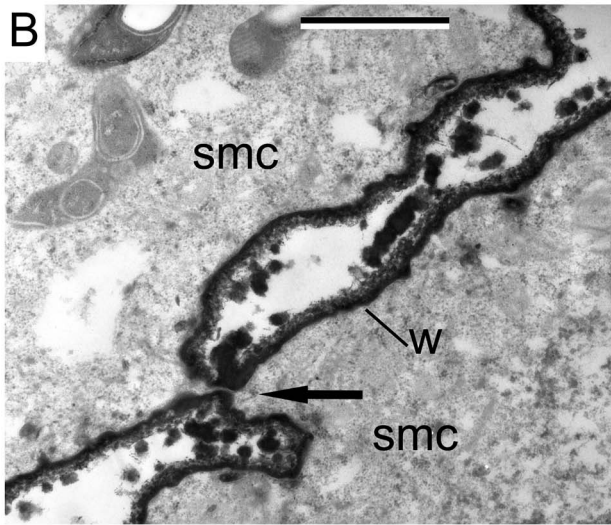
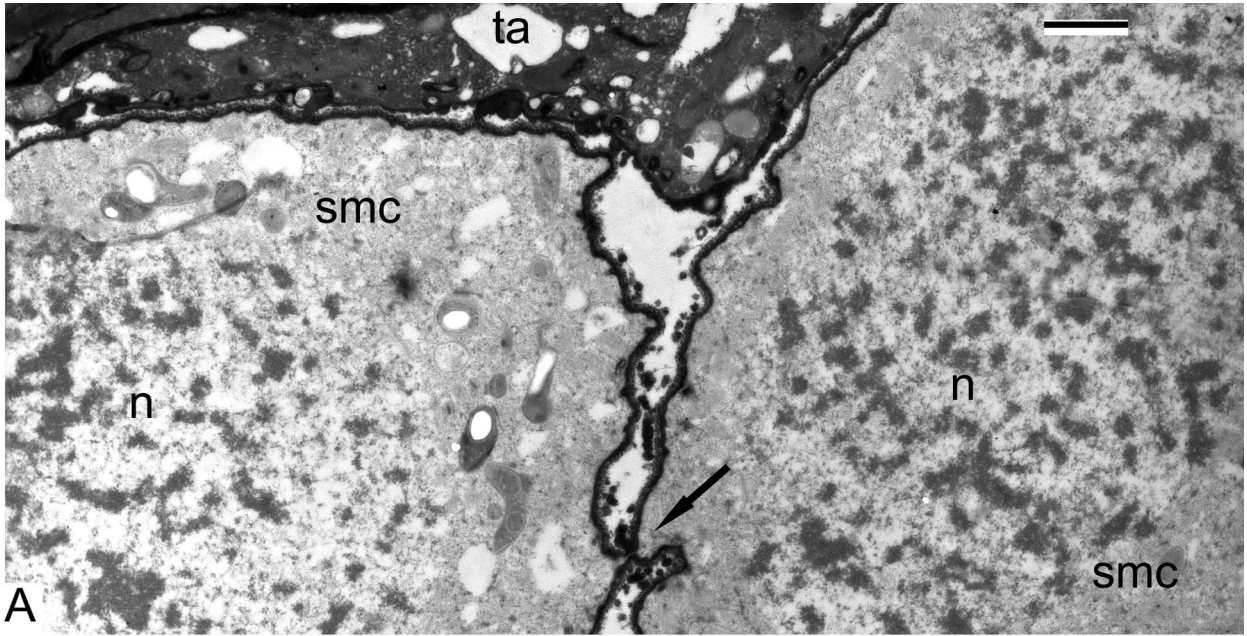
Sporangia of *Alsophila setosa* were collected during February 2009 in the Reserva de la Biósfera Yabotí, Misiones, Argentina. The material was fixed in the field using two fixatives: (1) 1% glutaraldehyde (GA) + 0.0025% ruthenium red (RR) + 0.1 M phosphate buffer (pH 7.4; 4 °C; 24 h), washed in phosphate buffer + 0.0025% RR, then post-fixed with 1% osmium tetroxide (OsO₄) + 0.0025% RR + 0.1 M phosphate buffer (pH 7.4; 4 °C; 2 h); (2) the same, but without RR. The material was dehydrated in an acetone series and then embedded in Spurr medium mixture. Ultrathin sections were stained with 1% uranyl acetate for 15 min followed by lead citrate for 5 min. The observations were made with a Hitachi H-600 TEM at the Komarov Botanical Institute, St. Petersburg.

Results

Pre-meiotic stages: sporogenous cells

The earliest stage that we managed to obtain in our material of *Alsophila* represented the stage at which the process of differentiation of the archesporial cells had been completed and sporogenous and tapetal cells were present. The sporogenous cells are tightly packed and have angular outlines and rather dark-contrasted cytoplasm with many ribosomes (Figure 1A, E). The common wall between the tapetum and sporogenous cells is represented by

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Figure 1. Pre-meiotic stages in *Alsophila setosa* and in *Psilotum nudum*: sporogenous cells. **A–E.** *Alsophila setosa*: **A.** Group of sporogenous cells, surrounded by tapetal cells; elongate tapetal cells contain large vacuoles with dark-contrasted precipitations; **B.** The border between tapetal cell and sporogenous cell; the middle lamella is high contrasted (arrow); all the tapetal organelles are aligned in the parietal layer of the cytoplasm with plasmodesma between tapetal and sporogenous cells (arrowhead); **C.** Clusters of plasmalemmasomes at the surface of sporogenous cell (asterisks); lipophilic accumulations penetrate from the tapetum to the sporogenous cell through plasmodesma (arrowhead); **D.** The border between the tapetal cell and the sporogenous cell with distinct middle lamella (arrows), plasma membrane of tapetal cell and plasma membrane of the sporogenous cell; the boundaries of mitochondria and dictyosome in the parietal cytoplasm of the tapetal cell are almost indistinguishable; plasmodesmata between the tapetal cell and the sporogenous cell become occluded (arrowheads); many outgrowths (plasmalemmasomes) of the plasma membrane of the sporogenous cell (asterisks) displaced the middle lamella closer to the tapetal cell; **E.** Outgrowths of the plasma membrane of the sporogenous cell (asterisk); a lipid substance penetrating from the tapetal to the sporogenous cell appears in the form of a distinct ordered substructure (arrowhead). **F, G.** *Psilotum nudum* (from Gabarayeva, 1984a, figure 3; reproduced with permission): **F.** Clusters of plasmalemmasomes at the surface of a sporogenous cell (asterisk) with ampoule-like dilation of the cisterna of the rough endoplasmic reticulum (arrow); **G.** The border between sporogenous cells and plasmodesmata; plasmalemmasomes at the surface of the cell (asterisk). Scale bars – 5 µm (A), 500 nm (B, F, G), 200 nm (C–E). Legend to all figures: ce – central element, ch – chromosome, cm – cytoplasm of meiocyte, cp – cup-like plastid, d – dictyosome, er – endoplasmic reticulum, fl – fibrillar layer, ga – Golgi apparatus, is – intercellular space, lg – lipid globule, me – meiocyte, mi – mitochondrion, mvb – multivesicular body, n – nucleus, ne – nucleus envelope, nu – nucleolus, p – plastid, pd – plasmodesma, pe – plastid envelope, pi – invagination of the plastid, pl – plasma membrane of the sporogenous cell, plta – plasma membrane of tapetal cell, rer – rough endoplasmic reticulum, s – young spore, sc – sporogenous cell, sco – synaptonemal complex, smc – spore mother cell, ss – sporangium space, ta – tapetal cell/tapetum, tet – tetrad, ts – tetraspore, v – vacuole, w – spore mother cell wall.



the middle lamella (Figure 1B). The latter is bordered with the plasma membranes of tapetal and sporogenous cells (Figure 1B, D). The plasma membrane of the sporogenous cells has many outgrowths, both small and large (plasmalemmasomes), which are seen in sections as clusters of small or large vesicles (Figure 1C–E). Note the displacement of the middle lamella towards the tapetal cell because of appearance of these plasmalemmasomes of the sporogenous cell (Figure 1D), when compared with the central position of the middle lamella at a slightly earlier stage (Figure 1B). Plasmodesmata between the tapetal cells and sporogenous cells are at first well pronounced (Figure 1B, C), but become partly occluded at a slightly later stage (Figure 1D).

The tapetal cells form a single layer around the sporogenous cells and have an elongate form (Figure 1A). Some of them have large vacuoles with dark contrasted precipitations (Figure 1A). The large vacuole squeezes the cytoplasm of tapetal cells out to the cell periphery, and all the organelles are aligned along the plasma membrane (Figure 1B, D). Nevertheless, the tapetal cells are active, they secrete an osmiophilic substance, probably lipophilic in nature, which accumulates outside the plasma membrane (Figure 1B, D). This osmiophilic substance penetrates into the sporogenous cells (Figure 1B–D) and in some places forms highly ordered substructures (Figure 1E).

At comparable stage in *Psilotum*, sporogenous cells also show clusters of plasmalemmasomes (Figure 1F, G) and numerous plasmodesmata (Figure 1G). The rough endoplasmic reticulum (RER) forms stacks of cisternae with dilations.

Pre-meiotic interphase: spore mother cells (SMCs)

SMCs of *Alsophila* are separated from each other by narrow spaces, but connected via cytotoxic channels (Figure 2A–C). The SMCs develop a dark contrasted envelope, which is slightly wavy; granules, most probably of tapetal origin, are accumulated in the space between the cells (Figure 2A–C). Large nuclei occupy a considerable part of the volume of the SMCs (Figure 2A). Plastids with starch grains are pleomorphic, their form varies from round to amoeboid (Figure 2B). The wall of SMCs has a jagged profile; a lipophilic substance penetrates from tapetum into SMCs (Figure 2D).

In *Psilotum*, SMCs are also connected by cytotoxic channels (Figure 2E) and multivesicular bodies; lipid globules and other organelles are in close vicinity, probably penetrating through the cytotoxic channels.

Meiosis, prophase I: leptotene

When entering meiosis, the SMCs of *Alsophila* retain an amoeboid form; the nucleus still occupies most of the cell volume (Figure 3A). The axial elements of chromosomes start to be evident (Figure 3A, B). Many vacuoles appear in the cytoplasm, the remaining organelles are poorly discernible, being poorly differentiated (Figure 3A, B). The tapetal cells have dark-contrasted cytoplasm and contain strongly osmiophilic plastids of ribbon-like or oval form (Figure 3A, B).

Meiosis, prophase I: zygotene

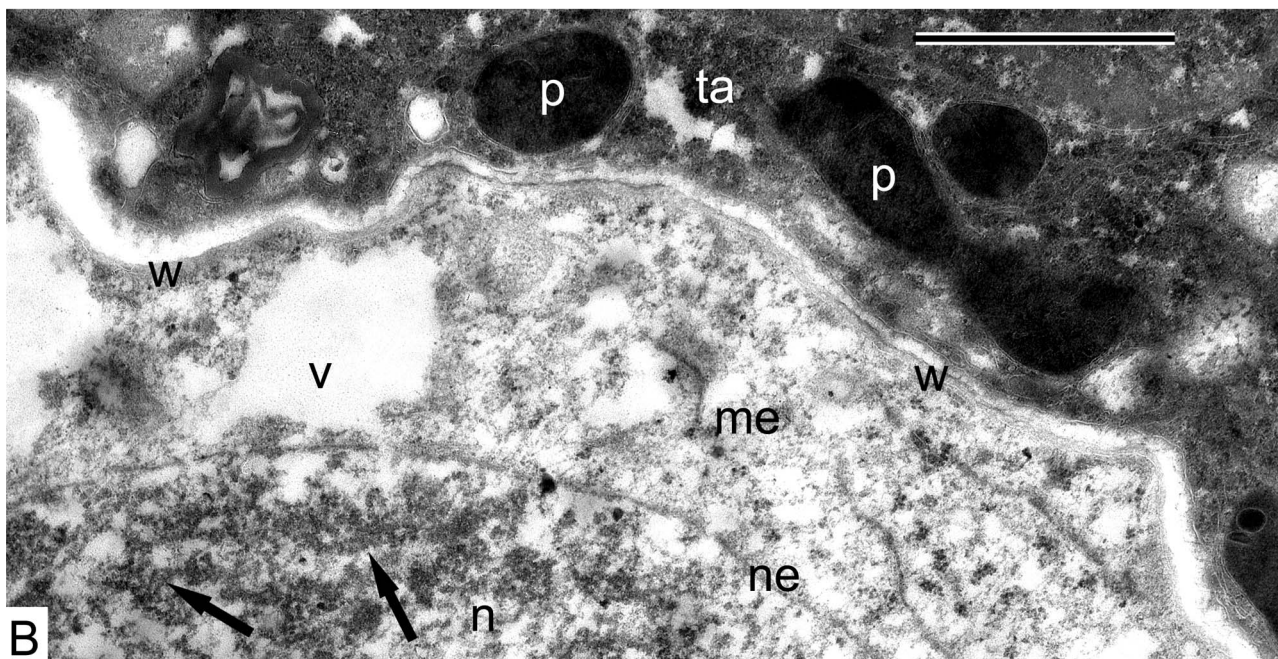
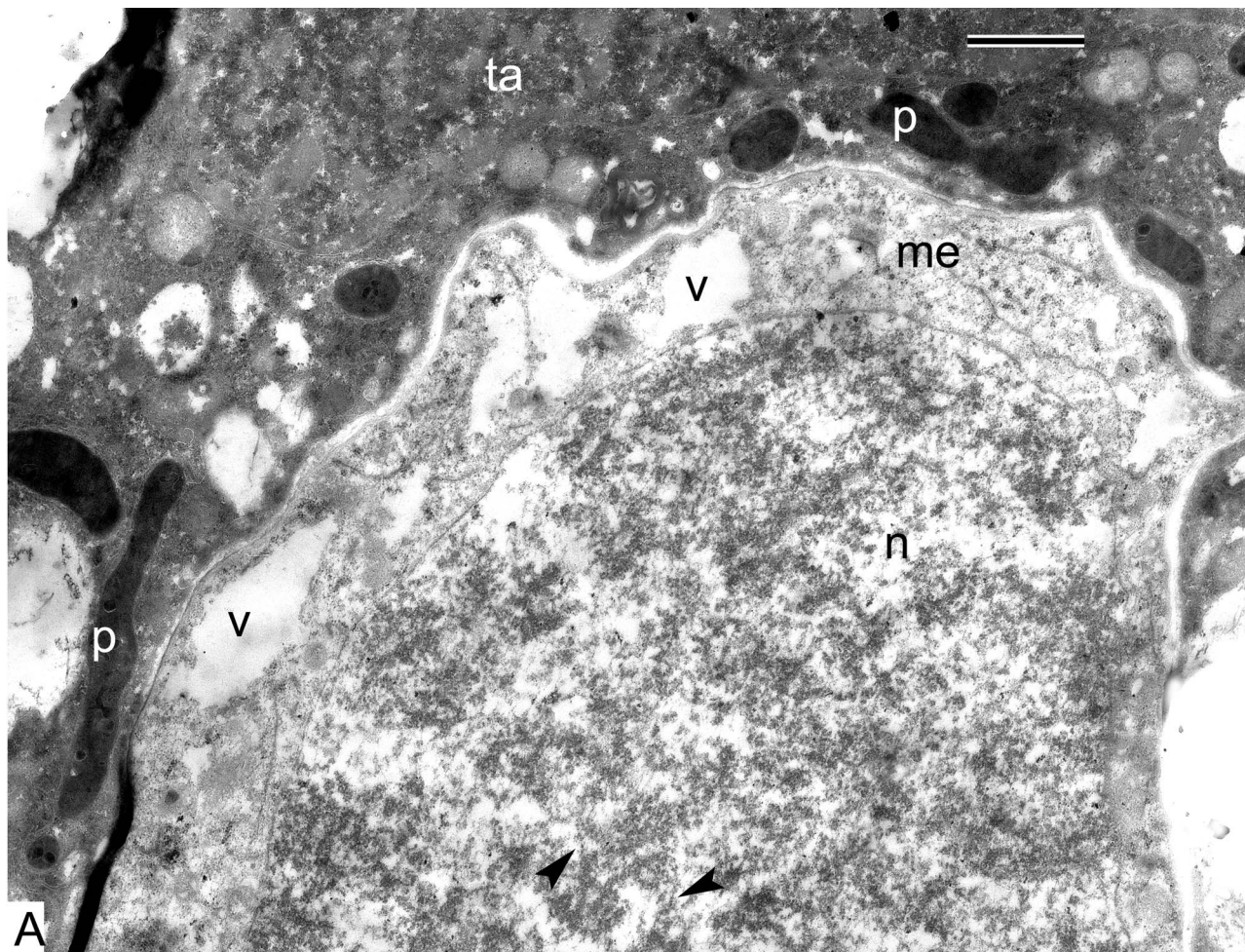
At this stage, the formation of synaptonemal complexes occurs. Pairs of chromosomes are clearly seen and lateral elements of the synaptonemal complexes are parallel to each other (Figure 4A, B, *Alsophila*); the central element is clearly seen at higher magnification (Figure 4D, *Psilotum*). The process of meiotic clarification of the cytoplasm is evident in both species: the increase of the number of autolytic vacuoles leads to the reduction of ribosome and organelles number, many multivesicular bodies are seen in the meiocyte cytoplasm of *Alsophila*, some of them are attached to the microspore wall (Figure 4C).

Meiosis, prophase I: early pachytene

Stronger condensation of the chromatin of the bivalents in synaptonemal complexes is typical for this stage in comparison with zygotene in *Psilotum* (Figure 4E) and *Alsophila* (Figure 5A). In those parts of synaptonemal complexes, which were sectioned longitudinally, it is possible to see coils of chromosome spirals (Figure 4E). The cytoplasm of meiocytes in both species is strongly clarified and contains many vacuoles. The membranes of organelles are either not well preserved or are poorly discernible,

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Figure 2. Pre-meiotic interphase in *Alsophila setosa* and *Psilotum nudum*: spore mother cells (SMCs). **A–D.** *Alsophila setosa*: **A.** Cytotoxic channels appear between the SMC (arrow); **B.** Two SMCs with a cytotoxic channel (arrow); the wall of the SMC is well pronounced; roundish particles are present in the space between the SMC; **C.** Three SMCs with a cytotoxic channel (arrow); **D.** The border between the tapetal cell and the SMC; the wall of the SMC has a jagged profile; penetration of lipoid substance from the tapetal cell into the SMC (arrowhead). **E.** *Psilotum nudum* (from Gabarayeva, 1984a, figure 4; reproduced with permission); a cytotoxic channel between the SMC (arrow); the multivesicular body is ready to penetrate the channel. Abbreviations: see Figure 1. Scale bars – 1 μm (A–C), 500 nm (D, E).



the envelope of meiocytes has differential contrast and looks three-layered with a jagged outer surface as in *Alsophila* (Figure 5B).

The condition of tapetal cells in *Alsophila* shows a sharp difference from that of the meiocytes. Tapetal cells are very active, their cytoplasm contains many organelles and all the membranes of organelles are very well preserved, and the contrast of tapetal cells continues to be negative (all the membranes appear white, but the contents of endoplasmic reticulum cisternae, of plastids and the cytoplasm appear black; Figure 5B).

In *Psilotum*, at the late pachytene stage, the compactness of the chromatin in the synaptonemal complexes is very high [Figure 6A, D; cross-sectioned complexes (A), longitudinally-sectioned complex with the central element (D)] and is not less intensive than that observed during metaphase. There are many cup-like plastids and close contact between some of these plastids and the nuclear envelope are a characteristic feature of this stage (Figure 6B). Many finger-like invaginations of the nuclear envelope are also observed (Figure 6C).

Meiosis I: telophase

At the late anaphase/early telophase in *Alsophila*, two groups of sister chromosomes are shared by the equatorial organelle plate, and the nuclear envelopes are indistinct (Figure 7B). This plate is a cluster of many organelles: mitochondria, plastids, lipid globules, small autolytic vacuoles (Figure 7C). Because the membranes of organelles are poorly visible, it is sometimes difficult to distinguish lipid globules from plastoglobules of plastids. No signs of the formation of a new equatorial cell wall is observed at this stage. The wall of *Alsophila* meiocytes is uneven; it becomes here and there rather thick (Figure 7B, D) and contains an electron transparent matrix with fine fibrillae (Figure 7D). Some tapetal cells, surrounding the four meiocytes in the sporangium, invade inside the loculus, partly embracing each meiocyte from the proximal side; two telophase meiocytes can be seen in this section (Figure 7A).

In *Psilotum*, the nuclear envelopes are clearly discernible at the advanced telophase stage, surrounding both groups of sister chromosomes and finger-like invaginations of the nuclear envelope

are preserved (Figure 8A). The organelle plate consists of readily recognisable plastids (many of them have a cup-like form), mitochondria, vacuoles, lipid globules and Golgi stacks (Figure 8A). Microtubuli penetrate the organelle plate and are attached to organelles (Figure 8B). Some ribbon-like plastids have two cup-like regions at their extremities (Figure 8B).

The tetrad period in Alsophila setosa

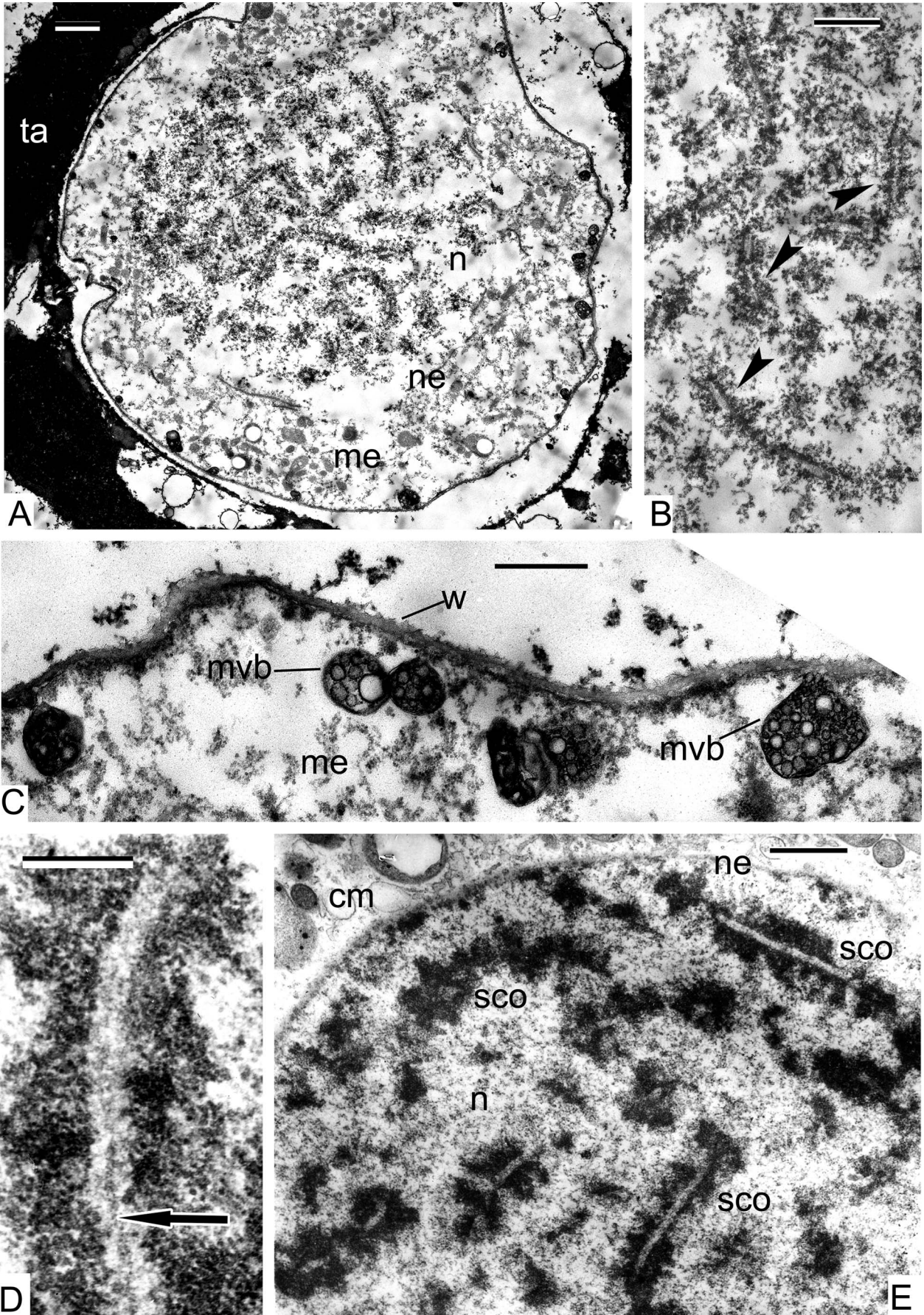
Early tetrad stage. — The tetrads are surrounded by elongate, binuclear tapetal cells, which may partly fuse together (Figure 9A, B). The tapetal cells maintain their parietal position and their periclinal walls are poorly discernible. Many osmiophilic spherical units accumulate between the tetraspores (Figure 9C). Long fibrils are discernible on the tetraspore surface (Figure 9D, E). It is difficult to judge whether these fibrillae transformed from the material of the meiocyte envelope (the primary wall of the sporocyte; Figure 7D), or are newly formed.

Tetrad stage in progress. — Sections through tetrahedral tetrads (Figures 10A, B) reveal that each tetraspore is enveloped in a fibrillar layer (Figure 10C). A dark thin unbroken sheet has appeared between the tetraspore plasma membrane and the fibrillar layer (Figure 10C–E). The cytoplasm of the tetraspores, observed at higher magnification, is quite different from that of the meiocytes and contains many ribosomes and polysomes (Figure 10E), although the membranes of organelles are still poorly defined. Some of the tapetal cells have normal contrast and contain large vacuoles with dark contrasted filaments (Figure 10C, D), whereas others retain a negative contrast (Figure 10E).

The end of the tetrad period. — Some microspores are retained together in tetrads (Figure 11A), but others start to be separated from each other, and some tapetal cells seem to be going to penetrate between the members of the tetrad (Figure 11B). A dark contrasted sheet, associated with fibrils, becomes more prominent and has a sinusoidal profile (Figure 11C–F). The tapetal cells maintain their parietal position (Figure 11A). Substances, secreted by the tapetum,

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Figure 3. Prophase I of meiosis in *Alsophila setosa*. **A.** The beginning of the prophase (leptotene stage): The axial elements of the chromosomes start to be visible (*arrowheads*); the cytoplasm of the meiocytes contains many autolytic vacuoles, elimination of ribosomes and dedifferentiation of plastids take place; the tapetal cells are dark-contrasted and have plastids of ribbon-like and oval form, which are especially osmiophilic. **B.** Magnified part of (**A**): Tapetal cells have so-called negative contrast: membranes appear white, but stroma of plastids and the cytoplasm are strongly osmiophilic; the wall of meiocytes is wavy; axial elements of chromosomes visible (*arrows*). Abbreviations: see Figure 1. Scale bars – 1 μm .



accumulate in the supporting liquid of the sporangial loculus. As a result, different kinds of osmiophilic accumulations are visible in the loculus of the sporangium including tripartite lamellae, spherical droplets and vesicles of different size and form (Figure 11G).

Very young free spores

Very young free spores are still surrounded by a parietal tapetum (Figure 12A). An outgrowth of the spore envelope, probably representing the initial proximal fold (Figure 12A), is discernible. Many accumulations of different form and size including vesicles, spherical particles and fragments of membranes fill the loculus of sporangium (Figure 12B): these could either be products of the tapetal synthesis or remnants of disintegrating tapetal cells. A magnified fragment of Figure 12A shows more details of the spore surface, with a membrane on the top of the fibrillar network and with the initial proximal fold visible (Figure 12C). In close vicinity to the tapetum, the distal fibrillar surface of the young spores is rather even and includes osmiophilic roundish particles (Figure 12D, F), but the proximal sides of young spores have a very uneven surface with a sinusoidal initial layer of sporoderm, parallel to the contour of the spore surface and separated from it by a gap (Figure 12E). The tapetum shows high activity and has a negative contrast (Figure 12D, F); its cisternae of endoplasmic reticulum pinch off secretory vesicles; the latter attach to the tapetal plasma membrane and release their contents into the sporangial loculus by exocytosis (Figure 12F).

Discussion

Compared with seed plants, ultrastructural data on pteridophyte spore ontogeny is far less extensive. This is explained by the considerable technical difficulties facing investigators, connected with the poor penetration of fixatives and the corresponding preservation of the study material. The challenges of investigating the extremely thick and dense sporopollenous envelopes of spores, which form as early as the beginning of the post-tetrad

period, make the members of this most interesting, polyphyletic group a kind of Cinderella group among researchers. Nevertheless, a small number of determined authors have continued to add to our knowledge of the ultrastructure and development of pteridophyte spore walls. We can therefore compare our new data on meiosis and early sporogenesis in *Alsophila* with previous data on meiosis (Gabarayeva, 1984a, 1984b, 1985) and sporoderm formation (Lugardon, 1979) in representatives of other fern groups including *Psilotum* (Psilotaceae) and with corresponding developmental events in other ferns and higher plants, such as gymnosperms and angiosperms.

Pre-meiotic intercellular connections

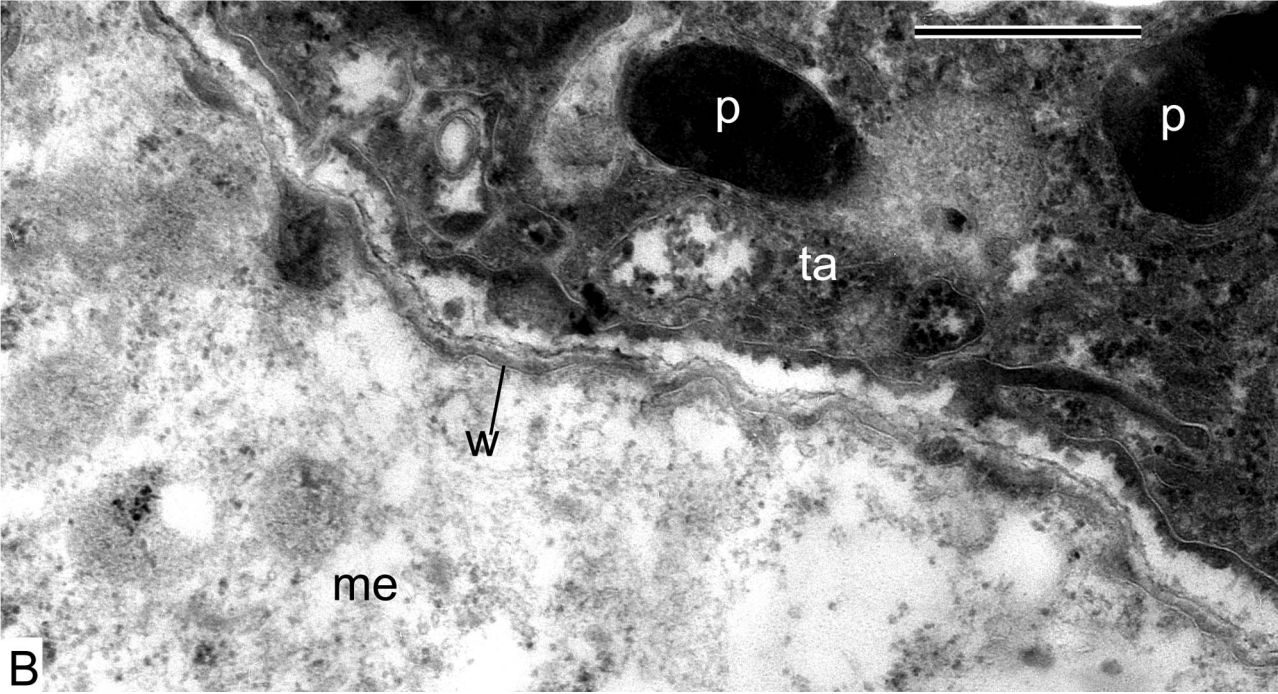
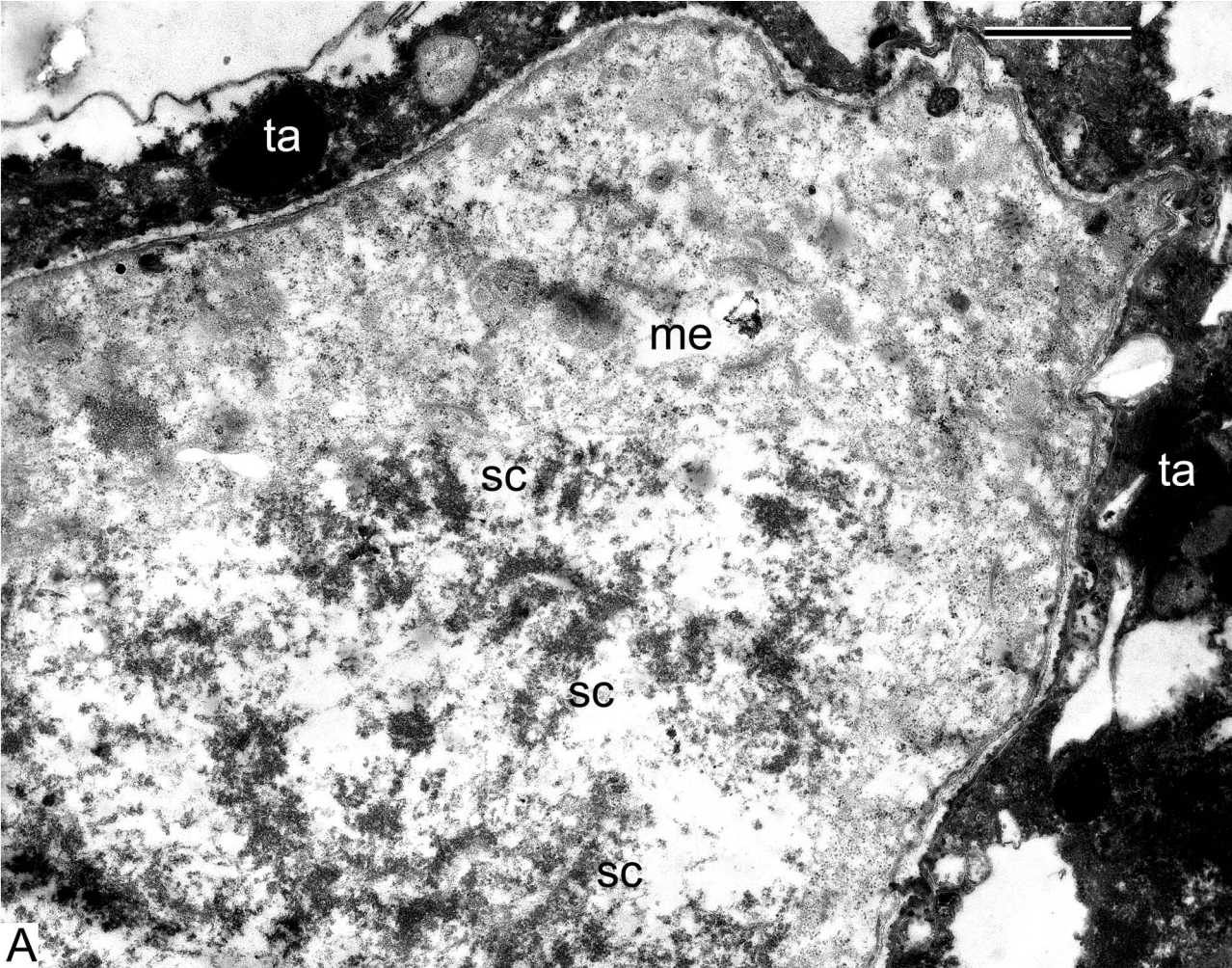
At the critical point of the transition from sporophyte to gametophyte, in addition to chromosome reduction, several features of the cells, involved in the transition, deserve special attention.

Clusters of plasmalemmasomes (outgrowths of the plasma membrane of different form) in sporogenous cells of *Alsophila* (Figure 1C–E) seem to be a typical feature for pre-meiotic cells of different plant groups. At the same point in ontogeny, the plasma membrane in *Psilotum nudum* sporogenous cells is also very wavy and forms clusters of plasmalemmasomes (Figure 1F). The latter were also observed in *Osmunda regalis* L., *Anemia phyllitidis* (L.) Sw. and *Lygodium japonicum* (Thunb.) Sw., as well as in many gymnosperms and angiosperms, for example, in *Stangeria eriopus* (Kunze) Baill. and *Helleborus foetidus* L. (Echlin & Godwin, 1968; Lugardon, 1968; Surova, 1981; Gabarayeva & Grigorjeva, 2002). It is likely that such an intensive folding of the plasma membrane is necessary for the rapid growth and rounding-up of the sporogenous cells that follows at the point of transition to SMCs.

Numerous plasmodesmata are characteristic for both sporogenous cells of *Alsophila* and *Psilotum* (Figure 1G). In *Alsophila*, plasmodesmata are observed not only between sporogenous cells, but also between sporogenous and tapetal cells. These intercellular connections in *Alsophila* (Figure 1B–D) are probably necessary for transition of some

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Figure 4. Prophase I of meiosis in *Alsophila setosa* and in *Psilotum nudum*. **A–C.** *Alsophila setosa* (zygotene stage): **A.** The formation of synaptonemic complexes; the process of meiotic clarification of the cytoplasm is in progress and the reduction of ribosome number, increasing of the number of autolytic vacuoles and formation of cup-like plastids are evident in meiocyte cytoplasm; **B.** Synaptonemic complexes (*arrowheads*) at higher magnification; lateral elements are visible; **C.** The cytoplasm of the meiocyte is essentially clarified; clusters of vesicles surrounded with a membrane (*arrows*) are located in the vicinity of the wall, some of them attached to it: these could be multivesicular bodies. **D, E.** *Psilotum nudum* (zygotene and pachytene stage): **D.** Zygotene synaptonemic complex of a chromosome dyad with a central element (*arrow*; from Gabarayeva, 1984b, figure 3; reproduced with permission); **E.** Pachytene synaptonemic complexes, most are cut longitudinally. Abbreviations: see Figure 1. Scale bars – 2 μm (A), 1 μm (B, E), 500 nm (C, D).



lipoid substance from the tapetum to sporogenous cells. Penetration of a lipid or some other hydrophobic surface-active substance is possible also in monomolecular form through the plasma membranes: in this case a hydrophobic substance, being excreted from a tapetal cell, comes into hydrophilic intercellular medium and forms micelle-like ordered structure (Figure 1E). Plasmodesmatal connections in pre-meiosis is a common feature for ferns (Sheffield & Bell, 1979) as well as for angiosperms (Dickinson & Heslop-Harrison, 1977): the latter authors suggested that up to a certain point in ontogeny, everything was coordinated between cells, because their cytoplasm was interconnected, but later, the closure of cytomictic channels allowed different lines of cells to follow different fates in the course of development.

Before the initiation of meiosis, most of the plasmodesmata become occluded and cytomictic channels appear between SMCs in both *Alsophila* (Figure 2A–C) and *Psilotum* (Figure 2E). Lugardon (1968) compared development of cytoplasmic connections in pre-meiotic and meiotic stages in homosporous ferns *Blechnum spicant* (L.) With. and *Osmunda regalis*: in *Blechnum*, pre-meiotic plasmodesmata are substituted for cytomictic channels in prophase I of the meiosis; the channels in *Osmunda* are wider than those of *Blechnum* and allow passage of such organelles as mitochondria and cisternae of endoplasmic reticulum. In *Psilotum*, the cytomictic channels are also wide enough to allow passage of multivesicular bodies and lipid globules, although we have not demonstrated the penetration of organelles through them (Figure 2E). The term ‘cytomictic’ itself has appeared to stress their function: to provide the flow of cytoplasm together with its organelles from cell to cell. Cytomictic channels in SMCs are closed earlier in pteridophyte ontogeny than those of angiosperms, and they are less numerous (Lugardon, 1968). Blackmore (1990) formulated that ontogeny in angiosperms is an extended process (i.e. has additional steps like the development of a thick primexine) compared to pteridophytes, and it could be that closing the cytomictic channels at an early stage is a characteristic of pteridophytes compared to seed plants. The substitution of plasmodesmata to cytomictic channels, which disappear later, was also shown in the heterosporous fern *Marsilea* (Bell, 1981). The same process is usual at the transition

from sporogenous cells to microspore mother cells for angiosperms (Heslop-Harrison, 1966; Pacini & Cresti, 1978; Gabarayeva et al., 1998, 2009a) and is a widespread feature. The time of the appearance of the cytomictic channels varies in different species: sometimes, they appear earlier between sporogenous cells as in *Chamaedorea* (Gabarayeva & Grigorjeva, 2010).

The meaning of these intercellular connections is to create a coenocyte-like organisation and to achieve synchronised meiosis in all the meiocytes of a sporangium. Depending on the number of the cytomictic channels between meiocytes, this synchronism can be complete or limited. In *Psilotum*, where the number of cytomictic channels is relatively low, incomplete synchronism of meiosis was observed (Gabarayeva, 1984a). Interestingly, the absence of cytomictic channels was shown for gymnosperms (Kozubov et al., 1982) and, as a consequence, the asynchronism of meiosis in their microsporocytes.

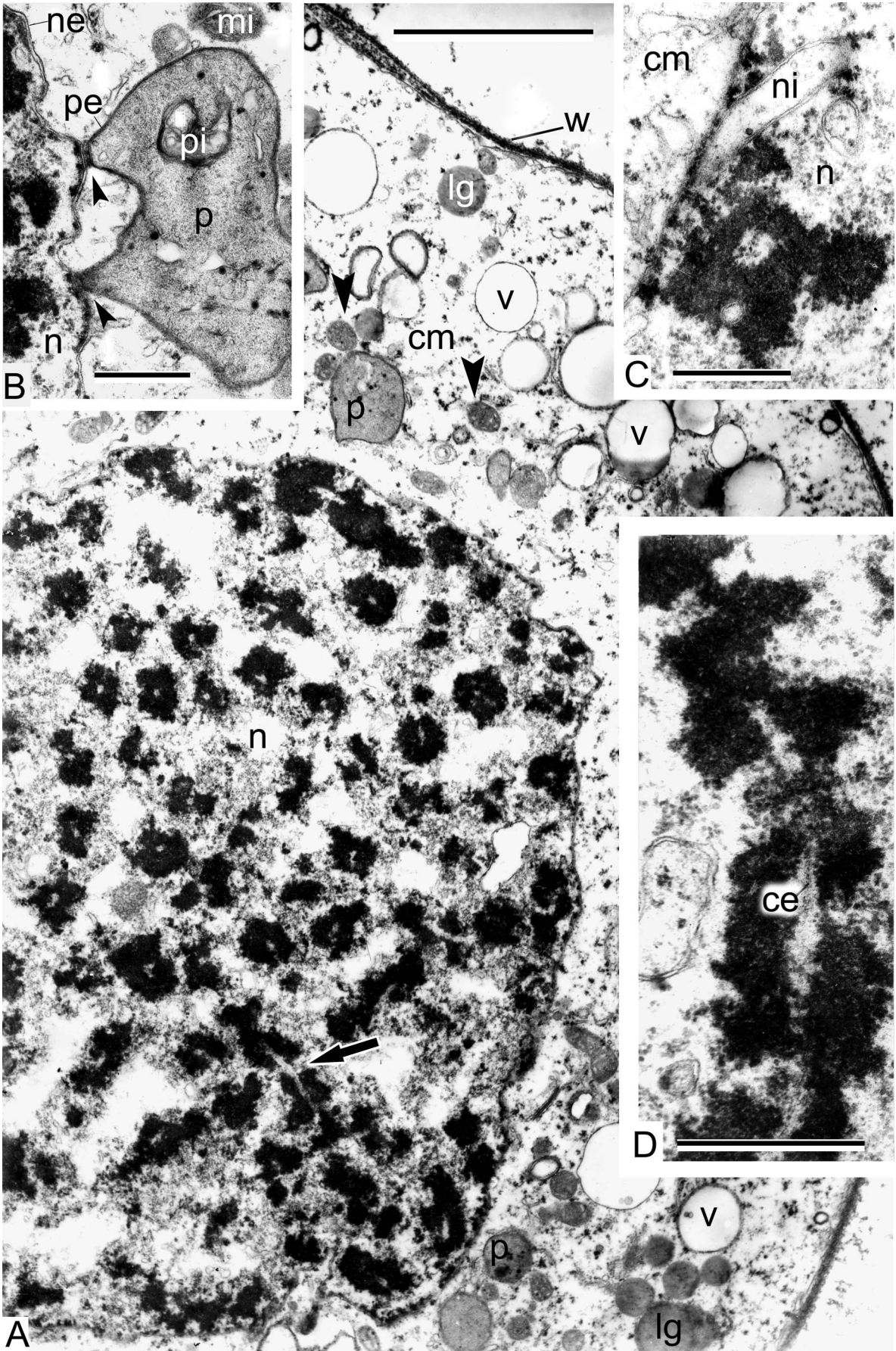
The walls of SMCs in *Alsophila* and in *Psilotum* are clearly discernible, but differ from each other. They are indented and associated with osmiophilic globules in *Alsophila* (Figure 2B–D), but smooth in *Psilotum* (Figure 2E). Many local dilations of stacked RER cisternae (one of them shown in Figure 1F) were described as connected with the formation of phytolysosomes in *Cucurbita pepo* L. (Coulomb & Coulon, 1972), but in ferns, Pettitt (1979) associated a secretory activity before meiosis with the formation of a fibrillar material of the meiocytes envelopes – the so-called special tetrad envelope. In *Hypnum rusciforme* Bridel (Bryophyta), a thick fibrillar layer is formed around sporogenous cells and meiocytes; histochemical data confirm its polysaccharide nature, namely callose (Genevès, 1971).

Meiosis, meiotic cytoplasmic clarification and re-organisation of organelles

At the transition to meiosis, a general re-organisation of the cytoplasm starts in both species. In the leptotene, when axial elements of chromosomes start to be discernible in *Alsophila* (Figure 3), we did not observe the formation of multimembraneous spheres, containing portions of the meiocyte cytoplasm with organelles and ribosomes (as is typical for angiosperms) but rather many autolytic vacuoles of

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Figure 5. Prophase I of meiosis in *Alsophila setosa*. Early pachytene stage. **A**. A part of the meiocyte surrounded by tapetal cells; chromosomes of the synaptonemal complexes are more condensed than they were at the zygotene stage; all organelles in the cytoplasm are dedifferentiated, their membranes are not discernible. **B**. Higher magnification of **(A)** evidences that the negative contrast of the tapetal cells is still preserved and the wall of the meiocyte becomes thicker and is differentiated into several layers of different contrast; the cytoplasm of meiocytes is clarified. Abbreviations: see Figure 1. Scale bars – 1 µm (A), 500 nm (B).



irregular form with electron-transparent contents are the characteristic feature of the cytoplasm. In parallel, the almost total disappearance of starch in *Psilotum* meiocytes and also of inner and outer membranes of plastids occurs in *Alsophila*. This was also reported for Polypodiophyta as a whole (Sheffield & Bell, 1979). Mitochondria are also poorly differentiated.

These changes of plastids and mitochondria during meiosis are so profound in *Alsophila* that these organelles are actually unrecognisable from the pachytene until the telophase I (Figures 3, 4A, C, 5, 7), but they are much better seen in *Psilotum* (Figures 6, 8). The hyaloplasm of meiocytes looks almost 'empty' because of the elimination of most of the ribosomes (Figures 3, 4C, 5, 6A). The stages of meiosis are well recognisable by the nuclear contents: in the leptotene, axial elements of chromosomes start to be discernible (Figure 3); in the zygotene, pairs of chromosomes form synaptonemal complexes (Figure 4) with a typical central element (Figure 4D); the pachytene is characterised by much stronger condensation of chromatin in bivalents (Figures 5A, 6A, D). After the metaphase and anaphase I stages, when homologous chromosomes have diverged, the organelle plate (band) forms in the telophase (Figures 7B, C, 8).

The main events during meiosis in both species are the same, but details are different. In *Psilotum*, many plastids invaginate and acquire a cup-like form; some of them are in close contact with the nuclear envelope (Figure 6B). We have not observed invaginations of the nuclear envelope in *Alsophila* during meiosis, but many finger-like invaginations were found in the pachytene in *Psilotum* (Figure 6C). The meaning of these invaginations is probably in that the surface of the contact of nucleus and cytoplasm increases considerably (therefore, nuclear-cytoplasmic interaction increases, which is connected with the beginning of another type of growth, in this case: gametophytic). A similar point of view was reported by Bell (1975), who observed another form of nuclear-cytoplasmic interaction: evaginations of the nucleus to the cytoplasm. This is important during the sporophyte-gametophyte transition, most probably in connection with the change of genome functioning and corresponding transfer of the information to the cytoplasm. The cytoplasm of the gametophytes is

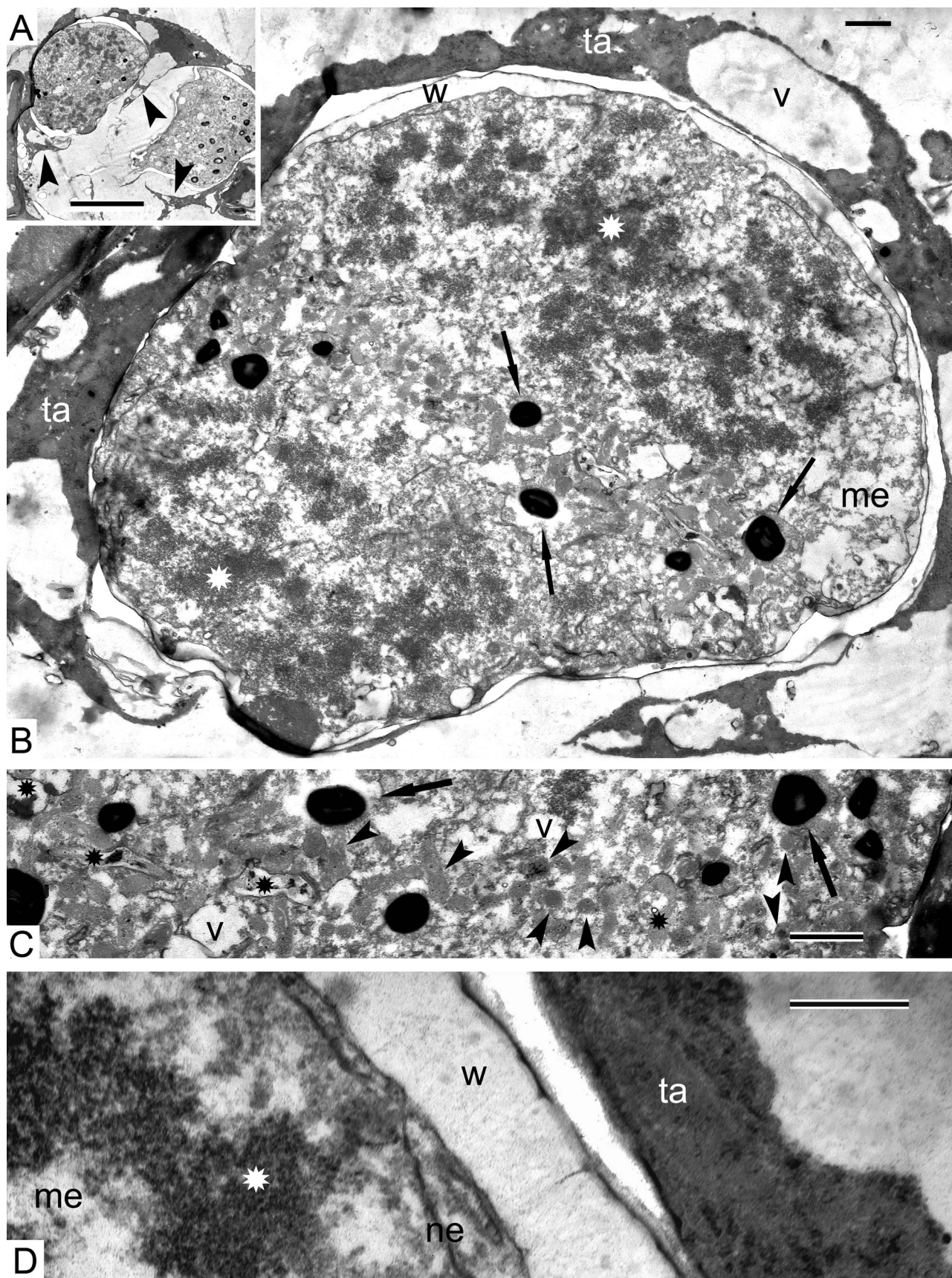
purged of sporophyte information in the process of cytoplasmic clarification (Heslop-Harrison, 1971). A similar intensification of the nucleo-cytoplasmic interaction is a common feature for all land plant groups.

The comparison of all the observed organelle changes with those in other, closely and remotely related plant groups reveals the same changes or variations of them, but in different combinations or confined to slightly different points in ontogeny. They were found in the homosporous ferns *Pteridium aquilinum* (L.) Kuhn and *Anemia phyllitidis* (Sheffield & Bell, 1979; Surova, 1981), in the heterosporous fern *Marsilea* (Bell, 1981), in the apogamous fern *Dryopteris borrieri* Newman (Sheffield et al., 1983), in mosses at sporogenesis (Brown & Lemmon, 1982a) and gametogenesis (Manohar Lal & Bell, 1977), in gymnosperms at microsporogenesis (Aldrich & Vasil, 1970; Dickinson & Bell, 1970; Kozubov et al., 1982) and in angiosperms at mega- and microsporogenesis (Bogdanov, 1983).

In particular, the contact of the plastid with the nuclear envelope in *Selaginella* during megasporogenesis and in young microspores was reported (Pettitt, 1971, 1977); invaginated plastids were described in *Isoetes* megasporogenesis (Pettitt, 1976). The monoplastidic type of meiosis (MTOC, in which the plastid is the site of microtubule organising centre) in eusporangiate ferns (Brown & Lemmon, 2001a) is considered to be more primitive than the polyplastidic type (Brown & Lemmon, 2001b), characteristic of the fern and seed plant clade. Novel meiotic spindle ontogeny in quadrilobed sporocytes of leafy liverworts, unique for bryophytes, was also reported (Brown & Lemmon, 2009). In *Isoetes sinensis* Palmer, a rare and endangered quillwort in China, irregular meiotic behaviour was reported (Wang et al., 2007).

Simultaneous meiosis in *Alsophila* and in *Psilotum* is accompanied by formation of an organelle plate (=organelle band), associated with microtubules, in the equatorial plane at the telophase I (Figures 7, 8). Distinct polarisation of the meiotic cell is observed at the previous stage, at the metaphase with its polar distribution of organelles, but this kind of polarisation changes to the equatorial in the telophase. The organelle plate represents a special mechanism for the equal distribution of organelles between

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Figure 6. Prophase I of meiosis in *Psilotum nudum*, pachytene stage (from Gabarayeva, 1984b, Figures 4, 1, 2, 4 and 5, 3; reproduced with permission). **A.** Part of a meiocyte, general view; most part of the synaptonemal complexes is cross-sectioned with the exception of one or two (arrow); the wall of the meiocyte is fibrillar and dark; many organelles, including mitochondria (arrowheads), are well discernible. **B.** Close contact between a plastid and the nuclear envelope (arrowheads). **C.** Finger-like invaginations of the nuclear envelope are characteristic for this stage. **D.** Magnified image of a pachytene synaptonemal complex with the central element. Abbreviations: see Figure 1. Scale bars – 10 µm (A), 1 µm (B–D).



the sister meiotic cells. The same behaviour of organelles was described in *Polypodium* (Marengo & Marengo, 1972) and *Onoclea* (Marengo, 1977), in *Pteridium* (Sheffield & Bell, 1979) and in the apogamous fern *Dryopteris borrieri* (Sheffield et al., 1983). An organelle plate forms in the moss *Rhynchostegium serrulatum* (Hedwig) A. Jaeger (Brown & Lemmon, 1982b); in the horsetails *Equisetum palustre* L. (Lewitsky, 1926) and *E. fluviatile* L. (Lehmann et al., 1984), in four other species of *Equisetum* (Bednara et al., 1986); in *Ginkgo biloba* L. (Wolniak, 1976); in the angiosperms *Helleborus foetidus* (Echlin & Godwin, 1968) and *Cypripedium californicum* A. Gray (Brown & Lemmon, 1996). Interestingly, the F-actin cytoskeleton plays an important role in meiosis: after experimental destruction of microfilaments in the *Psilotum nudum* meiocyte, the daughter nuclei were located too close to each other and an organelle plate did not form, causing abnormal meiosis (Tchórzewska & Bednara, 2011). During simultaneous meiosis in *Lavatera* (Malvaceae), an unusual type of microtubular cytoskeleton was described (Tchórzewska et al., 2008), in which plastids and mitochondria group around the nucleus forming a kind of envelope, surrounded by radially oriented microtubules at the end of the meiotic prophase I and maintain this position up to the late stages of sporoderm development. No organelle plate and fragmoplast were found in the simultaneous meiosis of the apomictic Asteraceae *Chondrilla juncea* L. (Koscinska-Pajak & Bednara, 2003).

Sporoderm development in the tetrad period in Alsophila setosa

Our main interest in the tetrad period is the sporoderm development. Summarising the main events in the tetraspore wall, we emphasise the appearance of a fibrillar layer and of dark thin sheet around each tetraspore (Figure 10C–E). A layer of clearly discernible fibrilles surrounding the tetraspores is most probably a primexine matrix, which might be polysaccharidic or glycoproteinous. If the latter is true, it could be called a glycocalyx, but only histochemical data could show its chemical composition. Until new data will be gathered, we use

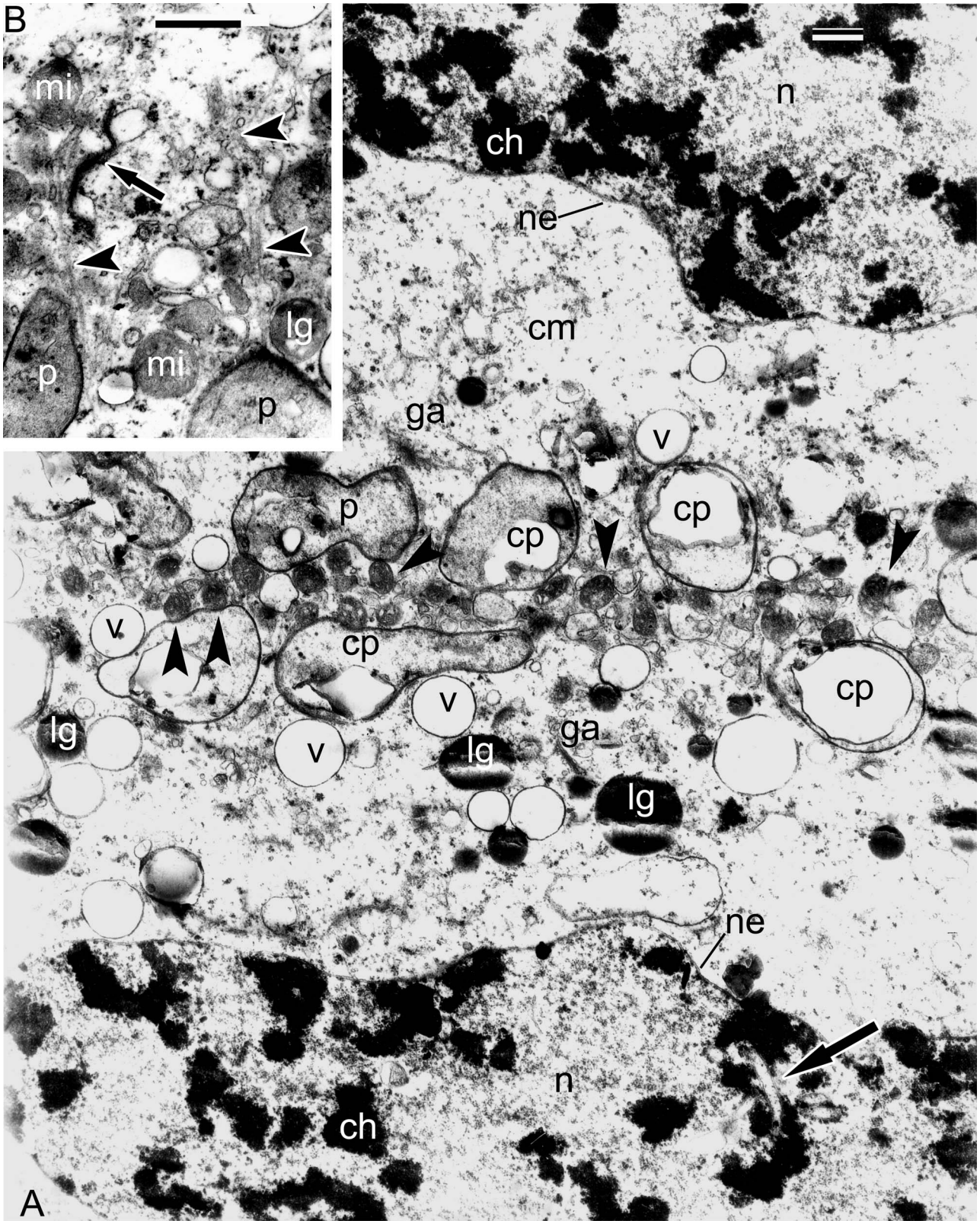
the more neutral term ‘primexine matrix’. No histochemical data exist so far on chemical composition of the surface coat (a coat of sporocyte plus a coat of tetraspore) of homosporous leptosporangiate ferns in the tetrad stage, whereas for all other groups of plants (beginning with some Bryophytes) such data exist (even if only fragmentary) that show the presence of glycoproteins comprising a glycocalyx (Gabarayeva & Hemsley, 2006). The latter plays a key role in exine development of seed plants (Rowley & Dahl, 1977; Rowley, 1990), and it is very important to clarify the role of the glycocalyx in sporoderm development of Pteridophyta. In particular, the presence of acid mucopolysaccharides (=glycoproteins) was confirmed histochemically for eusporangiate ferns (Pettitt & Jermy, 1974; Pettitt, 1979), but for leptosporangiate ferns, the presence of the glycocalyx is still in question.

The new tetraspore envelope, developing between the plasma membrane and the fibrillar layer, is very thin and sinusoidal (Figures 10, 11) and, importantly, its development is evidently connected with the primexine matrix. Lugardon (1990), describing the development of the sporoderm of *Oreopteris limbosperma* (All.) Holub., another homosporous leptosporangiate fern, has found a very similar sinusoidal thin sheet around each tetraspore – the initial exospore, but unlike *Alsophila*, the exospore layer in *Oreopteris* becomes fully developed during the tetrad period. The ‘feuilletts’ (leaf-like sheets) of the exospore were considered by Lugardon (1990) to be comparable to corresponding elements in the endexine of gymnosperms and angiosperms. Tryon and Lugardon (1991) have pointed out that in more derived ferns the exospore is reduced to a single sheet. Lugardon (1966) was very careful about naming initial layers in sporoderm development, preferring to call them by numbers in ontogeny and defining them only about maturity.

At present, we have observed only the first step of sporoderm development during the tetrad period and, following Lugardon, call it ‘layer 1’ or initial layer. As in *Alsophila*, the beginning of sporoderm development in *Equisetum fluviatile* starts very late (Lehmann et al., 1984), even later than in *Alsophila* in its free spore period. The authors concluded that

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Figure 7. The end of meiosis I in *Alsophila setosa*, late anaphase–early telophase. **A.** Sporangium, where two telophase meiocytes are visible; tapetum spreads outgrowths inside the loculus, embracing each meiocyte from the distal side (arrowheads). **B.** Overview of the meiocyte with two groups of sister chromosomes (asterisks) and organelle plate at the equatorial plane; nuclear envelopes are not completely restored; dark-contrasted globules could be plastoglobules or free lipid droplets in the cytoplasm (arrows); the tapetum partly embraces the meiocyte and includes large vacuoles; **C.** Magnified part of (B) with the organelle plate; plastoglobules (plastids are dedifferentiated, restricting membranes are not seen) or free lipid droplets (arrows), many mitochondria (arrowheads), cup-like plastids with remnants of autolysed cytoplasmic inclusions (asterisks) and small vacuoles crowd the equatorial plane of the cell. **D.** A border between the meiocyte and the tapetum; the meiocyte wall consisting of a network of fine fibrillae becomes thicker, probably as a result of swelling; a chromosome (asterisk) is still attached to a fragment of the nuclear envelope. Abbreviations: see Figure 1. Scale bars – 10 µm (A), 1 µm (B, C), 500 nm (D).



the development of the whole sporoderm is under tapetal influence. In *E. arvense* L., the appearance of the thin inner exospore (from roundish plate-like structures) starts at the late tetrad stage and from the proximal side only (Uehara & Kurita, 1989a). On the other side, in the eusporangiate fern *Ophioglossum thermale* Komarov and in the quillwort *Isoetes japonica* A. Braun and the liverwort *Lycopodium clavatum* L., all exospore development proceeds during the tetrad period (Uehara & Kurita, 1989b, 1991; Uehara et al., 1991).

We have not observed a well discernible proximal fold in the end of the tetrad period, just a long outgrowth of a thin initial spore envelope (Figure 12C, E). Blackmore and Crane (1998) mentioned that simultaneous cytokinesis, without a dyad stage, generally results in a tetrahedral tetrad, in which all four meiotic products are in direct contact and appear to be reflected in a distinct trilete aperture. Lugardon (1966, 1969), describing the formation of a proximal fold in the leptosporangiate homospore fern *Blechnum spicant*, calls the forming layers by numbers. Only the first layer is laid down during the tetrad period. It has initially a tripartite structure and later acquires a fibrillar-granular cover.

The sporoderm development was studied by *Psilotum triquetrum*. According to Lugardon (1979), the whole exospore in *Psilotum nudum* ($\equiv P. triquetrum$) develops during the tetrad period. The tetrads are covered by a special envelope (the envelope of the SMC) and an individual envelope covers every tetraspore, which is very thin from the distal side and thick from the proximal side. Both envelopes consist of slimy polysaccharides and have a fibrillar texture; both gradually disappear in the process of exospore formation. The first elements of the exospore are tripartite membranes. Later, they anastomose each other and join to a sheet. At the same time, a very thin proximal fold occurs. A continuous inner exospore layer is formed gradually from partly independent superficial sheets. Lugardon (1979) concluded that the sporoderm ontogenetic process in *Psilotum* is very similar to that of Filices, which is in accordance with recent genetic data (Qiu & Palmer, 1999), whereas it obviously differs from that of other Pteridophytes.

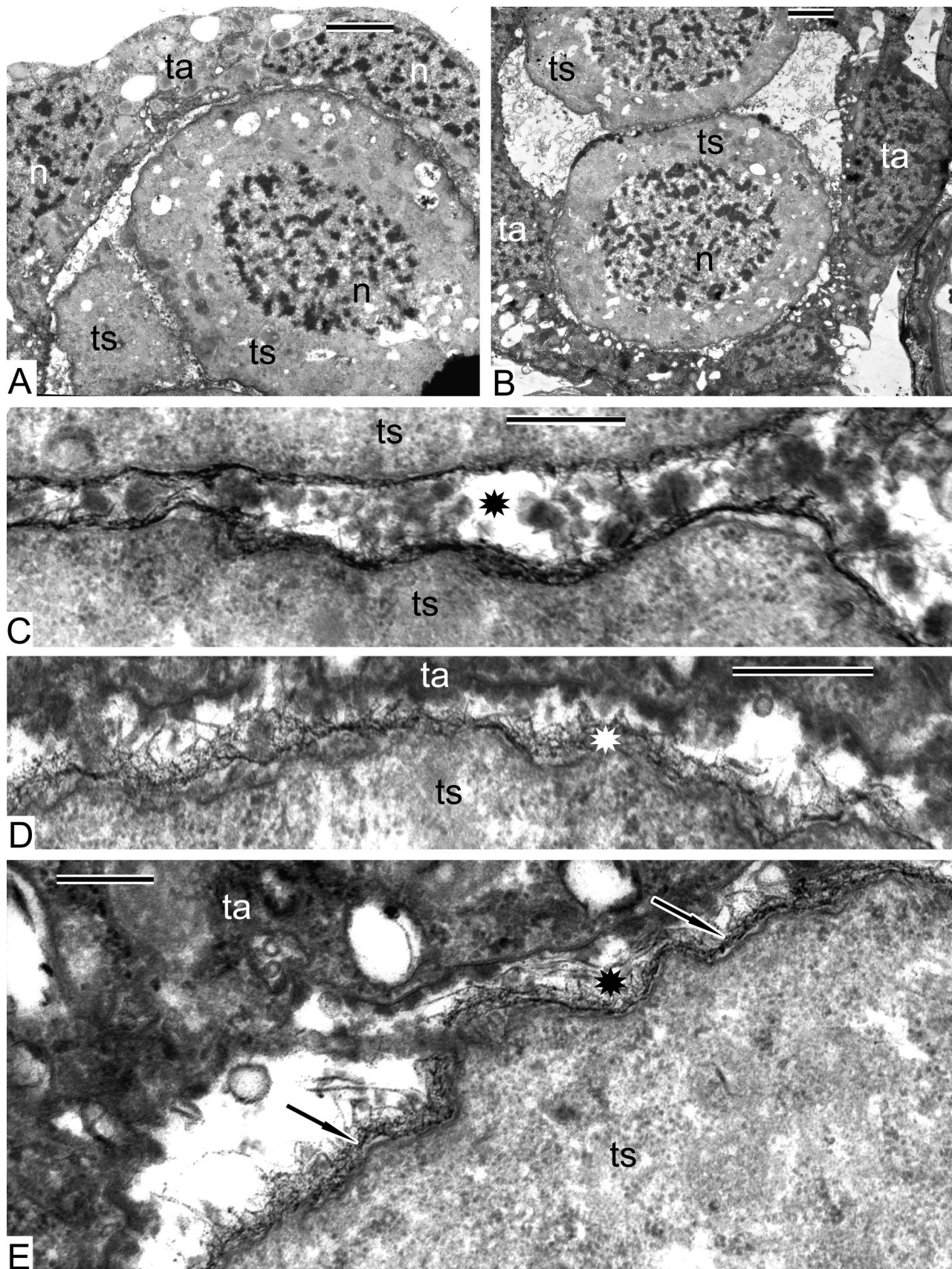
As is clear from the data obtained, the sporoderm ontogeny during the tetrad period in *Alsophila* and *Psilotum* has some common points, but also essential

differences. The main difference concerns the temporal aspect of unfolding events: whereas in *Alsophila* the end of the tetrad period is a starting point of exospore development, in *Psilotum*, the exospore is completed by this time. In its late starting point for exospore development, *Alsophila* is similar to *Equisetum*. Significant differences between the two species concern the tapetum. In *Alsophila*, we observed a layer of elongated tapetal cells enveloping first a compact complex of the four sporogenous cells (Figure 1A) and then every meiocyte (Figures 3A, 4A, 5A, 7B) and tetrad (Figures 9A, B, 10A, 11A). In *Psilotum*, the complex of sporogenous cells is highly branched and immersed into ceonocytic nutritive tissue, whereas the outer parietal layer lines the loculus of the sporangium. Later, penetration of the nutritive tissue between the fans of the branched complex of the tapetal cells in *Psilotum* leads to the isolation of several groups from the complex and, as a consequence, to the slightly asynchronous entry of meiocytes into the meiosis (Gabarayeva, 1984a). In contrast, *Alsophila* meiocytes develop simultaneously. Parkinson (1987, 1995) showed that in *Psilotum* (and also in the fern *Schizaeae*), the tapetum is of combination type: the outer layer consists of typical parietal secretory cells, which persist in their position until the end of the sporogenesis and form a sporopollenin tapetal membrane, but the inner layer turns into a periplasmodium.

It should be stressed that the type of the tapetum in *Alsophila* is difficult to determine precisely: the inner and outer plasma membranes of the tapetal cells are preserved (at least during the tetrad period), but the periclinal walls of the tapetal cells are poorly visible. The latter can be explained by high osmophilia and so-called negative contrast, when membranes of organelles appear white in TEM sections, but interiors of the organelles and the cytoplasm are intensively contrasted (Figures 2A, D, 3, 4A, 5, 6A, C, 7A, B, 10D). Considerable amounts of carotenoids cause also negative contrast as shown for sporocytes of *Lycopodium saururus* Lam. (Sersic, 1983). The negative contrast is also known as a kind of fixation artefact, resulting in disruption of the vacuole tonoplast and the exit of some phenolic substances from vacuoles (Mueller & Greenwood, 1978). This may be explained by the fact that phenolics are one of the building blocks of sporopollenin (Gubatz & Wiermann, 1992; Hemsley et al., 1996a; Niester-Nyveld et al., 1997).

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Figure 8. The end of meiosis I in *Psilotum nudum*, telophase. Nuclear envelopes are completely restored. **A.** The organelle plate is well pronounced at the equatorial plane and includes cup-like plastids, many mitochondria (*arrowheads*), vacuoles, lipid globules and dictyosomes; some nuclear invaginations are still present (*arrow*). **B.** A fragment of the organelle plate with microtubuli between organelles and attached to them (*arrowheads*) and ribbon-like double-invaginated plastid (*arrow*). Abbreviations: see Figure 1. Scale bars – 1 μ m.



Blackmore et al. (2000) have analysed studies on spore wall formation in their preliminary phylogenetic review of sporogenesis in pteridophytes and provided very useful data (Blackmore et al., 2000, appendix 1) on ontogenetic character states (total 25 characters). Ultrastructural information was available for 18 genera, representing most of the major extant plant lineages. The authors have extended the number of morphological characters available and emphasised that in sporogenesis, a limited number of ontogenetic processes generate a wide variety of sporoderm patterns because of interplay between processes in the haploid microspores and diploid tapetum. According to those character states (Blackmore et al., 2000), we summarise here selected characters of *Alsophila*, which we observed during meiosis, cytokinesis and the tetrad period and which would be useful for future phylogenetic analyses.

1. Sporangium ontogeny: leptosporangiate (1).
2. Tetrad wall: not formed (0).
3. Monoplastidic meiosis: not monoplastidic (1).
4. Mode of cytokinesis in meiosis (centripetal furrowing or centrifugal cell plate): ? (not evident)
5. Timing of cytokinesis: simultaneous, with organelle plate – no cell wall after meiosis I (0).
6. Aperture definition: by folding of plasma membrane (0).
7. Aperture type: proximal and trilete (3).
8. Exine (exospore) deposition: white-line centred lamellae (WLCL) not involved in the initial deposition, primexine (matrix) present (2).
9. Exine patterning: plasma membrane strongly undulating in sectional view, giving rise to parallel undulating first thin layer of the exospore (1).
10. Tapetal contribution to exine: exine formation definitely involves a tapetum-derived component, incorporated into tetraspore surface (1).
17. Spore output: four sporocytes give rise to 64 spores (1); (see Gastony (1974).
18. Spores: homosporous (0).
21. Tapetal thickness: tapetum of one layer of cells (0).
22. Tapetal activity: ambiguous. Some tapetal cells remain parietal through the tetrad period, but others invade the loculus (0/2). The type of tapetum differs from the combination type described by Parkinson (1987, 1995) in that

we did not observe both a true plasmodial tapetum, co-existing with a parietal one. It might be that later in *Alsophila* sporogenesis, in the post-tetrad period, plasmodial tapetum appears, but this is unclear at present.

(Information on characters 11–16, 19–20 and 23–25 is not available yet. We look forward to provide data after the study of the post-tetrad period of the sporoderm development in *Alsophila*.)

To compare characters of the sporogenesis in the species studied here with those of other ferns, we have put the data we have so far obtained on 14 characters of *Alsophila* alongside the corresponding data for *Psilotum*, based on the work of Lugardon (1973, 1979), Gabarayeva (1984a, 1984b, 1985, this study) and Parkinson (1987), and include them to the adapted data matrix of Blackmore et al. (2000, figure 1), from which we took only 14 characters and excluded lycophytes and heterosporous ferns. In result, we receive the following sequences (Table I). The comparison of the first two developmental sequences (*Alsophila* and *Psilotum*) shows that such characters as the timing of meiotic cytokinesis, aperture type and mode of its definition, and involvement of tapetal-derived material onto spore surfaces are present in both, but such important features as the presence of a well-defined tetrad wall, the involvement of WLCL and the configuration of the plasma membrane in young free microspores are not. These comparisons are inevitably preliminary; we need additional data on further stages of sporogenesis in *Alsophila* for a more complete comparison between the developmental features of these species. Interestingly, the initial spore wall layer is formed on WLCL in all pteridophytes taxa sampled by Blackmore et al. (2000); however, assuming that very thin glycocalyx is present in other pteridophytes, whereas this character only changes in taxa that either have a distinct primexine or lack a sporopollenin wall (Blackmore & Barnes, 1987). It appears that *Alsophila* with its distinct primexine matrix has moved into this exceptional group, and then the initial formation of ectexine is a new developmental process representing a non-terminal addition to sporoderm ontogeny, as was pointed out by Blackmore and Barnes (1987).

By comparing the two species under study with other ferns in the Blackmore et al. (2000) data matrix (Table I), one receives the next sequences: *Alsophila*

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Figure 9. Early tetrad stage in *Alsophila setosa*. **A**. Part of a tetrad (one of the tetraspores sectioned tangentially) surrounded by a binuclear tapetal cell. **B**. Section through two tetraspores of a tetrad; tapetal cells keep parietal position. **C**. Border between two tetraspores (proximal side); many spherical osmiophilic accumulations are seen in the slot (*asterisk*). **D, E**. Border between tetraspores and tapetum; long fibrills (*asterisks*) are visible on the tetraspore surface; initial dark contrasted sheet appears here and there (*arrows*). Abbreviations: see Figure 1. Scale bars – 2 µm (A, B), 300 nm (C–E).

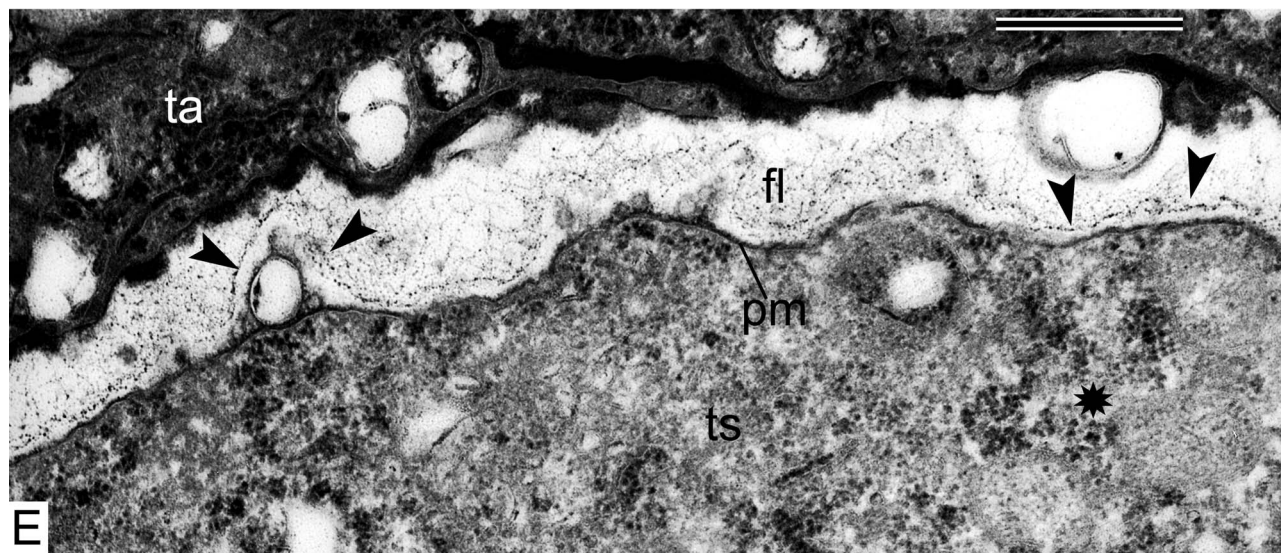
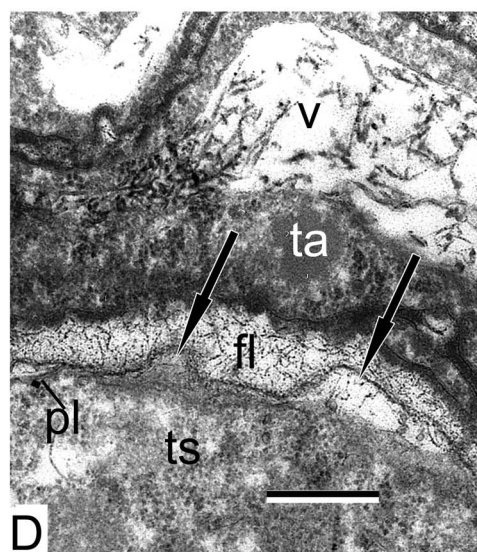
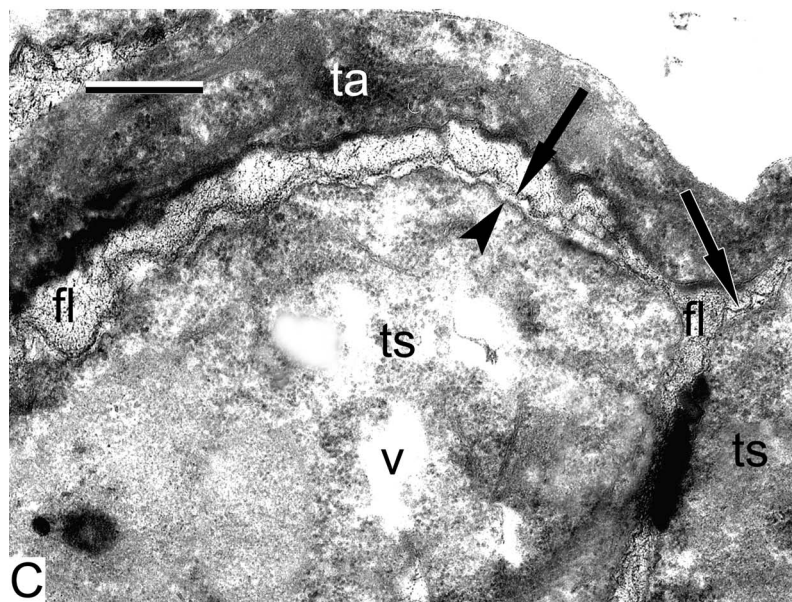
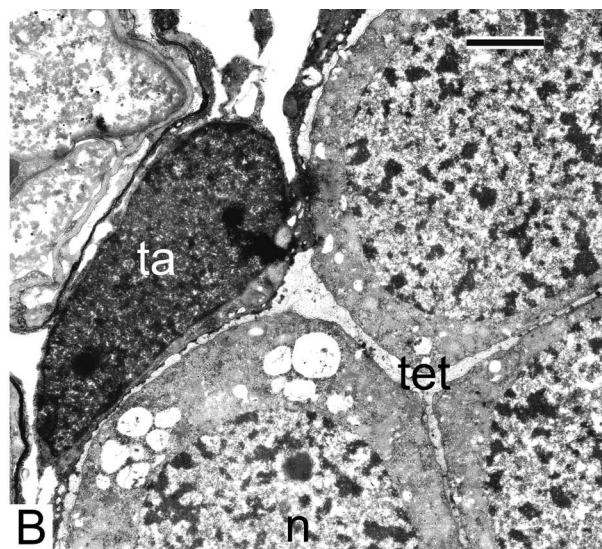
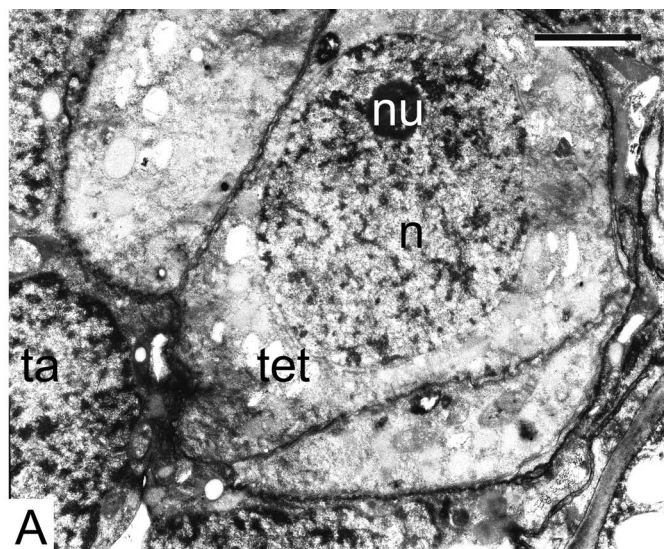


Table I. Data matrix of selected stages of sporogenesis (pre-tetrad and tetrad stage, some post-tetrad data) in *Alsophila setosa* and *Psilotum nudum*, based on character states of Blackmore et al. (2000, appendix 1), with some additions.

Character	1	2	3	4	5	6	7	8	9	10	17	18	21	22
<i>Alsophila</i>	1	0	1	?	0	0	3	2	1	1	1	0	0	0/2
<i>Psilotum</i>	0	1	1 ^a	1	0	0	3	0	0	1	0	0	1	1
<i>Anemia</i>	1	0	0	1	0	0	3	0	0	1	1	0	1	1
<i>Schizaea</i>	1	0	0	1	0	0	2	0	0	1	1	0	1	1
<i>Ophioglossum</i>	0	0	0	1	0	0	3	0	0	1	0	0	1	2
<i>Osmunda</i>	1	0	0	1	0	0	3	0	0	1	1	0	0	2
<i>Athyrium</i>	1	0	0	1	0	0	2	0	0	1	2	0	1	2
<i>Belvisia</i>	1	0	0	1	0	0	2	0	0	1	2	0	1	2
<i>Blechnum</i>	1	0	0	1	0	0	2	0	0	1	2	0	1	2
<i>Bolbitis</i>	1	0	0	1	0	0	2	0	0	1	2	0	1	2
<i>Drynaria</i>	1	0	0	1	0	0	2	0	0	1	2	0	1	2
<i>Equisetum</i>	0	0	0	1	0	1	1	0	0	1	0	0	1	2
<i>Microgramma</i>	1	0	0	1	0	0	2	0	0	1	2	0	1	2
<i>Oreopteris</i>	1	1	?	?	?	0	2	1	0	1	?	0		2
<i>Pteridium</i>	1	0	0	1	0	0	3	0	0	1	2	0	1	2

^aOur data do not confirm a monoplastidic type of meiosis in *Psilotum*.

has nine characters in common with *Osmunda*, eight with *Anemia*, seven with *Schizaea* and *Pteridium*, and six with remote genera such as *Ophioglossum* and *Athyrium* to *Microgramma* (in the latter all characters coincide; Table I), and four characters with *Equisetum*. *Psilotum* shares 11 common features with *Ophioglossum*, ten with *Anemia*, nine with *Schizaea*, *Equisetum* and *Pteridium*, and eight with the remaining genera from *Athyrium* to *Microgramma*.

A lot in these sequences appears surprising, but we should remember that this is pure numerical comparison; every character has its own 'specific ontogenetic gravity'. We also added data on *Oreopteris* (Lugardon, 1990) to the data matrix in Table I, although incomplete (data on meiosis stages are absent).

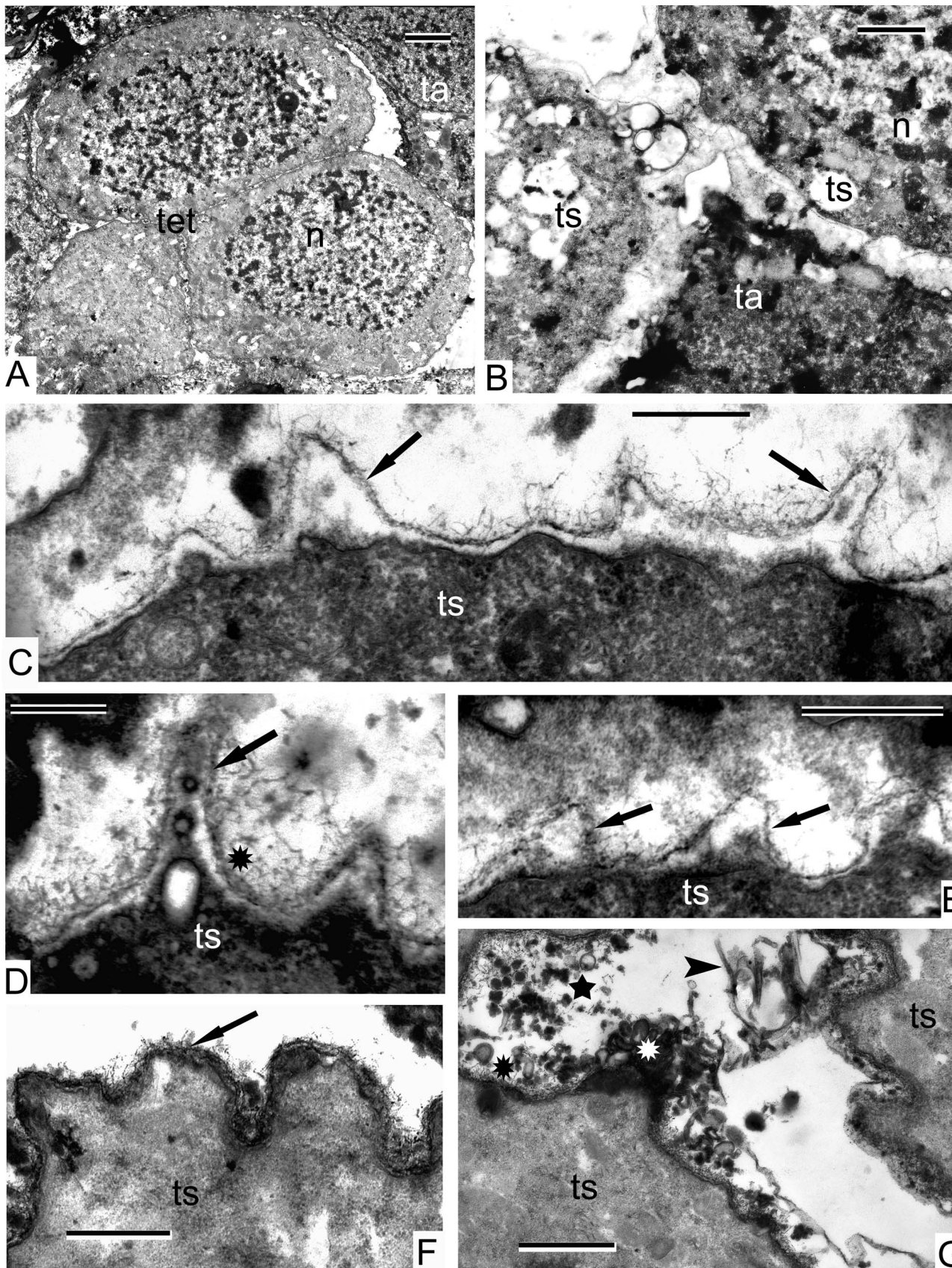
A thoughtful review on the origin, function and development of the spore wall in early land plants (Wellman, 2004) embraces many aspects, including the substructural organisation of the sporoderm and the role of molecular genetics in sporoderm development. Here, we touch on only some of these points. Our data on *Alsophila* has shown that a mode of sporoderm formation based upon a pre-patterned cell surface primexine matrix (glycocalyx?) may be not confined to the pollen of seed plants, but could also be characteristic of at least some pteridophytes. Two recent models of spore/pollen wall substructure, the model of universal tufts-units proposed by Rowley (e.g. 1990, 1995, 1996) and the

'micellar' (or self-assembly) model (Hemsley et al., 1992, 1994, 2000; Gabarayeva, 1993; Scott, 1994), discussed in the review of Wellman (2004), can now be regarded as united (Gabarayeva & Hemsley, 2006; Hemsley & Gabarayeva, 2007; Gabarayeva et al., 2009a, 2009b). This became possible after the conclusion that the tuft-units of Rowley are most probably the second mesophase in self-assembling micellar sequence. Cylindrical micelles and WLCL are so-called 'neat' micellae, bi-layers with a gap between the layers. Comparing pteridophytes with gymnosperms and angiosperms on their substructure, Rowley (1996) has found radially oriented units in some pteridophytes; Tryon and Lugardon (1991) described rodlet-like units (cylindrical micelles?) about 0.1 μm in diameter during the early perispore development. As to typical pteridophyte white-lined lamellae (=neat micelles), Rowley (1995) considered them to be similar in exospore of pteridophytes and in endexine of gymnosperms and angiosperms.

Information is gradually accumulating that the processes of self-assembly (which are initially under genetic control) interfere in some stages of (micro)sporogenesis, a hypothesis initiated by Wodehouse (1935) and continued by Heslop-Harrison (1972), van Uffelen (1991), Gabarayeva (1993) and worked out by Hemsley et al. (1992, 1994), including mimic experiments (Hemsley et al., 1996b, 1998; Griffiths & Hemsley, 2001; Moore

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Figure 10. Ongoing tetrad period in *Alsophila setosa*. **A.** Three members of a tetrad (lateral section) surrounded by tapetal cells. **B.** Tetrad (central section) and adjacent tapetal cell. **C.** Border between two tetraspores of a tetrad and an adjacent tapetal cell; the newcomer is a thin dark sheet (arrow) adjacent to the plasma membrane (arrowhead); a fibrillar layer extends around the tetraspores. **D.** Tetraspore of a tetrad and adjacent tapetal cell; the vacuole of the tapetal cell is filled with long-fibrillar contents; a sinusoidal dark contrasted layer (arrows) and a fibrillar layer are visible. **E.** Border between a tetraspore and tapetum with a sinusoidal thin dark sheet at the inner side of the fibrillar layer (arrowheads); the number of ribosomes is restored (asterisk). Abbreviations: see Figure 1. Scale bars – 2 μm (A, B), 500 nm (C–E).



et al., 2009) and regarding sporoderm development as unfolding system of micellar phases (Collinson et al., 1993; Gabarayeva & Hemsley, 2006; Hemsley & Gabarayeva, 2007). The latter idea received confirmation in ontogenetic studies of many angiosperms and some cycads (Blackmore et al., 2010; Gabarayeva & Grigorjeva, 2011; Gabarayeva et al., 2011, and references cited therein).

During the tetrad period in *Alsophila*, self-assembly processes participate in the development of high-ordered micelle-like connections between tapetal cells and sporogenous cells (Figure 1E), spherical lipid units, vesicles and tripartite membranes of tapetal origin (Figures 9C, 11G, 12B, F) and a fibrillar layer (primexine matrix) around tetraspores (Figures 9E, 10C–E, 12F). All of these are typical self-assembling structures. We were so far not able to observe cylindrical micelles in *Alsophila*: this fact seems to be meaningful (cylindrical micelles are indeed the framework for angiosperm columellae, which are not typical for pteridophytes). However, we intend to undertake the further investigations needed to trace development in the post-tetrad period.

Conclusions

Clusters of plasmalemmasomes in sporogenous cells as seen in *Alsophila* and *Psilotum*, are a typical feature for pre-meiotic cells of other pteridophytes, gymnosperms and angiosperms as well. They are probably necessary for the subsequent rapid growth and rounding-up of the sporogenous cells at the point of transition to SMCs.

Numerous plasmodesmata are characteristic for both sporogenous cells of *Alsophila* and *Psilotum*. In *Alsophila*, they are also present between sporogenous and tapetal cells suggesting that they are necessary for the transfer of some lipid substance from the tapetum. Plasmodesmatal connections in pre-meiosis are a common feature for both ferns and angiosperms.

Before the initiation of the meiosis, most plasmodesmata become occluded and cytotoxic channels appear between SMCs in both *Alsophila* and *Psilotum* to provide the flow of cytoplasm, together with its organelles, from cell to cell. The same process is usually observed at the transition from sporogenous cells to microspore mother cells

in angiosperms. The meaning of these intercellular connections is to secure coenocyte-like structure and, correspondingly, synchronisation of meiosis in all meiocyte of a sporangium/anther, which can be complete (*Alsophila*) or incomplete (*Psilotum*).

At the transition to meiosis, a general reorganisation of the cytoplasm starts in both species: meiotic cytoplasmic clarification and reorganisation of organelles occur. Many autolytic vacuoles are present and the membranes of plastids and mitochondria almost completely disappear in *Alsophila*, which is less pronounced in *Psilotum*; elimination of ribosomes, important in connection with the change of genome functioning, were also reported for Polypodiophyta in the whole. An organelle plate (band) forms in the telophase I of simultaneous cytokinesis in *Alsophila* and *Psilotum*. The main events during meiosis in both species are the same, but the details are different.

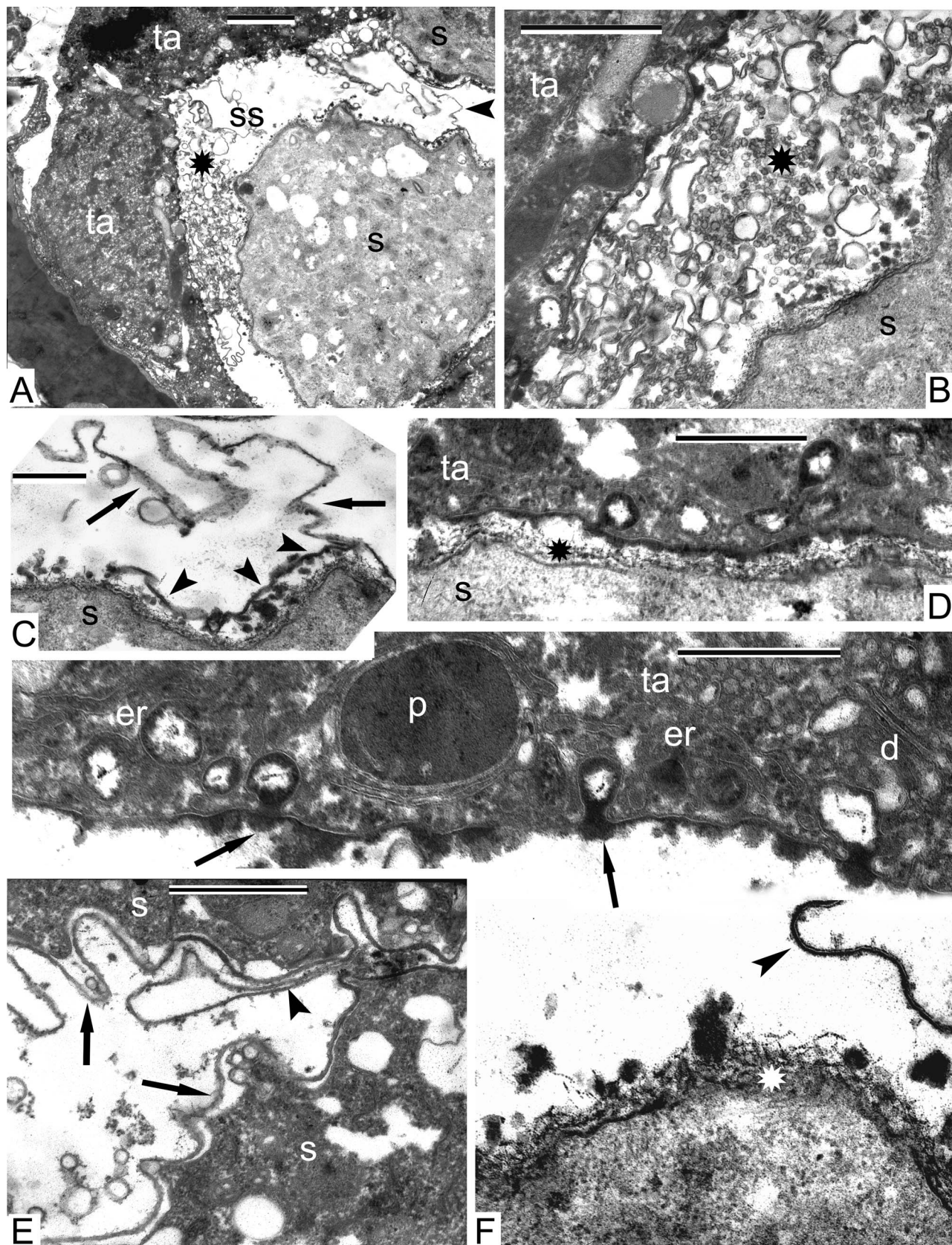
At the beginning of the tetrad period in *Alsophila*, a fibrillar layer, the primexine matrix, and a dark, thin, sinusoidal sheet associated with these fibrils, appear at the vicinity of the tetraspore plasma membrane. This initial sheet is not tripartite. Only this initial step of the sporoderm development is observed during the tetrad period in *Alsophila*, similar to *Equisetum fluviatile*, where sporoderm development starts also very late at the end of the tetrad period.

No clearly discernible proximal fold has been observed at the end of the tetrad period, just a long thin outgrowth of the initial spore envelope. This is similar to the formation of proximal fold in the leptosporangiate homosporous fern *Blechnum spicant*, where only the first layer is laid down during the tetrad period, but in *Blechnum* this layer has tripartite structure.

Sporoderm ontogeny during the tetrad period in *Alsophila* and *Psilotum* has some common points, but also essential differences. The main difference concerns the relative timing of the events: in *Alsophila* the end of the tetrad period is the starting point for the exospore development, while in *Psilotum* the exospore is completed by this point in ontogeny. Considerable differences between the two species concern the tapetum.

During the tetrad period in *Alsophila*, self-assembly processes participate in the development, judging on the presence of many spherical lipid units, vesicles and tripartite membranes of tapetal

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Figure 11. The end of the tetrad period in *Alsophila setosa*. **A.** Overview of a tetrad. **B.** Fragment of a tetrad at the disintegration point, proximal zone. **C–F.** Distal borders of tetraspores; a thin sinusoidal envelope (*arrows*), associated with the fibrillar layer (*asterisk*), is the initial sporoderm layer. **G.** Sporangia-supporting liquid between the tetraspores is full of accumulations of different forms: tripartite lamellae (*arrowhead*), spherical droplets (*star*) and vesicles (*black and white asterisks*). Abbreviations: see Figure 1. Scale bars – 2 μm (A), 1 μm (B, G), 500 nm (C, E, F), 300 nm (D).



origin in the sporangium loculus and the fibrillar primexine matrix around the tetraspores. These units correspond to spherical, laminate and filament micelles, but cylindrical micelles, typical for gymnosperms and angiosperms, were not observed.

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Figure 12. Very young free spores in *Alsophila setosa*. **A.** Neighbouring spores are separated from each other; tapetal cells maintain their parietal position; different kinds of accumulations (*asterisk*) are present in the sporangium space; an outgrowth of a spore envelope, probably the initial proximal fold (*arrowhead*). **B.** Vesicles of different size and form and spherical units (*asterisk*) in sporangia-supporting liquid between the tapetum and a young spore. **C.** Proximal side of a young spore with initial proximal fold (*arrows*) and tripartite membrane (*arrowheads*). **D.** Fibrillar envelope of a young spore in close vicinity to the tapetum. **E.** Two neighbouring young spores with wavy outlines and sinusoidal initial layer of the sporoderm (*arrows*); a loop of the proximal fold (*arrowhead*). **F.** A young spore, adjacent to tapetum; the tapetal cell has a negative contrast (white membranes and dark stroma of organelles); in the tapetum, plastids with large inclusion, wrapped in endoplasmic reticulum cisternae, dictyosomes, cisternae of endoplasmic reticulum and their large vesicles are visible, some vesicles open to the tapetal cell surface and release their content into the sporangium loculus (*arrows*); the surface of the spore is covered by a fibrillar envelope with addition of osmiophilic spherical units (*asterisk*); a fragment of tripartite membrane in sporangia-supporting liquid (*arrowhead*) is probably detached from the spore surface. Abbreviations: see Figure 1. Scale bars – 2 μm (A), 1 μm (B, E), 500 nm (C, D, F).

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