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ORIGINAL ARTICLE

Apoptosis and necrosis during the circadian cycle in the centipede midgut

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Abstract Three types of cells have been distinguished in the midgut epithelium of two centipedes, *Lithobius forficatus* and *Scolopendra cingulata*: digestive, secretory, and regenerative cells. According to the results of our previous studies, we decided to analyze the relationship between apoptosis and necrosis in their midgut epithelium and circadian rhythms. Ultrastructural analysis showed that these processes proceed in a continuous manner that is independent of the circadian rhythm in *L. forficatus*, while in *S. cingulata* necrosis is activated at midnight. Additionally, the description of apoptosis and necrosis showed no differences between males and females of both species analyzed. At the beginning of apoptosis, the cell cytoplasm becomes electron-dense, apparently in response to shrinkage of the cell. Organelles such as the mitochondria, cisterns of endoplasmic reticulum transform and degenerate. Nuclei gradually assume lobular shapes before the apoptotic cell is discharged into the midgut lumen. During necrosis, however, the cytoplasm of the cell becomes electron-lucent, and the number of organelles decreases. While the digestive cells of about 10 % of *L. forficatus* contain rickettsia-like pathogens, the corresponding cells in *S. cingulata* are free of rickettsia. As a result, we can state that apoptosis in *L. forficatus* is presumably responsible for protecting the organism against infections, while in *S. cingulata* apoptosis is not associated with the elimination of pathogens. Necrosis is attributed to mechanical damage, and the activation of this process coincides with proliferation of the midgut regenerative cells at midnight in *S. cingulata*.

Keywords Centipede · Midgut epithelium · Digestive cells · Ultrastructure · Cell death

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Introduction

Multicellular organisms have internal biological clocks that regulate their behavioral and physiological functions, thus establishing homeostasis between both the internal and external environments. It is composed of many periodic rhythms, e.g., seasonal, monthly, and/or circadian rhythms. The physiological reaction to the length of the day and night plays a role in regulating cell proliferation and tissue regeneration, hormone synthesis and secretion, reproduction (Lipovšek et al. 2002; Dunlap et al. 2003; Lee and Sankar 2011a, 2011b; Tomioka et al. 2012; Park et al. 2013). Myriapods, including centipedes (Chilopoda), are invertebrates whose life and behavior are synchronized with the day/night cycle (Minelli 1993). They are primarily nocturnal animals that hunt and feed during the night, while during the day they avoid the light by resting

under stones, in leaf litter, in crevices, or by burrowing into the soil. Therefore, many processes that occur in their body happen according to the day/night cycle (Lewis 1981; Minelli 1993). Circadian rhythms in centipedes affect, for example, the ecological niches that they occupy or the homeostasis of the animals (Mead 1970; Amouriq 1967; Lewis 1981; Tuf et al. 2006). During our previous studies, we described the proliferation of regenerative cells, which is dependent on (*S. cingulata*—Chajec et al. 2014) or is independent of the day/night cycle (*L. forficatus*—Chajec et al. 2012) as well as the process of autophagy, which proceeded in a continuous manner and did not depend on the day/night cycle (both *S. cingulata* and *L. forficatus*) (Rost-Roszkowska et al. 2015a). While analyzing the structure and ultrastructure of the endodermal region of the digestive system of the abovementioned species of centipedes (Chajec et al. 2012, 2014), we also observed signs of apoptosis and necrosis. Thus, as the next step of our studies, an analysis of the occurrence of apoptosis and necrosis according to the day/night cycle was performed.

Apoptosis has been recognized as the type of cell death that activates the genetically determined elimination of cells. There are many factors and conditions that can trigger apoptosis (e.g., irradiation, heavy metals, toxic substances, pathogens) (Norbury and Hickson 2001; Elmore 2007; Maghsoudi et al. 2012; Teixeira et al. 2013; Wilczek et al. 2014). Despite the fact that apoptosis is a type of cell death, no inflammatory reaction occurs. Inflammation is activated when apoptotic cells do not release their organelles and content into the extracellular space that surrounds neighboring tissues and where they are phagocytosed by the surrounding cells, e.g., macrophages and/or parenchymal cells (Kurosaka et al. 2003; Elmore 2007). In the midgut epithelium of invertebrates, cells that have phagocytic abilities do not appear and therefore, another mechanism is created—apoptotic cells or apoptotic bodies are discharged into the gut lumen where they are digested (Rost-Roszkowska et al. 2008, 2010, 2015b). To date, the dependence between apoptosis and circadian rhythms has been described in vertebrates (Ijiri and Potten 1988, 1990; Granda et al. 2005; Lee and Sankar 2011a, 2011b), while no information can be found in the literature about these processes in the gut epithelium of invertebrates. Thus, the aims of this study were (1) to answer the question of whether apoptosis and/or necrosis in two centipede species, *S. cingulata* and *L. forficatus*, depend on the circadian cycle; (2) to determine whether apoptosis and necrosis concern all types of midgut epithelial cells, i.e., digestive, secretory, and regenerative cells; (3) to describe apoptosis and necrosis in the midgut epithelium at the ultrastructural level in both males and females; and (4) to describe the role of these processes in the proper functioning of the midgut.

Material and methods

Material

L. forficatus (Chilopoda, Lithobiomorpha) is a widespread European species that occurs primarily in central and northern Europe. It lives in the upper layers of soil, under stones, in litter, under branches and trunks that are lying on the ground, in decaying wood. *S. cingulata* (Chilopoda, Scolopendromorpha) is widely distributed around the Mediterranean Sea in southern Europe and North Africa. It can be found under stones and trunks and in leaf litter. Both photophobic centipedes prefer a dark and damp environment.

Adult specimens of *L. forficatus* (males and females) were collected in southern Poland and in central and southern Bohemia (Czech Republic). Adult specimens of *S. cingulata* (males and females) were bought at boutiques that sell pets and were reared in plastic boxes (20×15×6 cm) at a temperature of 22 °C, fed ad libitum with the mealworm *Tenebrio molitor*, the house cricket *Acheta domesticus*, and adult specimens of the terrestrial isopod *Porcellio scaber*.

In order to determine whether apoptosis and necrosis are activated during any period of the day/night cycle, we analyzed midguts that were fixed every 6 h: at ~06:00, ~12:00, ~18:00, and ~00:00 of Central European Summer Time.

Methods

Light and transmission electron microscopy Adult specimens of *L. forficatus* (10 specimens at ~06:00, 20 specimens at ~12:00, 10 specimens at ~18:00, and 20 specimens at 00:00) and those of *S. cingulata* (5 specimens at ~06:00, 15 specimens at ~12:00, 5 specimens at ~18:00, and 15 specimens at 00:00) were decapitated and then fixed with 2.5 % glutaraldehyde in a 0.1 M sodium phosphate buffer (pH 7.4, 4 °C, 2 h). After postfixation in 2 % osmium tetroxide in a 0.1 M phosphate buffer (4 °C, 1.5 h), the material was dehydrated in a graded concentration series of ethanol (50, 70, 90, 95, and 100 %, each for 15 min), acetone (15 min), and embedded in epoxy resin (Epoxy Embedding Medium Kit; Sigma). Semi- (0.8-μm thick) and ultrathin (70 nm) sections were prepared using a Leica Ultracut UCT25 ultramicrotome. Semi-thin sections were stained with 1 % methylene blue in 0.5 % borax and observed using an Olympus BX60 light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate before being examined using a Hitachi H500 transmission electron microscope at 75 kV. Semi- and ultrathin sections were used in order to count the number of cells that had signs of apoptosis and necrosis in relation to the total number of midgut epithelial cells. The percentage of apoptotic and necrotic cells was determined by randomly counting 100 cells in all specimens analyzed at ~06:00, ~12:00, ~18:00, and 00:00.

TUNEL assay Pieces of the bodies of adult specimens of *L. forficatus* (10 specimens at ~12:00, 10 specimens at ~00:00) and of *S. cingulata* (5 specimens at ~12:00, 5 specimens at ~00:00) were embedded in a tissue-freezing medium (Jung) without fixation. Cryostat sections were cut (5-μm thick) and placed on 1 % gelatin-coated slides. After incubation in a permeabilization solution (0.1 % sodium citrate) (2 min on ice in 4 °C), the slides were washed in Tris-buffered saline (TBS) (3×5 min) and stained with a TUNEL reaction mixture (In Situ Cell Death Detection Kit, TMR red, Roche) (60 min at 37 °C in the dark). After the material was washed in TBS, it was labeled with DAPI (30 min in darkness). Slides were analyzed using an Olympus BX60 fluorescence microscope. Negative controls without terminal

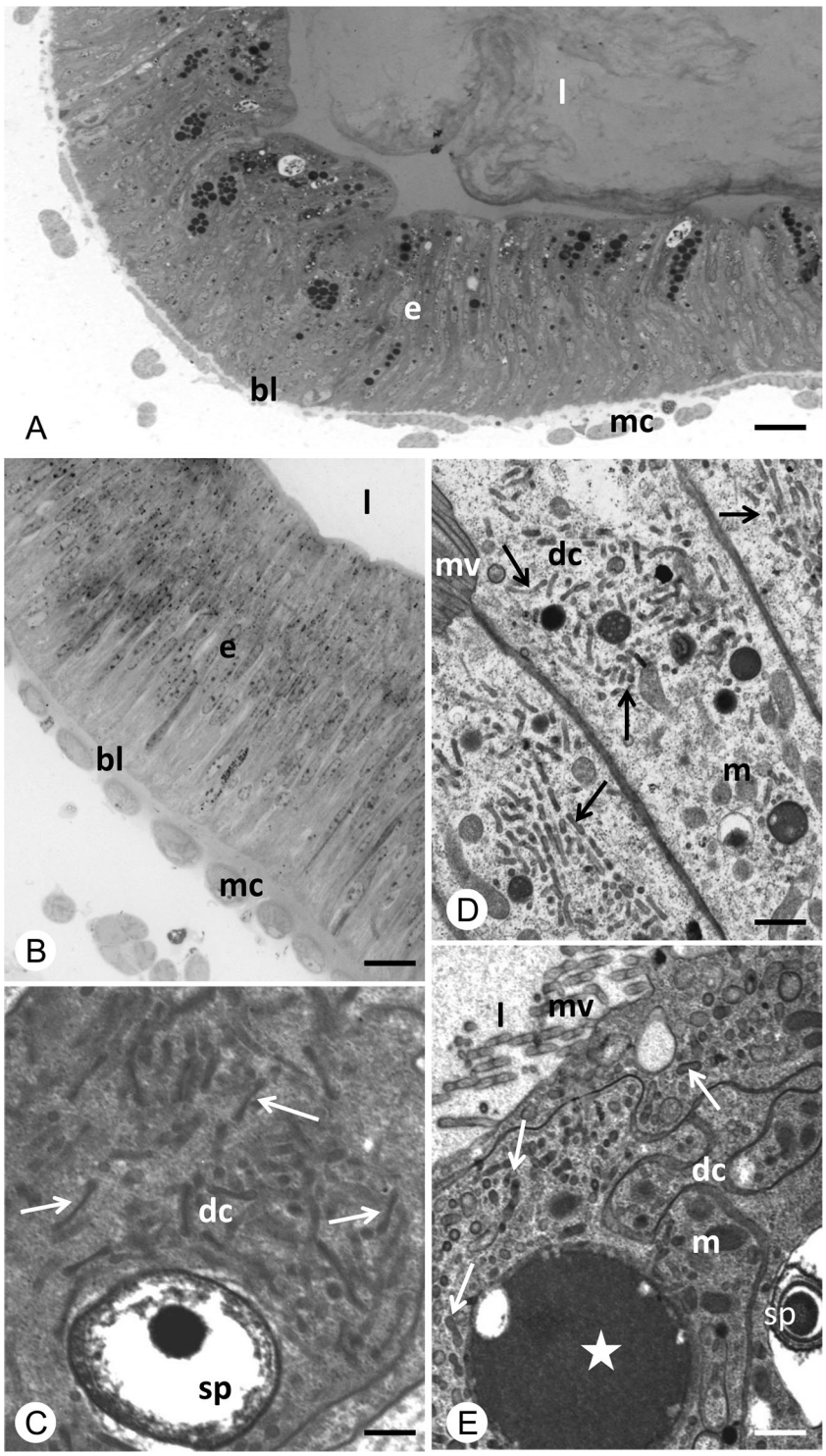

Fig. 1 **a** *L. forficatus*. **b** *S. cingulata*. Midgut pseudostratified epithelium (*e*). Midgut lumen (*l*), visceral muscles (*mc*), basal lamina (*bl*). LM. **a** *Bar*=31 μm. **b** *Bar*= 17 μm. **c** *L. forficatus*. The apical cytoplasm of the digestive cells (*dc*) with bacillus-like microorganisms (*arrows*). Spherite (*sp*). TEM. *Bar*=0.3 μm. **d** *S. cingulata*. The apical cytoplasm of the digestive cells (*dc*) with bacillus-like microorganisms (*arrows*). Mitochondria (*m*), microvilli (*mv*). TEM. *Bar*=0.4 μm. **e** *L. forficatus.* Rickettsia-like microorganisms (*arrows*) accumulated in the cytoplasm of digestive cells (*dc*). Mitochondria (*m*), spherite (*sp*), reserve material (*star*), midgut lumen (*l*). Microvilli (*mv*). TEM. *Bar*= 0.2 μm

deoxynucleotidyl transferase (TdT) were prepared according to the labeling protocol (In Situ Cell Death Detection Kit, TMR red, Roche).

Results

The pseudostratified midgut epithelium of *L. forficatus* (Fig. 1a) and *S. cingulata* (Fig. 1b) is composed of three types of cells—digestive, secretory, and regenerative. Their ultrastructure has previously been presented (Chajec et al. 2012, 2014; Rost-Roszkowska et al. 2015a). Numerous microorganisms were detected in the cytoplasm of the midgut epithelial cells in both of the centipedes that were examined.

All of the digestive cells of all of the analyzed specimens of *L. forficatus* and *S. cingulata* had bacillus-like microorganisms that accumulated in the apical cytoplasm (Fig. 1c, d). However, about 10 % of the specimens of *L. forficatus* that were examined had rickettsia-like microorganisms (Fig. 1e) present in the apical and perinuclear cytoplasm. Moreover, the rickettsia-like microorganisms were associated with an obvious decrease in the number of bacillus-like microorganisms (Fig. 1e) or these microorganisms completely disappeared. No rickettsia-like microorganisms were observed in *S. cingulata.*

The following description of apoptosis and necrosis in midgut epithelial cells relates to both species and both sexes that were studied, as no differences were observed between either the two species or the two sexes of a given species.

Apoptosis in centipedes

In *L. forficatus*, apoptosis occurred only occasionally in the digestive cells that were infected with the rickettsia-like microorganisms (Fig. 2a), while it did not occur in non-infected digestive, regenerative, or secretory cells. The number of digestive cells that showed signs of apoptosis in the cross-section of the midgut was less than 5 % in all of the infected specimens that were sectioned at about 06:00, 12:00, 18:00, and 00:00 (Table 1).

Apoptosis was detected in the digestive cells of the *S. cingulata* midgut (Fig. 2b), although it did not affect the secretory and regenerative cells. It occurred continuously during the day and night, although apoptosis proceeded at a higher frequency during the day when entire groups of

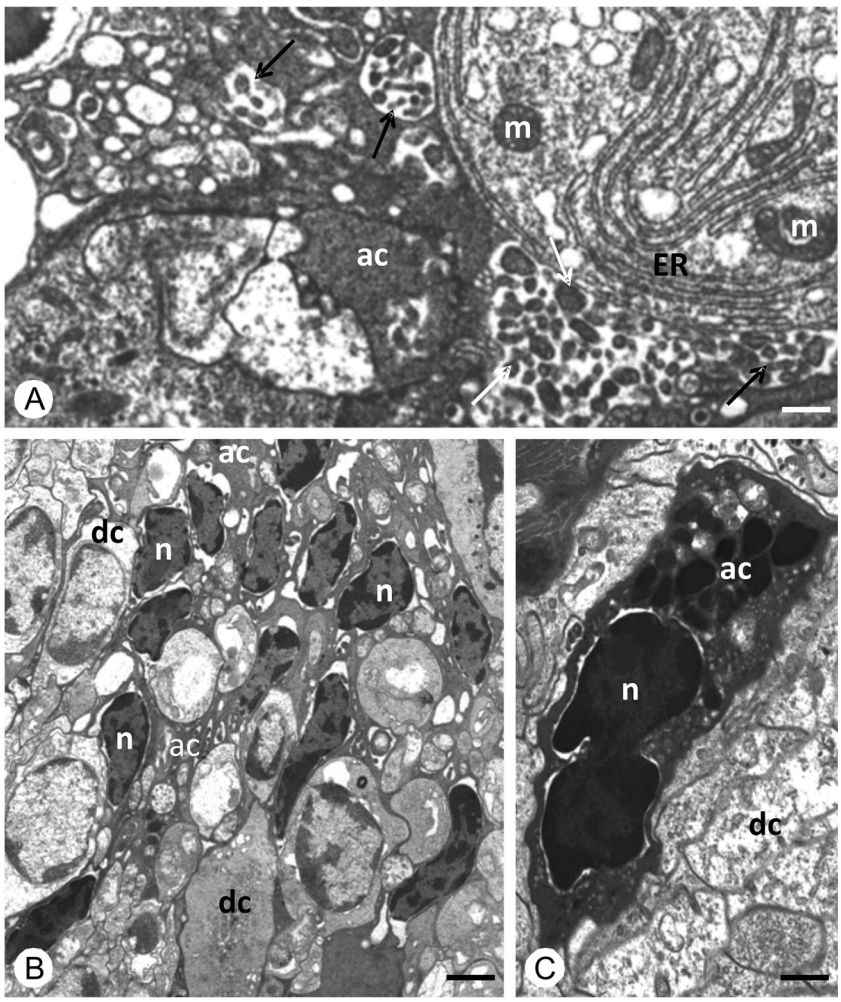

Fig. 2 **a** *L. forficatus*. Apoptotic cell (*ac*) infected with rickettsia-like microorganisms (*arrows*). Cisterns of the endoplasmic reticulum (*ER*), mitochondria (*m*). TEM. *Bar*=0.6 μm. (**b**–**c**) *S. cingulata*. Apoptotic cells (*ac*) with electron-dense cytoplasm and electron-dense karyoplasms in the nucleus (*n*) among digestive cells (*dc*). TEM. **b** *Bar*=1.2 μm. **c** *Bar*=0.9 μm

Table 1 The percentage (%) of apoptotic and necrotic midgut epithelial cells in centipedes (*L. forficatus* and *S. cingulata*) in digestive, secretory, and regenerative cells according to the circadian rhythms

	Digestive cells		Secretory cells		Regenerative cells	
	Apoptosis	Necrosis	Apoptosis	Necrosis	Apoptosis	Necrosis
Lithobius forficatus ~06:00	≤5	≤20	0	0	0	0
Mean	3.2	18.2	0	0	0	0
SE	0.84	3.11	0	0	0	0
Lithobius forficatus ~12:00	≤5	~20	0	0	0	0
Mean	3.2	19.6	0	0	0	0
SE	1.3	1.14	0	0	0	0
Lithobius forficatus ~18:00	≤5	~20	0	0	0	0
Mean	3.4	19.6	0	0	0	0
SE	1.67	1.95	0	0	0	0
Lithobius forficatus ~00:00	≤5	~20	0	0	0	0
Mean	3.6	19.6	0	0	0	0
SE	1.14	2.07	0	0	0	0
Scolopendra cingulata ~06:00	~20	≤5	0	0	0	0
Mean	21	3.6	0	0	0	0
SE	4.53	1.14	0	0	0	0
Scolopendra cingulata ~12:00	~20	≤5	0	0	0	0
Mean	21	3.2	0	0	0	0
SE	3.94	1.48	0	0	0	0
Scolopendra cingulata ~18:00	20	≤5	0	0	0	0
Mean	20	2.8	0	0	0	0
SE	3.39	1.48	0	0	0	0
Scolopendra cingulata ~00:00	~20	~15	0	0	0	0
Mean	20.8	13.4	0	0	0	0
SE	5.26	3.21	0	0	0	0

SE standard error

digestive cells died in an apoptotic manner (Fig. 2b). The number of apoptotic digestive cells in the cross-section of the midgut was about 20 % in all of the specimens that were sectioned at about 06:00, 12:00, 18:00, and 00:00 (Table 1).

At the beginning of apoptosis, the cells begin to shrink, creating the appearance of distinct extracellular spaces among them. The cytoplasm becomes electron-dense (Figs. 2b, c, 3a, b). The nucleus gradually achieves a lobular shape, the karyoplasm starts to become electron-dense, and the chromatin forms electron-dense patches that accumulate near the nuclear envelope (Figs. 2b, 3a, b). Mitochondria (Fig. 3a) and cisterns of the endoplasmic reticulum become extremely distended and start to swell (Fig. 3c, d). Additionally, the mitochondria lose their cristae (Fig. 3a, d). In some of apoptotic cells in *L. forficatus*, autophagosomes (Fig. 4a) or lamellar bodies (Fig. 4b) appear, while numerous vacuoles, vesicles with an electron-lucent content, autophagosomes, autolysosomes, and residual bodies accumulate in *S. cingulata* (Fig. 4c). At later stages, fragmentation of the nucleus begins (Fig. 4d) and the microvilli of the apical cell membrane gradually disappear (Fig. 4e). During its gradual shrinkage, the cell loses contact with the basal lamina because new intercellular junctions appear between the membranes of the neighboring cells in their basal regions. Apoptotic cell gradually moves towards the midgut lumen (Fig. 4e). Eventually, the apoptotic cell is discharged into the midgut lumen (Fig. 4f) where it undergoes degradation due to secondary necrosis.

DNA fragmentation, which occurs during apoptosis, was confirmed with a TUNEL assay (Fig. 5a–f): red signals show nuclei with DNA fragmented what results from apoptotic signaling cascade. A midgut epithelium that had been incubated without the TdT enzyme solution showed no signals (Fig. 5g, h).

Necrosis in centipedes

Necrosis occurred in the digestive cells of the *L. forficatus* midgut (Fig. 6a), although it did not affect the secretory and regenerative cells. It proceeded continuously and intensively during the day and night without any differences. The number of digestive cells that showed signs of necrosis in the cross-section of the midgut was about 20 % in all of the specimens that were sectioned at about 06:00, 12:00, 18:00, and 00:00

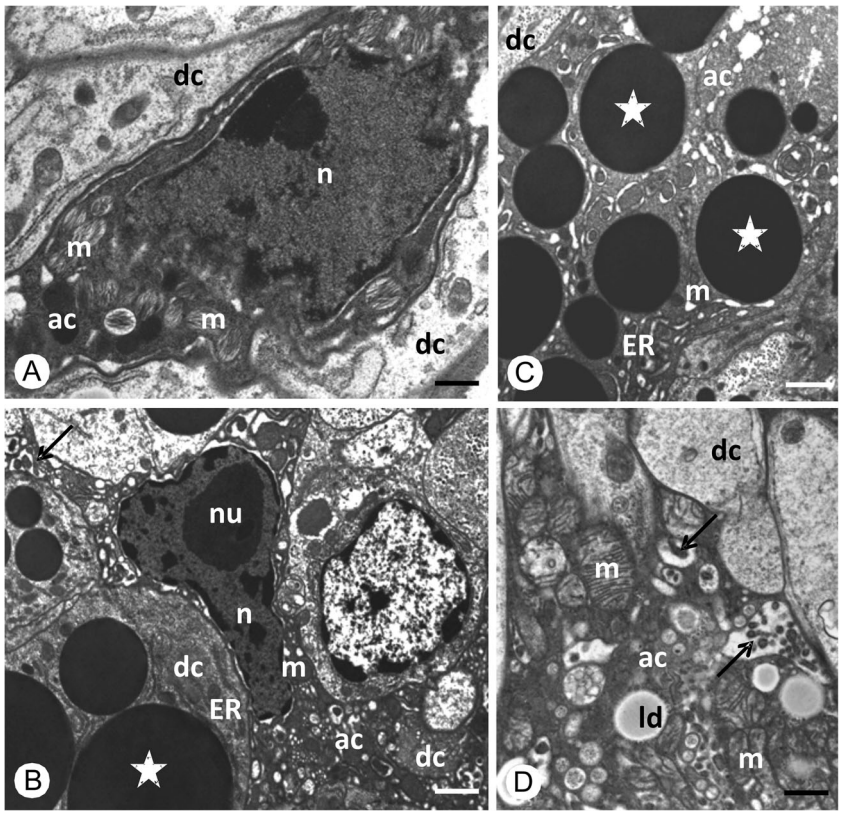

Fig. 3 **a** *S. cingulata*. Apoptotic cell (*ac*) with a lobular nucleus (*n*) that possesses electron-dense karyoplasm and transformed mitochondria (*m*). Adjacent digestive cells (*dc*). TEM. *Bar*= 0.55 μm. (**b**–**d**) *L. forficatus*. Apoptotic cell (*ac*) among digestive cells (*dc*). Cisterns of the endoplasmic reticulum (*ER*), mitochondria (*m*), nucleus (*n*), nucleolus (*nu*), reserve material (*stars*), lipids (*ld*), rickettsia-like microorganisms (*arrows*). **b** *Bar*=0.9 μm. **c** *Bar*=1.2 μm. **d** *Bar*=0.9 μm

(Table 1). However, necrosis in *S. cingulata* (Fig. 6b, c) occurred as an intensive process during the night, while it only occurred occasionally during the day (Table 1). The number of necrotic digestive cells in the cross-section of the midgut was about 5 % in all of the specimens that were sectioned at about 06:00, 12:00, and 18:00, but about 15 % in the specimens that were dissected at about 00:00 (Table 1).

The accumulation of spherites and/or pathogens (e.g., protists) (Fig. 6a) in the cytoplasm of the epithelial cells as well as abrasive food masses apparently damage the midgut epithelium and activate necrosis. During this process, numerous vacuoles accumulate in the electron-lucent cytoplasm and the cells start to swell. Simultaneously, the number of organelles decreases significantly (Fig. 6b). Numerous small vesicles with an electron-lucent content appear (Fig. 6a, c). Cisterns of the endoplasmic reticulum as well as the Golgi complexes and mitochondria begin to swell. Additionally, the mitochondria lose their cristae and their matrix becomes electron-lucent (Fig. 6c). Distinct changes were also observed in the nucleus, which included the disintegration of chromatin, the disappearance of the nucleolus, and the folding of the nuclear envelope. Finally, the apical membrane breaks (Fig. 6d) and the cytoplasm along with the remaining degenerated organelles are discharged into the midgut lumen (Fig. 6e), where they are digested. After the disintegration of the necrotic cells, they are replaced by the new epithelial cells that differentiated from the regenerative cells (Fig. 6f). The process of the differentiation of the regenerative cells was clearly described previously (Chajec et al. 2012, 2014).

Discussion

Cell death is responsible for the elimination of the damaged or unwanted cells that form the tissues of embryos or adults. Thus, it plays an important role in controlling the number of cells in each organ (Maghsoudi et al. 2012). Many stress factors that originate from the external environment can disrupt and damage cell organelles, causing the activation of the cell death pathways (Okuda et al. 2007; Menze et al. 2010; Maghsoudi et al. 2012; Jain et al. 2013; Teixeira et al. 2013; Lipovšek and Novak 2015). Among all of the processes of cell death that are known, only apoptosis, necrosis, and autophagy have been clearly described in the digestive epithelium of invertebrates (Parthasarathy and Palli 2007; Park and Takeda 2008; Park et al. 2009; Tettamanti et al. 2011; Franzetti et al. 2012; Rost-Roszkowska et al. 2008, 2010, 2012, 2015b; Lipovšek and Novak 2015). Additionally, functions of the digestive cells (e.g., enzymes synthesis, transport of ions and nutrients, storage of the reserve material and toxic substances) can cause the accumulation of reactive oxygen species (ROS), which trigger apoptosis and necrosis. These processes are all known to occur in the midgut epithelium of centipedes that were examined in this study. While central clocks that regulate

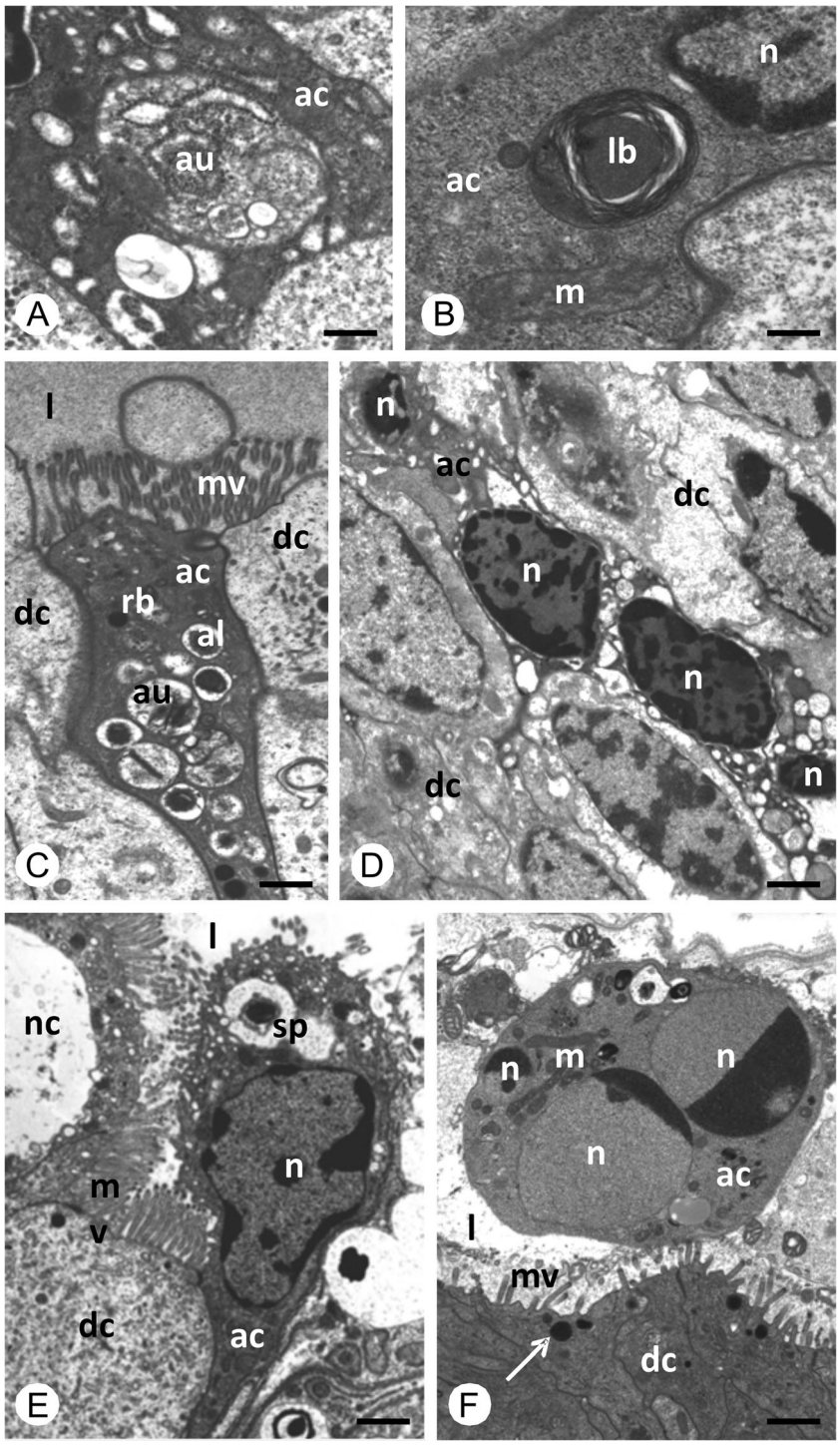

Fig. 4 **a**–**b** Apoptotic cell (*ac*) in *L. forficatus* with autophagosomes (*au*) (**a**) and lamellar bodies (*lb*) (**b**). Mitochondria (*m*), nucleus (*n*). TEM. **a** *Bar*=0.5 μm. **b** *Bar*= 0.4 μm. **c** Apoptotic cell (*ac*) in *S. cingulata* with autophagosomes (*au*), autolysosomes (*al*), and residual bodies (*rb*). Digestive cells (*dc*), midgut lumen (*l*), microvilli (*mv*). **c** *Bar*=0.7 μm. **d** *S. cingulata*. Apoptotic cell (*ac*) containing fragmented nucleus (*n*). Digestive cells (*dc*). TEM. *Bar*=1.4 μm. **e** *L. forficatus*. Apoptotic cell (*ac*) during discharged into the midgut lumen (*l*). Digestive cells (*dc*), microvilli (*mv*), necrotic cell (*nc*), spherite (*sp*), nucleus (*n*). TEM. *Bar*=1.12 μm. **f** *S. cingulata*. Apoptotic cell (*ac*) discharged into the midgut lumen (*l*). Fragments of the nucleus (*n*), mitochondria (*m*), reserved material (*arrow*), digestive cell (*dc*), microvilli (*mv*). TEM. *Bar*= 1.1 μm

behavior, physiology, and metabolism are located in the nervous system, peripheral clocks have also been described in the digestive and/or reproductive system (Tomioka et al. 2012; Wang et al. 2013; Park et al. 2013).

While autophagy has only been observed in the digestive cells of the midgut of *L. forficatus*, this process also occurred in the secretory and regenerative cells in the midgut of *S. cingulata* (Rost-Roszkowska et al. 2015a). Apoptosis and necrosis were observed only in the digestive cells in both of the species that were examined, but they never occurred in the secretory or regenerative cells. Secretory cells and regenerative cells are closed cell types whose cell membranes do not have contact with the midgut lumen and food masses (Chajec et al. 2012, 2014). The midgut lumen is the way that pathogens, metals, and toxic substances enter into the body of an animal. Therefore, the digestive cells, which are in contact

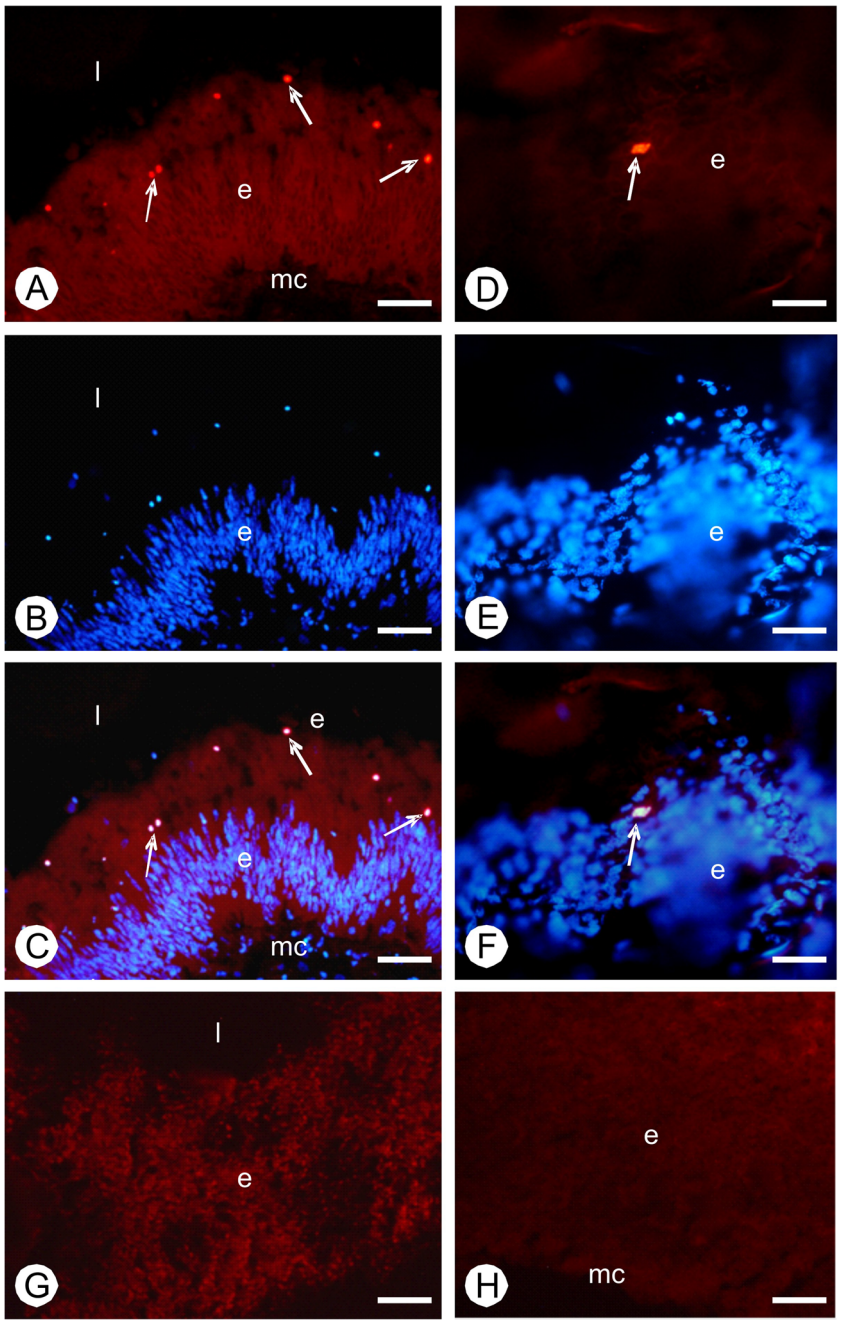

Fig. 5 Fluorescence micrograph of red TUNEL-positive signals (*arrows*) and DAPI staining in midgut epithelium (*e*) of *L. forficatus* (**a**–**c**) and *S. cingulata* (**d**–**f**). Midgut lumen (*l*), visceral muscles (*mc*). **a**–**c** *Bar*=12.3 μm. **d**–**f** *Bar*=9.8 μm. **g**–**h** Negative control of TUNEL assay. Midgut lumen (*l*), epithelium (*e*), visceral muscles (*mc*). **g** *Bar*=13.2 μm. **h** *Bar*= 10 μm

with the midgut lumen, are exposed to multiple stressors (Vaidyanathan and Scott 2006; Baton and Ranford-Cartwright 2007; Franzetti et al. 2012; Wilczek et al. 2014).

In our previous studies on the midgut of *L. forficatus* and *S. cingulata*, we analyzed the ultrastructure of the regenerative cells (midgut stem cells), their proliferation and differentiation (Chajec et al. 2012, 2014) as well as the activation of autophagy (Rost-Roszkowska et al. 2015a). The analysis of apoptosis and necrosis has revealed that these processes, as is the case with autophagy, also occur in a continuous manner and apparently are activated by other factors than the circadian rhythms. However, necrosis is activated at midnight in *S. cingulata*—about 15 % of the digestive cells showed signs of necrosis. This correlates with the activation of the proliferation of the regenerative cells and the intensive epithelial regeneration in the midgut of this species (Chajec et al. 2014). The correlation between cell proliferation and cell death has previously been described. Both of these processes control the cell number because the balance between cell proliferation, cell differentiation, and cell death is crucial for tissue-size homeostasis. However, apoptosis is primarily treated as the type of cell death that correlates with cell proliferation (Guo and Hay 1999; Maghsoudi et al. 2012).

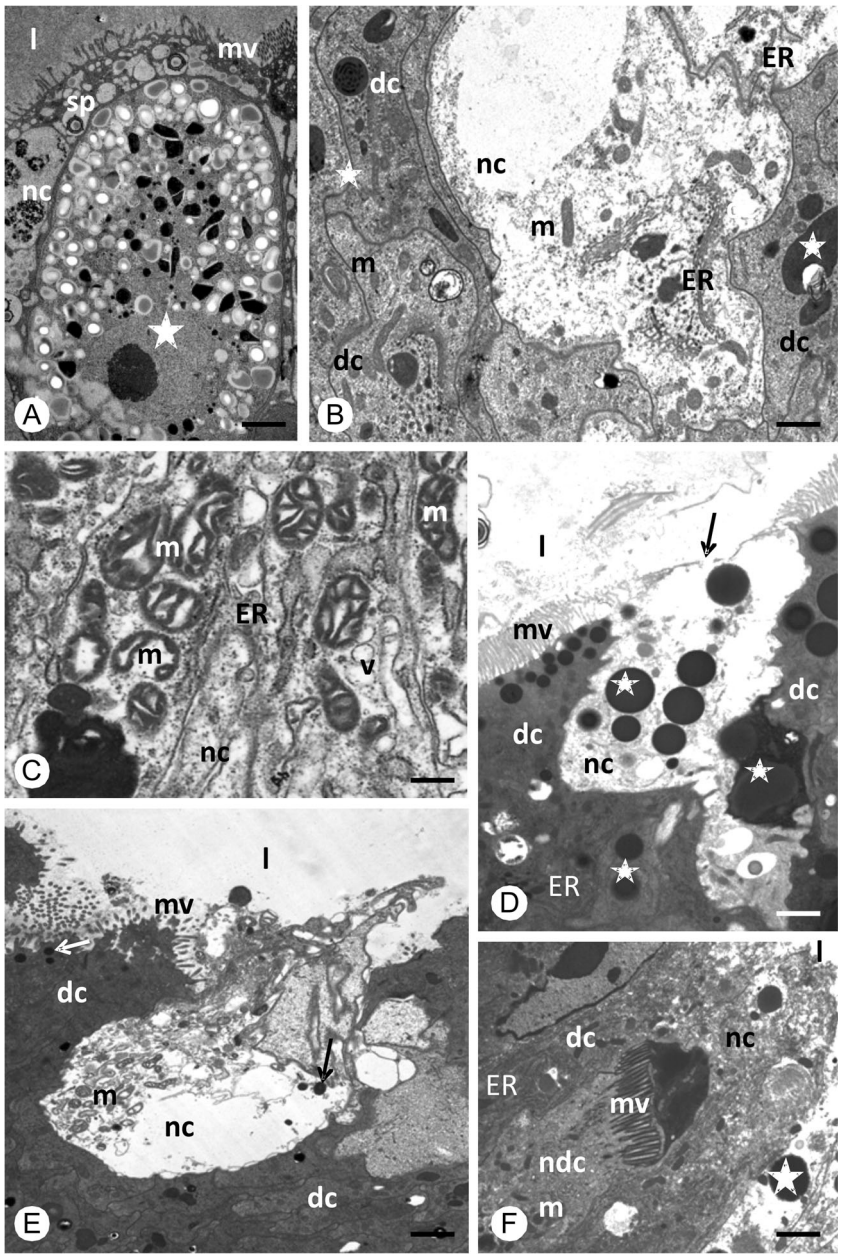

Fig. 6 **a** *L. forficatus*. Spherites (*sp*) and pathogen: protists (*star*) in the cytoplasm of necrotic cell (*nc*). TEM. Midgut lumen (*l*), microvilli (*mv*). *Bar*=1.4 μm. **b** *S. cingulata*. Necrotic cell (*nc*) with electron-lucent cytoplasm among digestive cells (*dc*). Cisterns of the endoplasmic reticulum (*ER*), mitochondria (*m*), reserve material (*stars*). TEM. *Bar*=0.6 μm. **c** *S. cingulata*. Transformation of mitochondria (*m*) in necrotic cell (*nc*). Vesicles with electron-lucent cytoplasm (*v*), cisterns of the endoplasmic reticulum (*ER*). TEM, Bar= 0.3 μm. **d** *L. forficatus*. The necrotic cell (*nc*) with the apical cell membrane broken (*arrow*) among digestive cells (*dc*). Midgut lumen (*l*), microvilli (*mv*), reserve material (*stars*), cisterns of the endoplasmic reticulum (*ER*). TEM. *Bar*=1.5 μm. **e** *S. cingulata*. The cytoplasm of necrotic cell (*nc*) with degenerated organelles gradually discharged into the midgut lumen (l). Digestive cells (*dc*), microvilli (*mv*), mitochondria (*m*), reserve material (*arrows*). TEM. *Bar*= 1.6 μm. **f** *L. forficatus*. New digestive cell (*ndc*) differentiated with distinct microvilli (*mv*). Digestive cell (*dc*), mitochondria (*m*), fragment of the necrotic cell (*nc*), reserve material (*stars*), cisterns of the endoplasmic reticulum (*ER*), midgut lumen (*l*). TEM. *Bar*=1.6 μm

The difference between both of the centipedes that were examined is that necrosis occurs more frequently than apoptosis in *L. forficatus* (about 20 % of digestive cells showed signs of necrosis), while less than 5 % of necrotic cells were observed in *S. cingulata* (except the activation of this process at midnight). This is probably caused by the structure of their digestive systems. The foregut in *L. forficatus* is short and straight. The food enters the midgut lumen quickly and causes damage to the midgut epithelium as manifested by cellular necrosis. Although this species is primarily carnivorous, it can also feed on litter (Lewis 1965). As is known, necrosis can be triggered by mechanical damage, external factors, or even an infection (Proskuryakov et al. 2003; Fink and Cookson 2005; Jain et al. 2013). The passage of the food mass can disrupt and damage the intestinal epithelium (Karasov et al. 2011), thereby activating necrosis. On the other hand, *S. cingulata* is an obligatory predator that does not feed when there is a lack of the food it hunts. Its foregut is long, and the food enters the midgut discontinuously. The quality of the food and the frequency with which it enters the crop or intestine have a significant impact on digestion and the functioning of the midgut (Mitra and Flynn 2007; Karasov et al. 2011).

About 20 % of the digestive cells in *S. cingulata* showed signs of apoptosis, while this process is rare in *L. forficatus* (less than 5 % of digestive cells were apoptotic). Moreover, apoptosis does not depend on the day/night cycle (see Table 1). Apoptosis, which is a continuation of the cell cycle that is initiated by cell division (mitosis), is triggered by

natural processes in tissues and organs. However, apoptosis can also be caused by external factors such as pathogens and environmental toxins (Elmore 2007; Parthasarathy and Palli 2007; Park and Takeda 2008; Park et al. 2009; Rost-Roszkowska et al. 2010, 2015b; Wilczek et al. 2014). In the digestive cells of *L. forficatus*, apoptosis affected only the cells that were infected with the rickettsia-like microorganisms. Thus, apoptosis represents a mechanism that protects against infection and inflammation in the different organs and tissues of the organism. Similar results have been described in the digestive epithelium of many arthropods (Baton and Ranford-Cartwright 2007; Rost-Roszkowska et al. 2008, 2010, 2015b). *S. cingulata* did not have any rickettsia-like microorganisms in the cytoplasm of the digestive cells. Our previous studies revealed that gregarines, which occupy the cytoplasm of digestive cells in about 10 % of adult specimens, are probably neutralized by the numerous hemocytes that enter the midgut epithelium (Chajec et al. 2014). Therefore, neither apoptosis nor necrosis is involved in protecting the midgut epithelium and ultimately the entire organism against infection.

Conclusions

The results of our studies have shown that (a) apoptosis and necrosis proceed in a continuous manner that is independent of the circadian rhythm in *L. forficatus*, while in *S. cingulata* necrosis is activated at midnight; (b) there are no differences in the course of apoptosis and necrosis between the males and females of both species; and (c) the activation of necrosis at midnight in *S. cingulata* correlates with the activation of the proliferation of the regenerative cells.

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Conflict of interest The authors declare that they have no competing interests.

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