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Title: Ultrastructural study of spermiogenesis and the spermatozoon of the proteocephalidean cestode *Barsonella lafoni* de Chambrier et al., 2009, a parasite of the catfish *Clarias gariepinus* (Burchell, 1822) (Siluriformes, Clariidae)

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Abstract: Spermiogenesis in the proteocephalidean cestode *Barsonella lafoni* de Chambrier et al., 2009 shows typical characteristics of the type I spermiogenesis. These include the formation of distal cytoplasmic protrusions forming the differentiation zones, lined by cortical microtubules and containing two centrioles. An electron-dense material is present in the apical region of the differentiation zone during the early stages of spermiogenesis. Each centriole is associated to a striated rootlet, being separated by an intercentriolar body. Two free and unequal flagella originate from the centrioles and develop on the lateral sides of the differentiation zone. A median cytoplasmic process is formed between the flagella. Later these flagella rotate, become parallel to the median cytoplasmic process and finally fuse proximodistally with the latter. It is interesting to note that both flagellar growth and rotation are asynchronous. Later, the nucleus enlarges and penetrates into the median cytoplasmic process. Finally, the ring of arching membranes is strangled and the young spermatozoon is detached from the residual cytoplasm.

The mature spermatozoon presents two axonemes of the 9+'1' trepaxonematan pattern, crested body, parallel nucleus and cortical microtubules, and glycogen granules. Thus, it corresponds to the type II spermatozoon, described in almost all Proteocephalidea. The anterior extremity of the gamete is characterized by the presence of an apical cone surrounded by the lateral projections of the crested body. An arc formed by some thick and parallel cortical microtubules appears at the level of the centriole. They surround the centriole and later the first axoneme. This arc of electron-dense microtubules disorganizes when the second axoneme appears, and then two parallel rows of thin cortical microtubules are observed. The posterior extremity of the male gamete exhibits some cortical microtubules. This type of posterior extremity has never been described in proteocephalidean cestodes. The ultrastructural features of the spermatozoon/spermiogenesis of the Proteocephalidea species are analyzed and compared.

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**Ultrastructural study of spermiogenesis and the spermatozoon of the proteocephalidean cestode *Barsonella lafoni* de Chambrier et al., 2009, a parasite of the catfish *Clarias gariepinus* (Burchell, 1822) (Siluriformes, Clariidae)**

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**Abstract**

Spermiogenesis in the proteocephalidean cestode *Barsonella lafoni* de Chambrier et al., 2009 shows typical characteristics of the type I spermiogenesis. These include the formation of distal cytoplasmic protrusions forming the differentiation zones, lined by cortical

1 microtubules and containing two centrioles. An electron-dense material is present in the  
2 apical region of the differentiation zone during the early stages of spermiogenesis. Each  
3 centriole is associated to a striated rootlet, being separated by an intercentriolar body. Two  
4 free and unequal flagella originate from the centrioles and develop on the lateral sides of the  
5 differentiation zone. A median cytoplasmic process is formed between the flagella. Later  
6 these flagella rotate, become parallel to the median cytoplasmic process and finally fuse  
7 proximodistally with the latter. It is interesting to note that both flagellar growth and rotation  
8 are asynchronous. Later, the nucleus enlarges and penetrates into the spermatid body. Finally,  
9 the ring of arching membranes is strangled and the young spermatozoon is detached from the  
10 residual cytoplasm.

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12 The mature spermatozoon presents two axonemes of the 9+1' trepaxonematan pattern,  
13 crested body, parallel nucleus and cortical microtubules, and glycogen granules. Thus, it  
14 corresponds to the type II spermatozoon, described in almost all Proteocephalidea. The  
15 anterior extremity of the gamete is characterized by the presence of an apical cone surrounded  
16 by the lateral projections of the crested body. An arc formed by some thick and parallel  
17 cortical microtubules appears at the level of the centriole. They surround the centriole and  
18 later the first axoneme. This arc of electron-dense microtubules disorganizes when the second  
19 axoneme appears, and then two parallel rows of thin cortical microtubules are observed. The  
20 posterior extremity of the male gamete exhibits some cortical microtubules. This type of  
21 posterior extremity has never been described in proteocephalidean cestodes. The  
22 ultrastructural features of the spermatozoon/spermiogenesis of the Proteocephalidea species  
23 are analyzed and compared.

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56 **Keywords:** spermiogenesis; spermatozoon; ultrastructure; *Barsonella lafoni*;  
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58 Proteocephalinae; Proteocephalidae; Proteocephalidea; Cestoda  
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## 1. Introduction

The Proteocephalidea Mola, 1928 have been recognized as an interesting group from an evolutionary point of view, because it was supposed to include the closest relatives of the ancestors of the Cyclophyllidea van Beneden in Braun, 1900 (Rego 1994, 1995). However, phylogenetically, they have also been regarded as a problematical group (Zehnder and Mariaux 1999, Škeříková et al. 2001, Scholz and de Chambrier 2003, de Chambrier et al. 2004). In fact, the systematics of Proteocephalidea is far from being suitably resolved (Rego 1994, 1995; Zehnder and Mariaux 1999; de Chambrier et al. 2004).

To date, there are two valid families, the Proteocephalidae La Rue, 1911 including six subfamilies (Gangesiinae Mola, 1929, Sandonellinae Khalil, 1960, Corallobothriinae Freze, 1965, Acanthotaeniinae Freze, 1963, Proteocephalinae Mola, 1929 and Marsypocephalinae Woodland, 1933) and the family Monticelliidae La Rue, 1911 also including six subfamilies (Monticelliinae Mola, 1929, Zygobothriinae Woodland, 1933, Nupeliinae Pavanelli & Rego, 1991, Ephedrocephalinae Mola, 1929, Peltidocotylinae Woodland, 1934 and Rudolphiellinae Woodland, 1935) (Rego, 1994).

*Barsonella lafoni* de Chambrier et al., 2009 is a recently described species belonging to the new genus *Barsonella* de Chambrier et al., 2009. This genus is included in the family Proteocephalidae and in the subfamily Proteocephalinae and occurs in a large area of Africa where it has been found in catfishes of the genus *Clarias* Scopoli, 1777.

Until now, only six species of Proteocephalidea (five Proteocephalidae and one Monticelliidae) have been subjected to ultrastructural spermatological studies (Świdorski 1985, 1996; Bâ and Marchand 1994; Sène et al. 1997; Bruňanská et al. 2003a,b,c, 2004a,b,c, 2005). Despite the small number of ultrastructural studies on the Proteocephalidea, some degree of incongruence has already been found among Proteocephalidea species such as the

1 observation of a type IV spermatozoon of Levron et al. (2010) in *S. sandoni* (Bâ and  
2 Marchand 1994), contrasting with the type II spermatozoa of Levron et al. (2010) observed in  
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4 the remaining species (Sène et al. 1997; Bruňanská et al. 2003a,c, 2004a,b). Therefore, further  
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6 studies on the spermatology of this group are necessary in order to clarify which types of  
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8 characters are representative of this group.  
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11 The present study presents new data concerning the ultrastructure of spermiogenesis and the  
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13 spermatozoon of another Proteocephalidea, *Barsonella lafoni*.  
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## 17 18 19 **2. Material and methods** 20

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22 Adult tapeworms of *Barsonella lafoni* were collected from the intestine of the catfish *Clarias*  
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24 *gariepinus* (Burchell, 1822) caught in Tana Lake at Bahir Dar (Ethiopia). Living cestodes  
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26 were placed in 0.9% NaCl solution and then fixed in glutaraldehyde (2.5%) in 0.1 M  
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28 phosphate buffer, pH 7.2, for a minimum of 2h at 4°C. After dissection, different portions of  
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30 mature proglottids were separated, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2,  
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32 postfixed in cold (4°C) 1% osmium tetroxide in the same buffer for 1h, rinsed in a 0.1 M  
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34 sodium cacodylate buffer at pH 7.2, dehydrated in an ethanol series and propylene oxide,  
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36 embedded in Epon, and then polymerised at 60°C for 48h. Ultrathin sections were obtained  
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38 using a Reichert-Jung Ultracut E ultramicrotome, placed on copper grids and double-stained  
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40 with uranyl acetate and lead citrate according to Reynolds (1963). Ultrathin sections were  
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42 examined using a Jeol 1010 transmission electron microscope in the “Centres Científics i  
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44 Tecnològics” of the University of Barcelona.  
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51 The Thiéry (1967) technique was used to emphasize the presence of glycogen particles. Gold  
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53 grids were treated in periodic acid, thiocarbohydrazide, and silver proteinate (PA-TCH-SP) as  
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55 follows: 30 min in 10% PA, rinsed in distilled water, 24hr in TCH, rinsed in acetic solutions  
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57 and distilled water, 30 min in 1 % SP in the dark, and rinsed in distilled water.  
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### 3. Results

#### 3.1. Spermiogenesis

Spermiogenesis in *Barsonella lafoni* is illustrated in Figures 1A-F, 2A-D and 3A-E.

The first clear evidence of the beginning of the spermiogenesis is the presence of the small cytoplasmic protrusion named *zone of differentiation* in the periphery of each spermatid (Fig. 1A). This zone of differentiation contains two centrioles, each associated with a pyramidal striated rootlet and separated with an intercentriolar body (Figs. 1A,B, 3A). The intercentriolar body is composed of a single electron-dense plate (Figs. 1B, 3A-C). Moreover, at this stage of spermiogenesis, the striated rootlets are situated tangentially to the long axis of the nucleus (Fig. 1A). Each centriole gives rise to a free flagellum (Figs. 1B, 3A). In the very early stage of spermiogenesis it is possible to observe an electron-dense material in the peripheral region of the zone of differentiation (Figs. 1A-C, 3A). Subsequently, a median cytoplasmic process is formed distal to the centriole region (Figs. 1D, 3B,C). In *B. lafoni*, typical striated rootlets may be occasionally accompanied by one additional striated rootlet associated to the same centriole (Fig. 1F). Both flagella grow and rotate asynchronously (Figs. 1D,E, 3B) thus becoming parallel to the longitudinal axis of the median cytoplasmic process (Fig. 2A). Arching membranes are visible at this stage of development (Fig. 2A). After the proximodistal fusion of the flagella with the median cytoplasmic process, the nucleus enlarges and begins its migration along the spermatid body (Fig. 2B). Cross-sections of late development spermatids at various levels reveal that cortical microtubules are arranged (i) as a semicircle lining the periphery in the proximal region containing one axoneme and (ii) in two opposite rows lining the periphery of sections with two axonemes or with one axoneme and nucleus (Fig. 2B). It is interesting to note that a striated rootlet is present in old spermatids (Fig. 2C). At the end of spermiogenesis, the ring of arching membranes narrows

and the spermatid is pinched off from the residual cytoplasm (Fig. 2D).

### 3.2. Spermatozoon

The mature spermatozoon of *Barsonella lafoni* is illustrated in Figures 4A-J, 5A-E and 6I-IV. It contains two axonemes of unequal length exhibiting the 9+‘1’ pattern of the Trepaxonemata, a single crested body, a parallel nucleus, parallel cortical microtubules, and electron-dense granules. From the anterior to posterior extremities of the spermatozoon, it is possible to distinguish four regions with distinctive ultrastructural characters.

Region I (Figs. 4A-G, 6I) corresponds to the anterior part of the gamete. It is characterized by the presence of an electron-dense apical cone that marks the anterior tip of the gamete (Figs. 4A,B, 6I). The apical cone is externally surrounded by a helical cord of electron-dense material that forms a single crested body 60-90 nm thick (Fig. 4A-C). Later, the first centriole becomes visible (Fig. 4D). It marks the beginning of the first axoneme. This axoneme is surrounded by some electron-dense tubular structures arranged in an arc (Fig. 4D-F). Thus, they form the so-called arc-like row of cortical microtubules and they are thick-walled and with an electron-lucent centre (Figs. 4E,F, 6I). At the end of this region, the crested body becomes thinner and subsequently disappears (Fig. 4G).

Region II (Figs. 4H-I, 5A, 6II) lacks crested body. It is characterized by the appearance of the second axoneme (Fig. 4H). At this level, nine scattered centriolar doublets are shown and the number of cortical microtubules lying beneath the plasma membrane increases (Fig. 4I). Also, it is possible to observe that cortical microtubules become thin-walled and are organized in two opposite and parallel sub-membranous layers (Figs. 4 I,J, 6II). Cross-sections of this region show an increase in the width of the male gamete and the appearance of glycogen

granules (Figs. 4 H,J, 5A).

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4 Region III (Figs. 5A-C, 6III) constitutes the nuclear region of the spermatozoon, in which two  
5 axonemes, granules of glycogen, two fields of thin cortical microtubules and nucleus coexist.  
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7 The nucleus, slightly electron-dense, exhibits a parallel disposition being localized between  
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9 the two axonemes (Fig. 5A,B). This parallel disposition extends into the area with a single  
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11 axoneme (Fig. 5C). Cross-sections show that the diameter of the nucleus increases towards  
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13 the middle part of the region (Fig. 5B). Later one of the axonemes disorganizes and  
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15 disappears (Figs. 5B, 6III) and the diameter of the nucleus decreases progressively (Fig. 5C).  
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17 At the end of region III, the nucleus disappears (Fig. 6III).  
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26 Region IV (Figs. 5D-G, 6IV) contains a single axoneme, cortical microtubules, and glycogen  
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28 granules. Towards the distal part of the male gamete, cross-sections show a decrease in the  
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30 size of the spermatozoon (Fig. 5D). There is also a decrease in the number of cortical  
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32 microtubules and granules of glycogen (Fig. 5D). In the posterior extremity of the male  
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34 gamete, the axoneme becomes disorganized (Figs. 5F,G, 6IV), the number of electron-dense  
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36 granules gradually decreases and only some cortical microtubules accompanied by some  
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38 granules of glycogen are present in the posterior tip of the spermatozoon (Fig. 5E-G).  
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## 46 **4. Discussion**

### 47 **4.1. Spermiogenesis**

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49 In Proteocephalidea, spermiogenesis has been studied in four species (Sène et al. 1997;  
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51 Bruňanská et al. 2003b, 2004c, 2005). In the present study we verified that spermiogenesis in  
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53 *Barsonella lafoni* is in accordance with the previously described basic pattern of  
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55 proteocephalideans. Spermiogenesis in *Barsonella lafoni* is characterized by the presence of  
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1 two flagella, flagellar rotation, and proximo-distal fusion. This pattern corresponds to the type  
2 I spermiogenesis of Bâ and Marchand (1995) and is also found in the Spathebothriidea  
3 (Bruňanská et al. 2006, Bruňanská and Poddubnaya 2010), in the Diphyllbothriidea (Levron  
4 et al. 2006a, 2009, 2011), in the Bothriocephalidea (Świderski and Mokhtar-Maamouri 1980;  
5 Bruňanská et al. 2001, 2010; Levron et al. 2005, 2006b; Šípková et al. 2010, 2011; Marigo et  
6 al. 2011a), in the Dyphyllidea (Azzouz-Draoui 1985, Azzouz-Draoui and Mokhtar-Maamouri  
7 1986/88, Marigo et al. 2011b), in the Tetraphyllidea-Onchobothriidae (Mokhtar-Maamouri  
8 and Świderski 1975, Mokhtar-Maamouri 1982, Marigo et al. 2011c), and in the  
9 Trypanorhyncha (Świderski 1976, McKerr 1985, Marigo et al. 2011d).

10 In some groups exhibiting the type I spermiogenesis, a condensation of electron-dense  
11 material is observed in the apical region of the differentiation zone during the early stage of  
12 the process. This dense material was described for the first time in *Eubothrium crassum*  
13 (Bloch, 1779) by Bruňanská et al. (2001) and is present in almost all the Bothriocephalidea  
14 (Bruňanská et al. 2001, 2010; Levron et al. 2005, 2006b; Šípková et al. 2010, 2011; Marigo et  
15 al. 2011a), in the Spathebothriidea (Bruňanská et al. 2006, Bruňanská and Poddubnaya 2010),  
16 in the Diphyllbothriidea (Levron et al. 2006a, 2009, 2011). Furthermore, electron-dense  
17 material in the apical region was also described in another group presenting type II  
18 spermiogenesis, the Caryophyllidea (Bruňanská and Poddubnaya 2006, Miquel et al. 2008,  
19 Bruňanská 2009, Bruňanská and Kostič 2011). The present study represents the first finding  
20 of this dense material in the spermiogenesis process of proteocephalideans. Therefore, our  
21 findings bring into question the restriction of this dense material to the basal cestodes  
22 proposed by Bruňanská and Poddubnaya (2010) on the base of available data at this time. In  
23 fact, the Proteocephalidea are considered the most closely related order to the Cyclophyllidea  
24 (Rego 1994, 1995).

25 Spermiogenesis of *B. lafoni* is also characterized by the asynchronous development of the  
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1 flagella resulting in the observation of two unequal flagella during spermiogenesis. A similar  
2 feature is described in other proteocephalideans such as *Nomimoscolex* sp. by Sène et al.  
3 (1997), *Proteocephalus torulosus* (Batsch, 1786) by Bruňanská et al. (2003b), and  
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7 *Proteocephalus longicollis* (Zeder, 1800) by Bruňanská et al. (2004c), in the  
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9 Bothriocephalidea *Eubothrium crassum* by Bruňanská et al. (2001), in the Diphyllidea  
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11 *Echinobothrium euterpes* (Neifar, Tyler and Euzet, 2001) by Marigo et al. (2011b), and in the  
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13 Tetraphyllidea-Onchobothriidae *Acanthobothrium crassicolle* Weld, 1855 by Marigo et al.  
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17 (2011c).

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19 In the cestodes, the intercentriolar body usually comprises a number of parallel disc-shaped  
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21 plates of different electron-density. In *B. lafoni* as in almost all proteocephalideans the  
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23 intercentriolar body consists of a single electron-dense plate (Sène et al. 1997; Bruňanská et  
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25 al. 2003b, 2004c, 2005). The presence/absence of an intercentriolar body has been used as a  
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27 character of phylogenetic importance in eucestodan studies (Hoberg et al. 1997; Justine 1998,  
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30 2001). It is considered to be a plesiomorphic character within the Eucestoda (Justine 1998).

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33 In *B. lafoni* two striated rootlets associated to the same centriole are viewed. This feature, also  
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35 mentioned in two other species belonging to the Caryophyllidea (Bruňanská and Poddubnaya  
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37 2006) and the Diphyllbothriidea (Levron et al. 2006a), could be a character of phylogenetic  
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40 importance in the future.

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43 Another particularity in *B. lafoni* spermiogenesis is the persistence of striated rootlets in the  
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45 very late spermatids. This character was already reported in other proteocephalideans such as  
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48 *C. solidum*, *P. torulosus*, *P. longicollis* and *Nomimoscolex* sp. (see Sène et al. 1997;  
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50 Bruňanská et al. 2003b, 2004c, 2005). While in *C. solidum* striated rootlets disappear just  
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52 after the nuclear migration into the median cytoplasmic process, in *B. lafoni* as in *P.*  
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55 *longicollis* striated rootlets persist in old spermatids. The observation of striated rootlets  
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58 during advanced stages of spermiogenesis were also reported in some Tetraphyllidea  
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1 *Phyllobothrium gracile* Weld, 1855, *Acanthobothrium filicolle* Zschokke, 1887,  
2 *Phyllobothrium lactuca* van Beneden, 1850 and *Acanthobothrium crassicolle* Weld, 1855 (see  
3 Mokhtar-Maamouri 1979, 1982; Sène et al. 1999; Marigo et al. 2011c) and in the  
4 Bothriocephalidea *Clestobothrium crassiceps* (Rudolphi, 1819) (see Marigo et al. 2011a).  
5 This pattern has recently been reported from an increasing number of cestode species,  
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7 indicating that the power and quality of observations are improving.  
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#### 10 11 12 13 14 15 16 17 **4.2. Spermatozoon**

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19 The present study shows that the basic pattern of ultrastructural organization of the mature  
20 spermatozoon of *B. lafoni* is similar to that reported in other proteocephalideans (see Sène et  
21 al. 1997; Bruňanská et al. 2003a,c, 2004a,b). It exhibits the type II spermatozoon of Levron et  
22 al. (2010) that includes the presence of two axonemes, crested body, and both parallel nucleus  
23 and cortical microtubules. In spite of this classic pattern, the spermatozoon ultrastructure of *B.*  
24 *lafoni* presents certain remarkable aspects.  
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34 In the Proteocephalidea, ultrastructural studies have been performed on spermatozoa of only  
35 six species. These are *Corallobothrium solidum*, *Proteocephalus longicollis*, *P. torulosus*,  
36 *Electrotaenia malopteruri* (Fritsch, 1886), *Sandonella sandoni* (Lynsdale, 1960) and  
37 *Nomimoscolex* sp. (see Bâ and Marchand 1994; Sène et al. 1997; Bruňanská et al. 2003a,c,  
38 2004a,b). Considering the six studied species, only *S. sandoni* (Bâ and Marchand 1994)  
39 presents a type IV spermatozoon, whereas the remaining species including *B. lafoni*, present  
40 type II spermatozoa (see Table I).  
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51 The anterior extremity of the mature spermatozoon of *B. lafoni* is characterized by the  
52 presence of an apical cone. This electron-dense structure has previously been described in  
53 only two proteocephalideans, namely *S. sandoni* and *Nomimoscolex* sp. (Bâ and Marchand  
54 1994, Sène et al. 1997).  
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1 The apical cone exhibits a helical crested body, externally coiled, which describes several  
2 turns around the apical cone and reaches the level of the axoneme. The crested body  
3 represents a structure of presumed phylogenetic importance (Justine 1998, 2001) and  
4 characterizes the anterior extremity of the spermatozoon of eucestodes (Bâ et al. 1991).  
5 According to Bâ and Marchand (1995), the presence of this structure represents a  
6 synapomorphy for the Eucestoda. However, during the last years, an increase of existing data  
7 on spermatology of eucestodes demonstrates its absence in several groups, such as  
8 caryophyllideans, spathebothriideans, diphyllbothriideans and trypanorhynchs (see reviews  
9 in Bruňanská 2010, Levron et al. 2010, Bruňanská & Poddubnaya 2010, Marigo et al. 2011d,  
10 Yoneva et al. 2011). To date, in the Proteocephalidea, crested body or bodies have been  
11 described in all studied species (Bâ et al. 1994; Sène et al. 1997; Bruňanská et al. 2003a,c,  
12 2004a,b). Like in most proteocephalideans (see Table I) only a single crested body was found  
13 in *B. lafoni*. The presence of this single crested body is considered a plesiomorphic condition  
14 for the Eucestoda (Justine 1998). Nevertheless, a particular pattern has been found in  
15 *Nomimoscolex* sp., which presents three helical crested bodies in the anterior tip of the male  
16 gamete (Sène et al. 1997). The presence of several crested bodies is not commonly described  
17 in “basal” cestodes. Thus, *Nomimoscolex* sp., presents a pattern only found in Cyclophyllidea.  
18 This feature could be an interesting character to demonstrate the close relationship between  
19 Proteocephalidea and Cyclophyllidea.

20 One of the most interesting characteristics found in the spermatozoon of *B. lafoni* is the  
21 arrangement of tubular structures in its anterior extremity. These cortical microtubules  
22 describe a sub-membranous arc surrounding the first axoneme. This arrangement, commonly  
23 named arc-like row of cortical microtubules, has been reported in several orders of Eucestoda.  
24 These are the Caryophyllidea (Arafa and Hamada 2004, Gamil 2008, Bruňanská 2009,  
25 Bruňanská and Kostič 2011, Yoneva et al. 2011), the Spathebothriidea (Bruňanská et al. 2006,  
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1 Bruňanská and Poddubnaya 2010), the Trypanorhyncha (Miquel and Świderski 2006, Miquel  
2 et al. 2007a, Marigo et al. 2011d), the Bothriocephalidea (Bâ et al. 2007), the  
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4 Diphyllbothridea (Justine 1986; Levron et al. 2006a, 2009, 2011), the Tetraphyllidea  
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6 (Marigo et al. 2011c), the Proteocephalidea (Bâ and Marchand 1994; Sène et al. 1997;  
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8 Bruňanská et al. 2003a,c, 2004a,b) and the Cyclophyllidea-Mesocestoididae (Miquel et al.  
9  
10 1999, 2007b). However, in *Nomimoscolex* sp. (Sène et al. 1997), due to the presence of three  
11  
12 helical crested bodies, this arc-like row is divided into two separated arcs. Moreover, in most  
13  
14 bothriocephalideans a complete ring of cortical microtubules replaces this arc-like row of  
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16 cortical microtubules (Świderski and Mokhtar-Maamouri 1980; Levron et al. 2005, 2006a,c;  
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18 Bruňanská et al. 2002, 2010; Šípková et al. 2010, 2011; Marigo et al. 2011a).

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24 In most cases, the cortical microtubules forming the arc-like row or the ring show a different  
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26 aspect in comparison to those present in posterior areas of the male gamete. The microtubules  
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28 forming the arc-like row or ring are thicker than microtubules in posterior areas of the  
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30 spermatozoon and therefore two types of cortical microtubules coexist in the male cell. These  
31  
32 two types are reported in all the species presenting an arc-like row or ring of cortical  
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34 microtubules, except in the caryophyllideans. In these species presenting two types of cortical  
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36 microtubules, the thick cortical microtubules are limited to the anterior region of the sperm  
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38 cell, whereas the thin ones occur after the appearance of the second axoneme, if the  
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40 spermatozoon exhibits two axonemes or in the posterior regions if the spermatozoon presents  
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42 only one axoneme.  
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49 The disposition and aspect of the nucleus are variable among the Proteocephalidea. These  
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51 facts have been reviewed by Bruňanská (2010). Thus, in *E. malopteruri* and *Nomimoscolex*  
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53 sp. (Sène et al. 1997, Bruňanská et al. 2004b) the nucleus appears before the second axoneme.  
54  
55 In *B. lafoni* as in *Nomimoscolex* sp. (Sène et al. 1997) the nucleus is rod-shaped and localised  
56  
57 between the axonemes. In *E. malopteruri*, *C. solidum* and *S. sandoni* (Bâ and Marchand 1994;  
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1 Bruňanská et al. 2004a,b) the nucleus is roughly circular and situated at the periphery of the  
2 cell. In *P. torulosus* and *P. longicollis* (Bruňanská et al. 2003a,c), the nucleus is initially  
3 circular and located between the axonemes, and posteriorly, it becomes horseshoe-shaped.  
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7 The posterior extremity of the spermatozoon in the proteocephalidean species generally  
8 shows the disorganisation of one of the axonemes (Bâ et al. 1994; Sène et al. 1997; Bruňanská  
9 et al. 2003a,c, 2004a,b). In *B. lafoni*, the posterior tip of the spermatozoon shows some  
10 cortical microtubules accompanied by some granules of glycogen. This type of posterior  
11 spermatozoon extremity is described for the first time in the Proteocephalidea.  
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## 10 Legends to figures

11 **Fig.1 A–F.** Spermiogenesis in *Barsonella lafoni*. (A) Longitudinal section of a zone of  
12 differentiation in the early stage of spermiogenesis showing the presence of a centriole (C),  
13 the nucleus (N), a striated rootlet (SR) and the dense material (DM). Scale bar = 0.5 µm. (B)  
14 Another longitudinal section of a zone of differentiation showing the growth of the two  
15 flagella (F). DM, dense material; IB, intercentriolar body. Scale bar = 0.5 µm. (C)  
16 Longitudinal section of a zone of differentiation during the flagellar rotation of the two  
17 flagella (F). DM, dense material; N, nucleus. Scale bar = 0.5 µm. (D) Longitudinal section of  
18 a zone of differentiation during the rotation of both flagella (F) showing their asynchronous  
19 growth. MCP, median cytoplasmic process; N, nucleus. Scale bar = 0.5 µm. (E) Another  
20 longitudinal section of a zone of differentiation during flagellar rotation showing the  
21 asynchronous rotation of the flagella (F). MCP, median cytoplasmic process; SR, striated  
22 rootlet. Scale bar = 0.5 µm. (F) Detail showing two striated rootlets (SR) associated to the  
23 same centriole (C). Scale bar = 0.3 µm.  
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46 **Fig. 2 A–D.** Spermiogenesis in *Barsonella lafoni*. (A) Longitudinal section of a zone of  
47 differentiation with two parallel flagella (F). Note the difference of length between the two  
48 flagella (F). AM, arched membranes; CM, cortical microtubules; MCP, median cytoplasmic  
49 process; SR, striated rootlet. Scale bar = 1 µm. (B) Several cross-sections of spermatids after  
50 proximodistal fusion showing the nucleus (N) and different types of cortical microtubules  
51 (CM). Scale bar = 0.3 µm. (C) Longitudinal section of a spermatid showing the presence of a  
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1 striated rootlet (SR) in the late stage of spermiogenesis. AM, arched membranes; Ax,  
2 axoneme. Scale bar = 1  $\mu\text{m}$ . (D) Longitudinal section of a spermatid in the final stage of  
3 spermiogenesis. AM, arched membrane; Ax, axoneme; CM, cortical microtubules. Scale bar  
4 = 0.5  $\mu\text{m}$ .  
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11 **Fig. 3 A–E.** Diagram showing the main stages of spermiogenesis in *Barsonella lafoni*. (A)  
12 Early stage of spermiogenesis showing the growth of the two flagella. (B) Stage of  
13 spermiogenesis showing the asynchronous rotation of the two free flagella. (C) Stage of  
14 spermiogenesis before the proximodistal fusion of the two flagella, (D) Stage of  
15 spermiogenesis after the proximodistal fusion of the two flagella and showing the migration  
16 of nucleus. (E) Final stage of spermiogenesis.  
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26 AM, arched membranes; Ax1, first axoneme; Ax2, second axoneme; C1, first centriole; C2,  
27 second centriole; CM, cortical microtubules; DM, dense material; F1, first flagellum; F2,  
28 second flagellum; IB, intercentriolar body; MCP, median cytoplasmic process; N, nucleus;  
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40 **Fig. 4 A–J.** Mature spermatozoon of *Barsonella lafoni*. (A) Longitudinal section of the  
41 anterior part of the spermatozoon showing the apical cone surrounded by the crested body  
42 (CB). C1, first centriole. Scale bar = 1  $\mu\text{m}$ . (B) Longitudinal section showing the anterior  
43 spermatozoon extremity (ASE). AC, apical cone; CB, crested body. Scale bar = 0.5  $\mu\text{m}$ . (C–  
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1 (CB) (arrowhead). Scale bar = 0.5  $\mu\text{m}$ . (H) Longitudinal section showing the presence of both  
2 axonemes (Ax1 and Ax2). Note the appearance of the second axoneme (arrowhead) and the  
3  
4 granules of glycogen (G). Scale bar = 0.5  $\mu\text{m}$ . (I) Cross-section at the level of arrowhead on  
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6 figure H. Scale bar = 0.3  $\mu\text{m}$ . (J) Cross-sections of region II showing both axonemes, thin  
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8 cortical microtubules (CM) and granules of glycogen (G). Scale bar = 0.3  $\mu\text{m}$ .  
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14 **Fig. 5 A–E.** Mature spermatozoon of *Barsonella lafoni*. (A) Longitudinal section of the  
15 transition area between regions II and III. G, granules of glycogen; N, nucleus. Scale bar = 0.5  
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17  $\mu\text{m}$ . (B) Two cross-sections at the nuclear area showing the increase of the nucleus (N)  
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19 diameter towards the posterior end of this region. Note the disorganisation of one of the  
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21 axonemes in the nuclear region (arrowhead). CM, cortical microtubules; G, granules of  
22  
23 glycogen. Scale bar = 0.3  $\mu\text{m}$ . (C,D) Consecutive cross-sections of the nuclear area of region  
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25 IV showing the gradual reduction of glycogen granules (G) and cortical microtubules (CM),  
26  
27 and the disappearance of the nucleus (N) in figure D. Scale bars = 0.3  $\mu\text{m}$ . (E–G) Cross and  
28  
29 longitudinal sections of the posterior area of the male gamete. Note the progressive decrease  
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31 of the glycogen amount (G) toward the posterior spermatozoon extremity (PSE) and the  
32  
33 presence of cortical microtubules (CM) at the posterior tip. Scale bars = 0.5  $\mu\text{m}$ , 0.3  $\mu\text{m}$ , 0.3  
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35  $\mu\text{m}$ , respectively.  
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46 **Fig. 6 I–IV.** Schematic reconstruction of the mature spermatozoon of *Barsonella lafoni*. To  
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48 simplify the diagram, the granules of glycogen are not shown in the longitudinal section. (I)  
49  
50 Anterior region of the mature spermatozoon showing the apical cone and the crested body.  
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52 (II) Second region of the mature spermatozoon showing the presence of the second axoneme.  
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54 (III) Nuclear region of the mature spermatozoon. (IV) Posterior region of the mature  
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56 spermatozoon.  
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1 AC, apical cone; ASE, anterior spermatozoon extremity; Ax1, first axoneme; Ax2, second  
2 axoneme; C1, first centriole; C2, second centriole; CB, crested body; CM, cortical  
3 microtubules; D, doublets; G, granules of glycogen; N, nucleus; PM, plasma membrane; PSE,  
4 posterior spermatozoon extremity.  
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11 **Fig. 7.** Cross-sections of the spermatozoon of *Barsonella lafoni* showing the presence of  
12 glycogen evidenced by the method of Thiéry (1967). G, granules of glycogen. Scale bar = 0.3  
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Family, subfamily and species References	Spermiogenesis							Spermatozoon						
	Type	IB	DM	SR	FR	PF	AxN	Type	AC	CB N T	ArcCM	AxN	G	PSE
<b>Monticelliidae</b>														
<b>Zygobothriinae</b>														
<i>Nomimoscolex</i> sp. Sène et al. (1997)	I	1	-	+	+	+	2	II	+	3 80	+	2	+	Ax
<b>Proteocephalidae</b>														
<b>Corallobothriinae</b>														
<i>Corallobothrium solidum</i> Bruňanská et al. (2004a, 2005)	I	1	-	+	+	+	2	II	-	1 30-200	+	2	+	Ax
<b>Gangesiinae</b>														
<i>Electrotaenia malopteruri</i> Bruňanská et al. (2004b)								II	-	1 60-150	+	2	+	Ax
<b>Proteocephalinae</b>														
<i>Barsonella lafoni</i> Present study	I	1	+	+	+	+	2	II	+	1 60-90	+	2	+	CM
<i>Proteocephalus longicollis</i> Świderski (1985, 1996) Bruňanská et al. (2003a, 2004c)	I	5 or 1	-	+	+	+	2	II	-	1 60-100	+	2	+	Ax
<i>Proteocephalus torulosus</i> Bruňanská et al. (2003b, 2003c)	I	1	-	+	+	+	2	II	-	1 80-100	+	2	+	Ax
<b>Sandonellinae</b>														
<i>Sandonella sandoni</i> Bâ and Marchand (1994)								IV	+	1 50-100	+	1	+	Ax

Table 1: Spermatological characters in the proteocephalidean cestodes.

Spermiogenesis characters: AxN = number of axonemes; DM = dense material; FR = flagellar rotation; IB = number of plates of intercentriolar body; PF = proximodistal fusion; SR = striated rootlets.

Spermatozoon characters: AC = apical cone; ArcCM = arc of cortical microtubules; AxN = number of axonemes; CB = crested body (N = number and T = thickness in nm); CM = cortical microtubules; G = glycogen; PSE = posterior spermatozoon extremity.  
+/-: presence/absence of character. Spermiogenesis types are considered according to Bâ and Marchand (1995). Spermatozoa types are considered according to Levron et al. (2010).

Figure 1  
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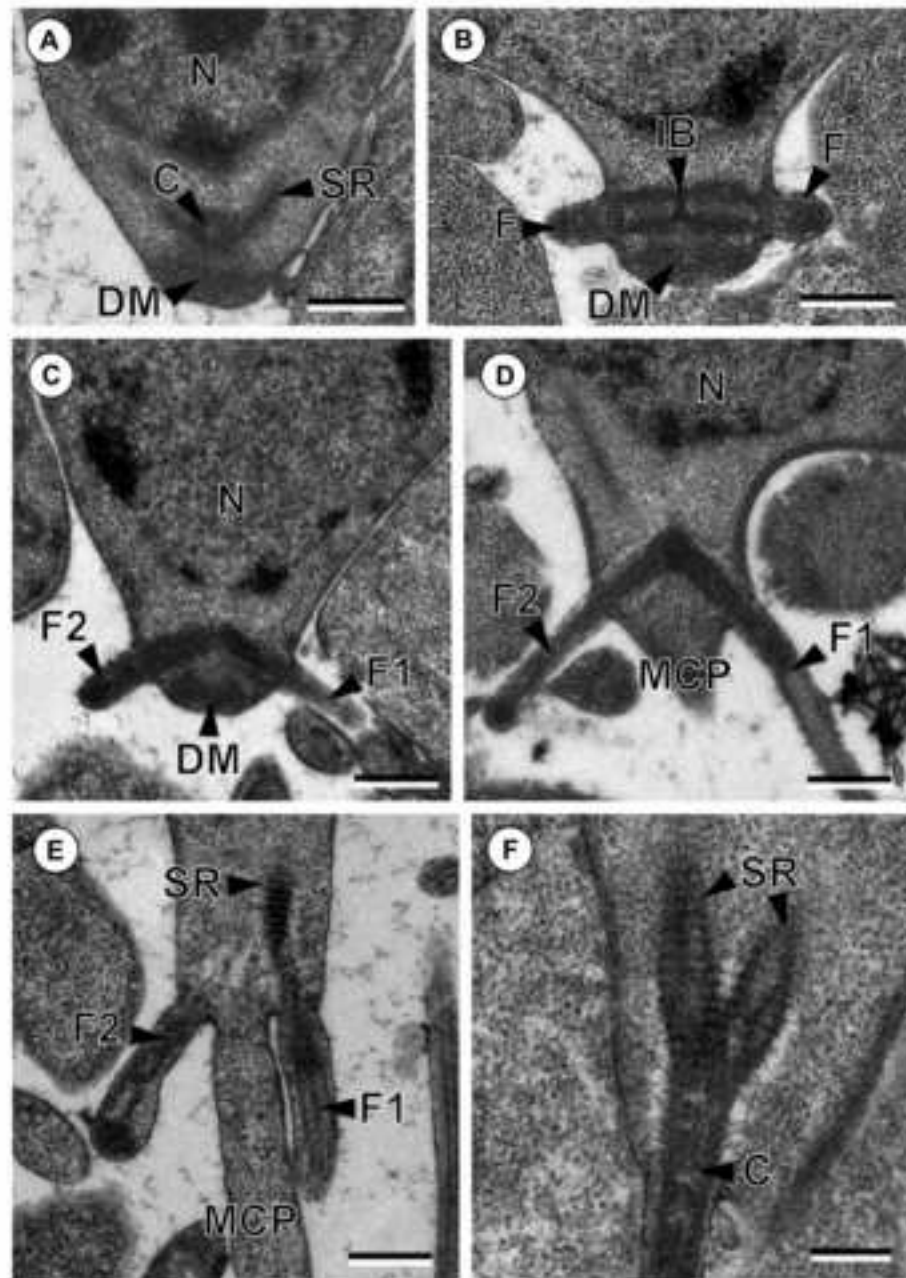


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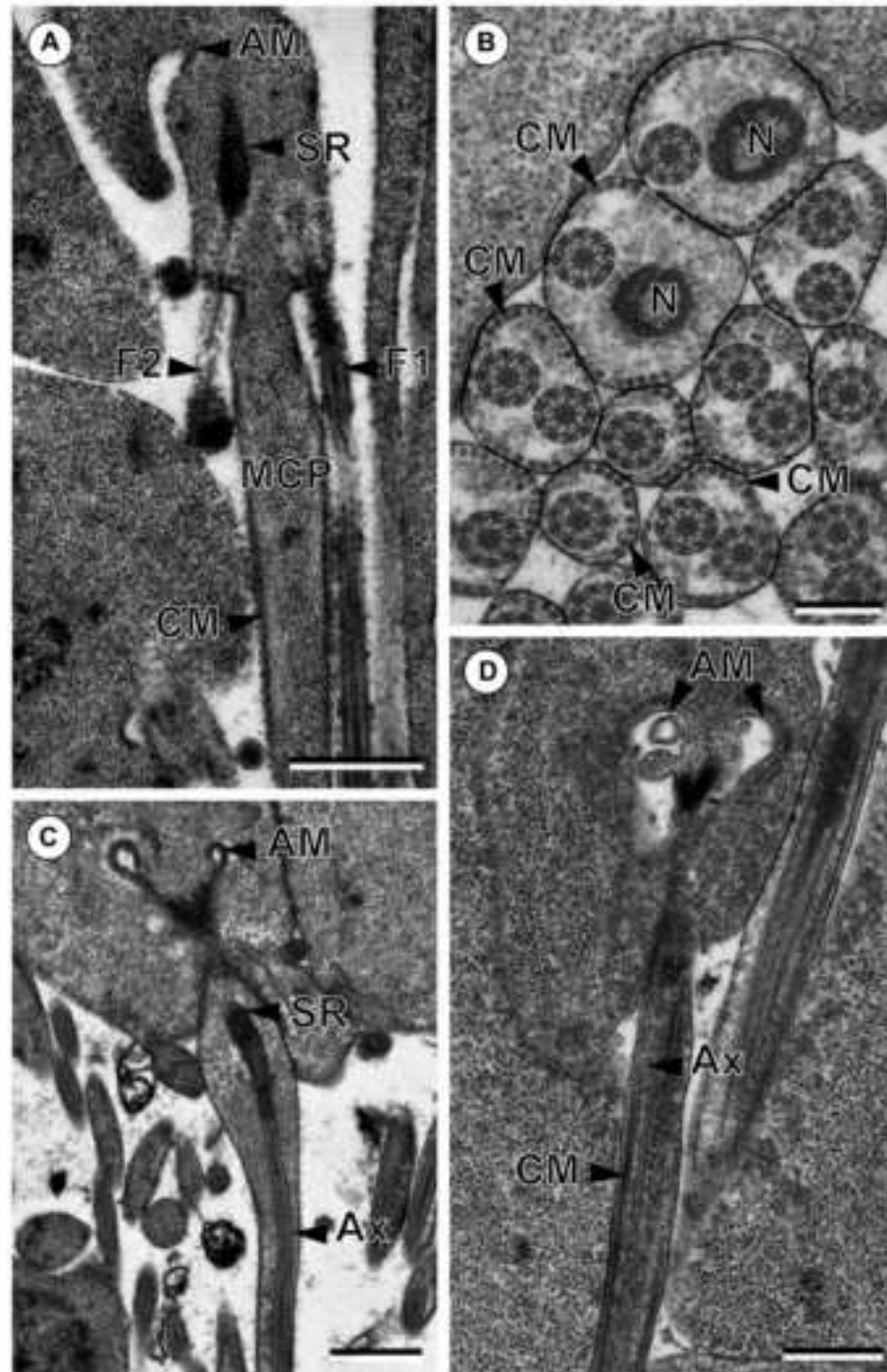


Figure 3

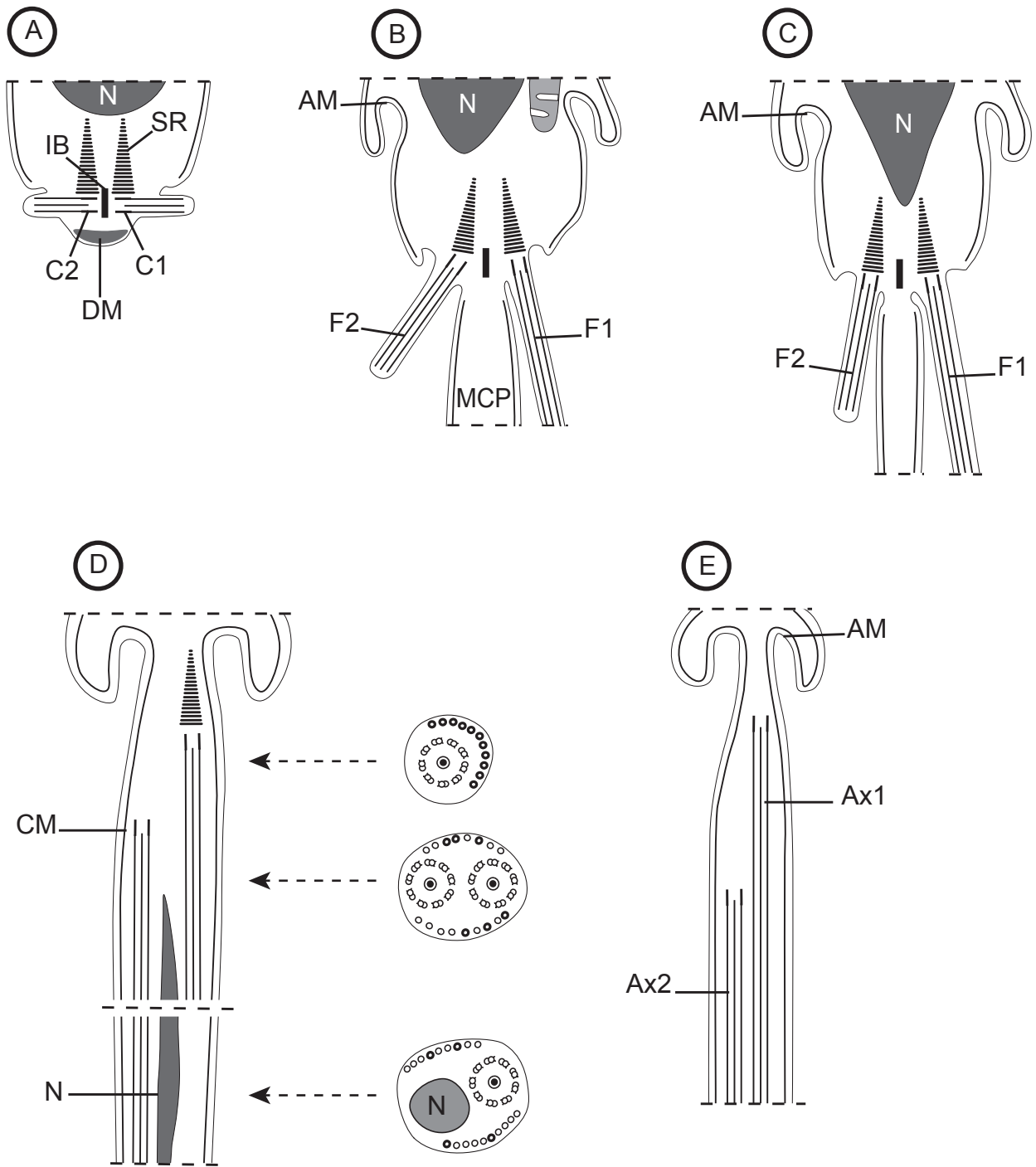


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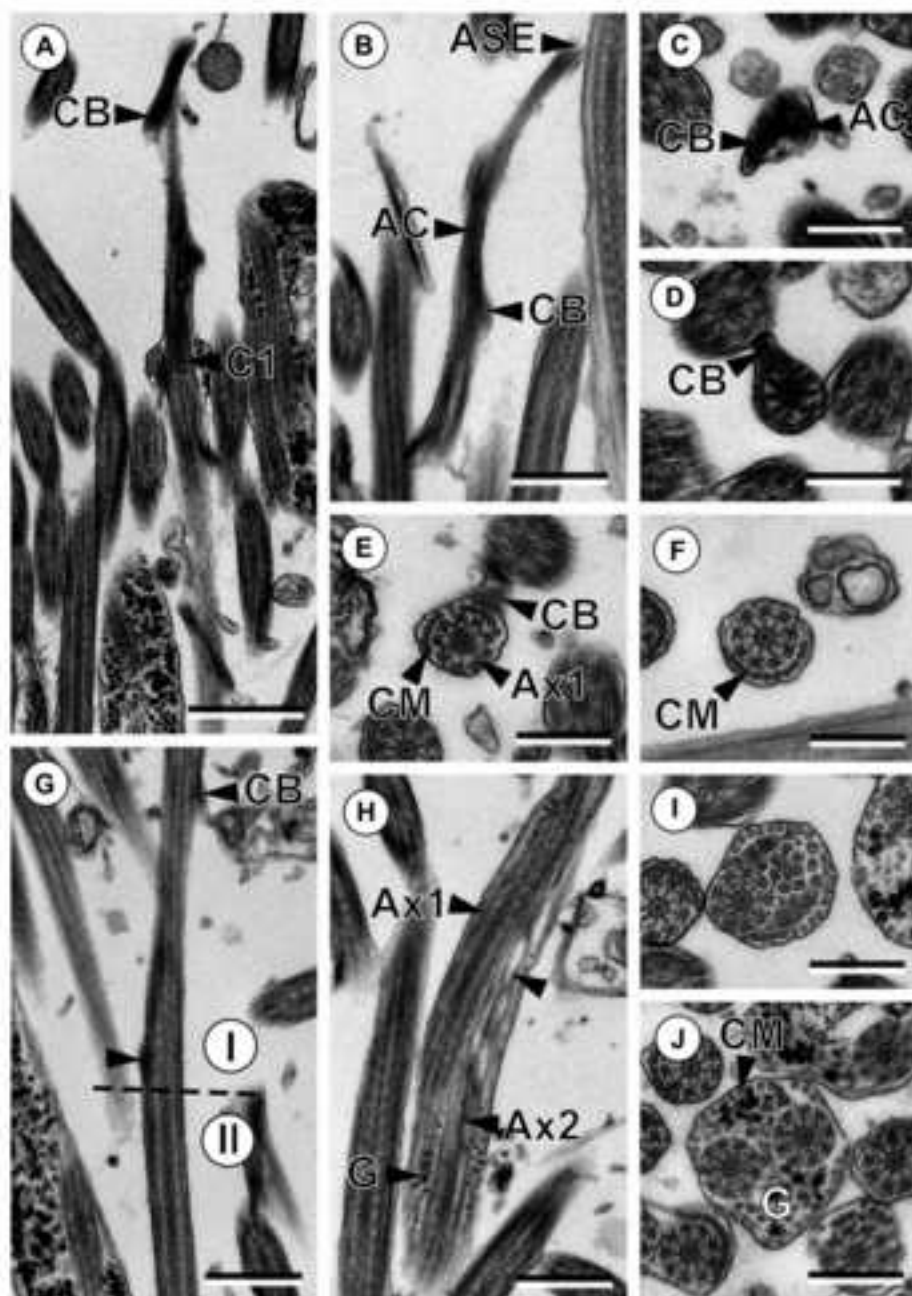




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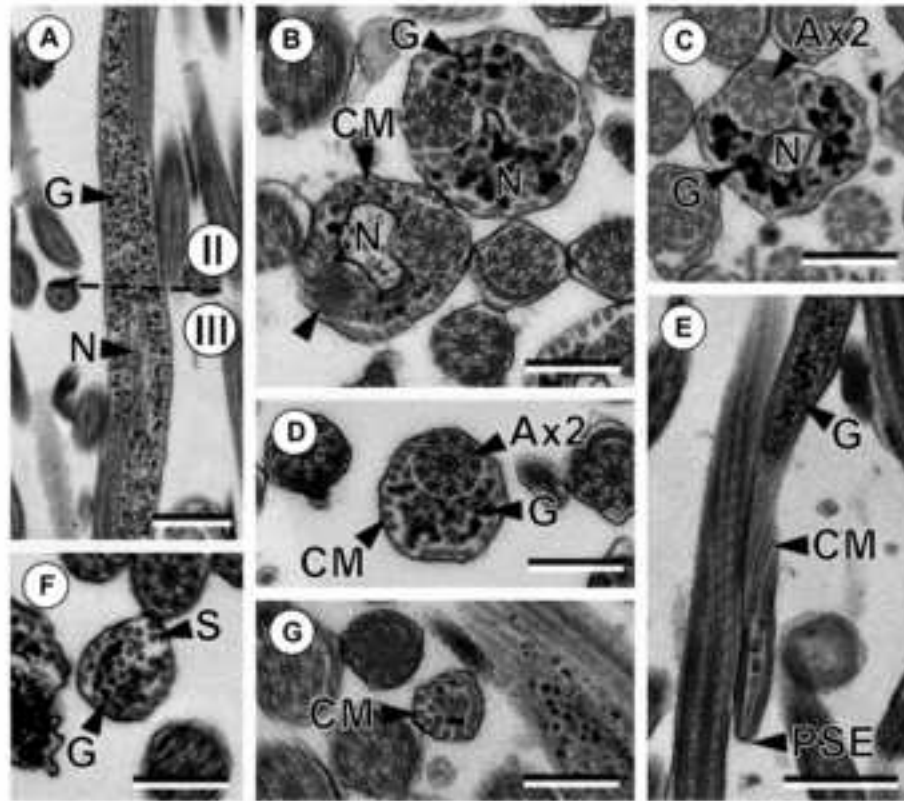
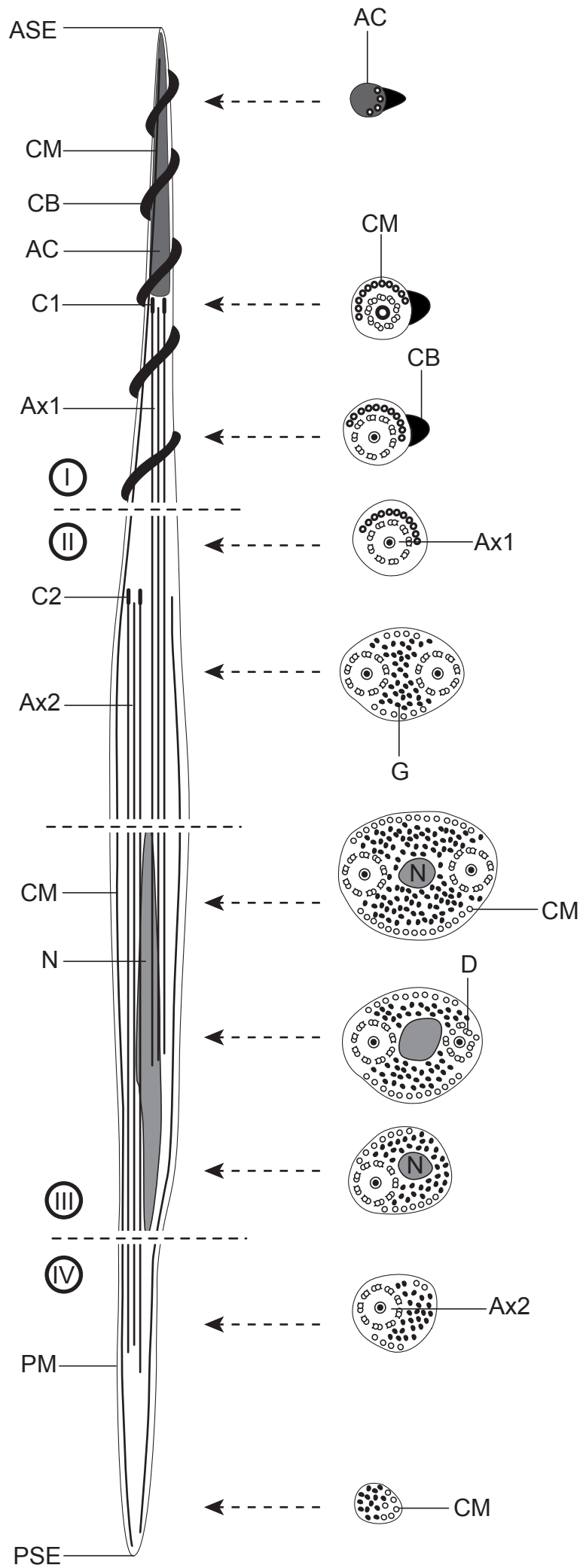




Figure 6



**Figure 7**  
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