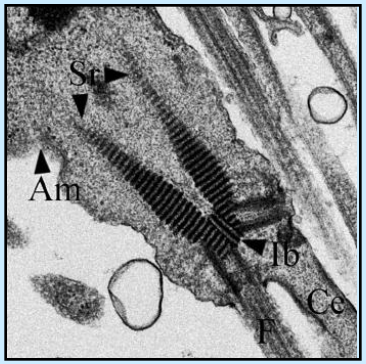

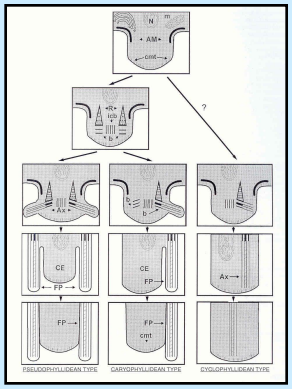

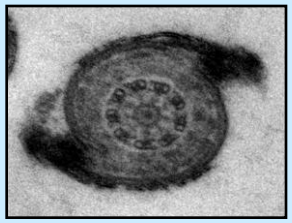

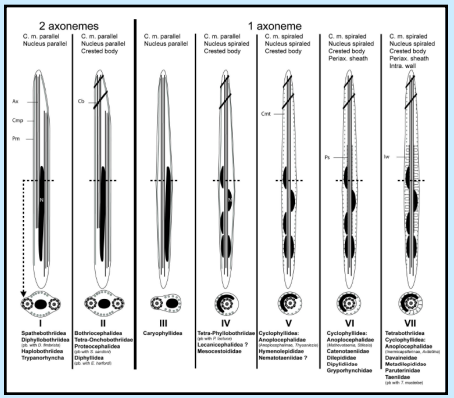

ULTRASTRUCTURE OF THE SPERMATOZOON IN THE CYCLOPHYLLIDEAN CESTODES

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IRBio, Fac. Biologia, Univ. Barcelona

Ultrastructure of the spermatozoon in the Platyhelminthes (flatworms)

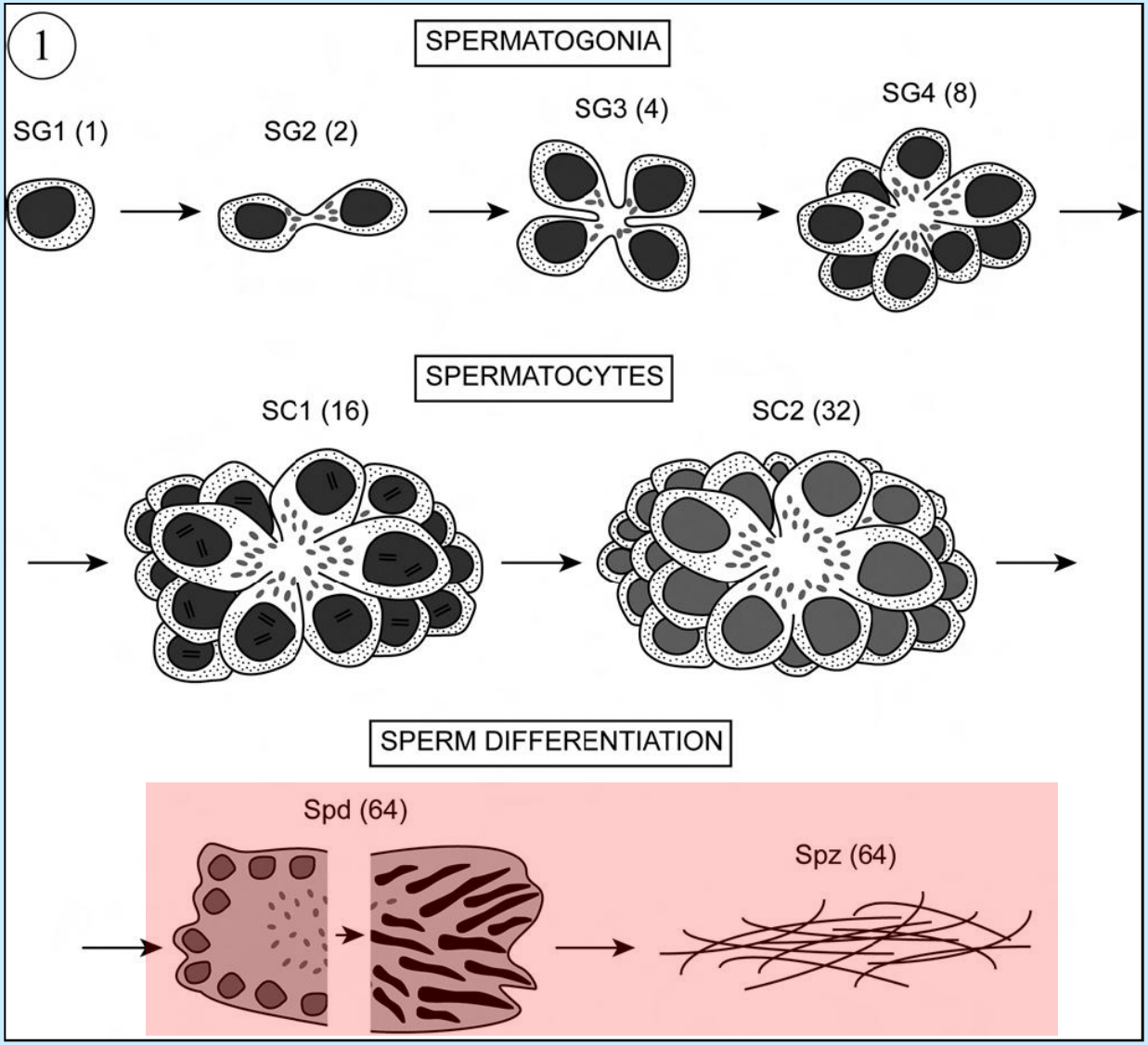
Spermatozoon ultrastructure is suitable for establishing phylogenetic relationships in the Platyhelminthes:

- Number of characters
- Variability
- Not linked to parasitism
- Not influenced by host specificity, host-parasite relationships, life cycle…

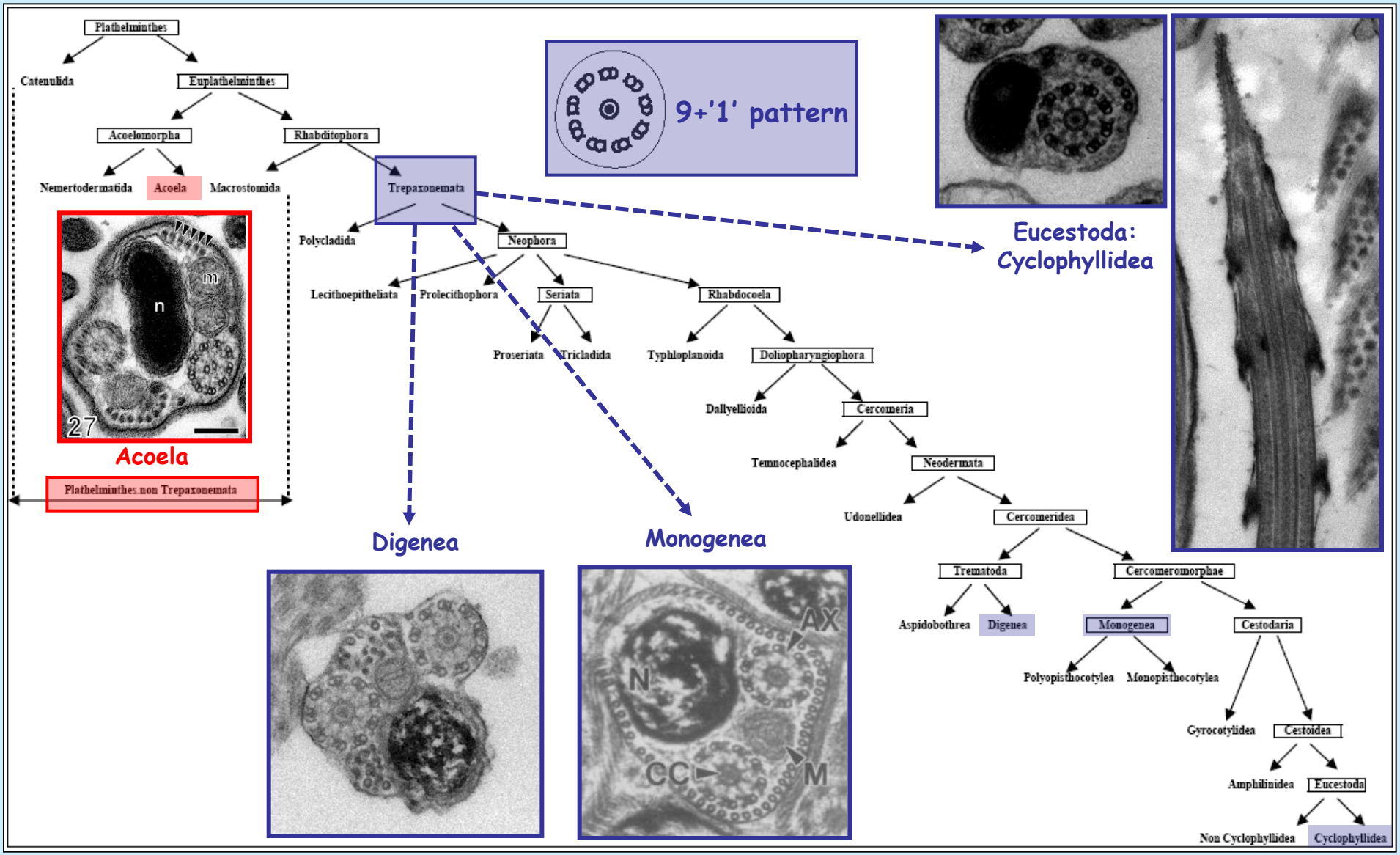
Usefulness of ultrastructural characters of the spermatozoon:

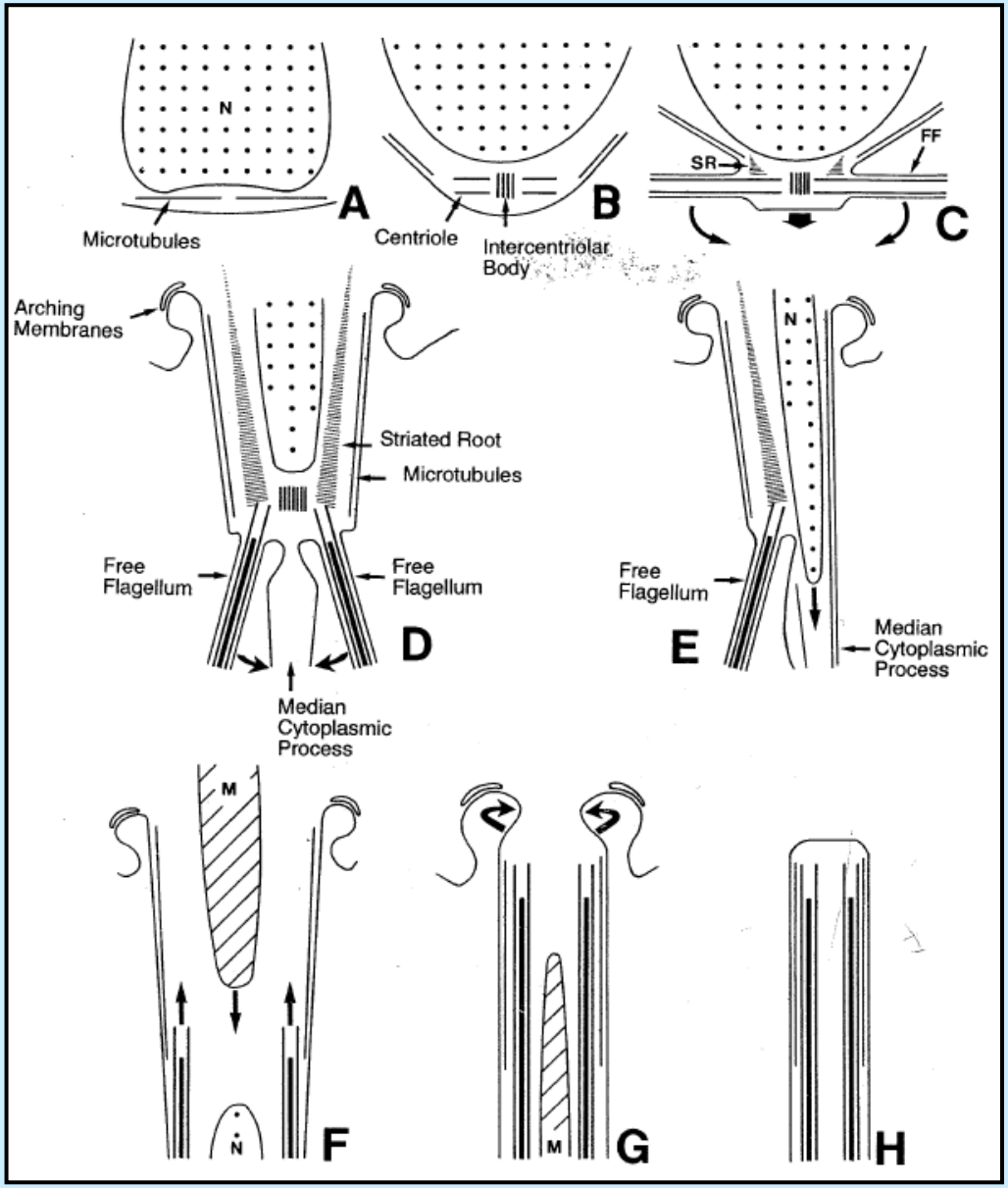
- Ehlers (1984): "Trepaxonemata"
- Świderski (1986): Cestoda
- Justine (1991, 1998, 2001): Platyhelminthes
- Bâ & Marchand (1995): Cestoda
- Hoberg *et al.* (1997): Eucestoda
- Levron *et al.* (2010): Eucestoda

Spermatogenesis

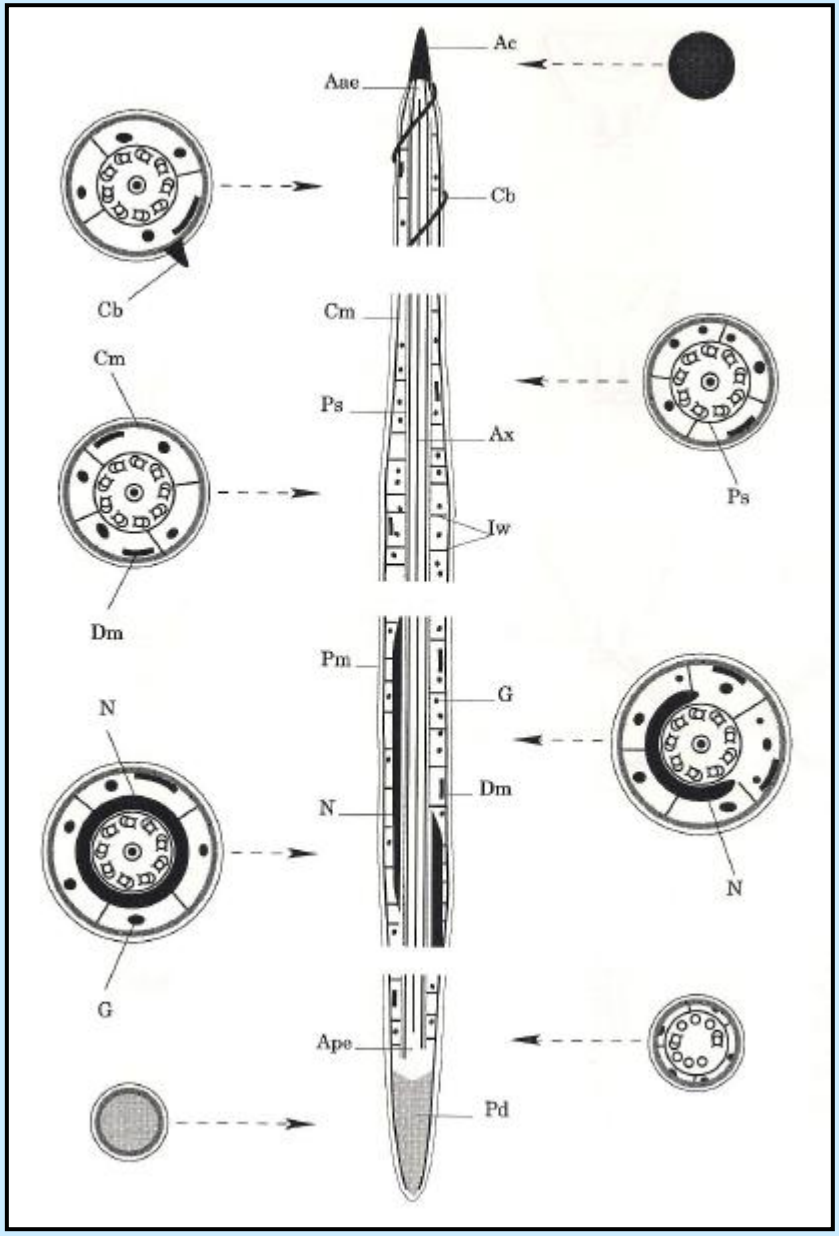


Ehlers (1984): Trepaxonemata ("axoneme in spiral")





(Justine 1991)



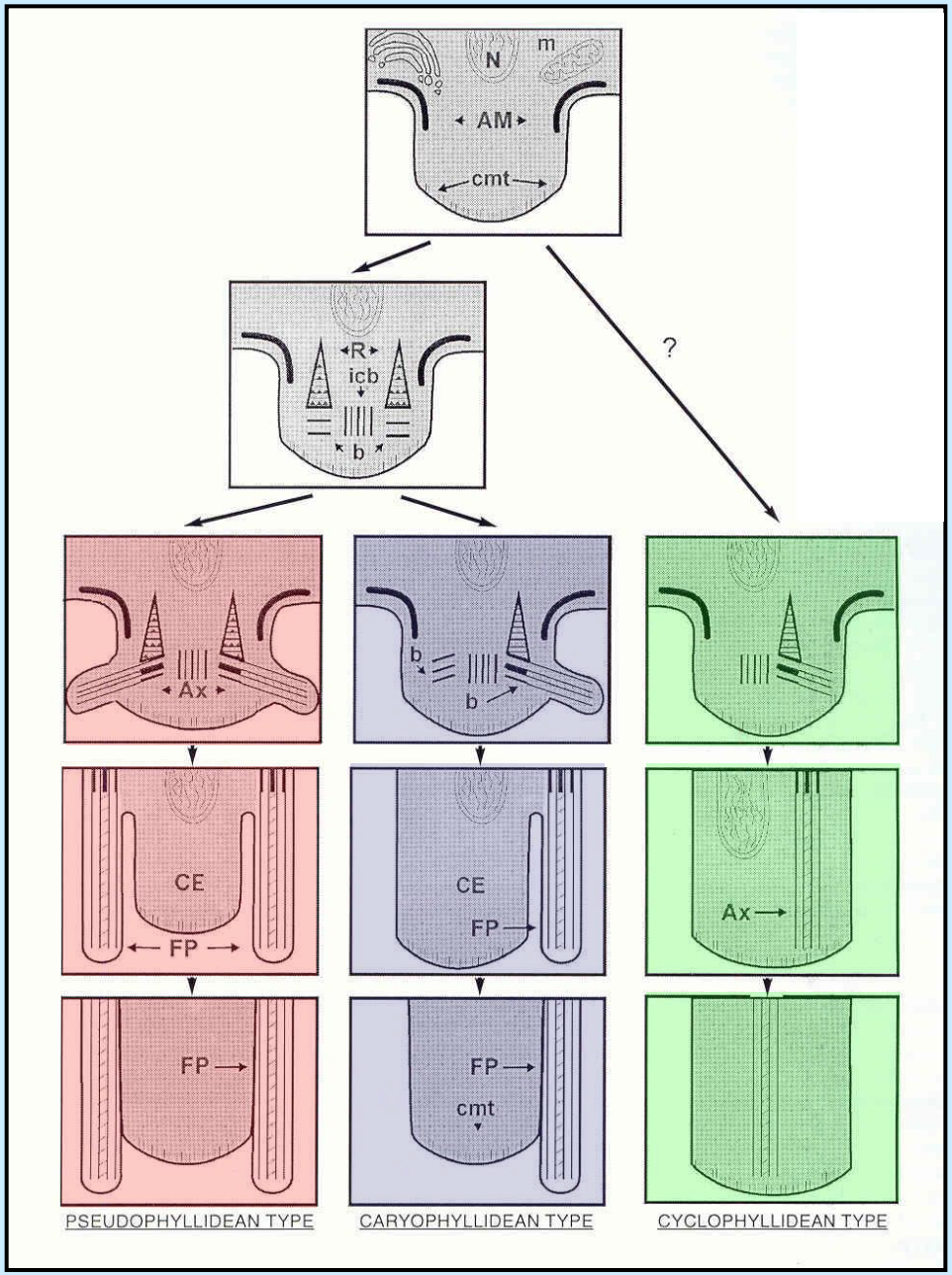
(Bâ & Marchand 1995)

Types of spermiogenesis (Świderski 1986):

Pseudophyllidean type
Acanthobothrium filicolle benedeni

Caryophyllidean type
Glaridacris catostomi

Cyclophyllidean type
Hymenolepis microstoma



Types of spermiogenesis (Bâ & Marchand 1995):

Type 1:

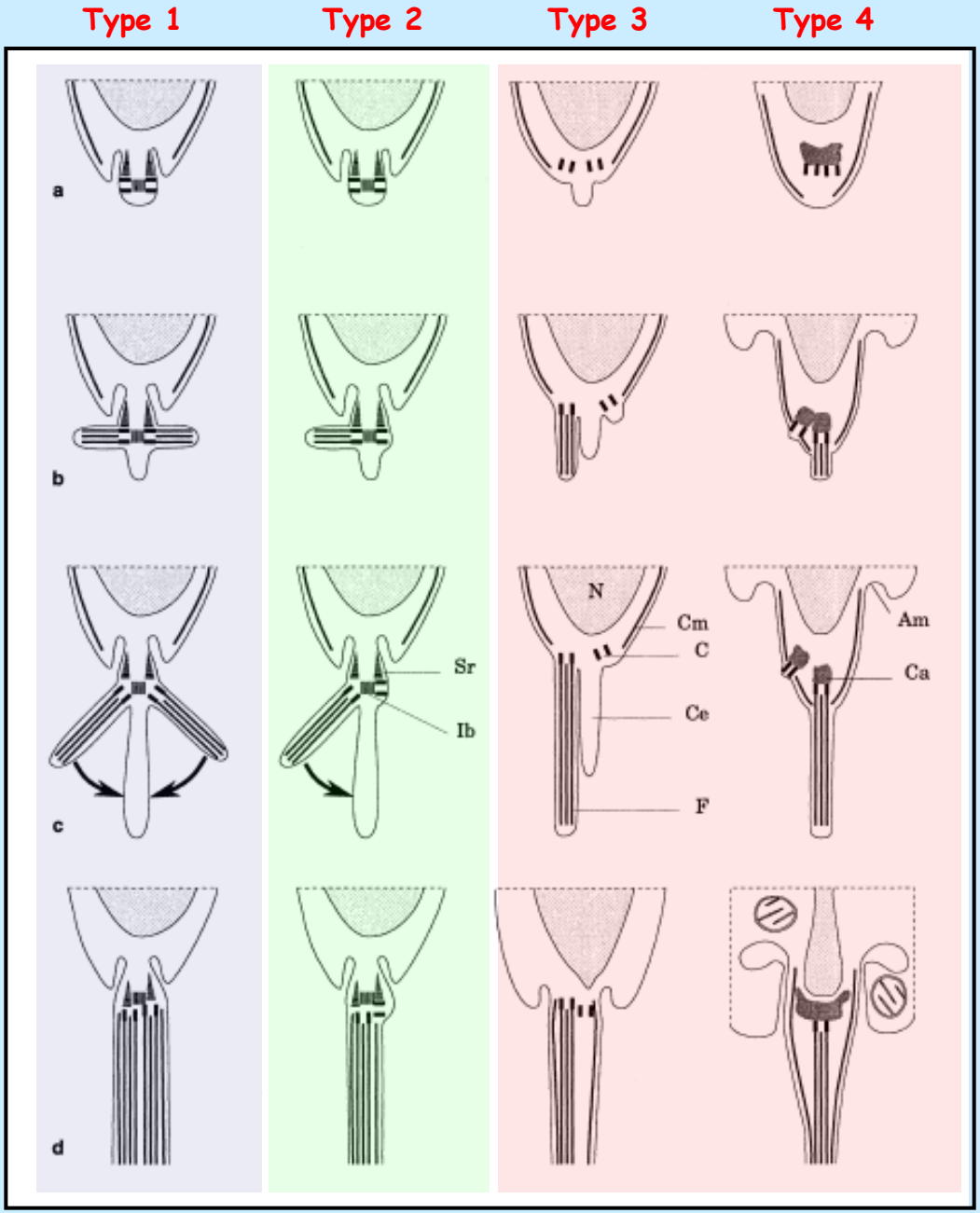
Spathebothriidea
"Pseudophyllidea"
Haplobothriidea
Diphyllidea?
Trypanorhyncha
Tetraphyllidea-Onchobothriidae
Proteocephalidea

Type 2:

Caryophyllidea
Tetraphyllidea-Phyllobothriidae?
Tetrabothriidea
Cyclophyllidea-Mesocestoididae

Type 3 and Type 4:

Cyclophyllidea



Phylogenetic hypothesis based on morphological characters including sperm ultrastructure (Hoberg *et al.* 1997)

Spermiogenesis:

- Type
- Striated rootlets
- Intercentriolar body

Spermatozoon:

- Mitochondrion
- Crested bodies
- Cortical microtubules
- Periaxonemal sheath
- Number of axonemes
- Type of cortical microtubules

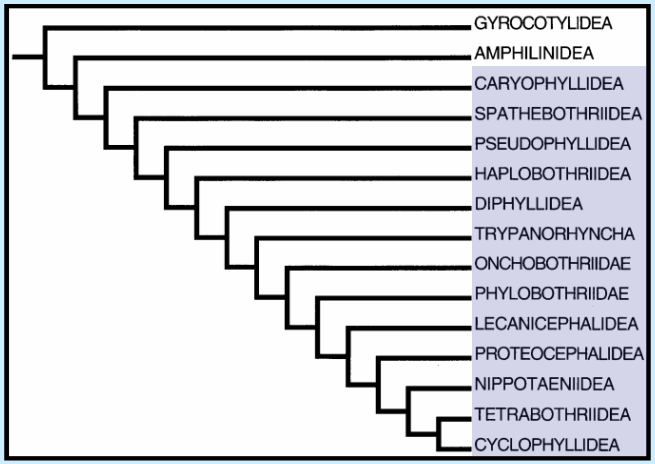


TABLE III. Consistency indices for individual characters used in analysis of the Eucestoda.

Character number	Character	CI
1	Uterus—development	0.75
2	Uterus–gravid	0.80
3	Uterine pore(s)	0.80
4	Uterus—position	1.0
5	Genital pore—position	1.0
6	Genital pore—structure	1.0
7	Holdfast—orientation	0.50
8	Holdfast—structure	0.80
9	Myzorhynchus	1.0
10	Rostellum	1.0
11	Bothridia—margins	0.60
12	Tentacles	0.50
13	Scolex hooks	1.0
14	Apical hooks	1.0
15	Hooks—structure	1.0
16	Scolex—ontogeny	1.0
17	Scolex—structure	0.667
18	Longitudinal musculature	1.0
19	Egg—development	1.0
20	Egg—operculum	0.75
21	Egg—tanning	1.0
22	Embryonic membranes	1.0
23	Protonephridium	1.0
24	Embryophore	0.714
25	Embryogenesis	0.667
26	Hexacanth embyo	1.0
27	Ovary—structure	1.0
28	Vitelline gland—position	0.80
29	Vitelline gland—form	1.0
30	Vitelline gland—ducts	1.0
31	Strobila—external segmentation	1.0
32	Strobila—internal segmentation	1.0
33	Strobila—structure	1.0
34	Testes—position	1.0
35	Cercoid—ontogeny	1.0
36	Cercoid—ontogeny	1.0
37	Metacestode—apical	0.80
38	Metacercoid—form	0.833
39	Sperm—Mitochondrion	1.0
40	Sperm—crested body	1.0
41	Sperm—microtubules	1.0
42	Spermiogenesis	0.857
43	Sperm—periaxonemal sheath	1.0
44	Sperm—axoneme(s)	0.833
45	Ontogeny	1.0
46	Apical organ	1.0
47	Sperm—straited roots	1.0
48	Sperm—intercentriolar body	1.0
49	Sperm—peripheral microtubules	1.0

SPERMIOGENESIS PATTERNS IN THE CYCLOPHYLLIDEA:

Type 2

- *Mesocestoides* spp. (Cyclophyllidea: Mesocestoididae)

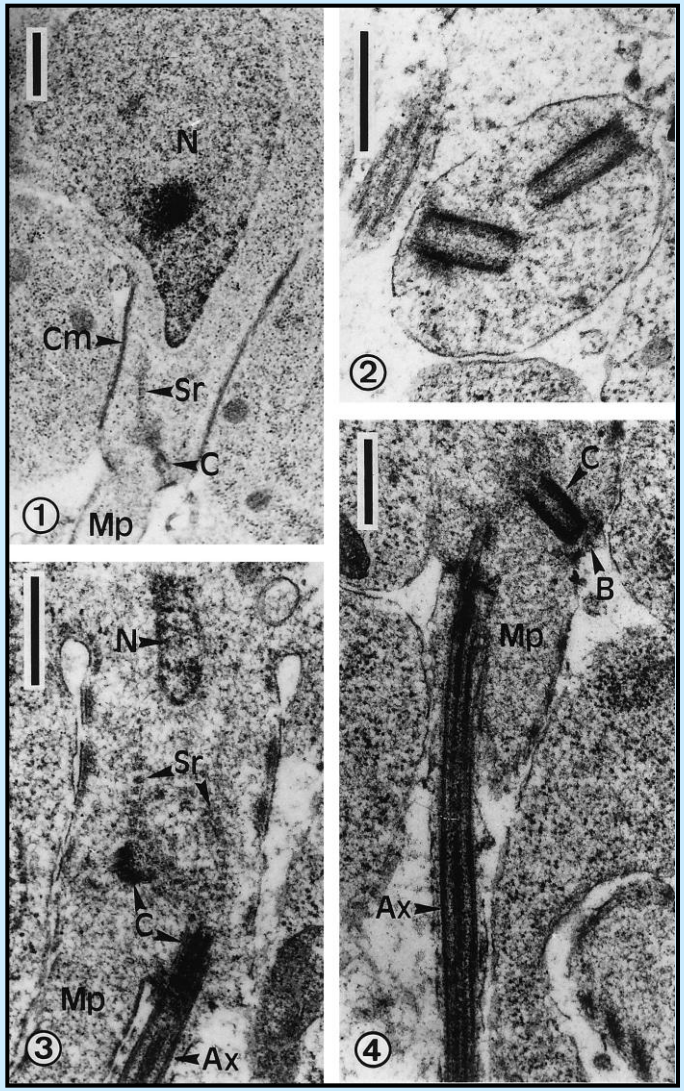
Type 3

- *Dipylidium caninum, Joyeuxiella* spp. (Cyclophyllidea: Dipylidiidae)

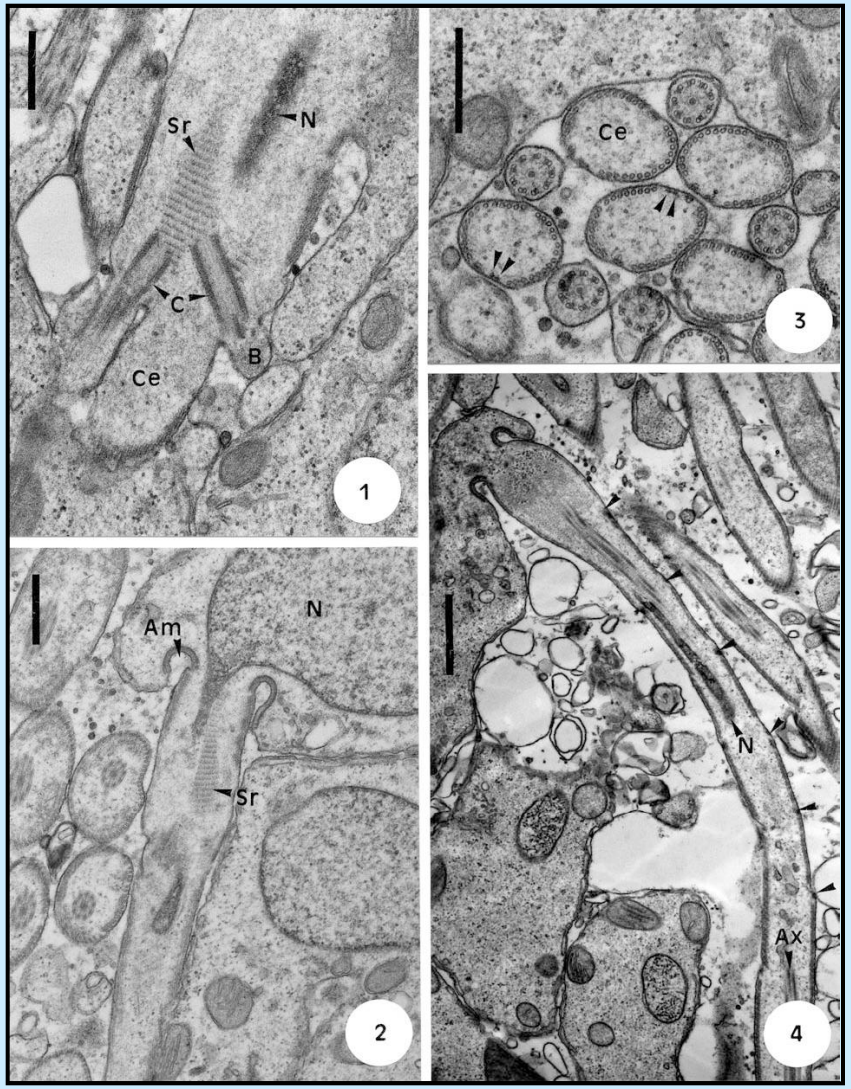
Type 4

- *Anoplocephaloides dentata* (Cyclophyllidea: Anoplocephalidae)

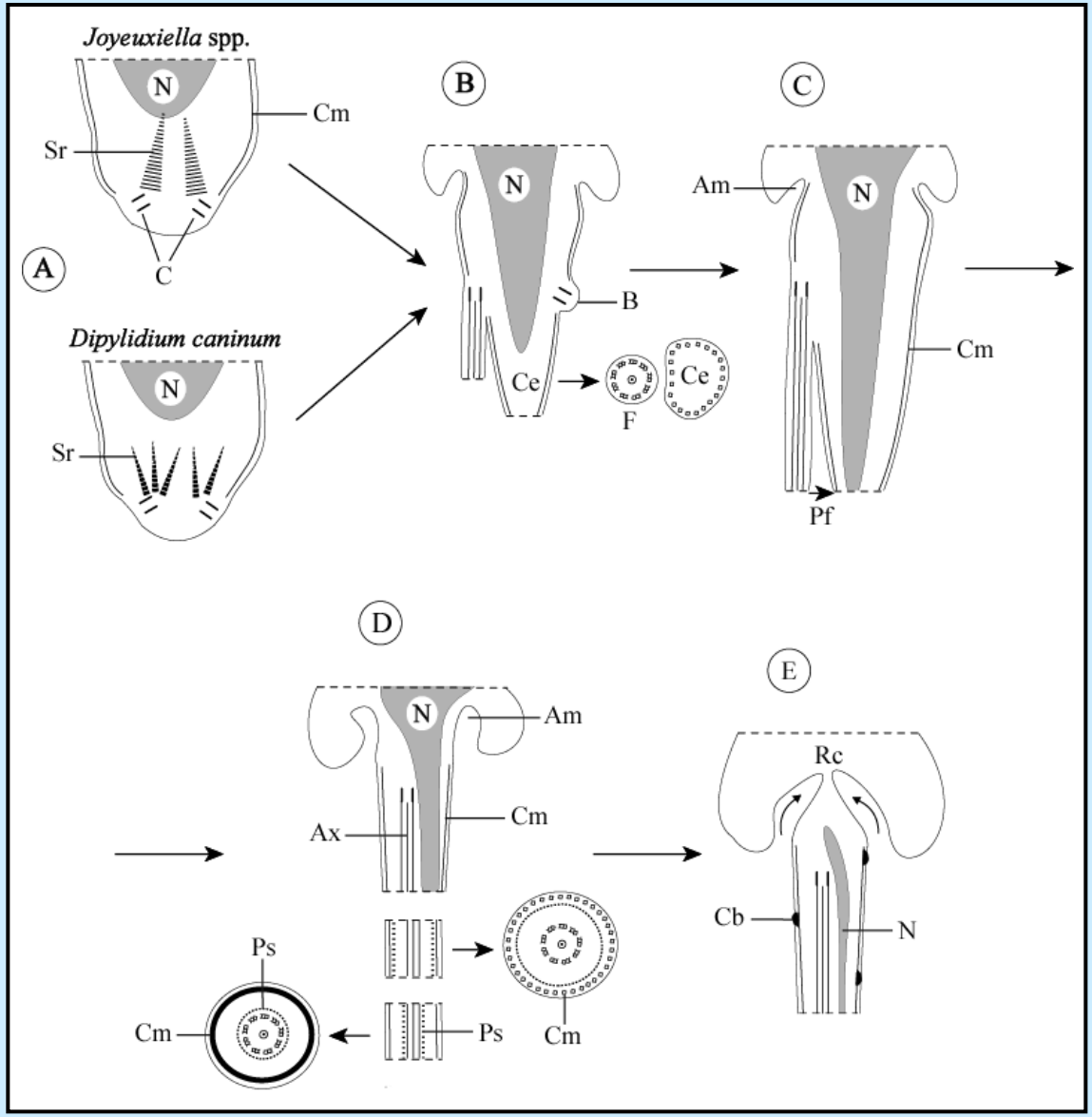
CESTODA SPERMIOGENESIS: Type 3



Dipylidium caninum
(Cyclophyllidea: Dipylidiidae)
(Miquel *et al.* 1998)

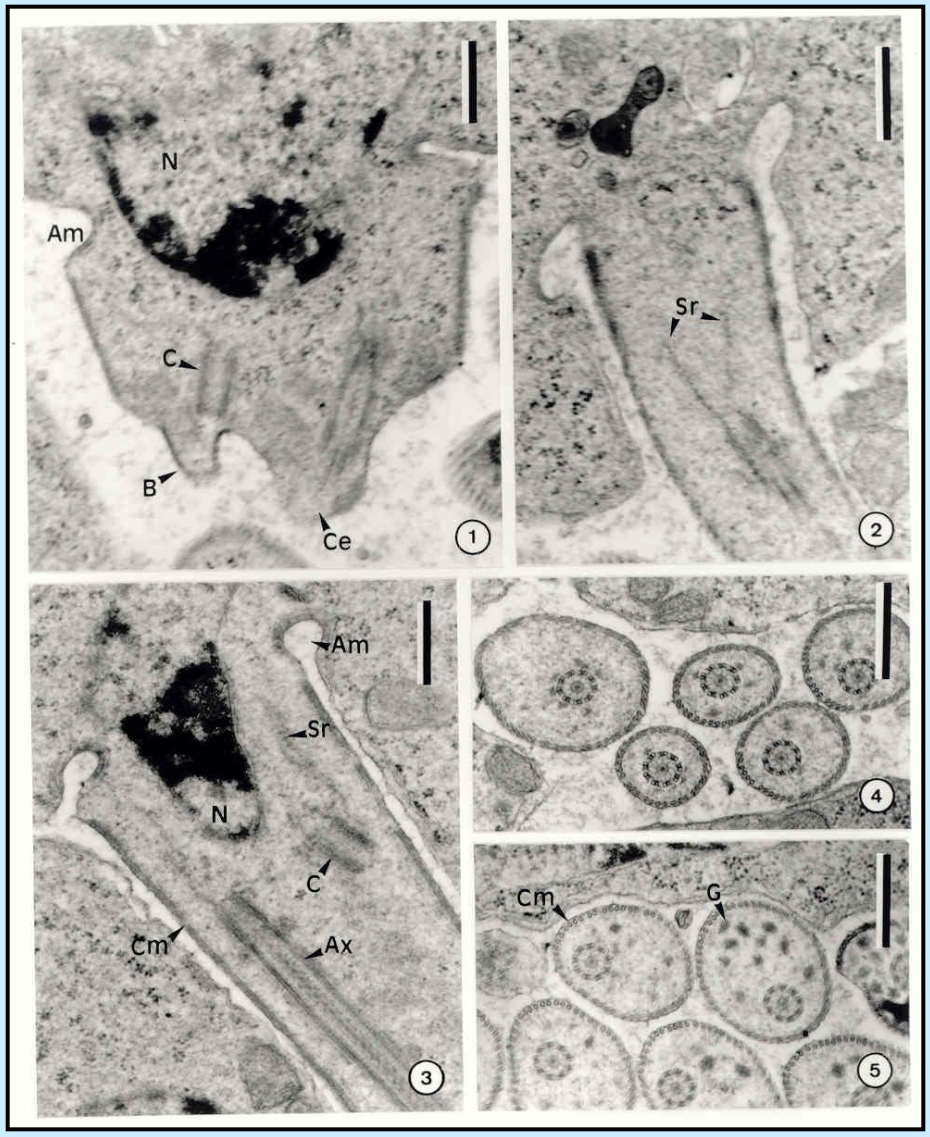


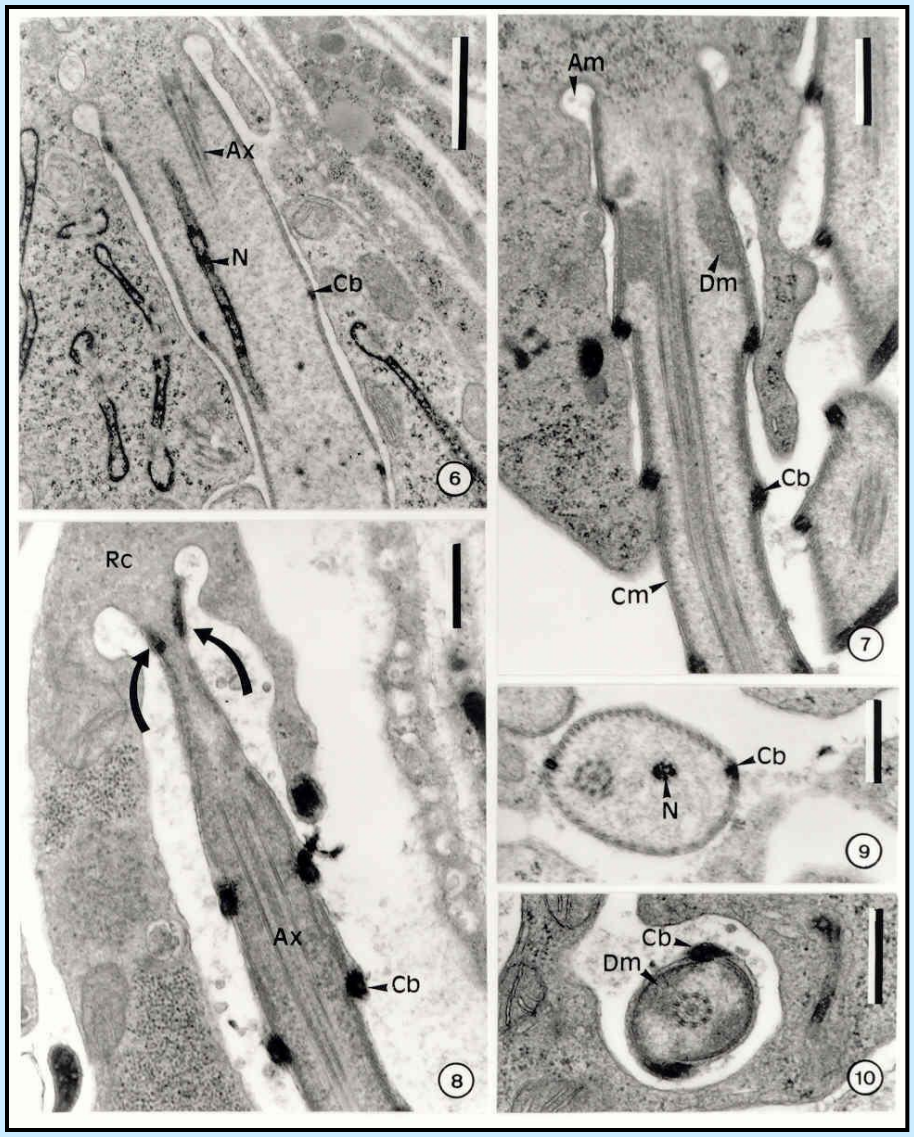
Joyeuxiella pasqualei, J. echinorhyncoides
(Cyclophyllidea: Dipylidiidae)
(Ndiaye *et al.* 2003)



Dipylidium caninum, Joyeuxiella pasqualei, J. echinorhyncoides
(Miquel *et al.* 2005)

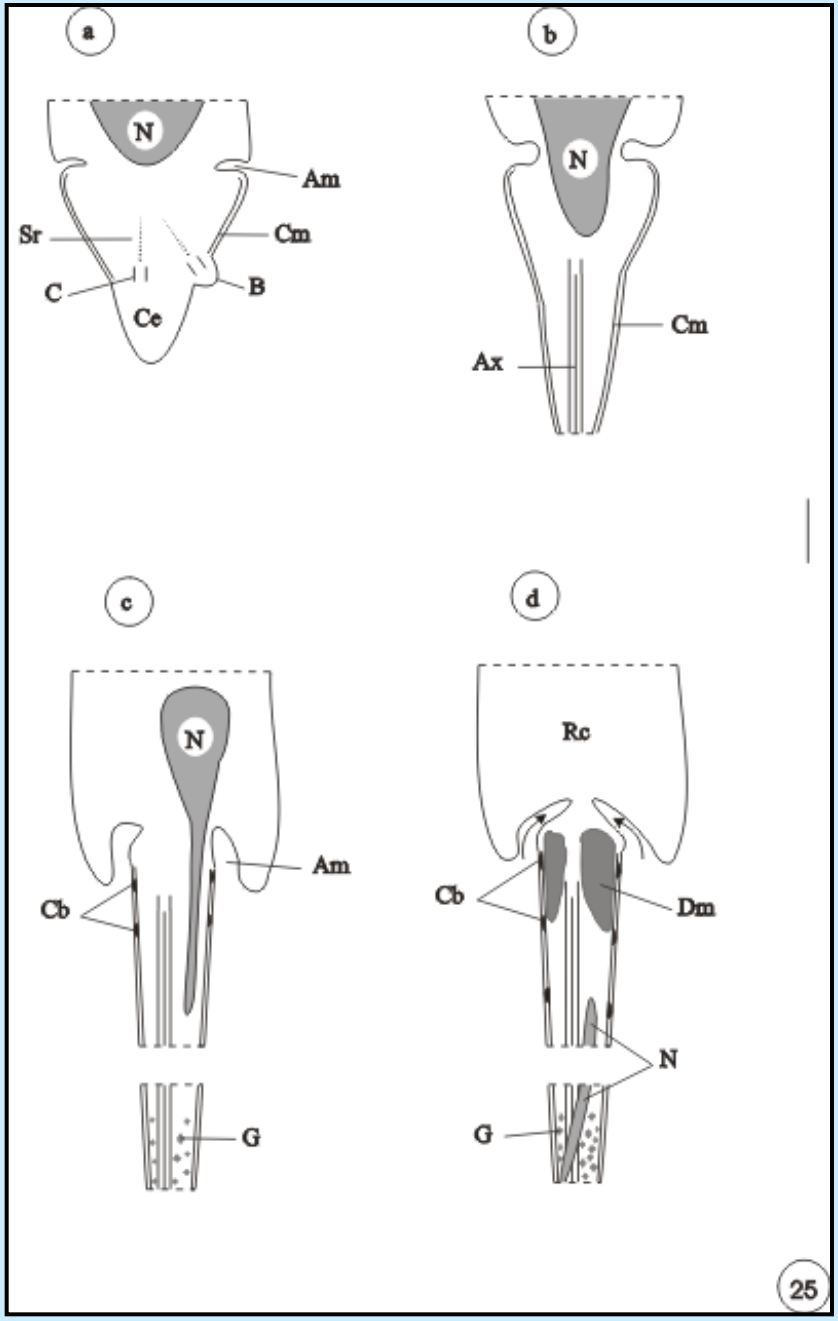
CESTODA SPERMIOGENESIS: Type 4





Anoplocephaloides dentata (Cyclophyllidea: Anoplocephalidae) (Miquel & Marchand 1998)

Anoplocephaloides dentata
(Miquel & Marchand 1998)



Spermatological characters in the Cyclophyllidea

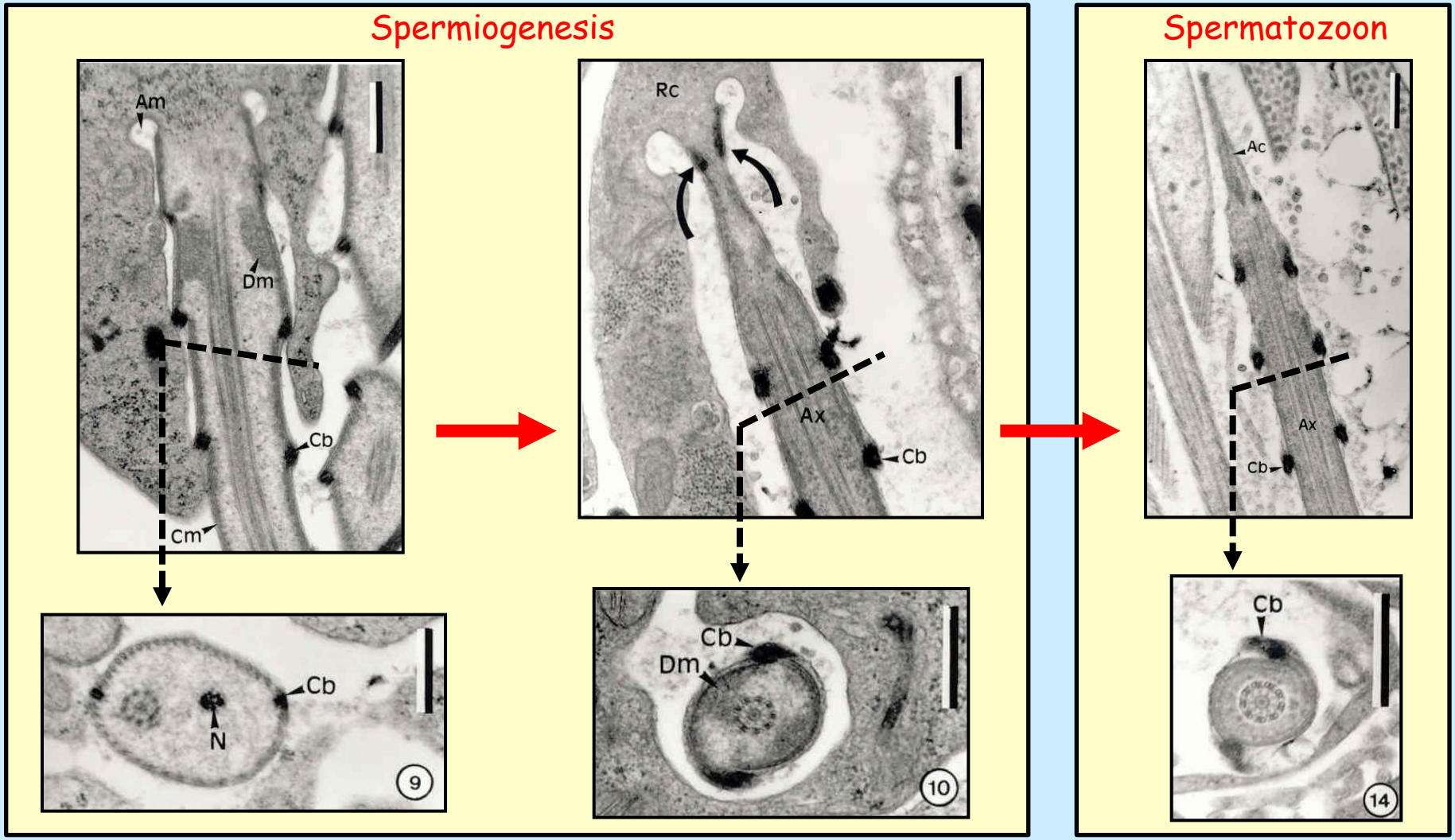
SPERMIOGENESIS:

- Type 3 or 4 (with or without proximodistal fusion)
- Absence of striated rootlets ?
- Absence of intercentriolar body ?
- Absence of flagellar rotation ?
- 1 flagellum/axoneme

SPERMATOZOON:

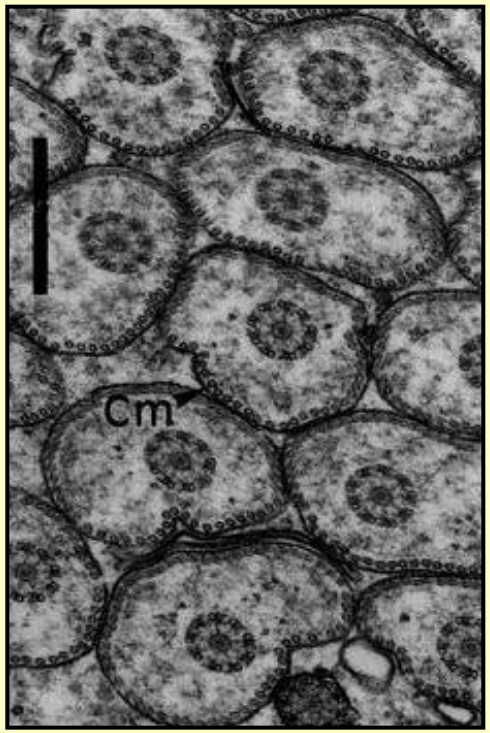
- Absence of mitochondrion
- 1 axoneme
- Crested body/ies
- Twisting of cortical microtubules ?

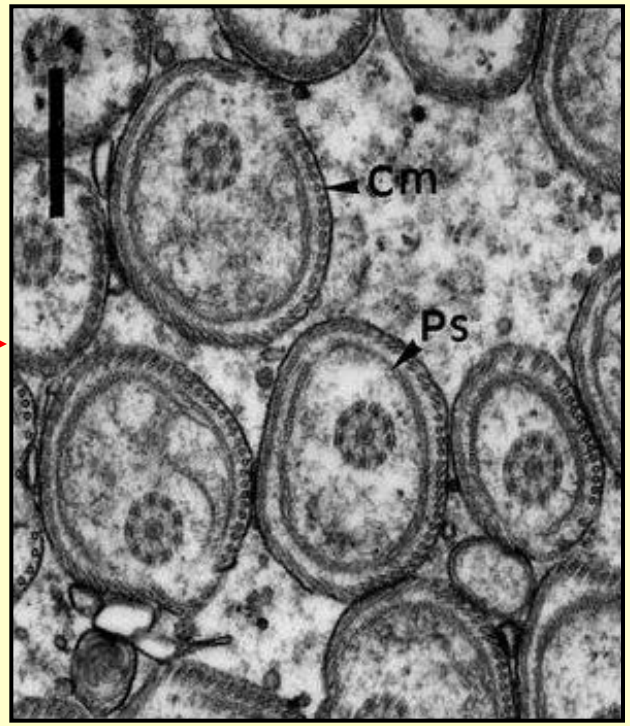
Structures present in the anterior spermatozoon extremity: apical cone and crested bodies

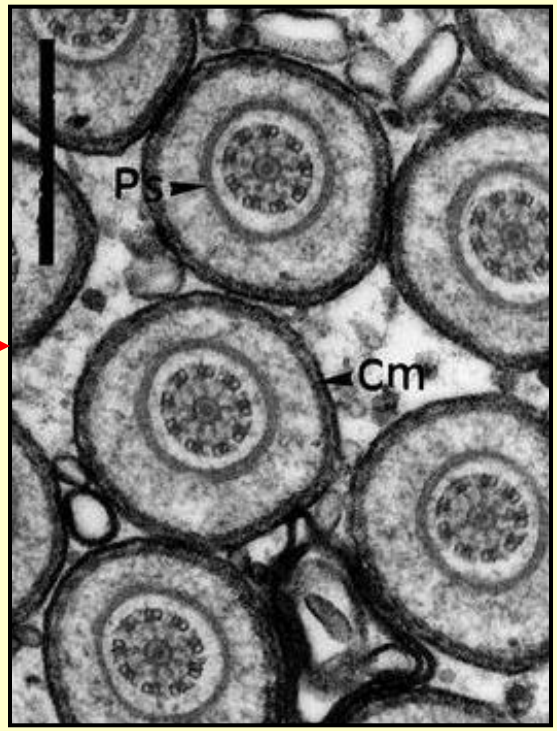


Anoplocephaloides dentata (Cyclophyllidea: Anoplocephalidae)
(Miquel & Marchand 1998)

Periaxonemal sheath







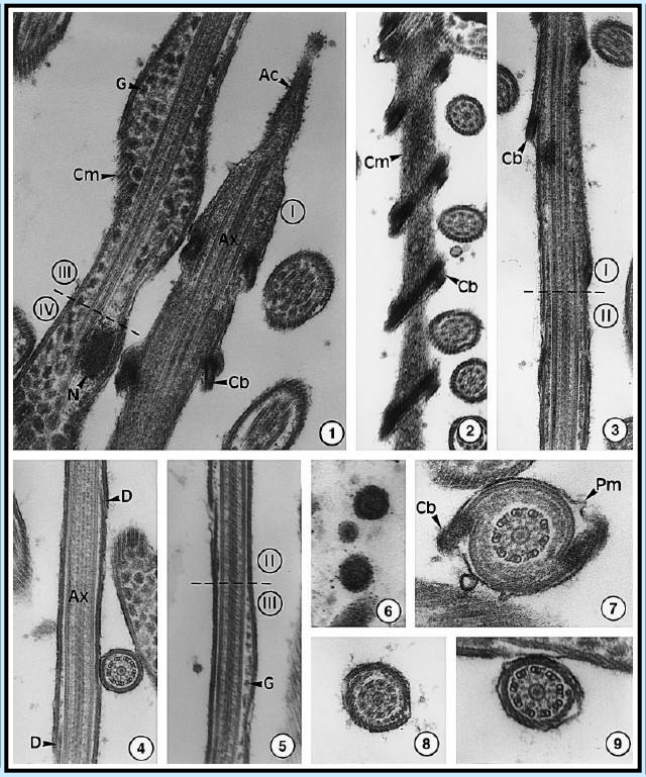
Spermiogenesis

Joyeuxiella echinorhyncoides
(Cyclophyllidea: Dipylidiidae)
(Ndiaye *et al.* 2003)

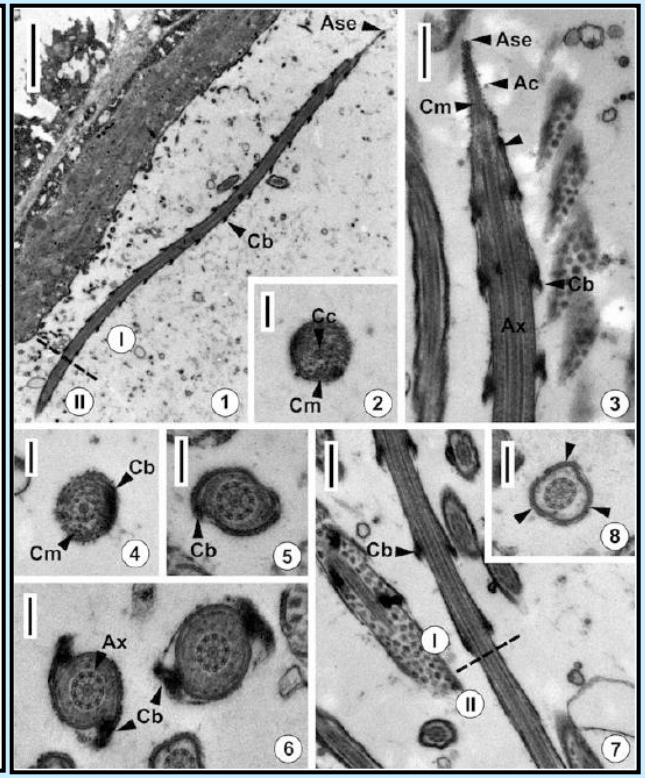
Spermatozoon

Ultrastructural similarities in the spermatozoon (Anoplocephalidae: Anoplocephalinae)

Anoplocephaloides dentata
(Miquel & Marchand 1998)

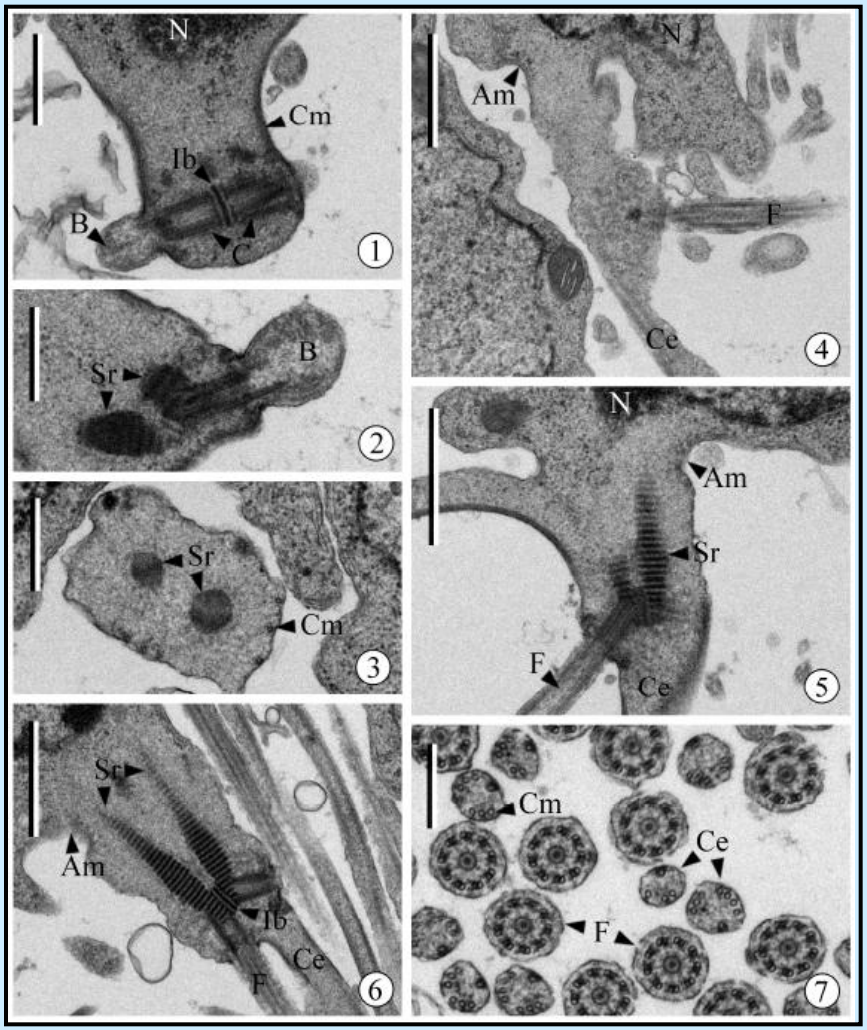


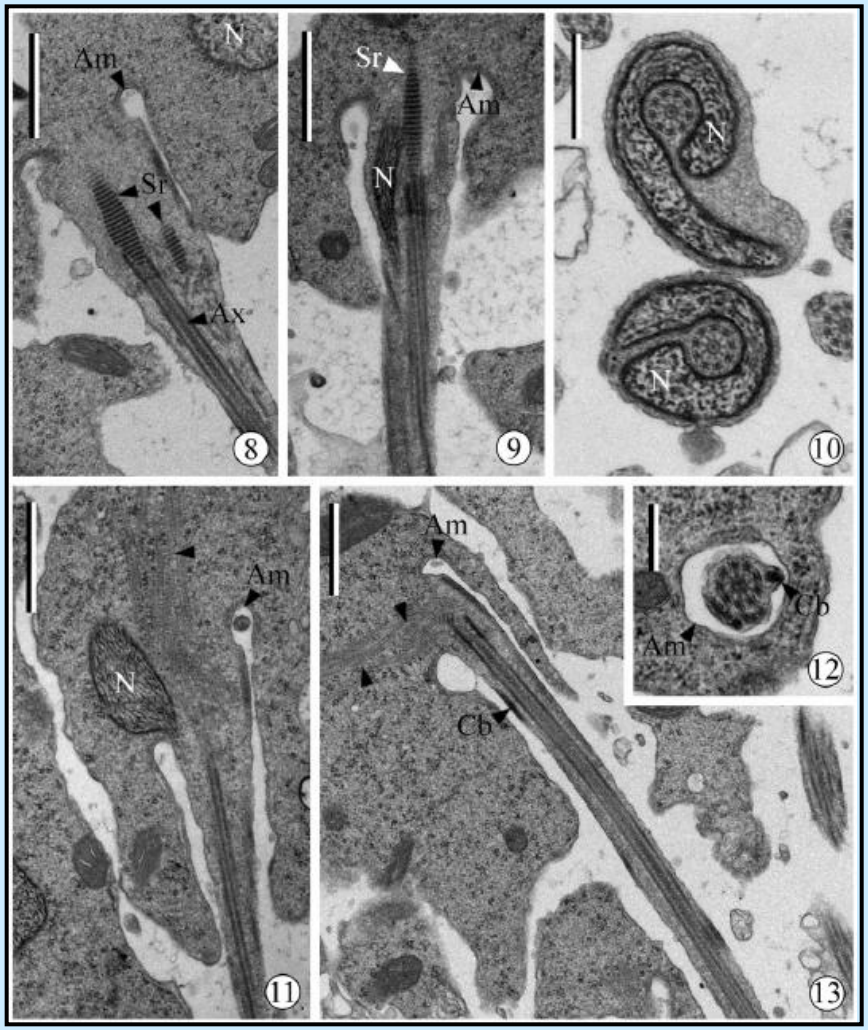
Paranoplocephala omphalodes
(Miquel & Marchand 1998)



Gallegoides arfaai
(Miquel *et al.* 2005)

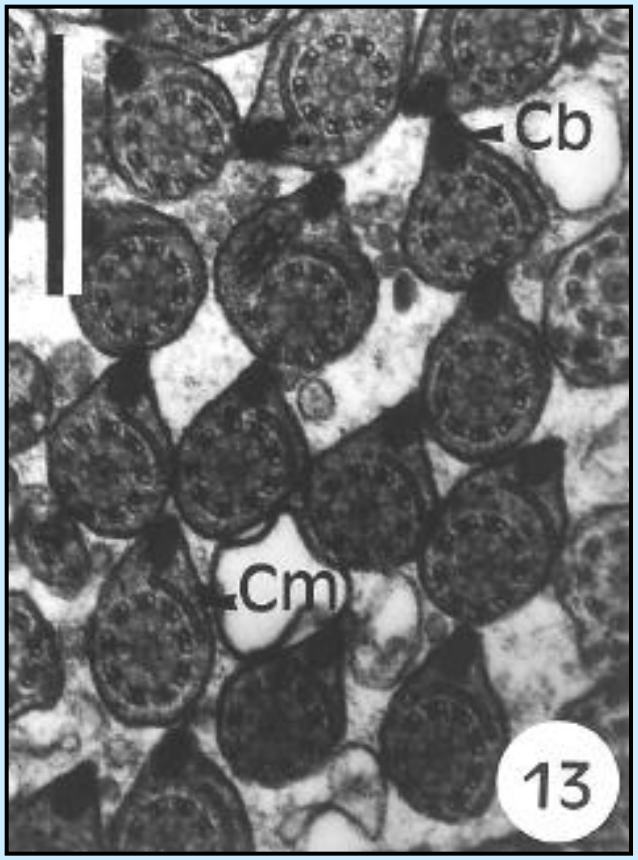
Plesiomorphic ultrastructural characters in the Mesocestoididae. Type 2 spermiogenesis

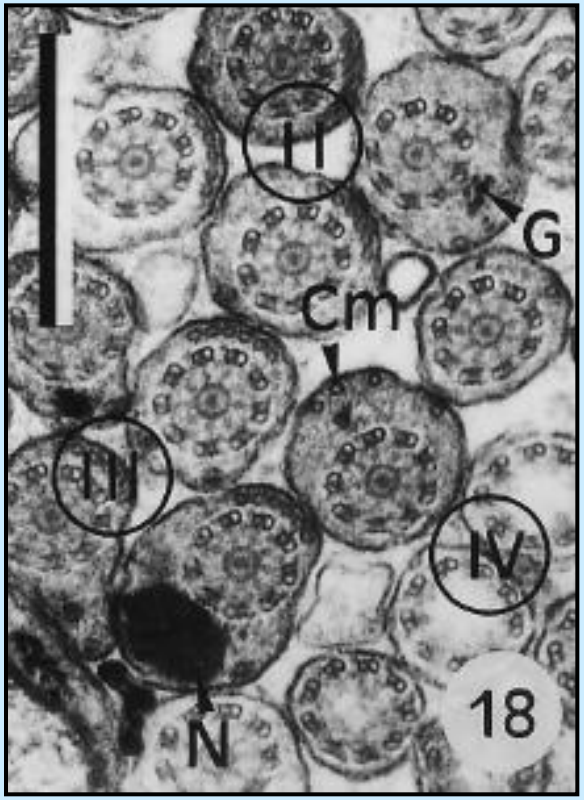




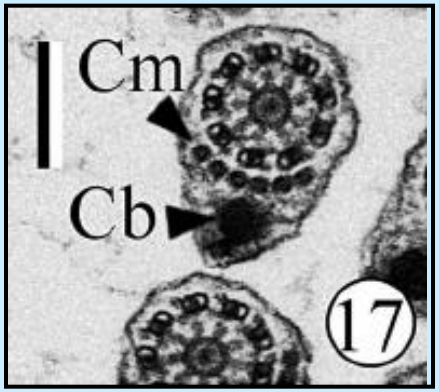
Mesocestoides lineatus (Cyclophyllidea: Mesocestoididae) (Miquel *et al.* 2007)

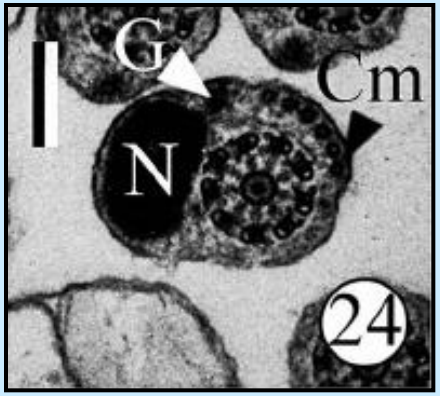
Plesiomorphic ultrastructural characters in the Mesocestoididae. Spermatozoon with parallel cortical microtubules





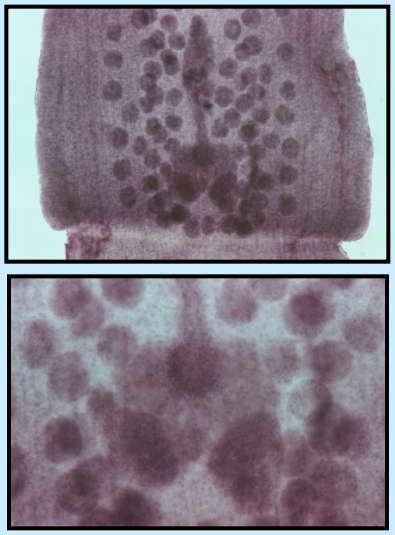
Mesocestoides litteratus
(Miquel *et al.* 1999)





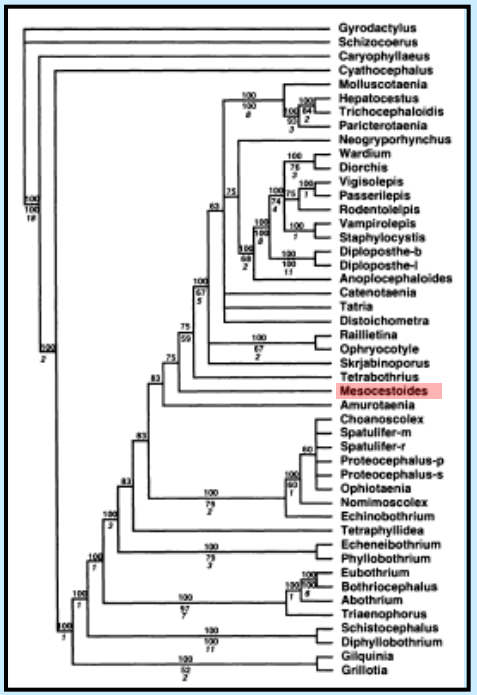
Mesocestoides lineatus
(Miquel *et al.* 2007)

Family Mesocestoididae:



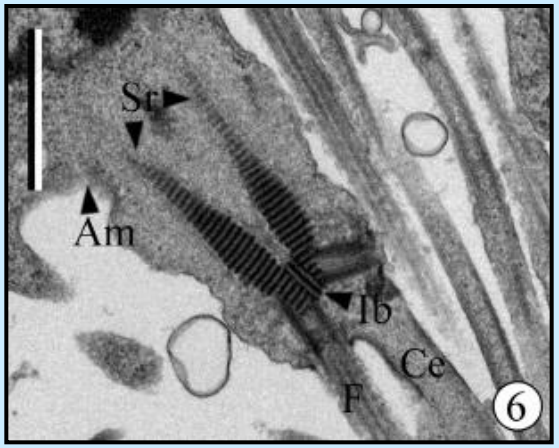
1. Morphology (Rausch 1994):
   - Mid-ventral genital pore
   - Bipartite vitelline gland

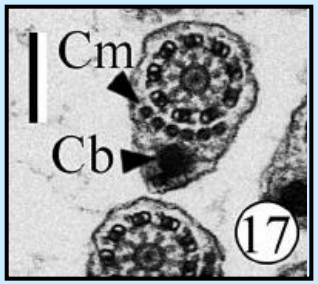
2. Biology:
   - Three hosts life cycle



3. Molecular phylogeny (Mariaux 1998):
   - Tetrabothriidea-Mesocestoididae

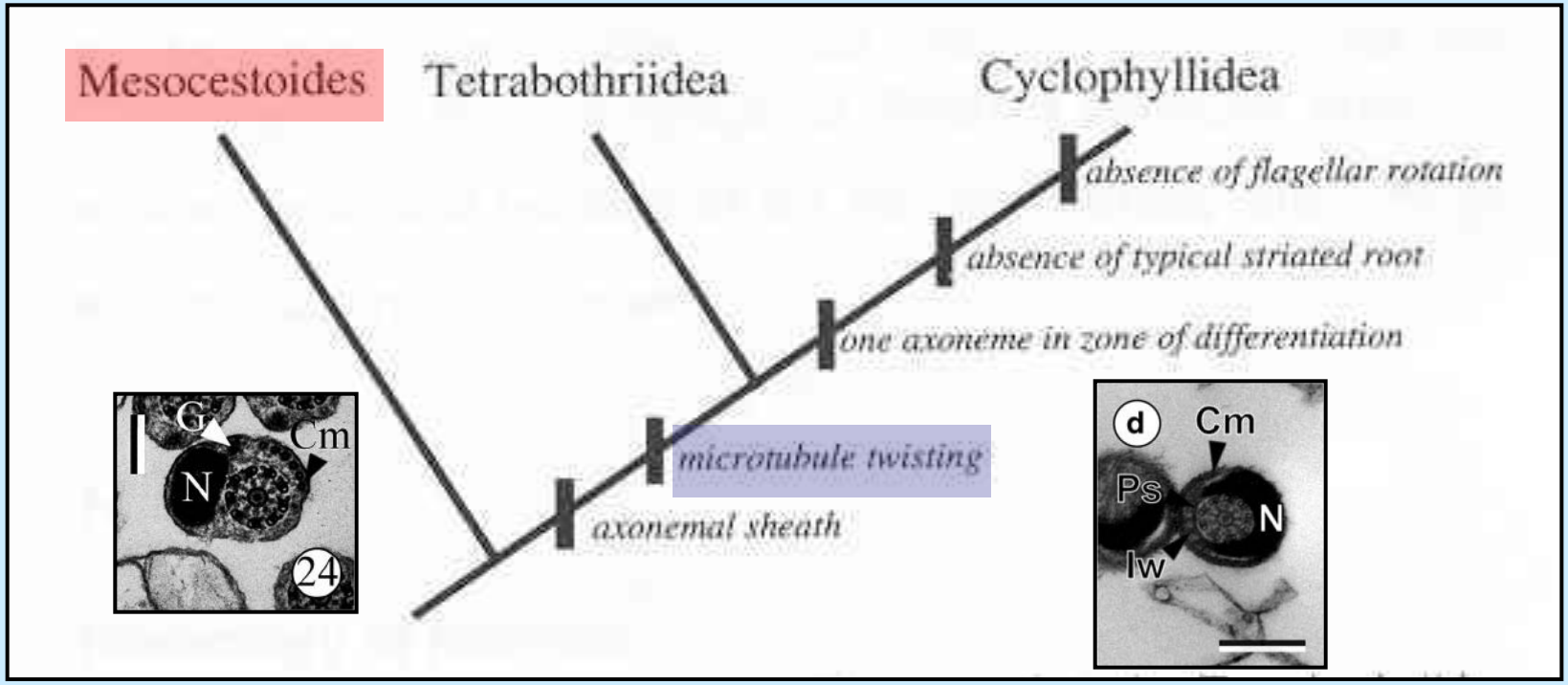
4. Sperm ultrastructure (Miquel *et al.* 1999, 2007)
   Plesiomorphic characters:
   - Type 2 spermiogenesis
     (Bâ & Marchand 1995)
   - Intercentriolar body
   - Striated rootlets
   - Type IV spermatozoon
     (Levron *et al.* 2010)
   - Parallel cortical microtubules





Position of the family Mesocestoididae as a basal group of the order Tetrabothriidea

Only ultrastructure of the spermatozoon:



Justine (2001)

Justine (2001). *In:* Interrelationships of the Platyhelminthes. Littlewood and Bray (eds.)

Character	Gy	Am	Ca	Spa	Pse	Hap	Dip	Try	Onc	Phy	Lec	Pro	Nip	Tet	Mes	Cyc	Remarks
Mitochondrion 0, present; 1, absent	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Synapomorphy for the Eucestoda
Crested body 0, absent; 1, present	0	0	?	?	1	?	1	?	1	1	1	1	?	1	1	1	Synapomorphy for the Eucestoda (or for a part of the Eucestoda only: basal to the Caryophyllidea, the Spathebothriidea, or the Pseudophyllidea?)
Intercentriolar body, binary coding 0, present; 1, absent	0	0	0	?	0	0	0	0	0	0	0	0-1	?	1	0	1	Also coded as multistate character below
Intercentriolar body, coded as a multistate character 0, present; 1, absent	0	0	0	?	0	0	0	0	0-1	0	1	0-1	?	2	1	2	Synapomorphy for higher groups, but polymorphism in several taxa
Typical striated root 0, present; 1, absent	0	0	0	?	0	0	0	0	0	0	0	0	?	0	0	1	Synapomorphy for the Cyclophyllidea
Peripheral microtubules 0, parallel; 1, twisted	0	0	0	0	0	0	0	0	0	0	0	0-1?	0?	1	0	1	Synapomorphy for the Tetrabothriidea + Cyclophyllidea
Periaxonemal sheath 0, absent; 1, present	0	0	0	?	0	0	0	0	0	0	0	0	?	1	0	0-1	Synapomorphy for the Tetrabothriidea + Cyclophyllidea, with reversal in certain Cyclophyllidea?
Flagellar rotation 0, present; 1, absent	0	0	0	?	0	0	0	0	0	0	0	0	?	0	0	1	Synapomorphy for the Cyclophyllidea
Number of axonemes in zone of differentiation 0, 2 axonemes; 1, 1 axoneme	0	0	0	?	0	0	0	0	0	0	0	0	?	0	0	1	Synapomorphy for the Cyclophyllidea
Number of axonemes in mature spermatozoon 0, 2 axonemes; 1, 1 axoneme	0	0	1	0	0	0	0-1	0	0	1	1	0-1?	1	1	1	1	A homoplastic character, with convergence in several unrelated groups.
Two types of cortical microtubules 0, 1 type; 1, two types	0	0	0	?	0	0	0	0	1	1	0	0	?	0	0	0	A character found only in the Onchobothriidae and Phyllobothriidae. Possible synapomorphy for the Tetraphyllidea?
Proximo-distal fusion 0, present; 1, absent	0	0	0	?	0	0	0	0	0	0	0	0	?	0	0	0-1	Synapomorphy for certain Cyclophyllidea?

Intercentriolar body

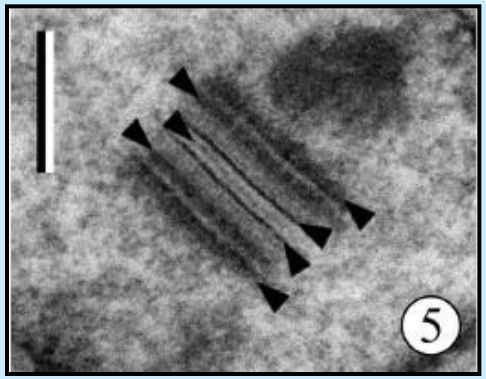
Present and well developed in Trematoda and primitive orders of Cestoda

Reduced in:

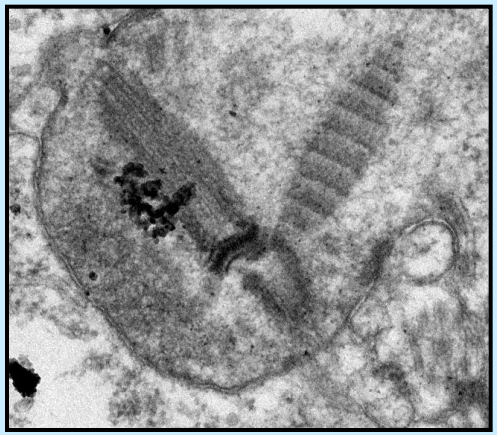
- Order Caryophyllidea
- Order "Pseudophyllidea"
- Order Tetraphyllidea
- Order Diphyllidea
- Order Proteocephalidea
- Order Lecanicephalidea
- Order Cyclophyllidea, Family Mesocestoididae

Absent in Tetrabothriidea and Cyclophyllidea

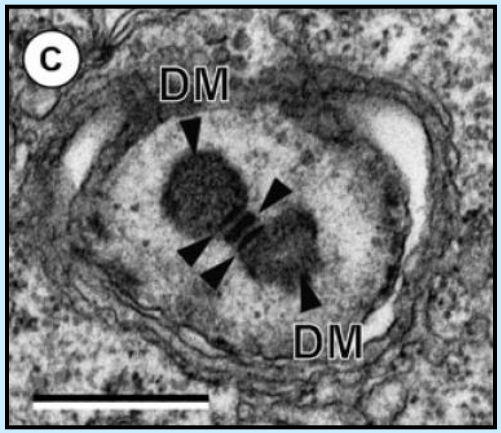
Intercentriolar body



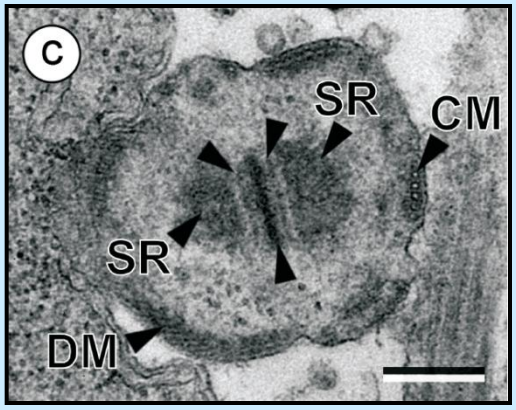
Troglotrema acutum
DIGENEA
(Miquel *et al.* 2006)



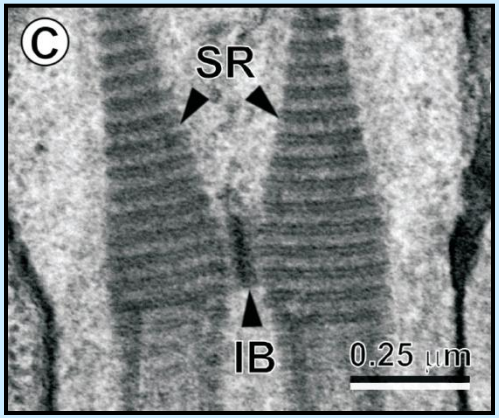
Wenyonia virilis
CARYOPHYLLIDEA
(Miquel *et al.* 2008)



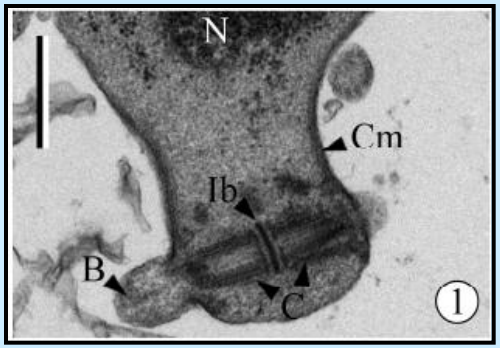
Echinobothrium euterpes
DIPHYLLIDEA
(Marigo *et al.* 2011)



Clestobothrium crassiceps
BOTHRIOCEPHALIDEA
(Marigo *et al.* 2012)



Acanthobothrium crassicolle
TETRAPHYLLIDEA
(Marigo *et al.* 2011)



Mesocestoides lineatus
CYCLOPHYLLIDEA
(Miquel *et al.* 2007)

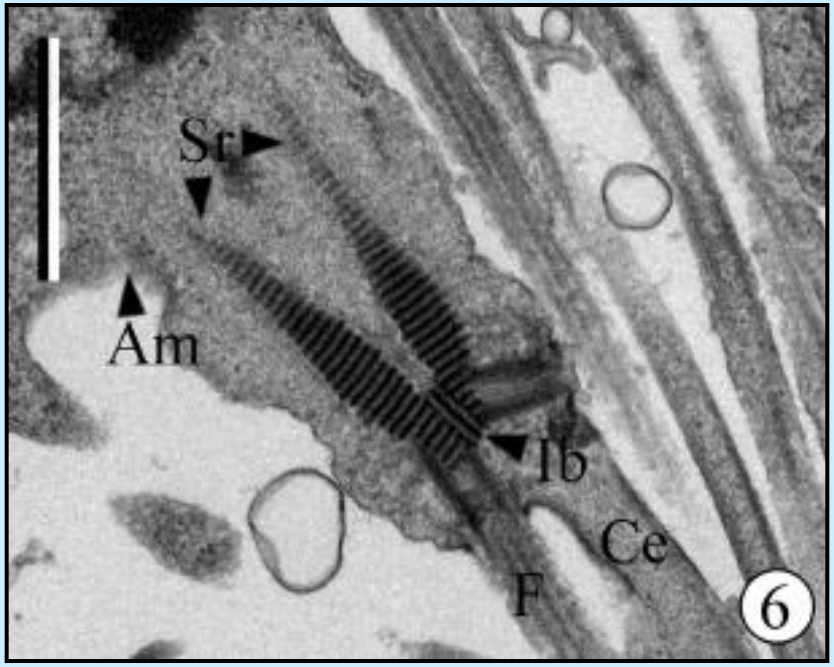
Striated rootlets

Plesiomorphic character present in some cyclophyllideans:

1. **Well developed:**
   - *Joyeuxiella* spp. (Dipylidiidae) (Ndiaye *et al.* 2003)
   - *Mesocestoides* spp. (Mesocestoididae) (Miquel *et al.* 1999, 2007)

2. **Reduced, "vestigial striated rootlets":**
   - *Anoplocephaloides dentata* (Anoplocephalidae) (Miquel & Marchand 1998)
   - *Moniezia expansa* (Anoplocephalidae) (Li *et al.* 2003)
   - *Gallegoides arfaai* (Anoplocephalidae) (Miquel *et al.* 2005)
   - *Mosgovoyia ctenoides* (Anoplocephalidae) (Eira *et al.* 2006)
   - *Dipylidium caninum* (Dipylidiidae) (Miquel *et al.* 1998)
   - *Anonchotaenia globata* (Paruterinidae) (Yoneva *et al.* 2008)
   - *Skrjabinoporus merops* (Metadilepididae) (Yoneva *et al.* 2006)
   - *Taenia taeniaeformis* (Taeniidae) (Miquel *et al.* 2009)

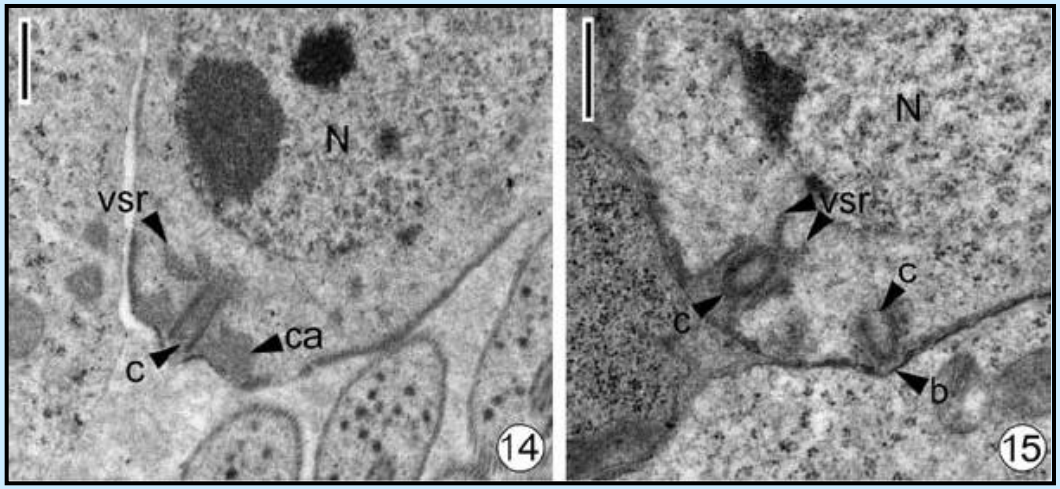
Typical striated rootlets

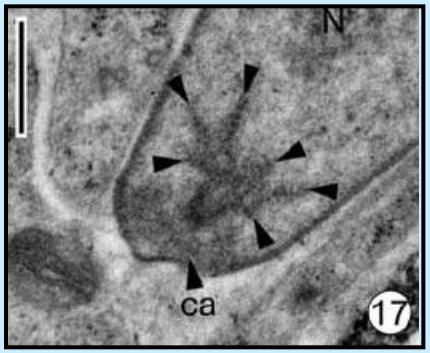


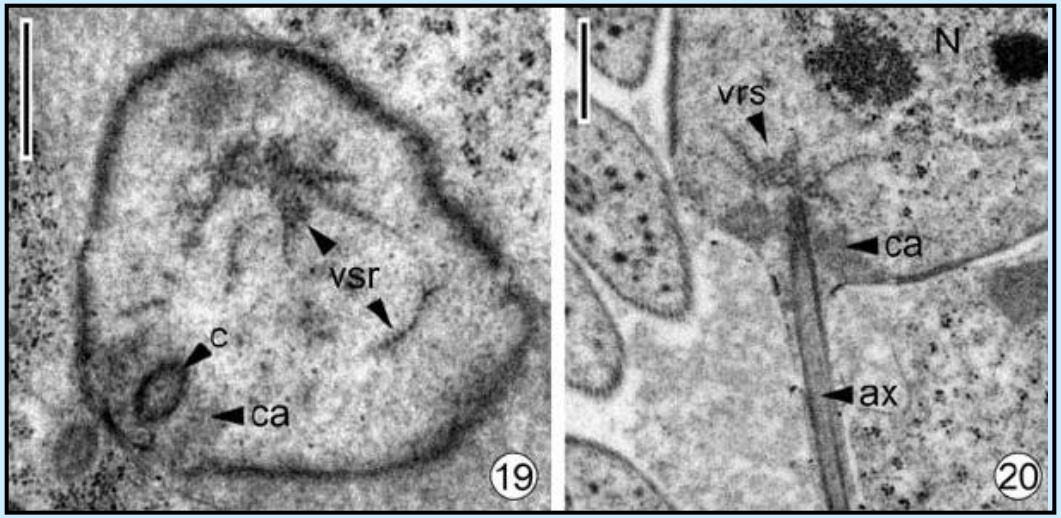
Mesocestoides lineatus
(Miquel *et al.* 2007)

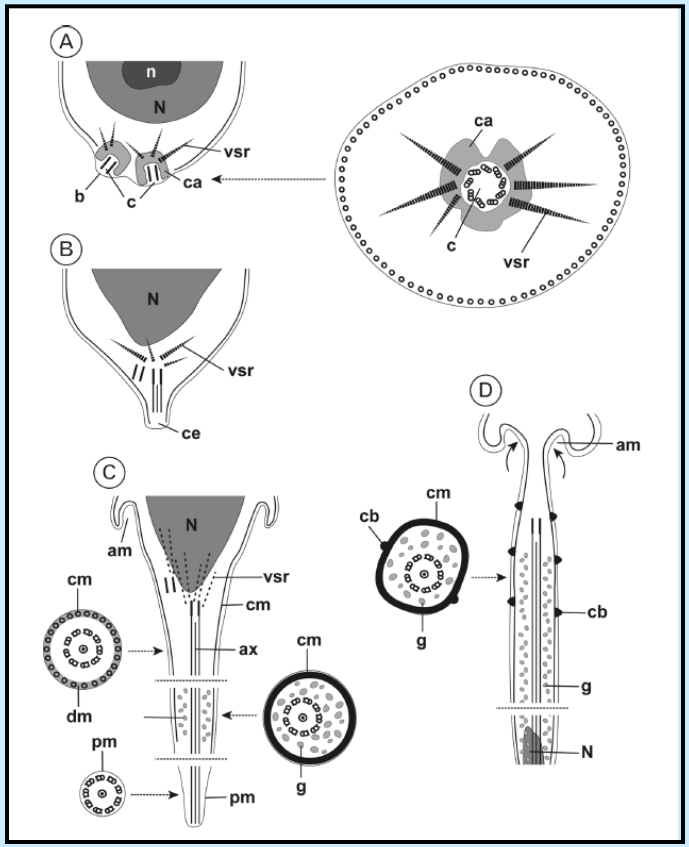
Joyeuxiella echinorhyncoides
(Ndiaye *et al.* 2003)

Vestigial striated rootlets









Gallegoides arfaai
(Miquel *et al.* 2005)

Variability in spermiogenesis in the Cyclophyllidea

Flagellar rotation around 45° in the Type 3:

Catenotaenia pusilla (Catenotaeniidae) (Hidalgo *et al.* 2000)
Anonchotaenia globata (Paruterinidae) (Yoneva *et al.* 2008)
Taenia spp. (Taeniidae) (Ndiaye *et al.* 2003, Miquel *et al.* 2009)

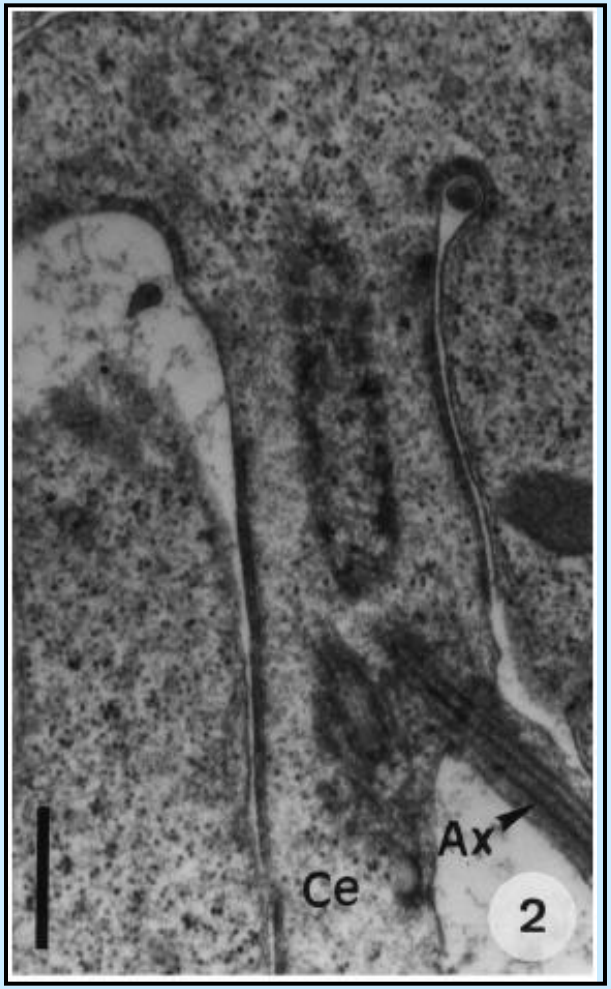
Striated rootlets in the Type 3:

Dipylidium caninum (Dipylidiidae) (Miquel *et al.* 1998, 2005)
Joyeuxiella spp. (Dipylidiidae) (Ndiaye *et al.* 2003, Miquel *et al.* 2005)
Skrjabinoporus merops (Metadilepididae) (Yoneva *et al.* 2006)
Anonchotaenia globata (Paruterinidae) (Yoneva *et al.* 2008)
Taenia taeniaeformis (Taeniidae) (Miquel *et al.* 2009)

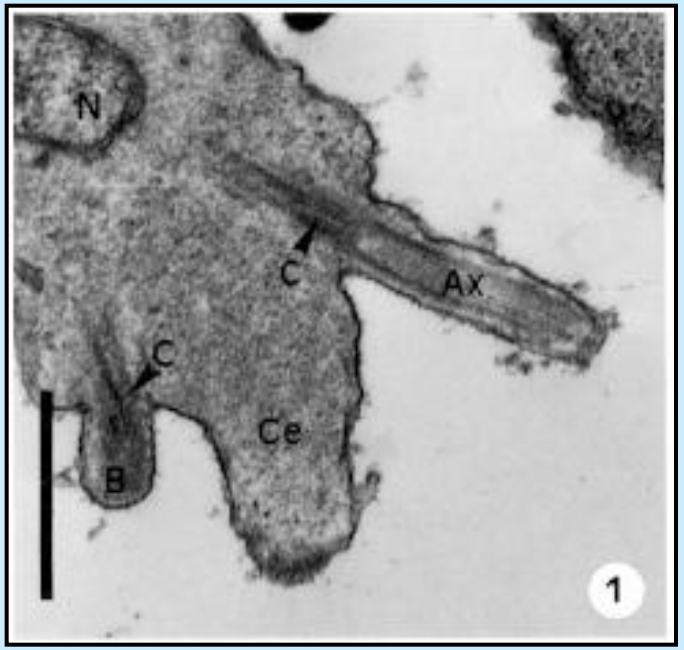
Striated rootlets in the Type 4:

Anoplocephaloides dentata (Anoplocephalidae) (Miquel & Marchand 1998)
Gallegoides arfaai (Anoplocephalidae) (Miquel *et al.* 2005)
Moniezia expansa (Anoplocephalidae) (Li *et al.* 2003)
Mosgovoyia ctenoides (Anoplocephalidae) (Eira *et al.* 2006)

Flagellar rotation ≈45°



Catenotaenia pusilla
(Hidalgo *et al.* 2000)



Taenia parva
(Ndiaye *et al.* 2003)

Flagellar rotation >90°

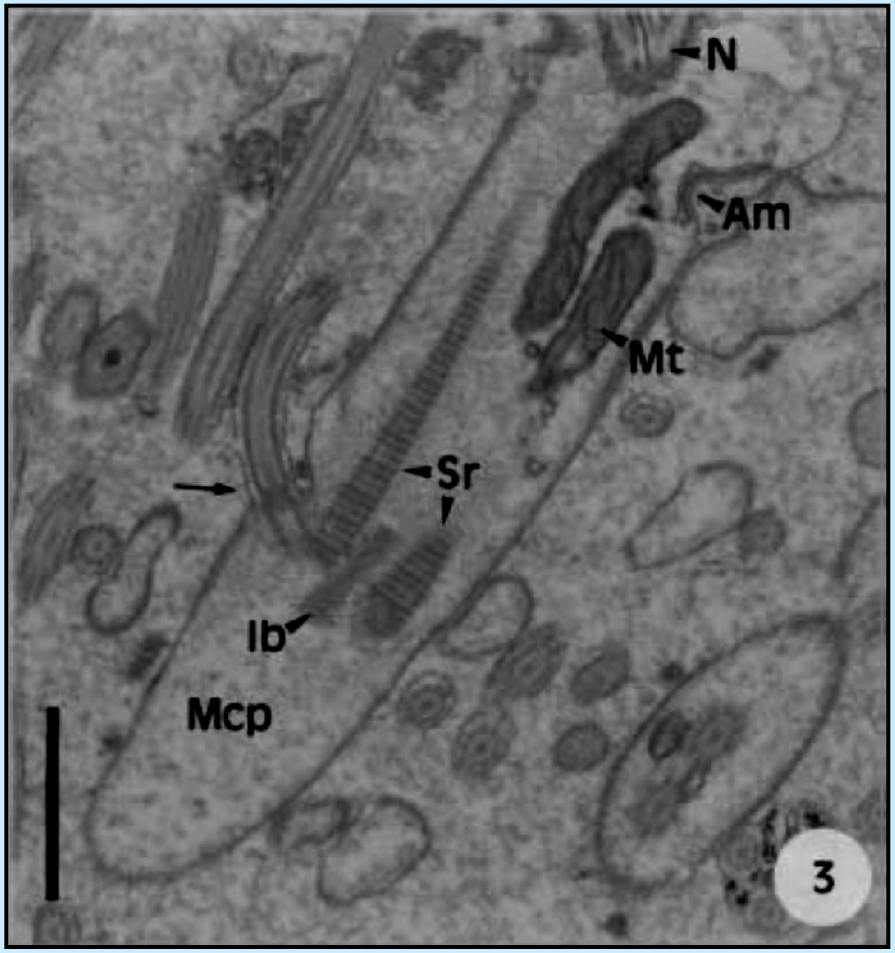
Digenea

Fasciola hepatica (Fasciolidae) (Ndiaye *et al.* 2003)
Helicometra fasciata (Opecoelidae) (Levron *et al.* 2003)
Nicolla wisniewskii (Opecoelidae) (Quilichini *et al.* 2007)
Monorchis parvus (Monorchiidae) (Levron *et al.* 2004)
Dicrocoelium hospes (Dicrocoeliidae) (Agostini *et al.* 2005)
Crepidostomum metoecus (Allocreadiidae) (Quilichini *et al.* 2007)

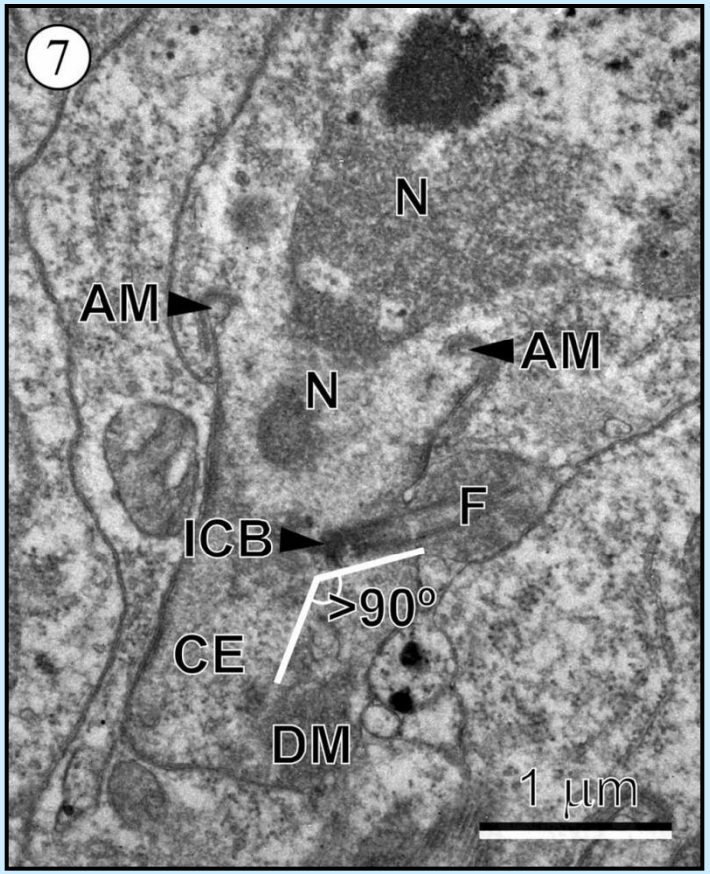
Cestoda

Wenyonia virilis (Caryophyllidea) (Miquel *et al.* 2008)
Cyathocephalus truncatus (Spathebothriidea) (Bruňanská *et al.* 2006)

Flagellar rotation >90°



Fasciola hepatica
DIGENEA
(Ndiaye *et al.* 2003)



Wenyonia virilis
CARYOPHYLLIDEA
(Miquel *et al.* 2008)

Presence of crested body/ies

Synapomorphy for the Eucestoda?

Order Caryophyllidea
- *Glaridacris catostomi* (Świderski & Mackiewicz 2002)
- *Wenyonia virilis* (Gamil 2008)
- *Khawia sinensis* (Bruňanská 2009)
- *Caryophyllaeus laticeps* (Bruňanská & Kostič 2012)
- *Breviscolex orientalis* (Yoneva *et al.* 2011)
- *Hunterella nodulosa* (Yoneva *et al.* 2012)
- *Lytocestus indicus* (Yoneva *et al.* 2012)

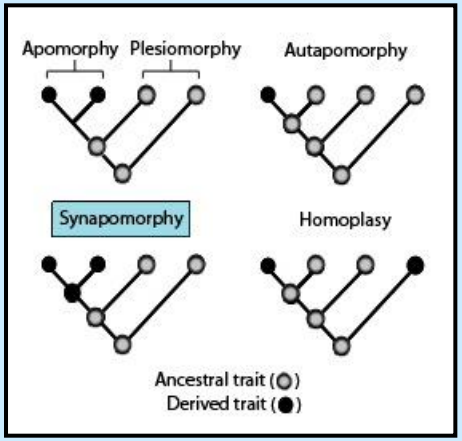
Order Spathebothriidea
- *Cyathocephalus truncatus* (Bruňanská *et al.* 2006)
- *Didymobothrium rudolphii* (Bruňanská & Poddubnaya 2010)

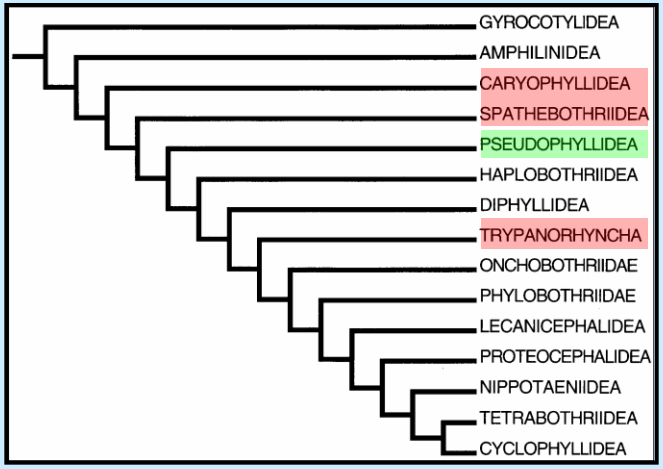
Order Diphyllobothriidea
- *Diphyllobothrium latum* (Levron *et al.* 2006)
- *Ligula intestinalis* (Levron *et al.* 2009)
- *Schistocephalus solidus* (Levron *et al.* 2013)
- *Duthiersia* spp. (Yoneva *et al.* 2013)

Order Haplobothriidea?

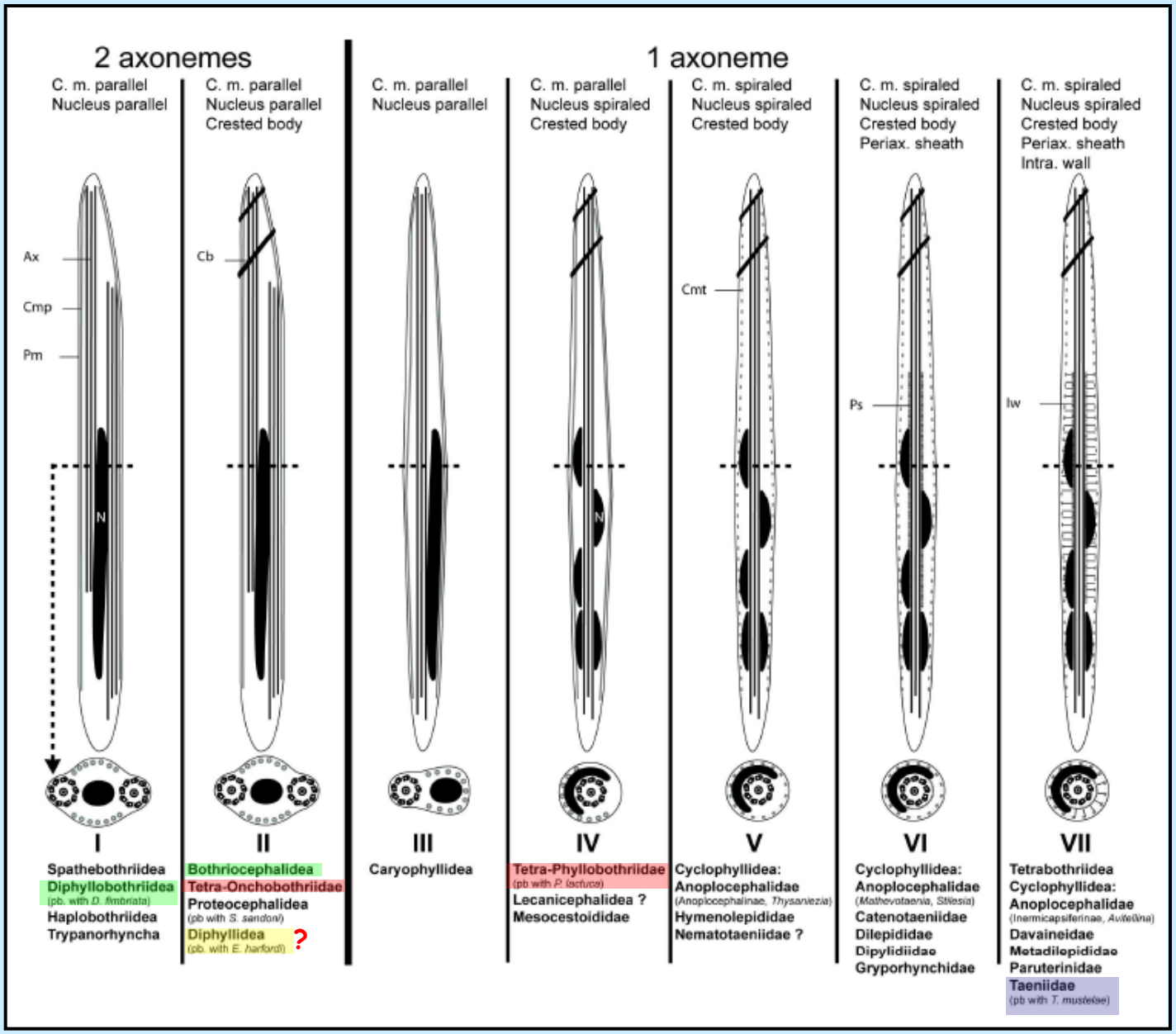
Order Trypanorhyncha
- *Dollfusiella spinulifera* (Miquel & Świderski 2006)
- *Parachristianella trygonis* (Miquel *et al.* 2007)
- *Aporhynchus menezesi* (Marigo *et al.* 2011)

Order Nippotaeniidea?





Types of spermatozoa in the Eucestoda (Levron *et al.* 2010)



-Number of axonemes
(1 or 2)

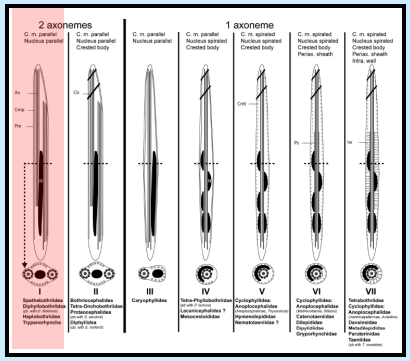
-Cortical microtubules
(parallel or spiralled)

-Nucleus
(parallel or spiralled)

-Crested body
(absent or present)

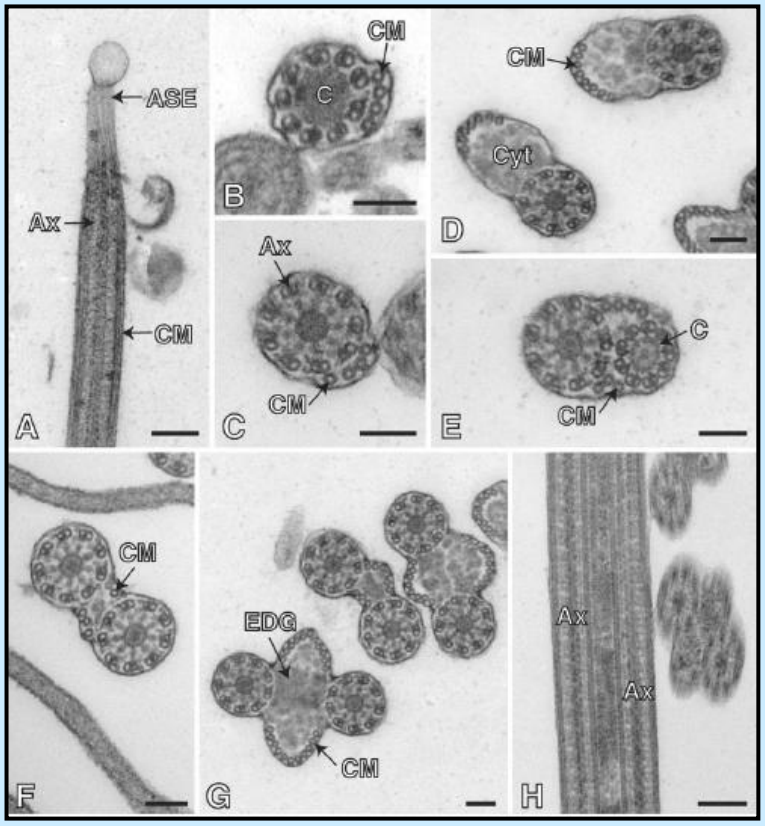
-Periaxonemal sheath
(absent or present)

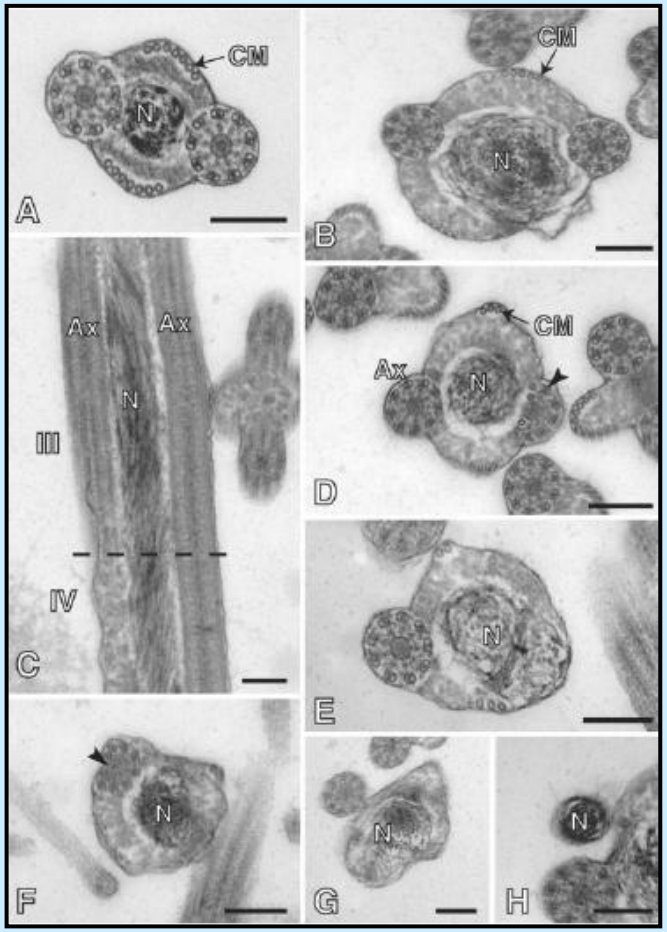
-Intracytoplasmic walls
(absent or present)

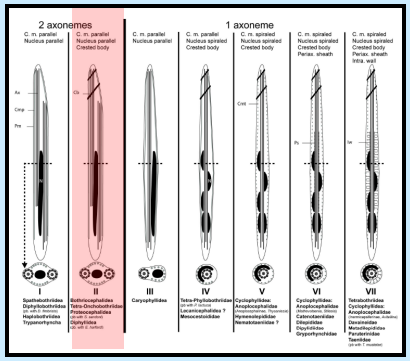


Type I spermatozoon

Diphyllobothrium latum (Diphyllobothriidea)
(Levron et al. 2006)

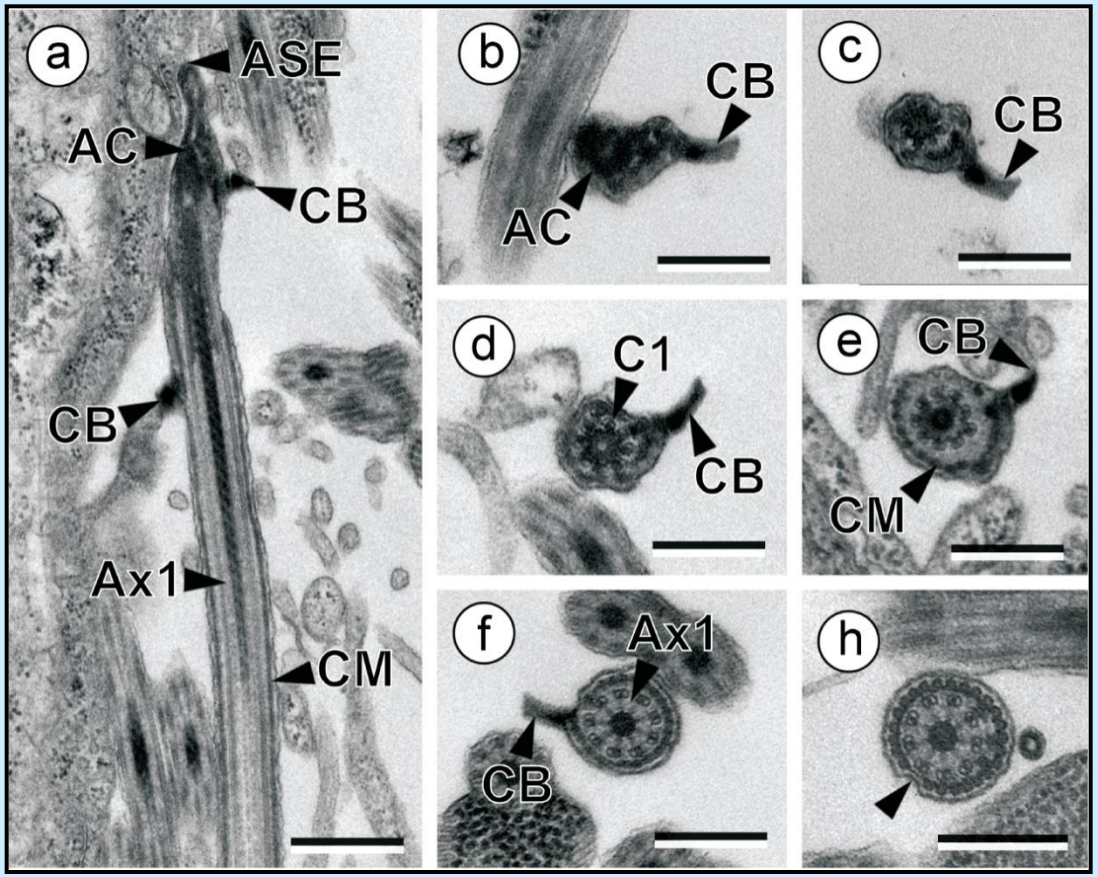


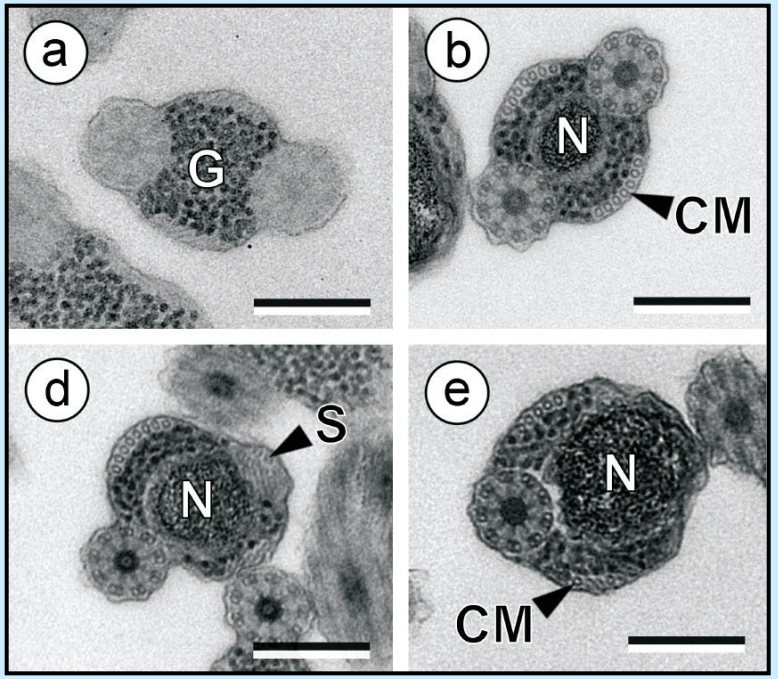




Type II spermatozoon

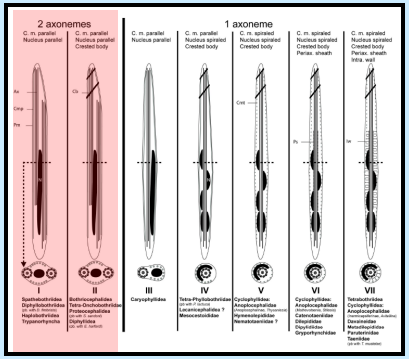
Clestobothrium crassiceps (Bothriocephalidea)
(Marigo *et al.* 2011)

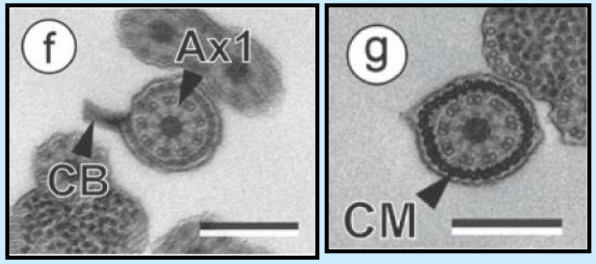


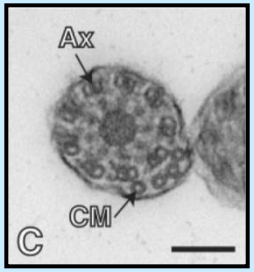


Suppression of the order Pseudophyllidea: Kuchta *et al.* (2008): Bothriocephalidea and Diphyllobothriidea

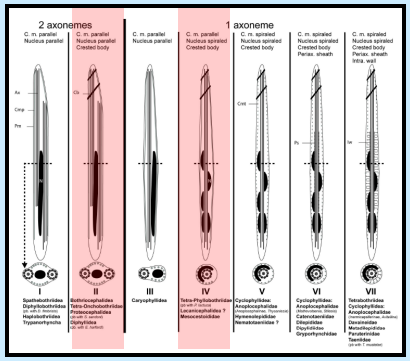
	Bothriocephalidea	Diphyllobothriidea
Genital pores (cirrus sac and vagina openings)	Dorsal surface (median, submedian or sublateral) or lateral	Ventral surface
Uterine pore	Anterior to genital pore	Posterior to genital pore
External seminal vesicle	Absent	Present
Uterine sac	Present	Absent
Definitive host spectrum	Fish, some taxa in newts	Tetrapods







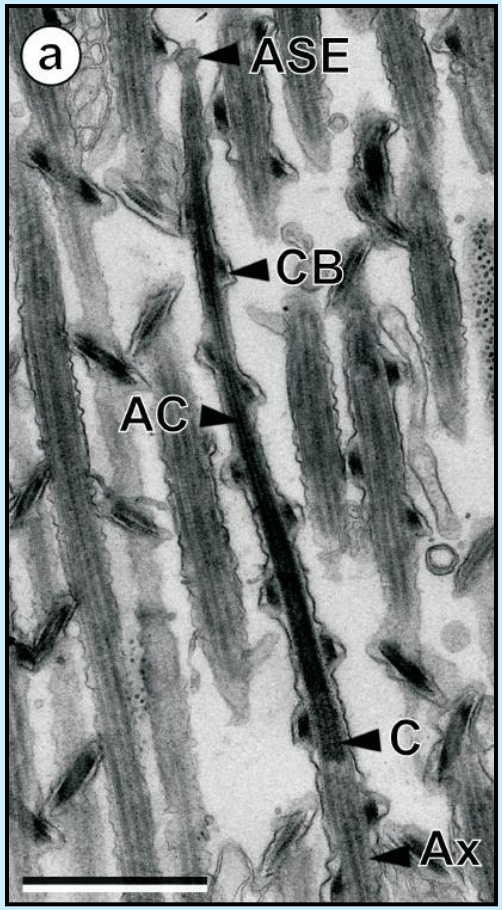
Spermatozoon ultrastructure: Type II vs Type I

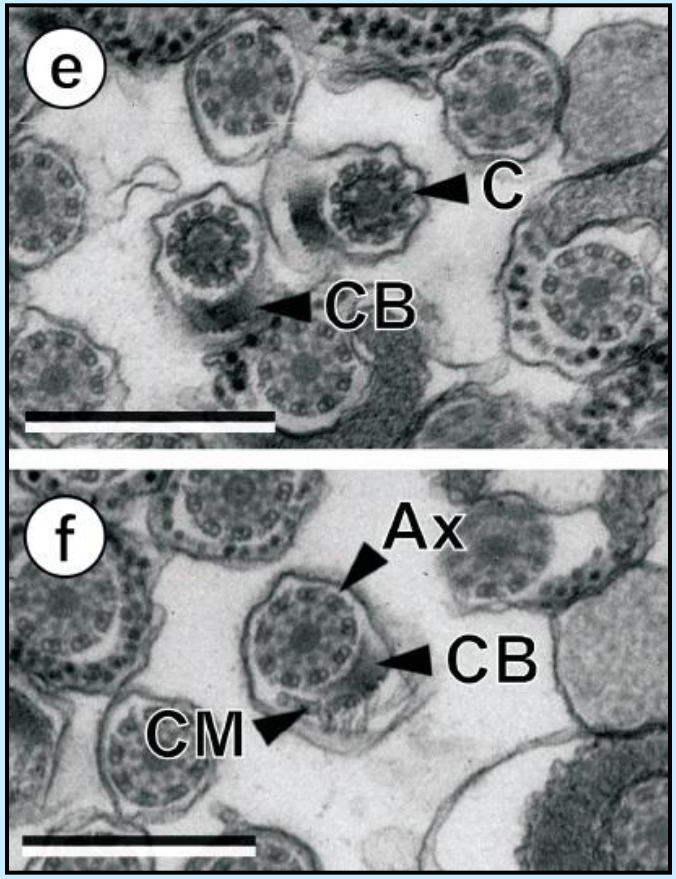


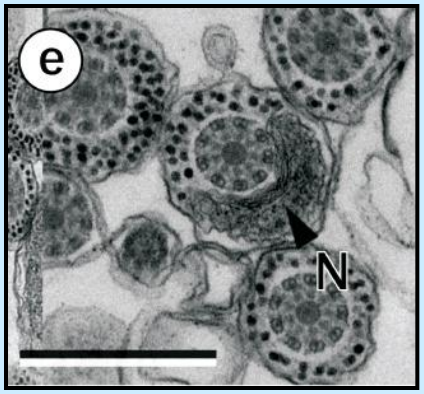
Type II or IV spermatozoon

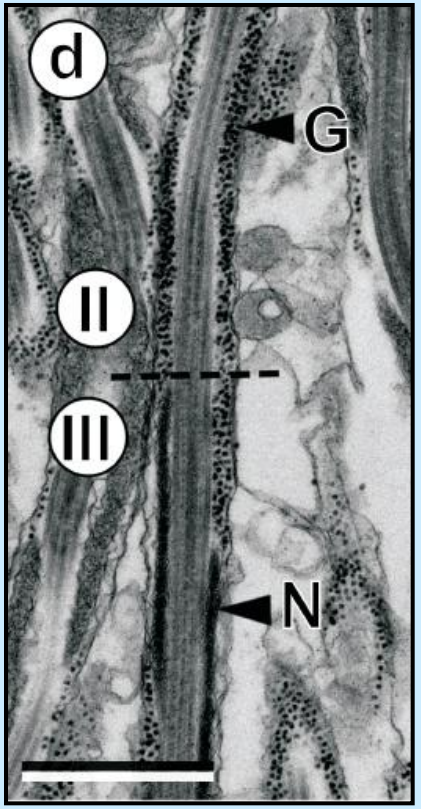
Echinobothrium euterpes (Diphyllidea)
(Marigo *et al.* 2011)

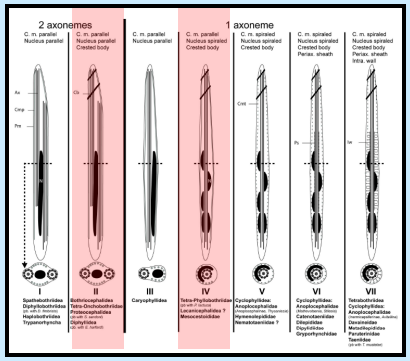
Diphyllidea – Type IV?









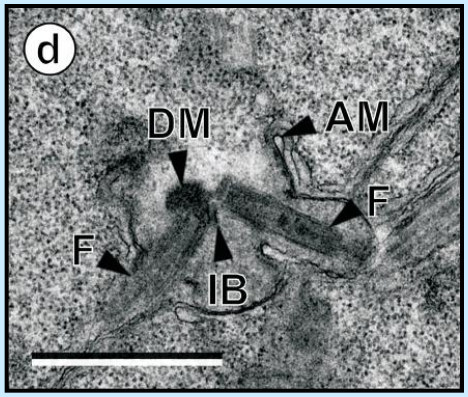


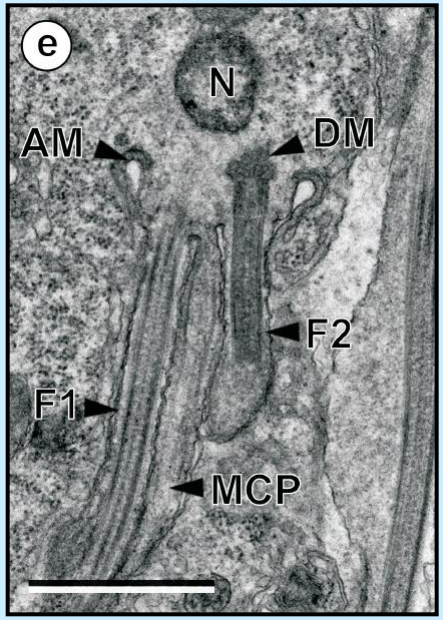
Type II or IV spermatozoon

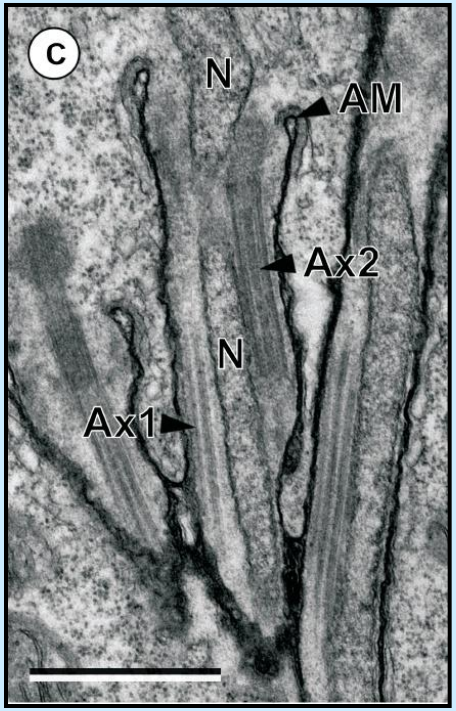
Echinobothrium euterpes (Diphyllidea)
(Marigo *et al.* 2011)

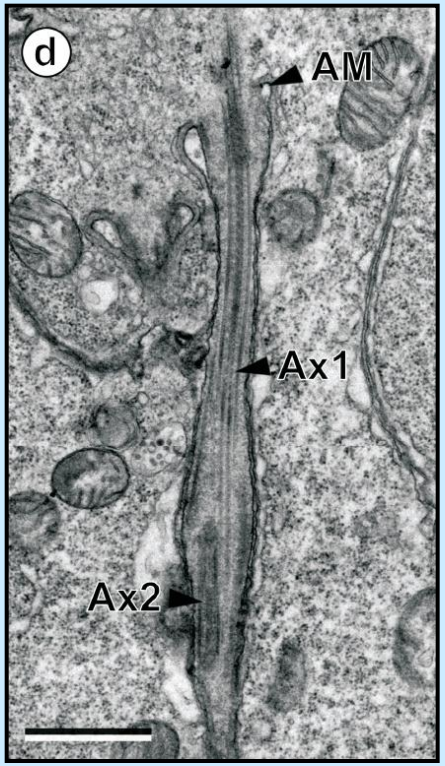
Diphyllidea

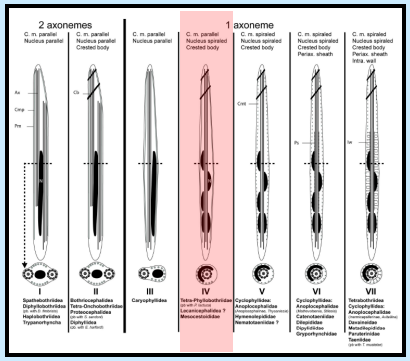
- **Type IV spermatozoon?**
- **Type 1 spermiogenesis**





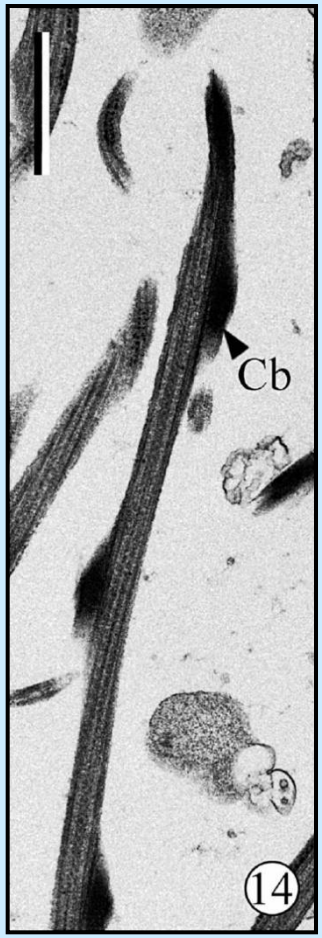


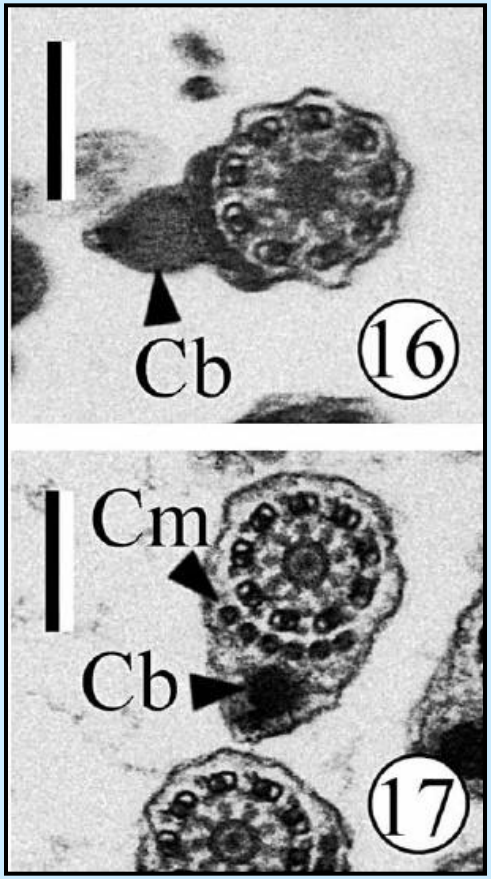


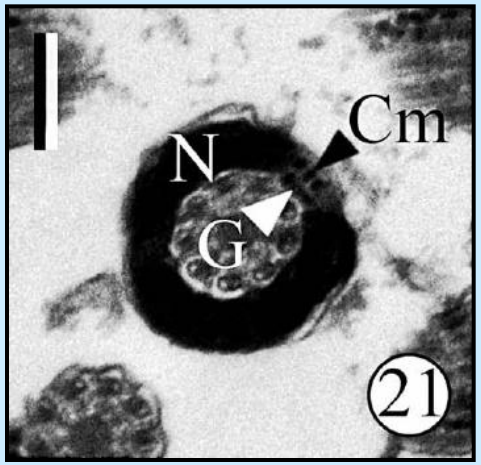


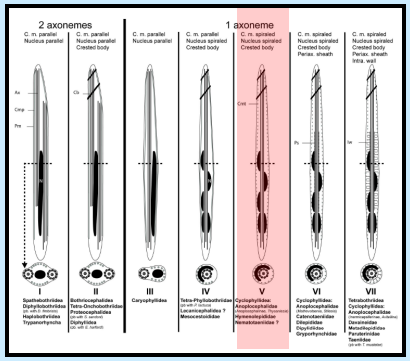
Type IV spermatozoon

Mesocestoides lineatus
(Cyclophyllidea: Mesocestoididae)
(Miquel *et al.* 2007)



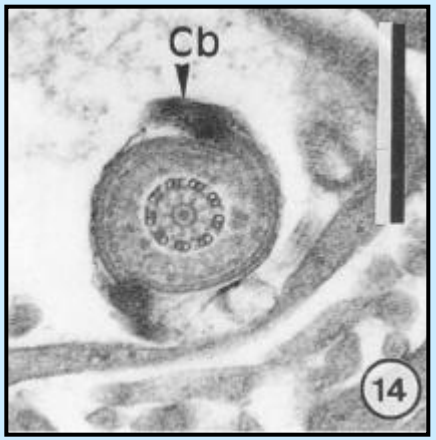


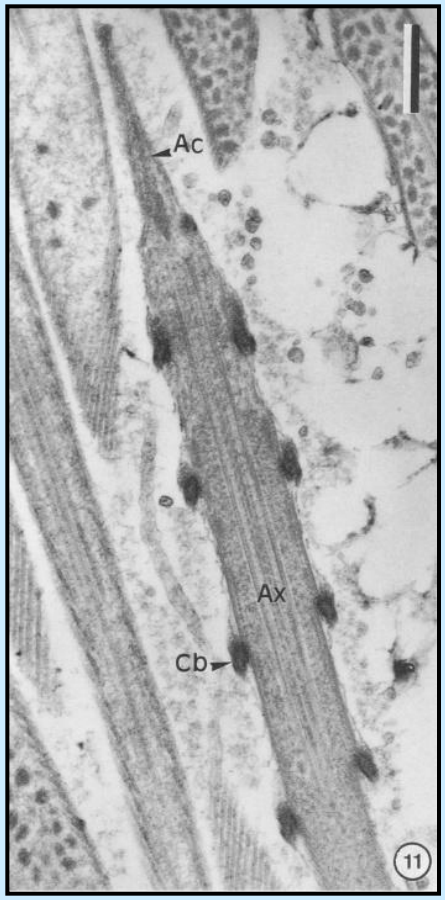


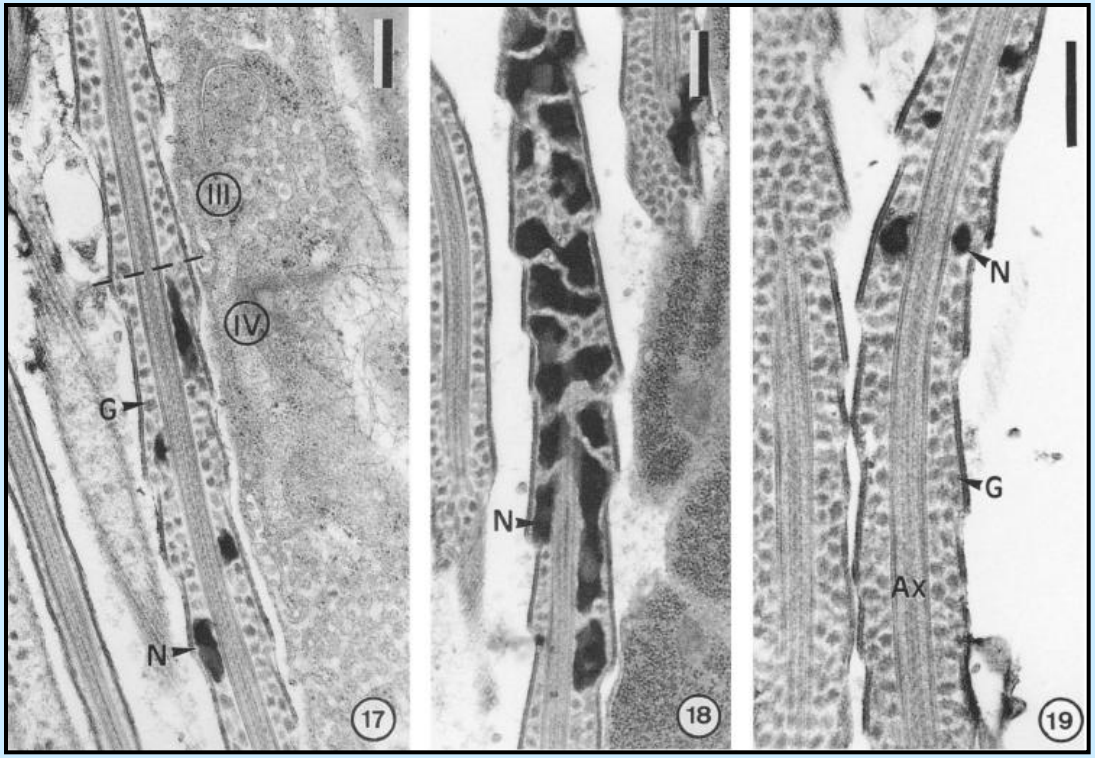


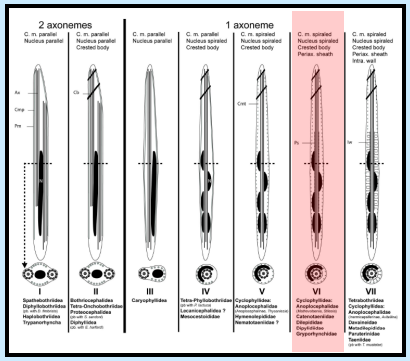
Type V spermatozoon

Anoplocephaloides dentata
(Cyclophyllidea: Anoplocephalidae)
(Miquel & Marchand 1998)



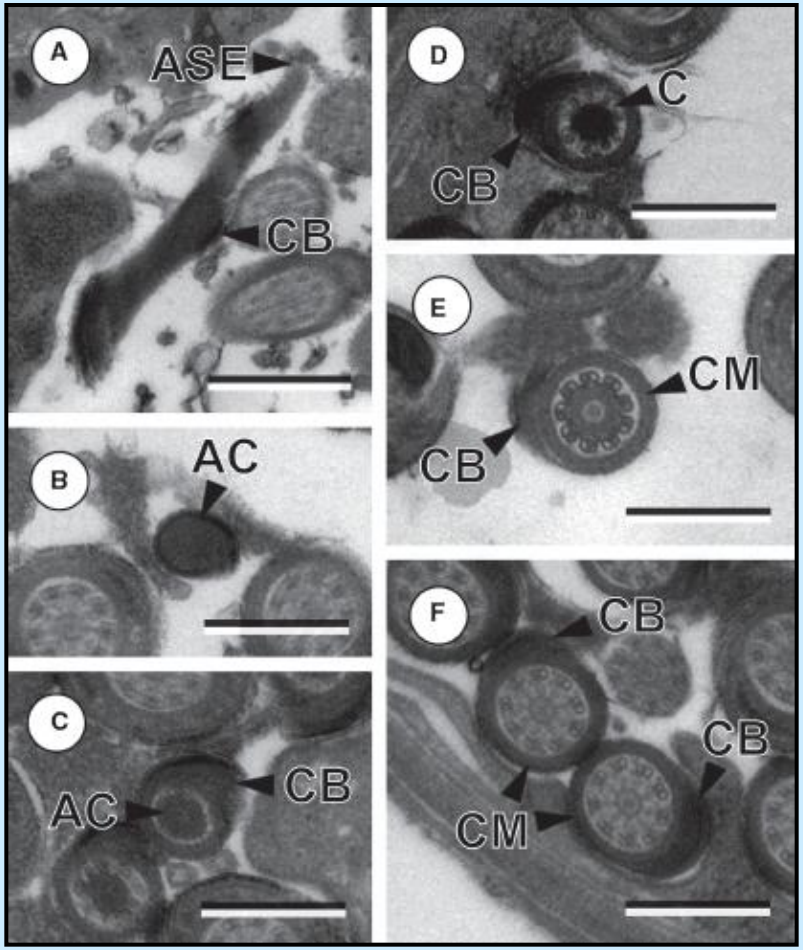


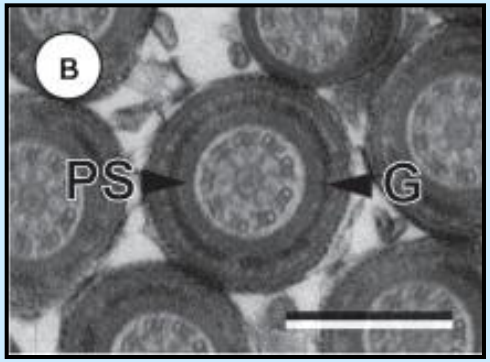


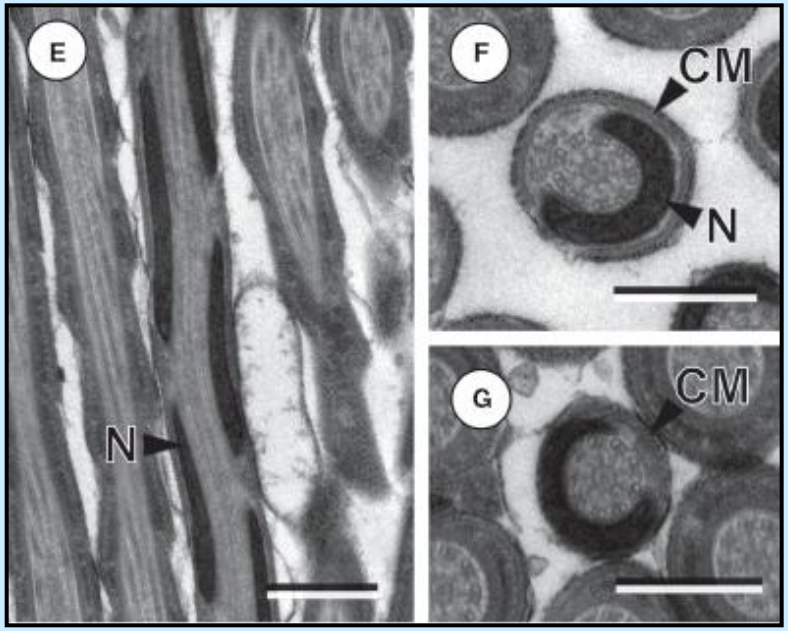


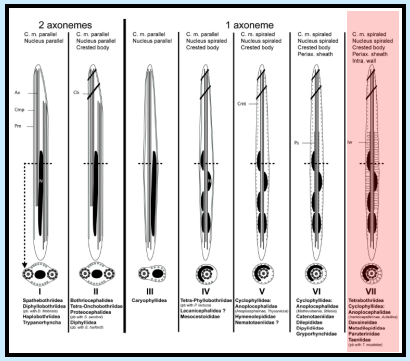
Type VI spermatozoon

Molluscotaenia crassiscolex (Cyclophyllidea: Dilepididae)
(Marigo *et al.* 2011)









Type VII spermatozoon

Taenia hydatigena (Cyclophyllidea: Taeniidae)
(Miquel *et al.* in press)