

Fine Structure of the Midgut Epithelium of *Nicoletia phytophila* Gervais, 1844 (Zygentoma: Nicoletiidae: Nicoletiinae) with Special Emphasis on its Degeneration*

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The midgut epithelium of *Nicoletia phytophila* is composed of columnar digestive cells and regenerative cells that form regenerative nests. The cytoplasm of midgut epithelial cells shows typical regionalization in organelle distribution. Two types of regenerative cells have been distinguished: cells which are able to divide intensively and cells which differentiate. Spot desmosomes have been observed between neighboring regenerative cells. The occurrence of intercellular junctions is discussed. The midgut epithelium degenerates both in an apoptotic and necrotic way. Necrosis proceeds during each molting period (cyclic manner), while apoptosis occurs between each molting, when the midgut epithelium is responsible for e.g. digestion. These processes of epithelium degeneration are described at the ultrastructural level. Our studies not only add new information about fine structure of the midgut epithelium of *N. phytophila*, but contribute to resolving the relationships within the Zygentoma. There are no doubts about the very close sister position of Nicoletiidae and Ateluridae. The midgut epithelium characters confirm their close relationship. However we do not recommend classifying the atelurid genera only within Nicoletiidae: Nicoletiinae.

Key words: Midgut epithelium, cell death, apoptosis, degeneration, ultrastructure.

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The primitively wingless insect taxon Zygentoma was originally classified in Thysanura Latreille, 1831 together with several other groups. The Zygentoma has been mostly accepted as an independent taxon within the Ectognatha (Insecta s. str.). The history of its classification was summarized by MENDES (2002a). The Archaeognatha, the second primitively wingless insect taxon, is a group closely related to Zygentoma. Their interrelationships and relationship to Entognatha and Pterygota are mostly resolved and accepted (e.g. GRIMALDI & ENGEL 2005; MACHIDA 2006; GAO *et al.* 2008). However, the interrelationships of the zygentoman subgroups are still unclear.

The most common and widespread zygentoman family, Lepismatidae, is generally accepted, as well as the family Maindroniidae with only one genus from South America. The family Lepidotrichi-

dae, containing one species from northern California, is a putative relict and its phylogenetic position has been variably assigned (e.g. GIRIBET *et al.* 2004). The classification of the two following taxa is not still clarified. MENDES (1988) accepted two independent families, Ateluridae and Nicoletiidae. He mentioned the history of their classification, concerning the rank of Nicoletiidae from a subfamily in Lepismatidae as a separate family. The family Ateluridae is the most diverse taxon of Zygentoma, with 56 genera and about 113 species (MENDES 1988, 2002a). The family Nicoletiidae was subdivided by MENDES (1988) into five subfamilies.

The phylogenetic relationships among these subfamilies were described as follows (MENDES 1994). The Protrinemurinae is a relict and the most plesiomorphic group, the closest taxon, Coletini-

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nae, are clearly isolated from the remaining taxa. The Nicoletiinae, characterized only by plesiomorphies, seems to be well individualized. The advanced Cubacubaninae and Subnicoletiinae are certainly closely related.

SMITH (1998) did not accept MENDES's (1994) proposal of the Zygentoma classification. He considered only four families, the Lepismatidae and closely related relict Maindroniidae, and relict Lepidotrichidae closely related to Nicoletiidae, occurring in all zoogeographical regions and including also former Ateluridae. As the Nicoletiidae are subdivided only into two subfamilies, Nicoletiinae and Atelurinae, the subfamilies of MENDES (1988, 1994) are treated as genera.

MENDES (2002b) as well other authors (e.g. KOCH 2003) accepted the lower rank of the Ateluridae as a subfamily in Nicoletiidae, and proposed a dendrogram of the five nicoletiid subfamilies in which Protrinemuridae was treated as an independent family. The Atelurinae are strictly related to Coletiniinae and both of them to the Nicoletiinae.

The most recent study on the comparative analysis of sperm structure in the Zygentoma (DALLAI *et al.* 2004) again treated Ateluridae and Nicoletiidae as separate families. The reconstruction of phylogenetic hypothesis suggests a close relationship between these two families, which seem to be the most advanced zygentomans. The basal family is the Lepismatidae, not the Lepidotrichidae, as the former authors accepted, if the Zygentoma is divided into two groups, Lepidotrichidae + Euzygentoma with the other families.

The subfamily Nicoletiinae, or *Nicoletia* group, is monotypic, with the species *Nicoletia phytophila* Gervais, 1844. This parthenogenetic species has recently become widely distributed almost worldwide, probably because of anthropogenic activities (SMITH 1998). *Nicoletia phytophila* was mentioned from Europe only from Italy (MOLERO-BALTANÁS *et al.* 2000), however, this old record was most probably a misidentification with *Coletinia* species. Besides the morphology, the ultrastructure of vitellogenesis in *Nicoletia phytophila* was studied (SZKLARZEWICZ 1989), with results confirming its relation to Lepismatidae.

The Zygentoma are mostly diurnal and omnivorous with the notable exception of the family Nicoletiidae which is principally subterranean and vegetarian after GRIMALDI & ENGEL (2005). Since the Nicoletiidae are vegetarian and the Ateluridae omnivorous, different structure of their midgut can be hypothesized, reflecting different kinds of food (e.g. TERRA *et al.* 1996). Otherwise the midgut characters are not influenced by envi-

ronmental constraints and therefore bear important phylogenetic information. Recently, KOCH & DOLGENER (2008) proposed an absence of midgut caeca as a new synapomorphy of nicoletiids and atelurids.

The zygentoman taxa were studied by the senior author, with co-authors respectively, concerning the comparative morphology of midgut epithelium ultrastructure (e.g. ROST 2006a; ROST *et al.* 2005; ROST-ROSKOWSKA *et al.* 2007b). Since *Atelura formicaria* Heyden, 1855, the nominotypical species of the taxon Ateluridae/Atelurinae, was also studied (ROST-ROSKOWSKA *et al.* 2010), a comparative description of the midgut epithelium of *Nicoletia phytophila*, the nominotypical species of the taxon Nicoletiidae, is warranted, and may contribute to resolving the interrelationships of the taxa Ateluridae and Nicoletiidae within the Zygentoma. Both recognized manners of midgut epithelial cell degeneration, apoptosis and necrosis, in these basal taxa of the Ectognatha are compared.

Material and Methods

Material examined

Adult specimens of *Nicoletia phytophila* Gervais, 1844 (Zygentoma: Nicoletiidae: Nicoletiinae) were collected from artificial termite nests of *Neotermes castaneus* (Burmeister, 1839) and *Neotermes cubanus* (Snyder, 1922) (Isoptera: Kalotermitidae) in Prague, Czech Republic, by Dr. Ivan Hrdý and Prof. Ivan Hrdý (October 2008). The termites originated from Cuba, Topes de Collantes. The determination of the material was confirmed by Prof. Luis Espinasa.

Methods

Light and transmission electron microscopy

About 20 adult specimens of *Nicoletia phytophila* were fixed in 2.5% glutaraldehyde (24 h, temp. 4°C), postfixed in 2% OsO₄ (2h, temp. 4°C), dehydrated in a graded series of ethanol (30, 50, 70, 90, 96 and 100%, each for 10 min) and acetone (15 min). After immersion in acetone – epon resin (1:1) overnight (room temperature), the material was embedded in Epon 812 and polymerized in 80°C. Semi- and ultra-thin sections were cut on a Leica Ultracut UCT25 ultramicrotome. Semi-thin sections were stained with 1% methylene blue in 0.5% borax and observed with an Olympus BX60 light microscope. Ultra-thin sections were stained

with uranyl acetate and lead citrate and examined with a Hitachi H500 transmission electron microscope at 75 kV.

Results

Fine structure of midgut epithelium in *N. phytophila*

The midgut of *N. phytophila* has a sack-like shape, which is devoid of midgut caeca, characteristic for insects. Its epithelium is composed of two types of cells: columnar digestive cells (e) (Fig. 1) and regenerative cells (r) which form regenerative nests (Fig. 2). Approximately 22-28 regenerative nests with several dozen regenerative cells in each occur in each transverse section through the midgut. The midgut epithelium lies on the non-cellular basal lamina (arrowhead), while it is separated from the nourishments by the peritrophic matrix (pm) (Fig. 1).

The cytoplasm of midgut epithelium cells shows regionalization in organelle distribution typical for digestive cells. The basal region is rich in mitochondria (m) (Fig. 3) and sporadic cisterns of rough endoplasmic reticulum (RER). Perinuclear cytoplasm (Fig. 4) possesses numerous Golgi complexes and cisterns of rough and smooth endoplasmic reticulum (SER), while many mitochondria and cisterns of RER occur in the apical cytoplasm. Numerous structures which resemble urospherites (Fig. 5) are accumulated in the cytoplasm of the each epithelial cell, while in some specimens also glycogen granules are observed (Fig. 4). Between adjacent epithelial cells smooth septate junctions (arrows) (in apical regions) (Fig. 5), gap junctions (gj) and pleated septate junctions (sj) (in perinuclear and basal regions) (Fig. 6) occur.

The cytoplasm of regenerative cells, in each period between moltings, possesses numerous mitochondria (m) and single cisterns of RER (Fig. 7) and SER, and Golgi complexes (d). Between neighboring regenerative cells spot desmosomes (maculae adhaerentes) (arrows) are present (Figs 8 and 8a). Regenerative cells, which occur in the central part of each regenerative nest, divide intensively (Fig. 9), while those which are located externally undergo differentiation into columnar digestive cells (Fig. 7). The more external the position of the regenerative cell in the regenerative nest, the more features of epithelial character the cell has. The first morphological sign of regenerative cell differentiation is the accumulation of mitochondria just above the nuclei in the part of the cytoplasm elongated toward the midgut lumen (Fig. 9).

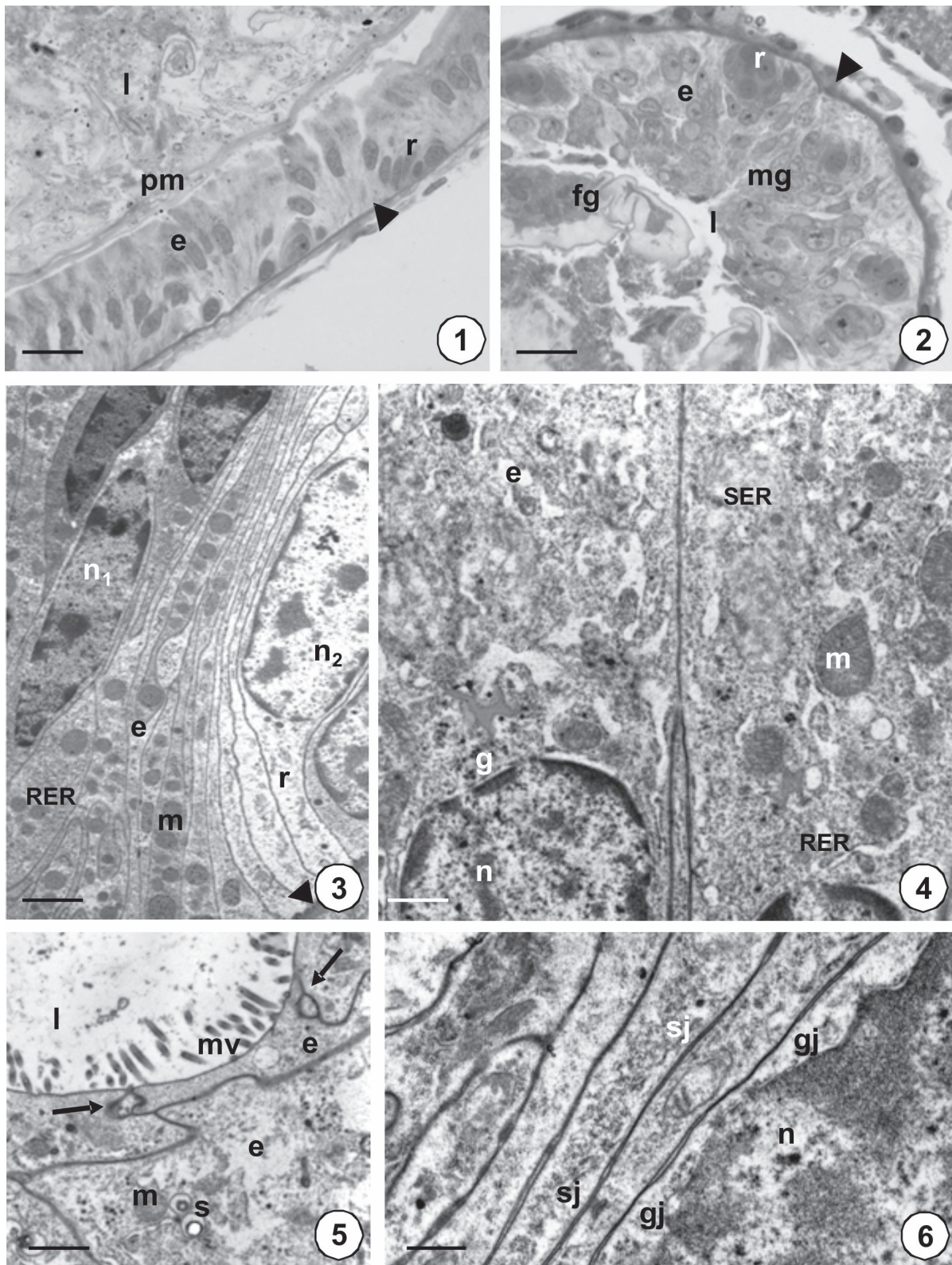
No differences in the ultrastructure of epithelial and regenerative cells were observed along the sack-shaped midgut.

Apoptosis and necrosis in the midgut epithelium of *N. phytophila*

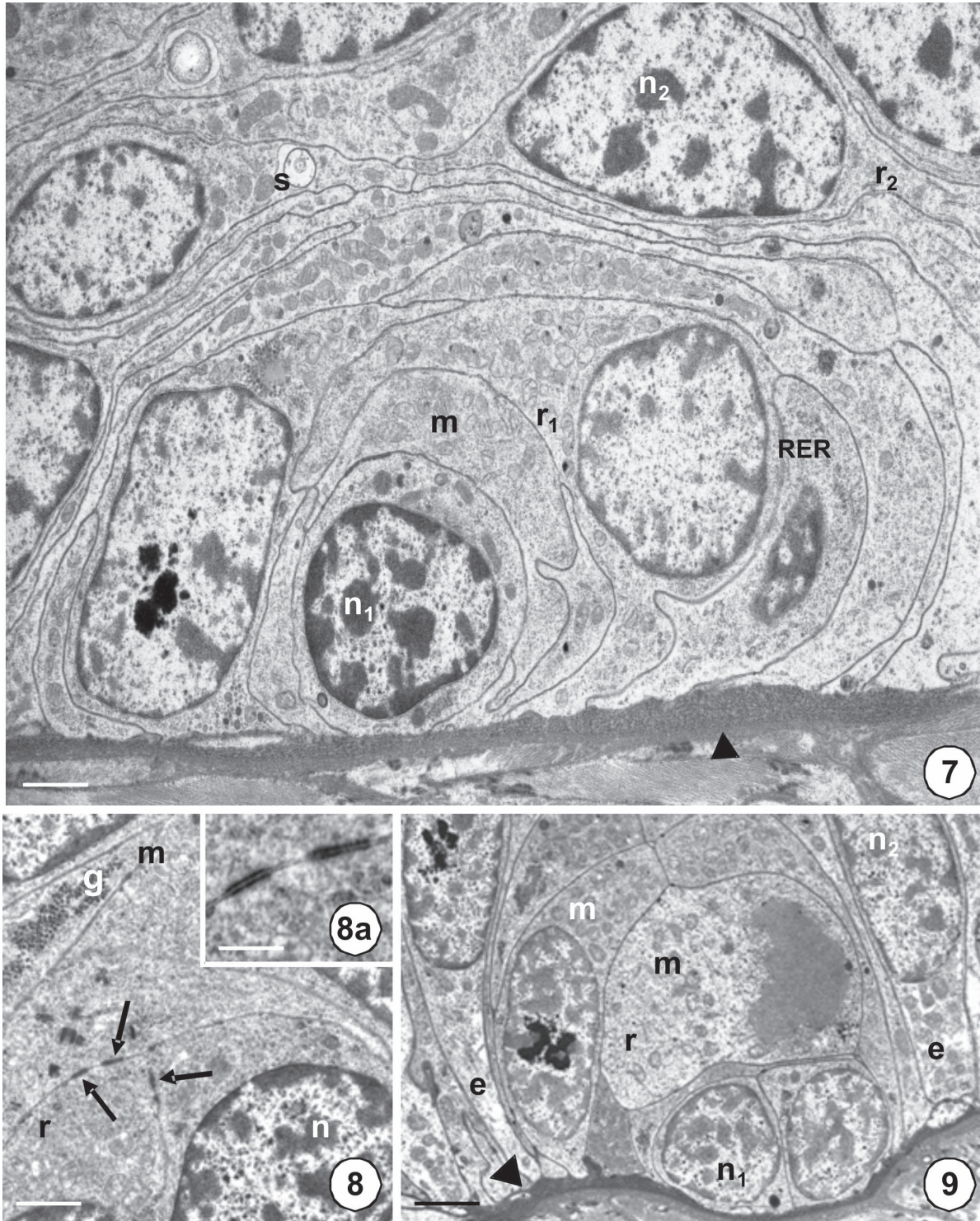
The midgut epithelium of *N. phytophila* degenerates both in an apoptotic and necrotic way. Necrosis occurs during each molting period (*N. phytophila* is a species which molts throughout adult life), thus it proceeds in a cyclic manner. Between each molting, when the midgut epithelium is responsible for digestion, secretion and absorption, the cells undergo apoptosis. Necrosis is a sporadic process during this period caused by nourishment accumulated in the midgut lumen.

The first morphological signs of the beginning of apoptosis are alterations in the electron density of the cytoplasm. The cell gradually shrinks and its cytoplasm becomes electron dense (Fig. 10). Distinct extracellular spaces (asterisks) appear between apoptotic and neighboring cells (Fig. 11). The chromatin of a lobular-shaped nucleus (n2) becomes electron dense and accumulates both near the nuclear envelope and in the central region of the nucleus (Fig. 11). Distinct autophagosomes (au) and spherites (s) appear in the cytoplasm of the apoptotic cell (Fig. 12). The apical membrane gradually loses microvilli and starts to form protrusions (arrow) into the midgut lumen (l) (Fig. 13). The simultaneously shrinking apoptotic cell is separated from the basal lamina, and eventually it is discharged into the midgut lumen just beneath the peritrophic matrix. Initially it contacts the midgut epithelium (Fig. 14) but gradually this contact is completely lost (Figs 15 and 16). Nuclear chromatin becomes electron lucent (Fig. 17) and the entire nucleus undergoes fragmentation. Subsequently the apoptotic cell membrane breaks near the large autophagosomes, the content of which is discharged into the midgut lumen and eventually the entire apoptotic cell proceeds to disintegrate (Fig. 18).

The first morphological sign of the incipience of necrosis in the midgut epithelium is a decrease of organelle number in epithelial cells cytoplasm, which gradually becomes electron lucent. Cisterns of RER and SER swell (Fig. 19). Meanwhile regenerative cells, undergoing rapid differentiation, form the new epithelium which completely separates the degenerated one from the basal lamina (Fig. 20). Initially apical membranes of the newly formed epithelium contact basal membranes of degenerated cells (Fig. 21). Eventually apical membranes of the new epithelium start to grow microvilli and distinct extracellular spaces appear



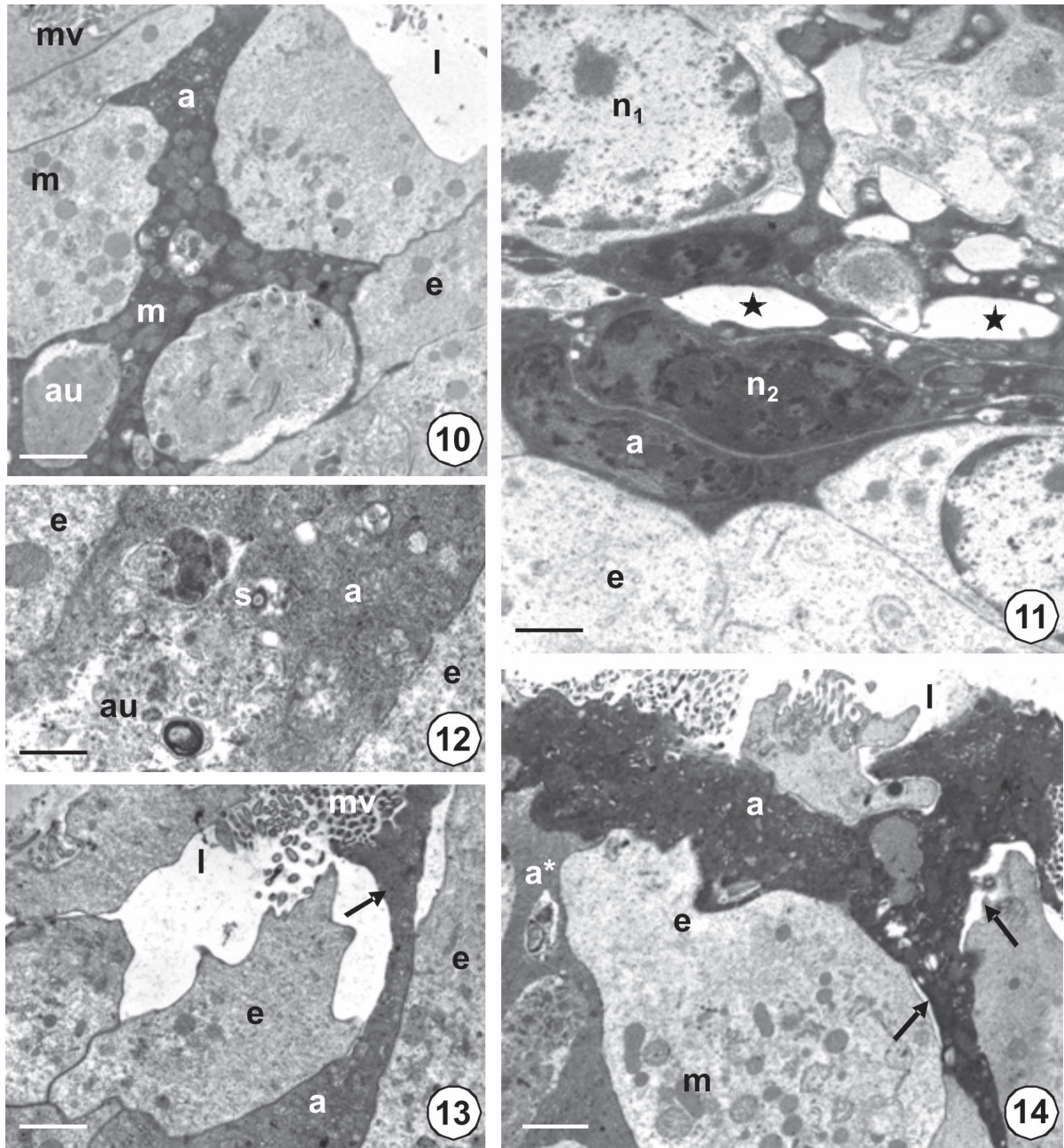
Figs 1-6. Fig. 1. Midgut epithelium of *N. phytophila* composed of epithelial (e) and regenerative (r) cells. Midgut lumen (l), peritrophic matrix (pm), basal lamina (arrowhead). Light microscope. Bar = 12.9 μm . Fig. 2. Regenerative cells (r) form regenerative nests. Foregut (fg), midgut (mg), epithelium cells (e), midgut lumen (l), basal lamina (arrowhead). Light microscope. Bar = 12.9 μm . Fig. 3. Basal regions of midgut epithelium cells (e) with numerous mitochondria (m). Cisterns of RER (RER), regenerative cells (r), nuclei of the epithelium cell (n₁) and regenerative cell (n₂). Basal lamina (arrowhead). TEM. Bar = 1.33 μm . Fig. 4. Perinuclear cytoplasm of epithelium cells (e) with cisterns of RER (RER) and SER (SER). Glycogen granules (g), single mitochondria (m), nucleus (n). TEM. Bar = 0.8 μm . Fig. 5. Smooth septate junction (arrows) between apical membranes of adjacent epithelium cells (e). Midgut lumen (l), microvilli (mv), mitochondria (m), spherites (s). TEM. Bar = 0.83 μm . Fig. 6. Gap junction (gj) and pleated septate junction (sj) between adjacent epithelial cells. Nucleus (n). TEM. Bar = 0.55 μm .



Figs 7-9. Fig. 7. Regenerative cells (r_1) with cytoplasm rich in mitochondria (m), cisterns of RER (RER). Nuclei of the regenerative cells in the central (n_1) and external regions (n_2) of the regenerative nest. External regenerative cells (r_2), spherites (s), basal lamina (arrowhead). TEM. Bar = $1.1 \mu\text{m}$. Fig. 8. Spot desmosomes (arrows) between regenerative cells (r). Glycogen (g), mitochondria (m), regenerative cell nucleus (n). TEM. Bar = $1 \mu\text{m}$. Fig. 8a. Higher magnification of spot desmosomes. TEM. Bar = $0.19 \mu\text{m}$. Fig. 9. Dividing regenerative cells (r). Epithelial cells (e), mitochondria (m), nuclei of regenerative (n_1) and epithelial (n_2) cells, basal lamina (arrowhead). TEM. Bar = $1.80 \mu\text{m}$.

between the newly formed epithelium and the degenerated one (Fig. 21). Electron dense structures appear in the apical cytoplasm of the newly formed

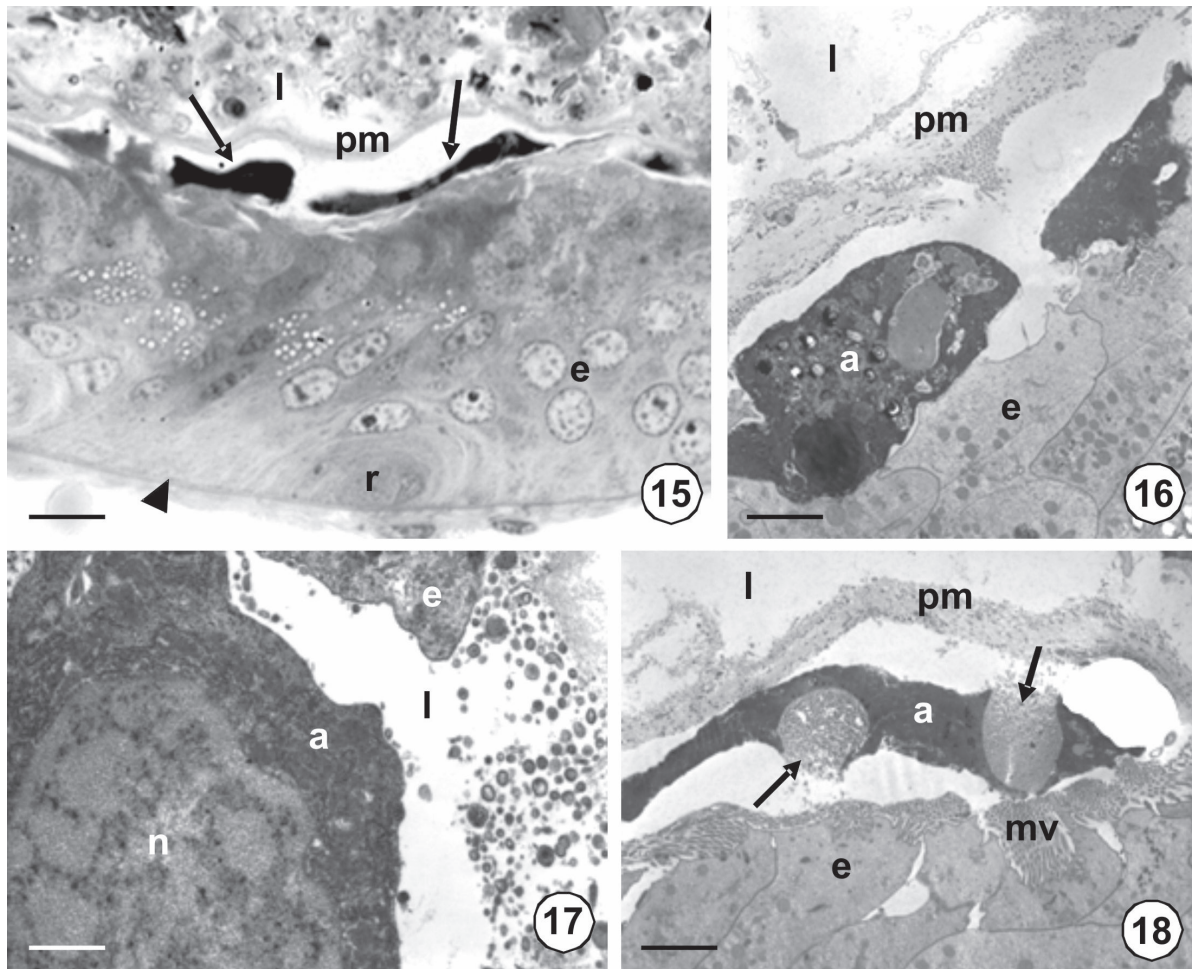
epithelium (Fig. 22). They are shifted towards the apical membrane, and their content is discharged into the midgut lumen. Then the basal membranes



Figs 10-14. Fig. 10. The cytoplasm of the apoptotic cell (a) becomes electron dense. Autophagosomes (au), mitochondria (m), midgut lumen (l), microvilli (mv), epithelial cell (e). TEM. Bar = 1.52 μm . Fig. 11. Distinct extracellular spaces (asterisks) between apoptotic (a) and neighboring epithelial cells (e). Nuclei of epithelial (n1) and apoptotic (n2) cells. TEM. Bar = 0.95 μm . Fig. 12. Autophagosomes (au) and spherites (s) in the cytoplasm of an apoptotic cell (a). Adjacent epithelium cells (e). TEM. Bar = 0.4 μm . Fig. 13. Protrusions (arrow) of the apoptotic cell (a) into the midgut lumen (l). Epithelium cells (e), microvilli (mv). TEM. Bar = 0.9 μm . Fig. 14. Apoptotic cell (a) in the midgut lumen (l) still contacts the midgut epithelium (e). Apoptotic cell at the beginning of apoptosis (a*), distinct extracellular spaces between cells (arrows), mitochondria (m). TEM. Bar = 1.39 μm .

of the degenerated epithelium break and microvilli of the new epithelium connect with the remains of the cytoplasm of degenerated cells (Fig. 22). Finally the whole degenerated epithelium is completely separated from the newly formed one and proceeds to disintegrate in the midgut lumen.

In some specimens the whole epithelium is degenerated, while the new one is not formed. Between regenerative nests only membrane remains of degenerated cells occur (Fig. 23). Probably this is a sign of approaching death of the entire organism, i.e. regenerative cells do not differentiate into



Figs 15-18. Figs. 15 and 16. Apoptotic cells (arrows and a) in the midgut lumen (l), midgut epithelium (e), peritrophic matrix (pm), basal lamina (arrowhead), regenerative nest (r). Fig. 15. Light microscope. Bar = $7.81\mu\text{m}$. Fig. 16. TEM. Bar = $2\mu\text{m}$. Fig. 17. Electron dense chromatin in apoptotic cell (a) nucleus (n). Midgut epithelium (e), midgut lumen (l). TEM. Bar = $0.73\mu\text{m}$. Fig. 18. Apoptotic cell (a) membrane breaks near autophagosomes (arrows). Midgut lumen (l), midgut epithelium (e), microvilli (mv), peritrophic matrix (pm). TEM. Bar = $2.27\mu\text{m}$.

epithelial cells and degeneration might affect all body tissues and organs.

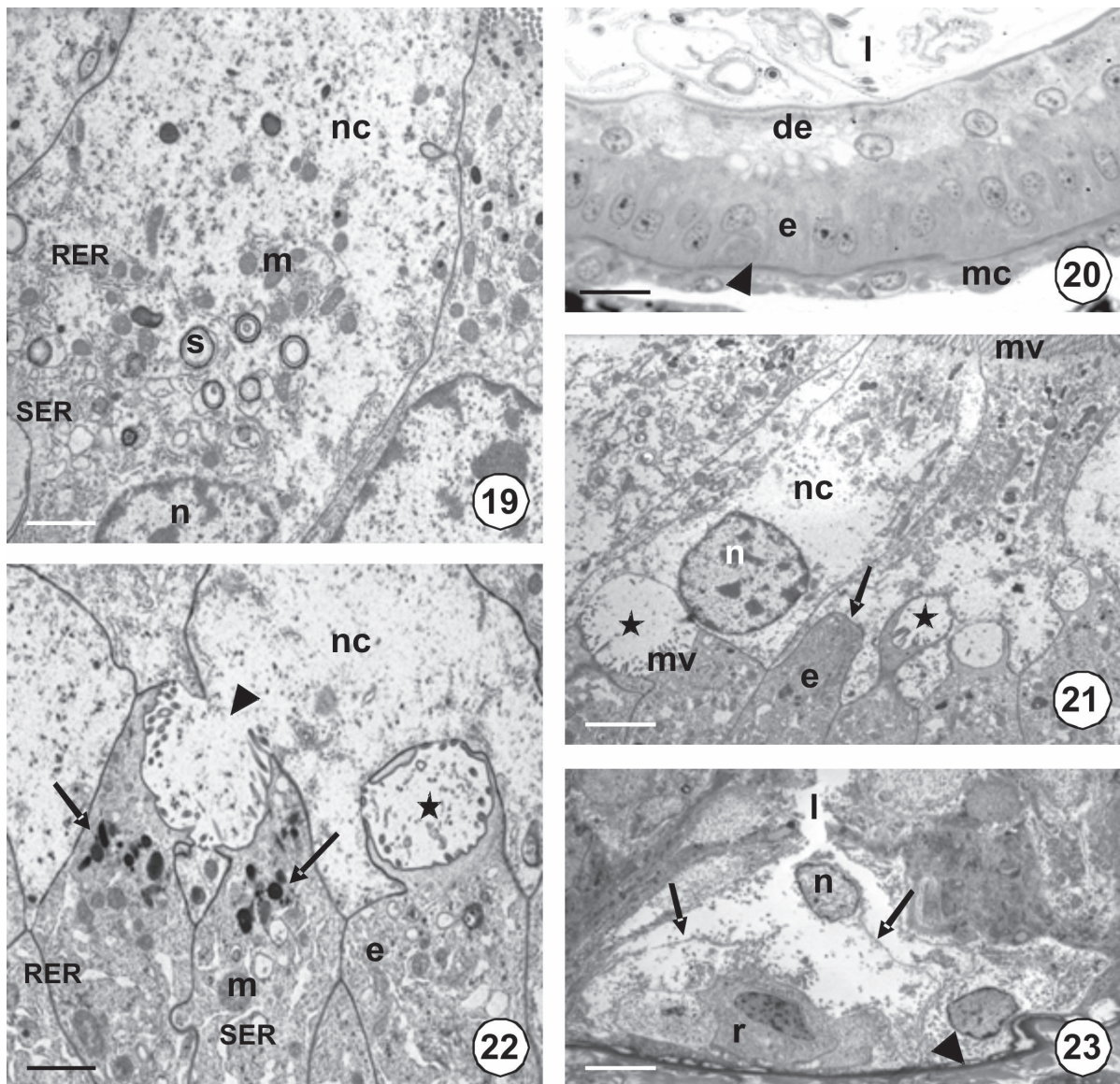
Discussion

The genera *Atelura* and *Nicoletia* represent two taxa at the family level, Ateluridae and Nicoletiidae, which are commonly accepted as closely related. However, they have been treated in different ways, for example as two separate families (MENDES 1988, 2002a; DALLAI *et al.* 2004) or Atelurinae as the subfamily of Nicoletiidae (SMITH 1998; MENDES 2002b). These two taxa are easily distinguishable morphologically (MENDES 1988, 2002a). The most conspicuous feature, the presence (Ateluridae) or absence (Nicoletiidae) of scales on the body, is used as a good diagnostic character at the family level. They also differ in

number of ovarioles (MENDES 1988), slightly in ultrastructure of sperm (DALLAI *et al.* 2004) and other characters.

Detailed data on the midgut epithelium ultrastructure of *Atelura formicaria* (ROST-ROSKOWSKA *et al.* 2010) is available, therefore it is possible to compare this structure with *Nicoletia phytophila*, and evaluate their phylogenetic relationships. The results from an ancestral reconstruction of the Hexapoda based on their midgut ultrastructure (unpublished), in which we studied and analyzed sixteen characters. The following conclusions are clear from the comparison of the midgut ultrastructure of the *Nicoletia* with *Atelura*.

Nicoletia and *Atelura* share with the lepismatid genera *Lepisma* and *Thermobia* (these genera share the same characters, thus are subsequently referred to as lepismatids) the following ancestral



Figs 19-23. Fig. 19. Electron lucent cytoplasm of necrotic cell (nc) in the midgut epithelium of *N. phytophila*. Cisterns of RER (RER) and SER (SER), mitochondria (m), nucleus (n), spherites (s). TEM. Bar = 1.9 μm . Fig. 20. The new epithelium (e) completely separates the degenerated one (de) from the basal lamina (arrowhead). Midgut lumen (l), visceral muscles (mc). Light microscope. Bar = 10.42 μm . Fig. 21. Apical membranes of the new epithelium (e) adhering to the basal membranes (arrow) of necrotic cells (nc) start to form microvilli (mv) and distinct extracellular spaces (asterisks) appear. Nucleus (n). TEM. Bar = 3.1 μm . Fig. 22. Appearance of electron dense structures (arrows) in the apical cytoplasm of the newly formed epithelial cells (e). Microvilli of the new epithelium connect with the remains of the cytoplasm of degenerated cells (arrowhead). Cisterns of RER (RER) and SER (SER), necrotic cell (nc), mitochondria (m). TEM. Bar = 1.39 μm . Fig. 23. Degeneration of the midgut epithelium. Membrane remains (arrows) of degenerated cells occur between regenerative nests (r). Basal lamina (arrowhead), midgut lumen (l), nucleus (n). TEM. Bar = 3.97 μm .

characters. The organelles in cells are distributed in a pattern of distinct regionalization; absence of endocrine cells, copper cells and goblet cells in midgut epithelium; midgut parts do not differ in ultrastructure from each other.

Nicoletia, *Atelura* and both lepisematids share two advanced characters: the digestive cells have a columnar shape and the regenerative cells are organized in special groups (regenerative nests) in a specific pattern.

The general shape of midgut (uniform x complex, with caeca developed) has been evaluated as ancestral in *Nicoletia* and *Atelura*, but is judged as advanced in lepisematids. KOCH and DOLGENER (2008) analyzed the absence of caeca as an apomorphy of Ateluridae and Nicoletiidae. However, the ancestor of the Hexapoda had a simple, sack-shaped midgut, the most parsimonious possible condition of this organ. In contrast, the following features are considered advanced in *Nicoletia* and

Atelura but ancestral in lepismatids: occurrence of apoptosis (not observed in lepismatids); existence of intercellular junctions between regenerative cells (not observed in lepismatids); high number of regenerative cells in nest, as well as nests in the epithelium (gradually lower in *Lepisma* and *Thermobia*).

Characters in *Zygentoma* with unclear evolutionary polarization are distributed as follows. In all *Zygentoma* two differentiated types of cells occur: the digestive and the regenerative cells. The ancestor of Hexapoda had either already developed more types of cells or possessed only one type of cell. Structures which resemble urospherites occur in *Nicoletia* and *Atelura*, while in lepismatids structures with excretion function in digestive cell are absent. The situation in lepismatids is probably ancestral for Ectognatha excluding Archaeognatha, based on maximum parsimony. However, it could as well be an advanced state.

The genera *Nicoletia* and *Atelura* differ each from one another in only one character studied, i.e. the manner of regeneration of midgut epithelium cells. The distribution of this character is mosaically distributed in *Zygentoma*. The putatively ancient continuous manner occurs in *Atelura* and *Thermobia*, the advanced cyclic manner in *Nicoletia* and *Lepisma*. Formerly, it was supposed that the manner of regeneration is connected with ametabolous or metabolous patterns of ontogeny. However, continuous regeneration was discovered also in paurometabolous insects whereas cyclic regeneration was described in several ametabolous insects (ROST-ROSZKOWSKA 2008a, 2008b).

Nicoletia and *Atelura* have been phylogenetically characterized as a group by unique feature, i.e. the proved occurrence of intercellular junctions between the regenerative cells, which represents the only known case in the Hexapoda.

The condition concerning intercellular junctions is peculiar to the Arthropoda. These junctions provide generally two main functions (LANE *et al.* 1996). Only one type of junction is known to cover intercellular communication – the gap junction localized approximately in central part of the cell wall. Two subtypes of this junction are associated with different septate junctions.

Smooth septate junctions, commonly existing in the Insecta, represent probably a variation of pleated septate junctions. Their synonyms are zonula continua and continuous junction. They occur only in endodermal-derived tissues. The general pattern may be altered in certain groups of insects. Clearly the septate junctions are involved in adherence between adjacent cells, they may also form a partial permeability barrier (LANE *et al.* 1996).

Smooth septate junctions have been described as the common intercellular junction between apical regions of the adjacent midgut epithelium cells in insects (LANE & SKAER 1980; LANE & SWALES 1982; LANE *et al.* 1996; ROST 2006a, 2006b).

Pleated septate junctions are supposed to exist only between cells in tissues of ectodermal origin (LANE & SWALES 1982; LANE *et al.* 1996). The midgut originated from endoderm, however, there is some evidence of pleated septate junctions in the insect midgut, e.g. only in the basal region in Coleoptera: Chrysomellidae (ROST-ROSZKOWSKA *et al.* 2007a); in perinuclear and basal regions in Collembola: *Allacma* (ROST-ROSZKOWSKA & UNDRUL 2008) and *Zygentoma*: *Atelura* (ROST-ROSZKOWSKA *et al.* 2010).

The two types of septate junctions mentioned above differ only by density of intercellular material, higher in smooth, lower in pleated junctions. BALDWIN and HAKIM (1987) studied ontogenetic development of junctions in the sphingid *Manduca sexta*. Septate junctions are treated as two different junctions, however, relatively little is known of their relationship. Crystalline gap junctions are found in association with smooth septate junctions whereas irregular gap junctions with pleated septate junctions. During ontogenesis the following changes occur: pleated septate junction + irregular gap junction → only gap junction → smooth septate junction replaces pleated septate junction → gap junction becomes crystalline in form → smooth septate junction + crystalline gap junction.

The current knowledge on the distribution of intercellular junctions within midgut epithelium in Hexapoda is as follows (LANE *et al.* 1996). Generally, smooth septate junctions form a belt apically in the cell, which is the most prominent structure. Gap junctions are usually developed beneath the belt. In the deep part of the cell fasciae or puncta adhaerentes are located, resembling the vertebrate desmosomes, and are extremely common in insect midgut. The basal plasma membrane can be modified in hemi-adhesing junctions.

Gap junctions are common in Hexapoda, however, we have only sporadic information about their detailed distribution. They never occur in the apical region (AR), but only in the perinuclear region (PR) and the basal region (BR). PR only: Collembola: *Xenylla* (e.g. KLAG *et al.* 1981). BR only: Diplura (e.g., XUE *et al.* 1990); Coleoptera: Chrysomellidae (ROST-ROSZKOWSKA *et al.* 2007a). PR + BR: Protura (e.g. DALLAI *et al.* 1987); Collembola (ROST 2006b; ROST-ROSZKOWSKA 2008a); Archaeognatha (our studies, not published); *Atelura* (ROST-ROSZKOWSKA *et al.* 2010); *Zygentoma*: *Nicoletia*.

The zygentomans *Atelura* (Ateluridae) (ROST-ROSZKOWSKA *et al.* 2010) and *Nicoletia* (Nicoletiidae) are the first hexapods in which intercellular junctions were recognized between the regenerative cells in regenerative nests. The junctions were found in all nests, thus they represent an unambiguous character, clearly advanced within all Hexapoda, and cannot be considered artifacts.

Pleated septate junctions were recognized in the regenerative nests of *Atelura*. They probably enable the maintenance of a large amount (several dozens) of regenerative cells in the nest. Spot desmosomes (maculae adhaerentes) were found between regenerative cells in the regenerative nests of *Nicoletia* in which also several dozens of regenerative cells were described.

The existence of intercellular junctions between regenerative cells in *Atelura* and *Nicoletia* characterized the clade in sister relation to all the other Ectognatha (ROST-ROSZKOWSKA *et al.* 2010). However, we could suppose that these junctions are more common in hexapods, but still remain unrecognized.

The general shape of the midgut should commonly depend on the kind of food (BILLINGSLEY 1990; BILLINGSLEY & LEHANE 1996; TERRA *et al.* 1996). However, the lepismatids and *Atelura* feed similarly but their midgut shapes strongly differ. In contrast, *Atelura* and *Nicoletia* use different food resources (omnivorous x vegetarian) and their midguts have a simple, probably ancestral shape (it could be advanced, developing by reduction). The explanation can be either that also Nicoletiidae use a wider range of food type or that the shape of the midgut is a more phylogenetically stable feature than was supposed. The midgut characters divided the studied representatives of *Zygentoma* into two clades in the phylogenetic analysis: Ateluridae + Nicoletiidae (= AN) and Lepismatidae (= L) (our studies, unpublished). The clade AN is defined by the existence of intercellular junctions between regenerative cells. The clade L shares with all other Ectognatha a more complex midgut shape, and with the Pterygota, of which they are the sister group, the absence of urospherites or similar structures. The clade L is defined by the absence of apoptosis (apoptosis not observed), presumably an ancestral condition, however, already the hexapodan ancestor could have had a predisposition to apoptosis, then the situation in clade L can be a reduction. In both zygentoman clades the genera differ from each other by the manner of cell regeneration. We are not able to explain such a mosaic pattern of this character distribution.

Even if some small differences between *Atelura* = Ateluridae and *Nicoletia* = Nicoletiidae are recognized, besides morphological characters also

different food, number of ovarioles (MENDES 1988) – plesiomorphic in Nicoletiidae = 5, apomorphic in Ateluridae = 3, and manner of midgut cell regeneration, these taxa are very similar in most features. There are no doubts about their sister position. The midgut epithelium characters confirm their close relationship. If we accept the Ateluridae either as a family or subfamily of Nicoletiidae, it is in each case an independent taxon. We do not recommend classifying the atelurid genera only within Nicoletiidae: Nicoletiinae.

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