

CLADISTIC STUDY IN THE MONOGENEA (PLATYHELMINTHES), BASED UPON A PARSIMONY ANALYSIS OF SPERMIOGENETIC AND SPERMATOZOAL ULTRASTRUCTURAL CHARACTERS

JEAN-LOU JUSTINE

Laboratoire des Vers, Muséum national d'histoire naturelle, 61 rue Buffon, 75231 Paris cedex 05, France

(Received 2 February 1991; accepted 16 May 1991)

Abstract—JUSTINE J. -L. 1991. Cladistic study in the Monogenea (Platyhelminthes), based upon a parsimony analysis of spermiogenetic and spermatozoal ultrastructural characters. *International Journal for Parasitology* 21: 821–838. Characters concerning spermiogenesis and spermatozoon ultrastructure in the Monogenea were analysed with the program PAUP. A synapomorphy for the entire Monogenea (Polyopisthocotylea + Monopisthocotylea) could not be defined on the basis of a spermatozoal character. In the polyopisthocotylean Monogenea, spermatozoon ultrastructure is relatively homogeneous and a parsimony analysis was not attempted. In the monopisthocotylean Monogenea, 18 unordered characters (17 binary, one multistate) were used. A parsimony analysis was done for 23 taxa (21 monopisthocotylean taxa, the Polyopisthocotylea were considered as one taxon, and the Digenea was used as the outgroup); among the Monopisthocotylea, nine taxa were redundant, generally because of incomplete data. A semi-strict consensus tree was obtained (consistency index 0.645). Monophyly was established on the basis of spermatozoal and spermiogenetic ultrastructural characters in the following groups: the Polyopisthocotylea (one synapomorphy), the Monopisthocotylea (three synapomorphies), the Capsalidae/Dionchidae (two synapomorphies), the Loimoidae/Monocotylidae (one synapomorphy), and a group (two synapomorphies) which contains all Monogenea with uniflagellate spermatozoa grouped in a polychotomy (Ancyrocephalidae, Calceostomatidae, Pseudodactylogyridae, Diplectanidae, Tetraonchidae and Amphibdellatidae). Autapomorphies were recognized for the Amphibdellatidae and the Calceostomatidae. Not only spermatozoal ultrastructure, but also ultrastructure of spermiogenesis, should be used for defining synapomorphies. The tree herein obtained was compared with the most recent classifications of the Monogenea, based on morphology (Lebedev, 1988, *Angewandte Parasitologie* 29: 149–167; Malmberg, 1990, *Systematic Parasitology* 17: 1–65); important similarities with Lebedev's classification were observed.

INDEX KEY WORDS: Monogenea, Platyhelminthes; Polyopisthocotylea; Monopisthocotylea; phylogeny; cladistics; systematics; classification; parsimony analysis; ultrastructure; spermatozoon; spermiogenesis.

INTRODUCTION

PHYLOGENY and classification of the Platyhelminthes were recently reviewed using cladistic methods (Ehlers, 1984, 1985a, b, 1986, 1988; Ax, 1987; Brooks, 1982, 1989a, b, c; Brooks, O'Grady & Glen, 1985; Rohde, 1990; Rohde, K. 1990. Abstract in *Bulletin de la Société Française de Parasitologie* 8, Suppl. 1: 278). In these works, much emphasis was placed on ultrastructural characters. Among these, spermatozoal and spermiogenetic characters were recently analysed and criticized (Justine, J. -L. 1990. Abstract in *Bulletin de la Société Française de Parasitologie* 8, Suppl. 1: 193; Justine, in press a, b).

In the Monogenea, spermatozoal ultrastructure was used by several authors for analysing phylogenetic relationships (Justine, Lambert & Mattei, 1985; Timofeeva, T. A., unpublished, First International Symposium on Monogenea, České Budejovice, 1988; Lebedev, 1988; Malmberg, G. 1990. Abstract in *Bulletin de la Société Française de Parasitologie* 8,

Suppl. 1: 274; Malmberg, 1990; Malmberg & Afzelius, 1990; Tappenden & Kearn, 1990) but, until now, not with cladistic methods.

Two synapomorphies for the Monogenea were accepted in all three recent systems for the Platyhelminthes (Ehlers, 1985a; Brooks, 1989a; Rohde, 1990): the presence of three rows of epidermal ciliary bands in the oncomiracidium, and two pairs of rhabdomic eyespots. Brooks (1989a) added two morphological synapomorphies, one was a homoplasy with the Trematoda, and Rohde (1990) added one synapomorphy based on the monogenetic life-cycle. Since there is general agreement on the monophyly of the Monogenea, it is possible to study phylogenetic relationships within this taxon. Within the Cercomeridea, which display a rather homogeneous spermatozoal structure (Justine, in press a, b), the Monogenea are outstanding because of the great diversity of their sperm structure, which allows recognition of numerous synapomorphies and allows a parsimony analysis. However, no synapomorphy for

TABLE 1—LIST OF THE 56 MONOGENEAN TAXA IN WHICH SPERMATOZOON ULTRASTRUCTURE IS KNOWN, AND ORIGIN OF THE DATA

Taxon	Origin of data	Taxon	Origin of data
Polyopisthocotylea		Monopisthocotylea	
<i>Atriasier sargui</i>	Justine * †	<i>Acanthocotyle lobianchi</i>	Malmberg & Afzelius, 1990; Tappenden & Kearns, 1990*
<i>Axine</i> sp.	Justine & Mattei, 1985b * †	<i>Amphibdella paronaperugiae</i>	Justine & Mattei, 1988 * †
<i>Cemocotyle</i> sp.	Justine & Mattei, 1985c * †	<i>Amphibdelloides vallei</i>	Justine & Mattei, 1983c*
<i>Choricotyle pagelli</i>	Ktari ‡; Tuzet & Ktari, 1971b	<i>Amphibdelloides</i> sp. 1	Justine & Mattei, 1983c*
<i>Diclidophora merlangi</i>	MacDonald & Caley, 1975; Halton & Hardcastle, 1976, 1977	<i>Amphibdelloides</i> sp. 2	Justine & Mattei, 1983c*
<i>Diclidophora</i> sp.	Ktari ‡; Tuzet & Ktari, 1971b	<i>Caballerocotyla</i> sp.	Justine ; Justine & Mattei, 1987*
<i>Diplozoon gracile</i>	Justine, Le Brun & Mattei, 1985a,b	<i>Calceostoma herculanea</i>	Justine & Mattei, 1986b*
<i>Erpocotyle catenulata</i>	Ktari ‡; Tuzet & Ktari, 1971b	<i>Cichlidogyrus halli</i>	El-Naggar, Khidr & Kearns, 1990
<i>Gotocotyla secunda</i>	Rohde, 1980	<i>Cleitharticus beninensis</i>	Justine & Mattei, 1982b*†
<i>Gotocotyla acanthura</i>	Justine & Mattei, 1985a * †	<i>Cleitharticus</i> sp.	Justine & Mattei, 1982b;*†
<i>Heteraxine</i> cf. <i>mexicana</i>	Justine & Mattei, 1985c * †	<i>Dionchus remorae</i>	Justine, Lambert & Mattei, 1985; Justine & Mattei, 1986a, 1987*
<i>Heteraxinoides</i> sp.	Justine & Mattei, 1985c * †	<i>Diplectanum</i> sp.	Justine & Mattei, 1984b*; Justine & Mattei, 1982b
<i>Hexostoma</i> sp.	Justine & Mattei, 1984a	<i>Diplectanum banyulensis</i>	Justine *†
<i>Kuhnia</i> sp.	Justine & Mattei, 1985b	<i>Diplectanum aequans</i>	Oliver, personal communication*
<i>Microcotyle erythrini</i>	Ktari ‡; Tuzet & Ktari, 1971b	<i>Encotyllabe</i> sp.	Present study*
<i>Microcotyle mormyri</i>	Ktari ‡; Tuzet & Ktari, 1971a*,b	<i>Euzetrema knoeppfleri</i>	Fournier §*
<i>Microcotyle</i> sp.	Justine & Mattei, 1985b; Justine * †	<i>Furnestinia echeneis</i>	Justine, Lambert & Mattei, 1985; Justine *†
<i>Microcotyle chrysophrii</i>	Justine & Mattei, 1985b; Justine * †	<i>Gyrodactylus eucaliae</i>	Kritsky, 1976*
<i>Plectanocotyle gurnardi</i>	Ktari‡; Tuzet & Ktari, 1971b	<i>Heterocotyle</i> sp.	Justine & Mattei, 1983b
<i>Polystoma integerrimum</i>	Bekkouche, Fournier & Peyrière ¶	<i>Lamellodiscus ignoratus</i>	Justine, Lambert & Mattei, 1985; Justine *†
<i>Polystomoides malayi</i>	Rohde, 1971, 1975	<i>Lamellodiscus elegans</i>	Justine, Lambert & Mattei, 1985; Justine *†
<i>Polystomoides asiaticus</i>	Rohde, 1971, 1975	<i>Lamellodiscus ergensi</i>	Justine, Lambert & Mattei, 1985; Justine *†
<i>Polystomoides bourgati</i>	Justine	<i>Loimosina wilsoni</i>	Justine & Mattei, 1985d*
<i>Pseudomazocraes</i> cf. <i>monsivaisae</i>	Justine & Mattei, 1985b	<i>Megalocotyle grandiloba</i>	Justine & Mattei, 1983a*; Justine, 1983*
<i>Pterinotrema</i> sp.	Justine *†	<i>Megalocotyle hexacantha</i>	Justine, Lambert & Mattei, 1985; Justine & Mattei, 1987*†
<i>Pyragraphorus pyragraphorus</i>	Justine & Mattei, 1985b * †	<i>Myxinidocotyle californica</i>	Malmberg & Afzelius, 1990
<i>Sphyranura</i> sp.	Oliver & Euzet, personal communication*	<i>Pseudodactylogyrus anguillae</i>	Le Brun, Lambert & Justine, 1986*†; Mehlhorn, 1988
		<i>Tetraonchus monenteron</i>	Justine *†
		<i>Trochopus pini</i>	Ktari‡; Tuzet & Ktari, 1971b

* Data on spermiogenesis; † unpublished observations added to published data; ‡ unpublished thesis, University of Montpellier, France, 1971; § unpublished thesis, University of Perpignan, France, 1980; || unpublished thesis, University of Montpellier, France, 1985; ¶ III International Congress of Parasitology, Munich, 1974.

the entire taxon Monogenea could be defined on spermatozoal characters (Justine, in press a, b). Also, Baverstock, Fielke, Johnson, Bray & Beveridge (1991) in a study of partial sequencing of 18S ribosomal RNA of parasitic Platyhelminthes could not define a basis for the monophyly of the Monogenea.

A parsimony analysis based on a limited number of monogenean taxa and sperm ultrastructural characters was previously published (Justine, in press b); the availability of new data (Malmberg & Afzelius, 1990; Tappenden & Kearns, 1990) led the author to perform a new parsimony analysis on a larger set of characters and taxa, which is presented herein.

MATERIALS AND METHODS

Material. Data origin is shown in Table 1. Most data were taken from the literature, but some supplementary unpublished observations were also used. Unpublished observations concerning *Encotyllabe* sp., a gill parasite of *Plectorhynchus mediterraneus*, caught near Dakar, Senegal, were also used.

Character polarity and outgroup. Character polarity was determined according to outgroup criterion (Watrous & Wheeler, 1981; Wiley, 1981; Ax, 1987). In some cases (such as character 18) ontogenetic criteria were also used. The outgroup used for the monogenean study was a digenean, *Haematoloechus* sp. This is one of the most commonly studied digeneans (Shapiro, Hershenov & Tulloch, 1961; Burton, 1972; Justine & Mattei, 1982a); it shows a spermatazoal morphology which may be considered 'general' for the Digenea. A Gyrocotylidae or an Amphilinidae could also have been chosen, since sperm structure in the Cercomeridea (Digenea, Gyrocotylidae, Amphilinidae), although derived as compared to other Platyhelminthes, may be considered plesiomorphic compared to the Monogenea (Rohde & Watson, 1986; Xylander, W. E. R., unpublished thesis, University of Göttingen, Germany, 1986; Xylander, 1989; Justine, in press a, b). Spermatozoal structure in the Eucestoda has autapomorphies and thus is less convenient for comparisons (Euzet, Swiderski & Mokhtar-Maamouri, 1981; Justine, in press a, b).

Parsimony analysis programs. The programs MAC-CLADE 2.1 (Maddison & Maddison, 1987), FACTOR, MIX, PENNY and CONSENSE from the PHYLIP 3.1 package (Felsenstein, 1988), and PAUP 3.0k (Swofford, 1990) were used for this analysis on Apple Macintosh Computers (either Plus or SE). After comparative tests, PAUP was preferred to the other programs, and results published herein were those obtained with PAUP; for a comparison of parsimony programs, see Fink (1986) and Platnick (1987, 1989).

Simplification of matrix by removal of redundant taxa. An attempt at parsimony analysis by a branch-and-bound method of the matrix used herein (23 taxa, 18 characters) was revealed to be impossible on an Apple Macintosh with 1 Megabyte RAM; the number of trees to be kept in the memory was too high, probably because of the presence of redundant taxa and unknown character states in the matrix, which led to numerous polychotomies. For this reason, the matrices were first simplified by removal of redundant taxa, a parsimony analysis was performed with PAUP, and then redundant taxa were added to the tree obtained.

PAUP assumptions and options. The reproducibility of parsimony analysis is possible only if PAUP's options are

known. Options for branch-and-bound parsimony analysis were default options, i.e. initial upper bound:compute via stepwise, keep: minimal trees only, collapse zero-length branches: on, addition sequence: furthest. For analysis of the data matrix given in Table 4, the following options were used. Comments are given between square brackets.

BEGIN ASSUMPTIONS;

OPTIONS DEFTYPE = unord PolyTcount = MINSTEPS;

TYPESET * CURRENT = unord: 1-18;

WTSET * CURRENT = 1: 1-18;

END;

BEGIN PAUP;

OUTGROUP Digenea;

DELETE

Gyrodactylus Myxinidocotyle Trochopus [redundant to Euzetrema]

Caballerocotyla Encotyllabe Dionchus [redundant to Megalocotyle]

Furnestinia [redundant to Diplectanum]

Amphibdelloides [redundant to Amphibdella]

Cichlidogyrus [redundant to Tetraonchus];

SET MAXTREES = 1600;

set torder = right; [shows trees as ladders]

chartype unord: 1-18; [all characters unordered]

END;

RESULTS

The first part is a summary of our current knowledge about ultrastructure of spermiogenesis and spermatozoa in the outgroup, the Digenea, in order to allow recognition of synapomorphies in the Monogenea. Then, the phylogenetic analysis of the Monogenea has two parts: the first is research of ultrastructural autapomorphies for each of the two taxa traditionally recognized in the Monogenea, namely the Polyopisthocotylea and the Monopisthocotylea; the second is a parsimony analysis of the Monopisthocotylea.

A summary of current knowledge about ultrastructure of spermiogenesis and spermatozoa in the outgroup, the Digenea

A detailed bibliography and description may be found in Justine (in press a). In the Cercomeridea, the 32 (or 64, according to the group) spermatids are fused to form a common cytoplasmic mass, and spermiogenesis takes place at the periphery of this mass, in 32 (64) bulging and elongating structures called the zones of differentiation. Each zone of differentiation is a cone-shaped structure with cortical longitudinal microtubules, and has three lengthening elements at its distal extremity: one middle cytoplasmic process containing cortical microtubules and two free flagella (Fig. 1). Later in spermiogenesis, these three elements fuse; the characteristic of the Cercomeridea (Justine in press a, b) is that this fusion progresses from the proximal extremity to the distal extremity ('proximo-distal fusion'). The consequence of the proximo-distal fusion is that two regions may be recognized in the mature spermatozoon: the spermatozoon's *anterior*

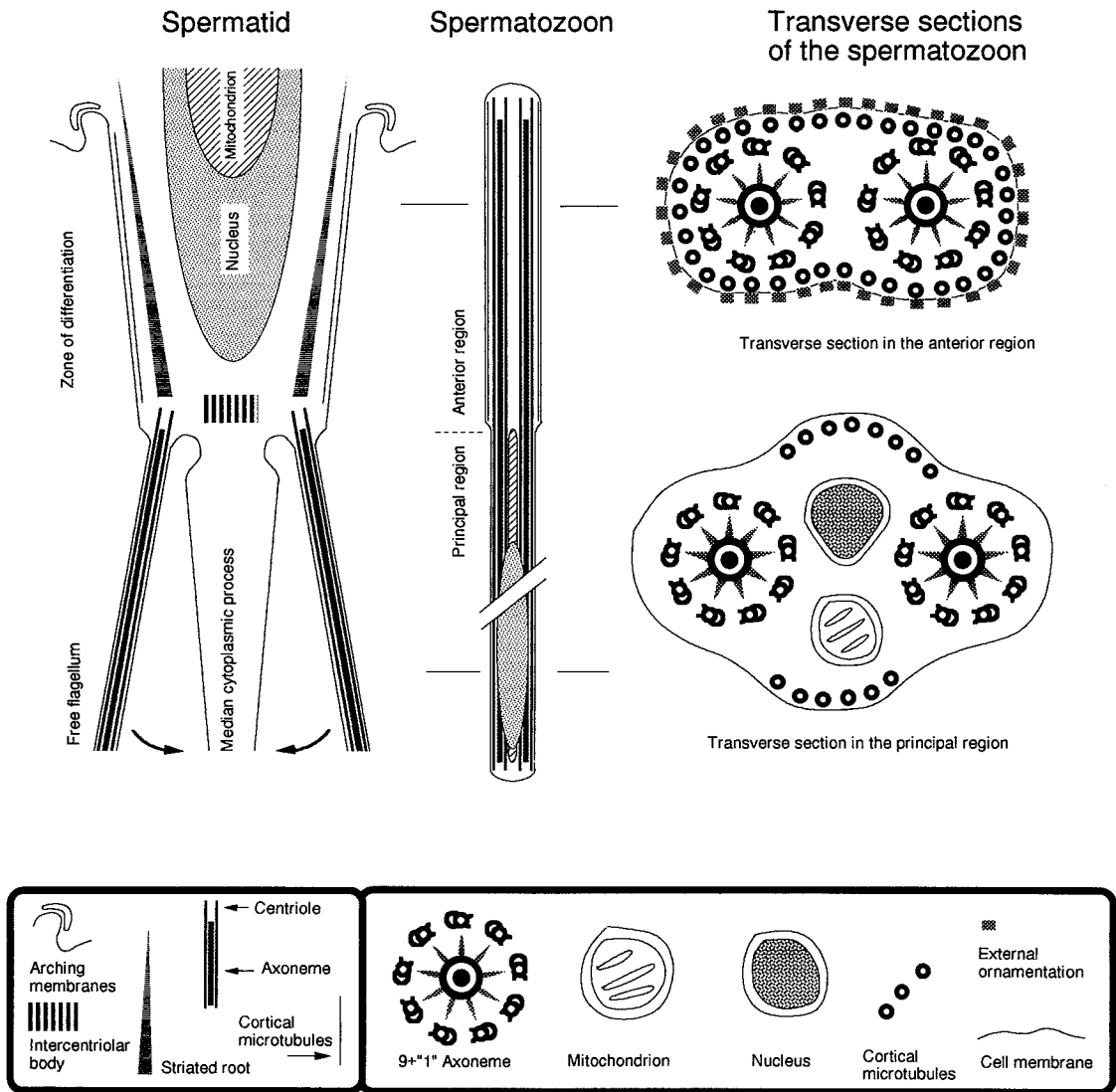


FIG. 1. Zone of differentiation and mature spermatozoon in the outgroup, the Digenea. The zone of differentiation produces the spermatozoon's anterior region; the middle cytoplasmic process and the two free flagella fuse ('proximo-distal fusion') to produce the spermatozoon's principal region, which contains the nucleus. Diagrams with length and width not to the same scale.

region is generally very short, originates from the spermatid's zone of differentiation, and has a continuous row of peripheral longitudinal microtubules often associated with external ornamentation on the cell membrane; the spermatozoon's *principal region* is generally very long and originates from the fusion of the median cytoplasmic process and the two free flagella. The principal region has peripheral longitudinal microtubules on the ventral and dorsal faces, originating from the median cytoplasmic process, but has no microtubules on the lateral faces, where the axonemes are located. Justine (in press a, b) con-

sidered this characteristic a synapomorphy for the Cercomeridea, and a consequence of the proximodistal fusion. The orientation of the Digenea spermatozoon (and that of other Cercomeridea) differs from that known in most (but not all) phyla, where the spermatozoon nucleus is anterior. During spermiogenesis in the Cercomeridea, the nucleus and mitochondrion become slender and migrate into the zone of differentiation. The nucleus then migrates to the distal extremity of the lengthening spermatid. Since the centrioles are located in the proximal extremity, this proximal extremity should be

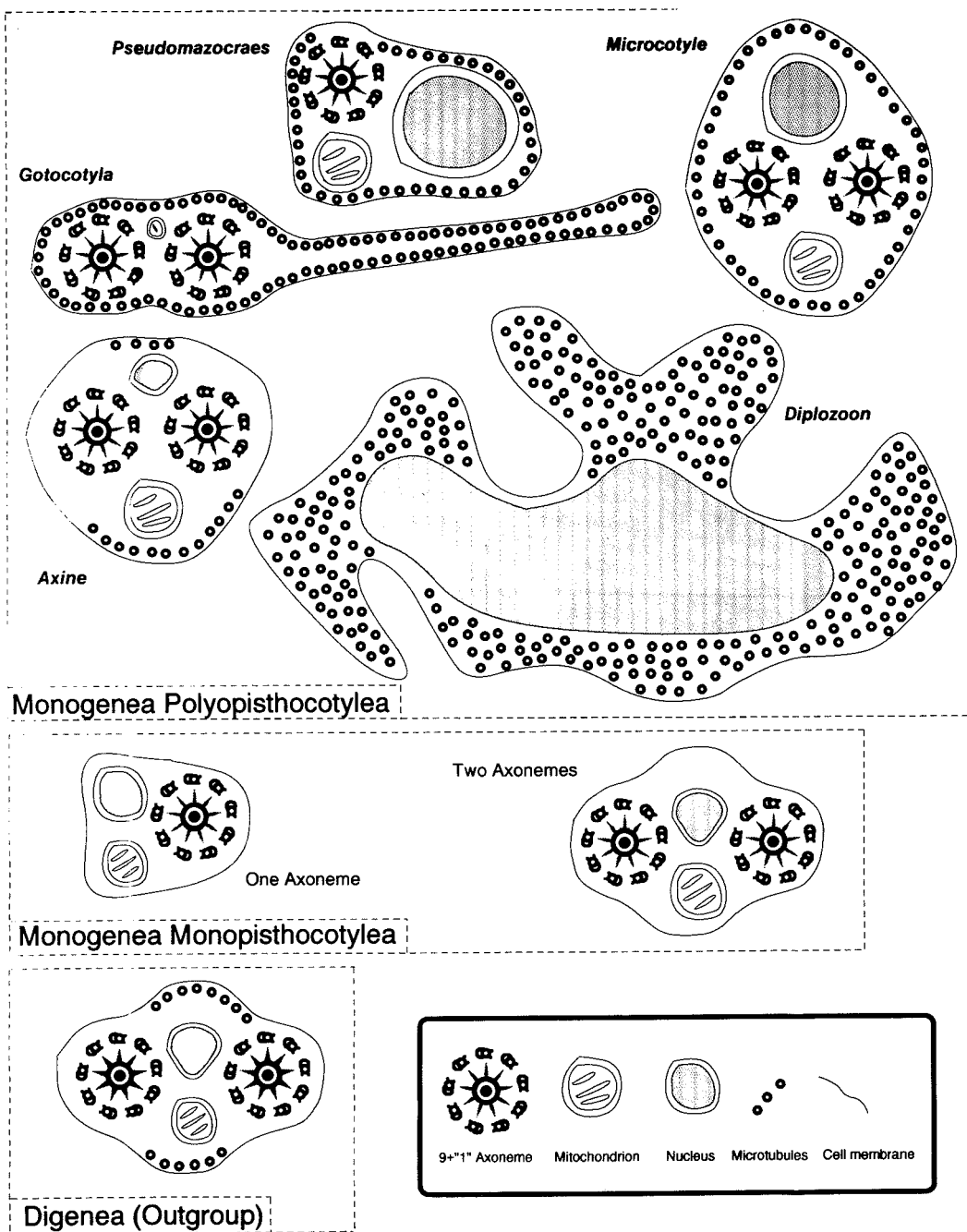


FIG. 2. Transverse sections of the spermatozoon's principal region in some Monogenea and in the outgroup, the Digenea. The Digenea have the sympleisiomorphic structure found in the Cercomeridea; microtubules are present only on the dorsal and ventral faces of the spermatozoon. The Polyopisthocotylea have supplementary microtubules, located on the lateral faces, along the axonemes; this is interpreted here as a synapomorphy for the group. The polyopisthocotylean common structure is shown here in *Microcotyle*. Other structures are autapomorphic. In *Pseudomazocraes*, one of the two axonemes is shorter than the other, and thus a part of the sperm body length shows only one axoneme. In *Axine*, lateral microtubules are absent: this is interpreted here as a reversal of the synapomorphy for the Polyopisthocotylea. In *Gotocotyla*, a motile undulating membrane, containing numerous cortical microtubules, is attached to the right side of the spermatozoon. *Diplozoon* is the single case of aflagellate spermatozoon in the Neodermata; the sperm body contains numerous cortical and cytoplasmic microtubules. The Monopisthocotylea have no dorsal and ventral microtubules; this is interpreted here as a synapomorphy for the group. Some taxa of the Monopisthocotylea secondarily lose one of their axonemes. Orientation of spermatozoa follows the convention of Sato, Oh & Sakoda (1967): nucleus dorsal, mitochondrion ventral.

considered anterior; moreover, observations of the fertilization process confirm this orientation (Justine & Mattei, 1984c). Therefore, the nucleus, located in the distal extremity (the principal region) of the spermatozoon, is posterior; the spermatozoon is considered 'inverted'.

Synapomorphies for the Monopisthocotylea and the Polyopisthocotylea

Two characters were recognized (Fig. 2)

Synapomorphy for the Polyopisthocotylea. Presence of lateral microtubules (along the axonemes) in the spermatozoon's principal region. This characteristic exists in no other Cercomeridea, except some Eucestoda where it probably arose by convergence. In the Polyopisthocotylea, this character was found in all species studied (more than 20) except *Axine* and *Pterinotrema*. This character is interpreted as a synapomorphy for the Polyopisthocotylea, with reversals in *Axine* and *Pterinotrema*. In *Diplozoon* the spermatozoon ultrastructure is so highly modified (aflagellate with numerous microtubules) that this character cannot be recognized.

Synapomorphy for the Monopisthocotylea. Absence of dorsal and ventral microtubules in the spermatozoon's principal region. This character is found in all taxa. Microtubules in the spermatozoon's principal region are completely lacking, since the lateral microtubules (characteristic of the Polyopisthocotylea) are also absent. This character (*absence* of dorso-ventral microtubules in the spermatozoon's principal region) is considered a synapomorphy for the Monopisthocotylea, whereas the *presence* of these microtubules is considered a synapomorphy for a higher ranking group, the Cercomeridea. Since the Monogenea are considered a monophyletic group within the Cercomeridea, this character state is interpreted as a reversal in the monopisthocotylean Monogenea. Also, since this character concerns the mature spermatozoon and not spermiogenesis, it is easy to observe. Some remarks: (a) In *Loimosina* and *Heterocotyle*, some peripheral microtubules are present. These microtubules are visible during spermiogenesis in the proximal region of the spermatid, or zone of differentiation. (b) In the Capsalidae and Dionchidae, microtubules are present in the zone of differentiation at the beginning of spermiogenesis, but later disappear. In these first two cases, the microtubules are homologous to the zone of differentiation's microtubules found in the Cercomeridea and not to the peripheral microtubules found in the spermatozoon's principal region. (c) In *Diplectanum* (Diplectanidae) Justine & Mattei (1982b) erroneously reported the presence of peripheral microtubules, which in fact are singlet microtubules originating from the distal extremity at eight out of nine axonemal doublets (Justine & Mattei, 1984b). These microtubules are also not homologous to the peripheral microtubules found in the spermatozoon's principal region.

The Polyopisthocotylea and the Monopisthocotylea thus may each be defined on the basis of one

synapomorphy and may be considered monophyletic, allowing a parsimony analysis to be performed within each of these two groups.

Spermiogenesis and spermatozoa in the Polyopisthocotylea

In the Polyopisthocotylea, spermatozoa and spermiogenesis are very homogeneous, and show the symplesiomorphic characters of the Cercomeridea. It is therefore difficult to perform a phylogenetic analysis from these structures. However, some autapomorphies may be cited; these could be useful for future phylogenetic analysis if the same character states are found in other taxa. The autapomorphies are (Fig. 2): (a) the undulating membrane of *Gotocotyla*; (b) the absence of lateral peripheral microtubules in *Axine* and *Pterinotrema* (reversal of the synapomorphy for the Polyopisthocotylea); (c) the presence of a single axoneme along part of the sperm body's length (this was described in *Pseudomazocraes*; the same structure exists in *Plectanocotyle*, where Tuzet & Ktari (1971b) interpreted it, probably erroneously, as the co-existence of two types of spermatozoa, uniflagellate and biflagellate); and (d) the aberrant case of *Diplozoon*. In this animal, the zone of differentiation, middle cytoplasmic process and flagella are absent during spermiogenesis. The spermatozoon is aflagellate and contains small mitochondria, endoplasmic reticulum, and hundreds of both peripheral and internal longitudinal microtubules. These peculiarities prohibit the use of this sperm structure for phylogenetic analysis. The aberrant pattern of the spermatozoon of *Diplozoon* may be correlated with an aberrant reproductive biology: the two hermaphroditic members of a pair are permanently fused together and the sexual ducts are anastomosed.

Spermiogenesis and spermatozoa in the Monopisthocotylea, and a parsimony analysis

This taxon shows a wide variety and a high degree of evolution compared to the symplesiomorphic cercomeridean sperm pattern. The characters used in this analysis are described in Table 2 and diagrammed in Figs. 3–5.

A parsimony analysis was made from the data matrix shown in Table 3 (23 taxa, 18 characters). This matrix contains nine redundant taxa (taxa are considered 'redundant' when their matrix line is similar; this does not mean that it is the same taxon, since characters used here deal only with spermiogenesis). The matrix also contains seven taxa in which spermiogenesis is incompletely known; these are the taxa with '?' in the matrix, except for character 18 for which '?' means uncodable.

Because of the limits imposed by the hardware (see the Materials and Methods), the parsimony analysis was performed on the 14 non-redundant taxa; this simplified matrix is given in Table 4. A branch-and-bound parsimony analysis (see PAUP options in the Materials and Methods) of this simplified matrix produced 400 trees each 25 steps long. A strict

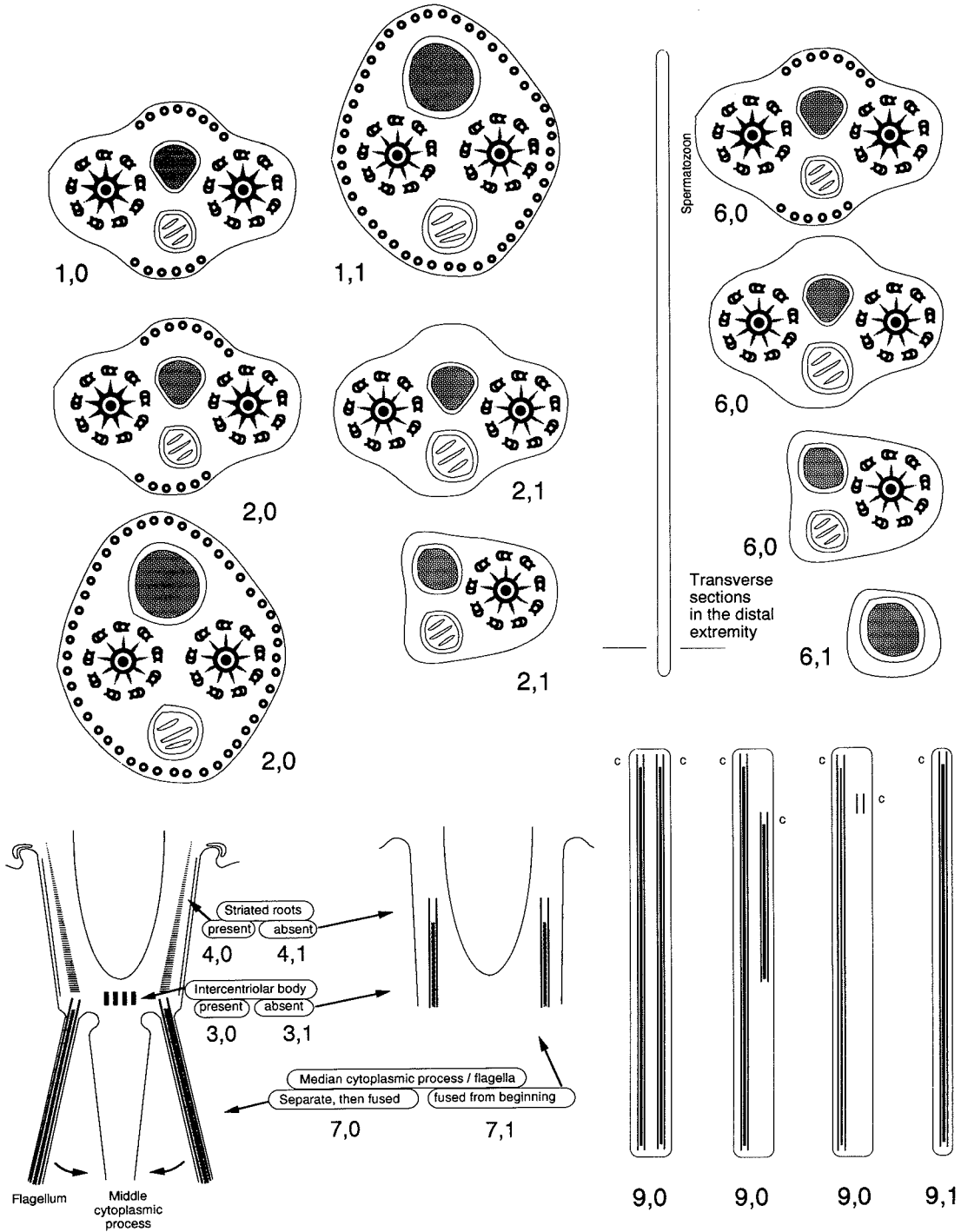


FIG. 3.

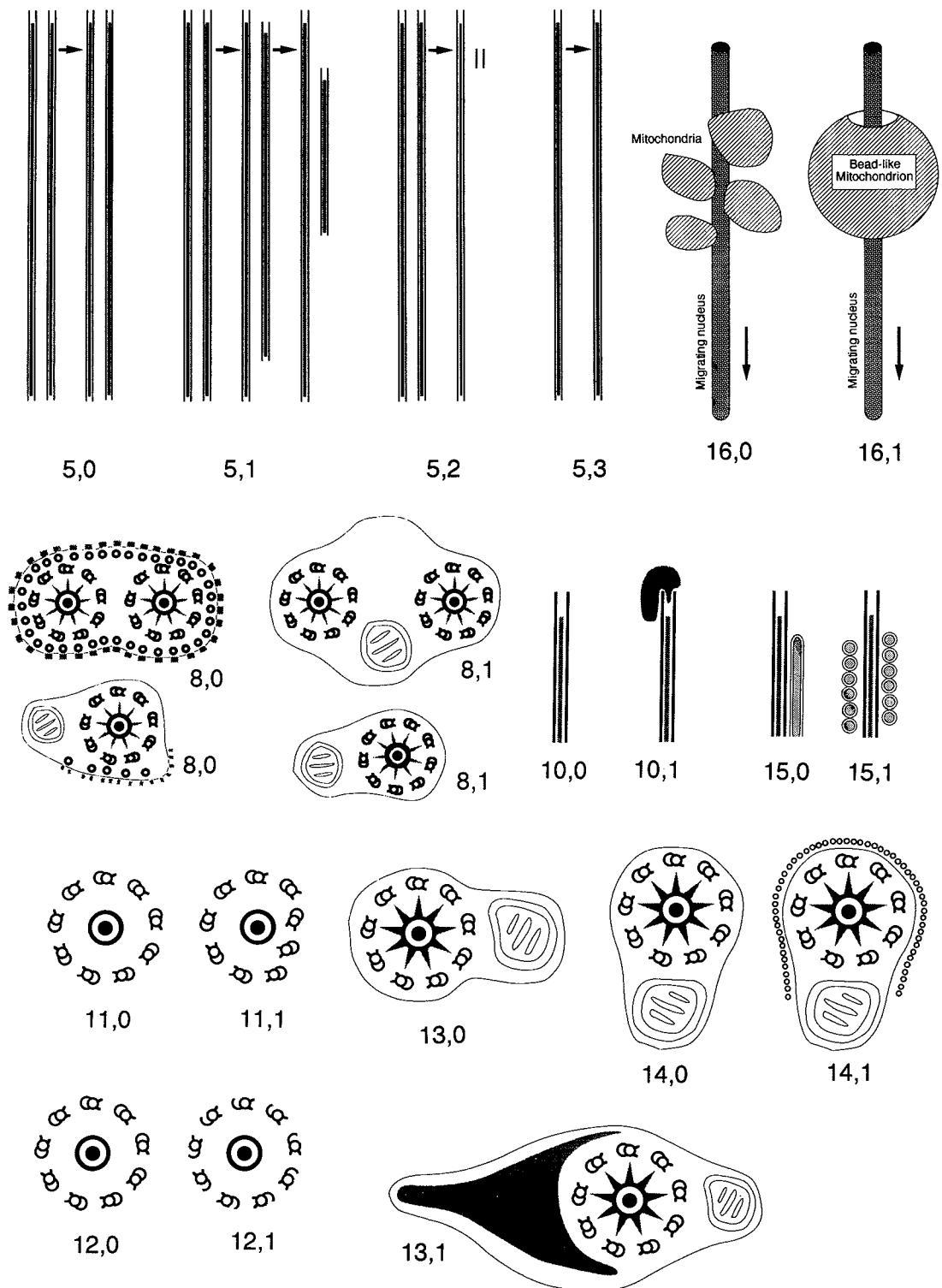


FIG. 4.

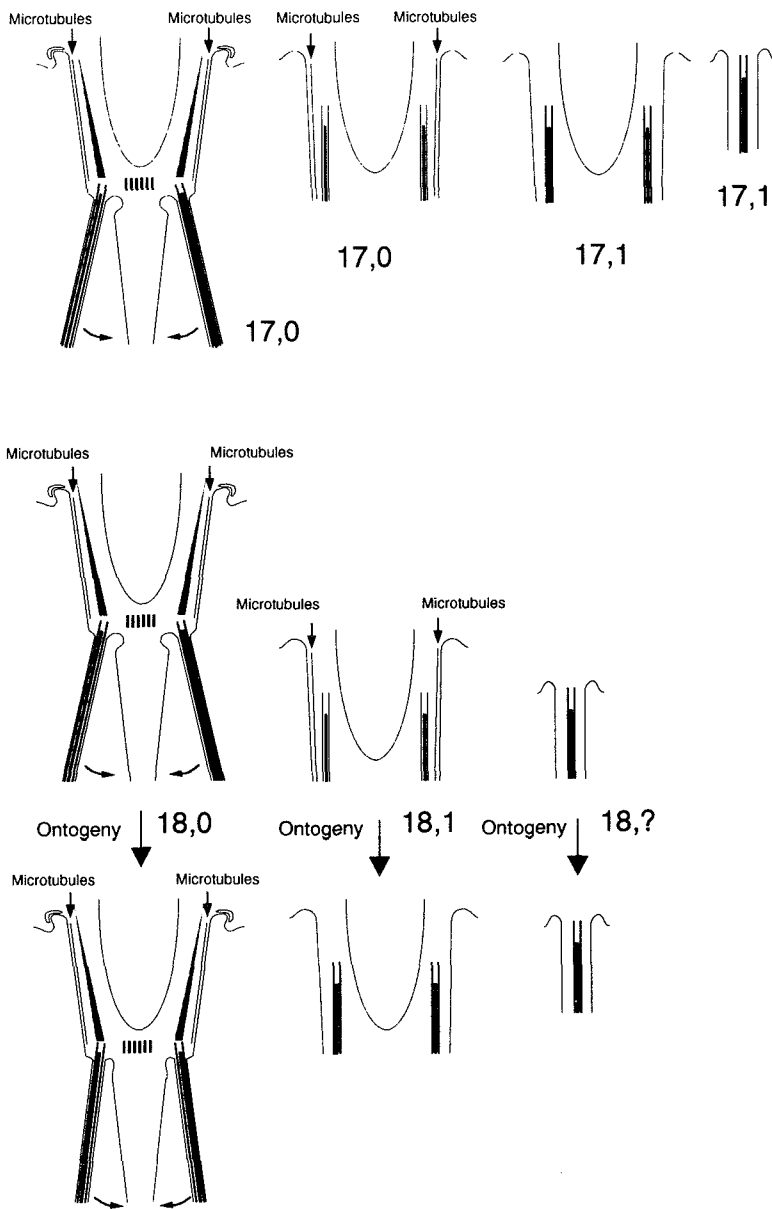


FIG. 5.

consensus tree (length 32 steps, consistency index 0.625) and a semi-strict consensus tree (length 31 steps, consistency index 0.645) were computed from these 400 trees (Fig. 6). The two consensus trees differed only in the isolation of a group containing *Diplectanum* + *Lamellodiscus* in the semi-strict consensus tree, which was present in 90% of the trees; this difference is probably due to the presence of several '?' in the *Lamellodiscus* data matrix line.

A cladogram was constructed from the semi-strict

consensus after addition of the redundant taxa (Fig. 7).

Some characters were considered 'uninformative' by PAUP. These were the autapomorphies for a single taxon. Characters 13 and 14, which are autapomorphies of *Calceostoma*, were however conserved here because they could have an important comparative role when other data become available. Character 1 was considered 'uninformative' by PAUP, but it was only because the Polyopisthocotylea were considered together as a single taxon.

TABLE 2—LIST OF CHARACTERS ANALYSED (SEE FIGS. 3–5)

No.	Character	Character states
1	Lateral microtubules in the spermatozoon's principal region	0: absent; 1: present
2	Dorsoventral microtubules in the spermatozoon's principal region	0: present; 1: absent
3	Intercentriolar body	0: present; 1: absent
4	Striated roots	0: present; 1: absent
5	Number of axonemes during spermiogenesis	0: 2 axonemes; 1: 1 axoneme + 1 altered axoneme 2: 1 axoneme + 1 disappearing axoneme 3: 1 axoneme from beginning
6	Distal region containing only the nucleus in mature spermatozoon	0: absent; 1: present
7	Cytoplasmic middle process and flagella	0: separate, then fused 1: fused from the start
8	External ornamentation of the cell membrane	0: present; 1: absent
9	Number of centrioles in the spermatozoon	0: 2 centrioles; 1: 1 centriole
10	Centriole adjunct	0: absent; 1: present
11	Axoneme structure in mature spermatozoon	0: circular; 1: non-circular
12	Axonemal b microtubules during spermiogenesis	0: complete; 1: incomplete
13	Lateral crest on mature spermatozoon	0: absent; 1: present
14	External microtubules associated with the spermatid	0: absent; 1: present
15	Anterior region of the nucleus	0: not coiled; 1: coiled
16	A bead-like giant mitochondrion	0: absent; 1: present
17	Microtubules in the spermatid's zone of differentiation	0: present; 1: absent
18	Ontogeny of microtubules in the zone of differentiation	0: persisting; 1: disappearing

All characters are binary unordered characters, except character 5, a multistate unordered character. Characters 3, 4, 5, 7, 12, 14, 16, 17, 18 concern spermiogenesis; the others concern the mature spermatozoon.

DISCUSSION

In the tree presented in Fig. 7, the position of some taxa comes from the lack of data concerning spermiogenesis. For example, *Trochopus* is clearly a

Capsalidae, but however is not included in the Capsalidae/Dionchidae group because the synapomorphies of spermiogenesis for this taxon have not been described. Also, the positions of *Myxinidocotyle* (not

FIGS. 3–5. Characters used in this paper for a parsimony analysis of the Monogenea. Characters are not placed into numerical order due to spacing.

FIG. 3. Characters 1, 2, 3, 4, 6, 7 and 9 (characters 3, 4 and 7 are shown on the same diagram). Character 1, lateral microtubules in the spermatozoon's principal region: 1,0: absent; 1,1: present. Character 2, dorsoventral microtubules in the spermatozoon's principal region: 2,0: present; 2,1: absent. Character 3, intercentriolar body: 3,0: present; 3,1: absent. Character 4, striated roots: 4,0: present; 4,1: absent. Character 6, distal region only with the nucleus in mature spermatozoon: 6,0: absent (various morphologies are shown); 6,1: present. Character 7, cytoplasmic middle process and free flagella: 7,0: separate, thus fused; 7,1: fused from the start of spermiogenesis. Character 9, number of centrioles (c) in the spermatozoon: 9,0: two centrioles; 9,1: one centriole.

FIG. 4. Characters 5, 8, 10, 11, 12, 13, 14, 15 and 16. Character 5, number of axonemes during spermiogenesis (multistate unordered character): 5,0: two axonemes; 5,1: one axoneme and one altered axoneme; 5,2: one axoneme and one disappearing axoneme; 5,3: only one axoneme from the start of spermiogenesis; arrows indicate a change during ontogeny (spermiogenesis). Character 8, external ornamentation on cell membrane: 8,0: present; 8,1: absent. Character 10, centriole adjunct: 10,0: absent; 10,1: present. Character 11, axoneme structure in the mature spermatozoon: 11,0: circular; 11,1: non-circular. Character 12, axonemal b microtubules during spermiogenesis: 12,0: complete b microtubules; 12,1: incomplete b microtubules. Character 13, lateral crest on the mature spermatozoon: 13,0: absent; 13,1: present. Character 14, external microtubules associated with the spermatid: 14,0: absent; 14,1: present. Character 15, Nucleus in its anterior part: 15,0: not coiled; 15,1: coiled. Character 16, one bead-like giant mitochondrion with the nucleus going through it: 16,0: absent; 16,1: present.

FIG. 5. Characters 17 and 18. Character 17, microtubules in the spermatid's zone of differentiation: 17,0: present; 17,1: absent. Character 18, ontogeny of microtubules in the zone of differentiation: 18,0: microtubules persist; 18,1: microtubules disappear; 18,?: uncodable state if state 17,1 is present; arrows indicate a change during ontogeny (spermiogenesis).

TABLE 3—DATA MATRIX OF THE 23 TAXA STUDIED

Taxon	Character states																Remarks (redundant taxa)
	1	2	3	4	5	6	7	8	9	1	1	1	1	1	1	1	
Acanthocotyle	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	1	?
Amphibdella	0	1	1	1	3	0	1	1	1	1	0	0	0	1	0	1	?
Amphibdelloides	0	1	1	1	3	0	1	1	1	1	0	0	0	1	0	1	?
Caballerocotyla	0	1	1	1	0	0	1	1	0	0	0	0	0	0	1	0	1
Calceostoma	0	1	1	1	3	1	1	1	1	0	1	1	1	1	0	0	1
Cichlidogyrus	0	1	?	?	3	?	?	?	?	?	0	?	0	0	?	?	?
Cleitharhicus	0	1	1	1	3	0	1	0	1	0	0	1	0	0	0	0	1
Digenea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dionchus	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	1	0
Diplectanum	0	1	1	1	3	1	1	1	1	1	0	0	0	0	0	1	?
Encotyllabe	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	1	0
Euzetrema	0	1	?	?	0	0	?	?	0	?	0	?	0	0	0	?	?
Furnestinia	0	1	1	1	3	1	1	1	1	1	0	0	0	0	0	0	1
Gyrodactylus	0	1	?	?	0	0	?	?	0	?	0	?	0	0	0	?	?
Heterocotyle	0	1	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0
Lamellodiscus	0	1	?	?	3	1	?	?	?	1	0	?	0	0	0	?	?
Loimosina	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Megalocotyle	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	1	0
Myxinidocotyle	0	1	?	?	0	0	?	?	0	?	0	?	0	0	0	?	?
Polyopisthocotylea	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudodactylogyrus	0	1	1	1	3	0	1	1	1	1	0	1	0	0	0	0	1
Tetraonchus	0	1	?	?	3	?	?	?	?	?	0	?	0	0	?	?	?
Trochopus	0	1	?	?	0	0	?	?	0	?	0	?	0	0	0	?	?

Taxa are in alphabetical order. Reference taxa for redundant taxa were chosen arbitrarily. 'spe?' indicates taxa in which spermiogenesis is unknown.

placed close to *Acanthocotyle*, which belongs to the same family Acanthocotylidae), *Euzetrema* and *Gyrodactylus* do not indicate their real phylogenetic position because of a lack of data on their spermiogenesis. Hence, with this analysis, these four taxa can only be placed within the Monopisthocotylea, but not placed within a subgroup of the Monopisthocotylea.

The following monophyletic taxa may be defined on the basis of spermatozoal and spermiogenetic synapomorphies:

- Polyopisthocotylea (synapomorphy: 1).
- Monopisthocotylea (synapomorphies: 2, 3, 4).
- Loimoidae/Monocotylidae (synapomorphy: 5, state 1).
- Capsalidae/Dionchidae (synapomorphies: 16, 18). These synapomorphies concern spermiogenesis; *Trochopus*, which is also a Capsalidae, is not included here because of the lack of data on its spermiogenesis. It is remarkable that *Dionchus* (family Dionchidae) cannot be distinguished from the three other genera (*Megalocotyle*, *Caballerocotyla*, *Encotyllabe*), which belong in the family Capsalidae. The close phylogenetic relationships of the Capsalidae and Dionchidae were also proposed in the classifications of Llewellyn (1970), Beverley-Burton (1984) and Bychowsky (1957), but not in that of Sproston (1946) and Baer & Euzet (1961).
- Monogenea with uniflagellate spermatozoa (synapomorphies: 5 state 3, 9). This group is polychotomous because various apomorphic characters

(centriole adjunct, incomplete b tubules) conflict with each other. Within this group, the Amphibdellatidae (*Amphibdella* and *Amphibdelloides*) may be defined on the basis of synapomorphy 15. *Calceostoma* (Calceostomatidae) possesses two autapomorphies (13, 14).

The separation of the Monopisthocotylea from the Polyopisthocotylea is one of the most striking results of this analysis. Thus, spermatological data confirm this dichotomy, based primarily on morphological and anatomical data: the structure of the attachment organ, the haptor, and the genito-intestinal canal. In contrast, protonephridia ultrastructure, which has a high phylogenetic value for larger taxa (Rohde, 1990), does not allow the Monopisthocotylea and the Polyopisthocotylea to be distinguished (Rohde, Justine & Watson, 1989).

As a general rule, it is not recommended to define the phylogeny of a group only on the basis of the characters from a single organ or a single structure, such as the spermatozoon ultrastructure herein. The phylogenetic conclusions expressed here should be tested against data coming from the analysis of other characters. The best would be to utilize a cladistic analysis of the morphological characters (haptor, oncomiracidium) of the Monogenea, and then add this matrix to the matrix herein from ultrastructural data. However, as far as known, such an analysis does not exist, although some attempts have been made on some families or smaller taxa (Klassen & Beverley-Burton, 1987, 1988; Kritsky

TABLE 4—DATA MATRIX AFTER REMOVAL OF REDUNDANT TAXA

Taxon	Character states																Remarks
	1	2	3	4	5	6	7	8	9	1	1	1	1	1	1	1	
Acanthocotyle	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	1	?
Amphibdella	0	1	1	1	3	0	1	1	1	1	0	0	0	0	1	0	?
Megalocotyle	0	1	1	1	0	0	1	1	0	0	0	0	0	0	0	1	0
Calceostoma	0	1	1	1	3	1	1	1	1	0	1	1	1	1	0	0	?
Tetraonchus	0	1	?	?	3	?	?	?	?	?	?	?	?	?	?	?	?
Cleitharthicus	0	1	1	1	3	0	1	0	1	0	0	1	0	0	0	0	?
Digenea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?
Diplectanum	0	1	1	1	3	1	1	1	1	1	0	0	0	0	0	0	?
Euzetrema	0	1	?	?	0	0	?	?	?	?	?	?	?	?	?	?	?
Heterocotyle	0	1	1	1	2	1	0	0	0	0	0	0	0	0	0	0	?
Lamellodiscus	0	1	?	?	3	1	?	?	?	?	?	?	?	?	?	?	?
Loimosina	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	?
Polyopisthocotylea	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?
Pseudodactylogyrus	0	1	1	1	3	0	1	1	1	1	0	1	0	0	0	1	?

'spe?' indicates taxa in which spermiogenesis is unknown.

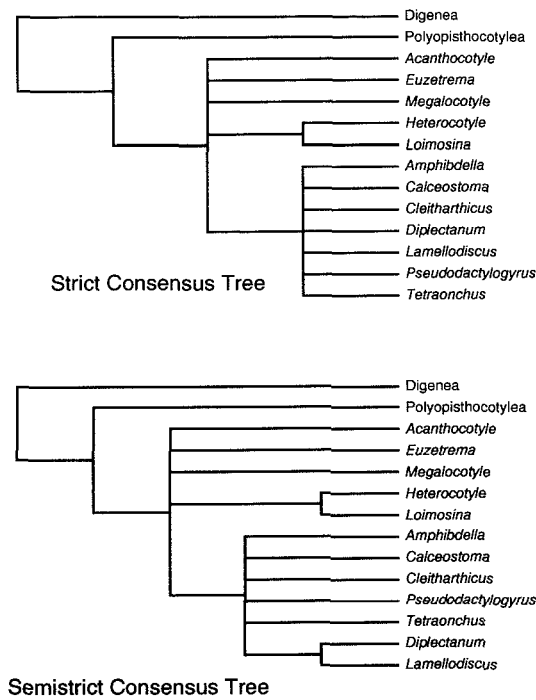


FIG. 6. Consensus trees obtained after a branch-and-bound parsimony analysis of the data matrix shown in Table 4. These trees do not include redundant taxa. They were computed from 400 trees of 25 step lengths. Strict consensus tree: with a length of 32 steps; consistency index: 0.625. Semi-strict consensus tree: with a length of 31 steps; consistency index 0.645.

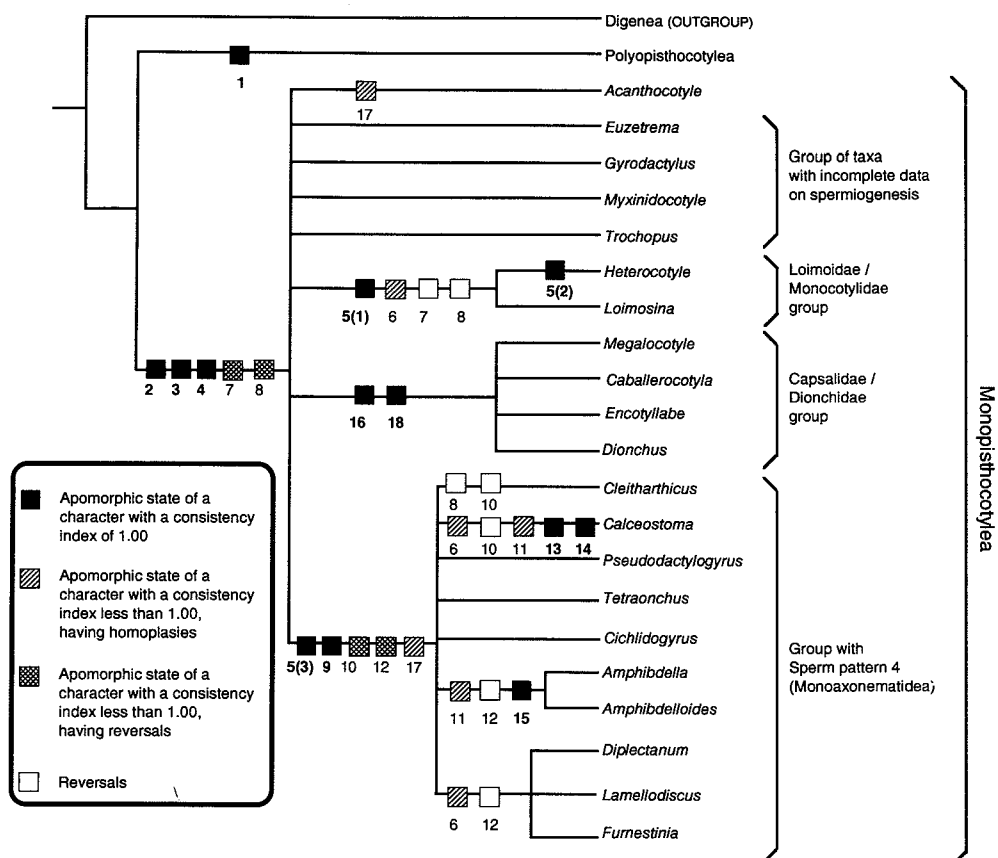


FIG. 7. Cladogram for the Monogenea, based on the semi-strict consensus tree computed after a branch-and-bound parsimony analysis of the 18 spermiogenetic and spermatozoal ultrastructural characters. Redundant taxa are included in this cladogram. Tree length has 31 steps; consistency index 0.645; consistency index excluding 'uninformative' characters: 0.607. Synapomorphies and autapomorphies are indicated.

& Boeger, 1989; Boeger & Kritsky, 1989; Wheeler & Beverley-Burton, 1989; Beverley-Burton & Klassen, 1990; Measures, Beverley-Burton & Williams, 1990).

Three previous studies used spermatozoon ultrastructure for analysing phylogeny: Justine, Lambert & Mattei (1985), Lebedev (1988), and Malmberg (1990); they are hereafter analysed and commented upon.

In the paper by Justine, Lambert & Mattei (1985), a good agreement was noticed between the results of comparative spermatology and Lambert's (1980) classification, mainly based on oncomiracidia chaetotaxy. This study of the Monogenea included *Udonella*. Later ultrastructural studies (Kornakova, 1987, 1988; Xylander, 1988; Rohde, Watson & Roubal, 1989) indicated that this taxon should be excluded from the Monogenea. In cladistic classifications, it is now a sister-group to either the Neodermata (Brooks, 1989a; Justine, in press a) or the Cestoda (Rohde, 1990).

In this 1985 study, only two characters were used: the number of axonemes and the presence/absence of cortical microtubules. The character of "the number

of axonemes in the mature spermatozoon" in fact concerns both spermiogenesis and mature spermatozoon, because the concept of axoneme alteration during spermiogenesis was used (for *Heterocotyle* and *Loimosina*). A classification of spermatozoa into four types (1, 2, 3 and 4) was proposed in this paper. A fifth type (aberrant) was later defined for *Diplozoon* (see Justine, Le Brun & Mattei, 1985a, b). This classification seems to be followed by other authors (Malmberg & Afzelius, 1990; Malmberg, 1990; Tappenden & Kearn, 1990).

The classification into five patterns may be retained, with the following slight modifications:

—The Polyopisthocotylea are characterized by pattern 1, with one aberrant case (pattern 5); the Monopisthocotylea are characterized by patterns 2, 3 and 4.

—The polyopisthocotylean type 1 (two axonemes and cortical microtubules) differs from the digenean spermatozoon by the presence of lateral microtubules in the spermatozoon's principal region.

—Type 3 (one axoneme + one altered axoneme + cortical microtubules in the anterior region) is retained; it is exclusively found in the Monocotylidae and Loimoidae.

—Type 2 (two axonemes, cortical microtubules absent) does not seem to correspond to a taxonomic unit. This option was already expressed by Justine, Lambert & Mattei (1985) and Justine & Mattei (1987). However, Malmberg & Afzelius (1990), Malmberg (1990) and Tappenden & Kearn (1990) used the presence of Type 2 in the Acanthocotylidae as a phylogenetic argument. For the purpose of clarity, it is hereby proposed to divide this type into three subtypes. (a) Subtype 2a, with the spermiogenetic synapomorphies, a bead-like mitochondrion and the loss of microtubules in the zone of differentiation during spermiogenesis. This subtype probably corresponds to a phylogenetic unit (Dionchidae + Capsalidae in our present state of knowledge). (b) Subtype 2b, with microtubules absent from the start of spermiogenesis, which characterizes only *Acanthocotyle* in our present state of knowledge. (c) Subtype 2c, without apomorphies, found in *Euzetrema* and *Gyrodactylus*; this subtype 2c is expected to be broken up later when detailed studies of spermiogenesis allow the description of apomorphies. *Trochopus*, a Capsalidae, is included in this subtype only because its spermiogenesis is unknown. In our present state of knowledge (Fig. 7), subtype 2c should be considered the symplesiomorphic pattern for the Monopisthocotylea (it only has the synapomorphies for the Monopisthocotylea).

—Type 4 (one axoneme, cortical microtubules absent) is retained and is thought to characterize a monophyletic taxon.

—Type 5 (no axoneme, numerous cortical and cytoplasmic microtubules) characterizes only *Diplozoon* (Polyopisthocotylea, Diplozoidae). This aberrant type is probably correlated with its reproductive biology rather than with phylogeny; however, to ascertain this, data are needed from the families close to the Diplozoidae and with normal reproductive biology. This extraordinary case illustrates relationships between sperm structure and the biology of reproduction (Franzén, 1956; Baccetti & Afzelius, 1976; Afzelius, 1983; Jamieson, 1987; Holland, 1990; and references therein).

Lebedev's classification (1988, 1989) uses classical morphology and updates Bychowsky's classification (1957). Spermatozoon ultrastructure is used as a complementary argument for the morphological analysis in some cases, such as the phylogenetic position of *Gotocotyla* (= *Cathucotyle*), but not for the phylogenetic relationships between Capsalidae and Dionchidae. Lebedev (1988, p. 154) cites data on the ultrastructure of the Microbothriidae erroneously, since spermatozoon ultrastructure has not been studied in this family.

If the type of spermatozoon is indicated for each family in this classification (Table 5), it appears that

this classification remarkably coincides with the data from comparative spermatology, particularly in the higher ranking taxa proposed by Lebedev. The superorder Monocotylina Lebedev, 1988 is characterized by sperm pattern 3. The order Capsalidea Lebedev, 1988 is characterized by sperm pattern 2 (subtypes 2a and 2b), and the superfamily Capsaloidae contains the Capsalidae and Dionchidae (sperm pattern 2a), and the Iagotrematidae (*Euzetrema*, sperm pattern 2c). The Gyrodactylidae (sperm pattern 2c), are placed in another suborder, and this fits well with the non-monophyly of Monogenea with sperm pattern 2. However, spermatological arguments for a separation of the Sphyrnauridae and Polystomatidae from the Polyopisthocotylea (as in Lebedev's classification) are lacking, since they share sperm pattern 1.

One of the most interesting points is that all spermatozoa with sperm pattern 4 are found in only two orders: Dactylogyridae and Tetraonchidea. These two orders thus are likely to constitute a monophyletic taxon. The name **Monoaxonematidea** is proposed for a taxon uniting these two groups (etymology: one single axoneme).

Malmberg's classification (1990) differs from all others by the fact that Malmberg claims that the haptor's evolution is progressive (hook number increases during evolution), although other authors consider the number of hooks to decrease during evolution. In Malmberg's text, the sperm pattern is indicated for each family, but apparently is not used for the erection of the higher ranking taxa recognized in this classification. The superorder Laterohaptanchorea Malmberg, 1990, which contains the families with sperm patterns 1, 2 and 3, is an example showing the disagreement of Malmberg's classification with the results of comparative spermatology. This superorder cannot be accepted as monophyletic if spermatological data are considered.

Superorder Laterohaptanchorea Malmberg, 1990

Family	Spermatozoon pattern
Hexabothriidae	1
Polystomatidae	1
Sphyrnauridae	1
Iagotrematidae	2c
Capsalidae	2a
Dionchidae	2a
Neodactylodiscidae	?
Monocotylidae	3
Loimoidae	3
Montchadskyellidae	?

In Malmberg's work, spermatozoon type 2 is used for inferring phylogenetic relationships, and this contradicts the opinions expressed herein and in previous papers (Justine, Lambert & Mattei, 1985; Justine & Mattei, 1987).

Two conclusions may be drawn from the present analysis, and may serve as guides for future research. They concern the simplification in the evolution of spermiogenesis in the Monopisthocotylea, and the

TABLE 5—SPERMATOZOON PATTERN AND LEBEDEV'S CLASSIFICATION (1988) FOR THE MONOGENEA

Taxon	Sperm pattern
Class Monogenoidea	
Subclass Polyonchoinea	
Superorder Microbothria	
Order Microbothrida	
Family Microbothriidae	?
Superorder Dactylogyria	
Order Dactylogyridea	
Suborder Dactylogyrynea	
Family Dactylogyridae*	4
Family Diplectanidae	4
Family Ancyrocephalidae	4
Family Neodactylodiscidae	?
Suborder Calceostomatinea	
Family Calceostomatidae	4
Order Tetraonchidea	
Family Tetraonchidae	4
Family Amphibdellatidae	4
Family Tetraonchoididae	?
Family Bothitrematidae	?
Order Capsalidea	
Superfamily Capsaloidea	
Family Capsalidae	2a
Family Dionchidae	2a
Family Iagotrematidae	2c
Superfamily Acanthocotyloidea	
Family Acanthocotylidae	2b
Superorder Monocotylia	
Order Monocotylinea	
Suborder Monocotylinea	
Family Monocotylidae	3
Family Loimoidae	3
Suborder Montchadskiellinae	
Family Montchadskiellidae	?
Superorder Gyrodactylia	
Order Gyrodactylidea	
Family Gyrodactylidae	2c
Subclass Polystomatinea	
Order Polystomatidea	
Family Polystomatidae	1
Family Sphranuridae	1
Subclass Oligonchoinea†	
Most families	1
Family Diplozoidae	5

* Family Dactylogyridae includes the Pseudodactylogyridae in this system.

† Oligonchoinea not separated into subgroups.

relative value of spermiogenesis and spermatozoa for phylogenetic analysis.

Many synapomorphies used in the present analysis indicate a simplification in evolution: the plesiomorphic character state has numerous organelles, and the apomorphic character states progressively lose these organelles. However, some characters seem to be new acquisitions (i.e. the apomorphic structure is more complicated than in the outgroup): the centriole adjunct of some spermatozoa with pattern 4, the single bead-like mitochondrion in sperm pattern 2a, and the lateral crest and extracellular tubules of *Calceostoma*.

The ontogenetic process (spermiogenesis) contains more information than only the result of the process (the mature spermatozoon). An attempt at parsimony analysis of Table 4 data matrix after excluding spermiogenetic characters resulted in a highly polychotomous tree (not shown here). Transient structures seen only during spermiogenesis, but not in the mature spermatozoon, provide numerous useful characters. Various spermiogenetic patterns may lead to similar (converging) spermatozoon morphologies. Some taxa (such as the Capsalidae/Dionchidae) cannot be recognized if only the spermatozoon characters are used.

Thus, the study of spermiogenesis is indispensable to correctly define synapomorphies.

Acknowledgements—Electron microscope observations were performed at the Centre de Microscopie Électronique, Faculté des Sciences, Université de Dakar, Senegal, and at the Service d'Accueil de Microscopie Électronique du Centre National de la Recherche Scientifique, Paris, France. Profs L. Euzet, B. Afzelius and G. Malmberg, and Dr G. Oliver communicated unpublished observations. Prof. L. Euzet and Dr A. Fournier provided specimens; Dr I. Beveridge and Profs K. Rohde and G. Malmberg provided manuscripts in press. Drs B. Seret, D. Goujet and G. Dingerkus, and Profs L. Euzet, A. Chabaud and K. Rohde made helpful comments on the manuscript. Dr G. Dingerkus kindly edited the English. The software PHYLIP, programmed by Felsenstein, was obtained for free from Dr W. Ellis (Instituut voor Taxonomische Zoölogie, Zoölogisch Museum, Universiteit van Amsterdam afd. Entomologie, Plantage Middenlaan 64, 1018 DH Amsterdam, The Netherlands). MACCLADE 2.1 is a free program distributed by its authors, W. P. Maddison and D. R. Maddison, Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, U.S.A.

Note added in proof—Since submission of this paper, three studies of monogenean spermatozoa have been published (Schmahl & Obiekieze, 1991; Tappenden & Kearn, 1991 a, b). Data reported in these articles agree with the phylogenetic analysis proposed in the present paper.

REFERENCES

- AFZELIUS B. A. 1983. Sperm structure in relation to function and phylogeny. In: *The Sperm Cell* (Edited by ANDRÉ J.), pp. 385–394. Martinus Nijhoff, The Hague.
- AX P. 1987. *The Phylogenetic System. The Systematization of Organisms on the Basis of their Phylogenesis*. Wiley, Chichester.
- BACCETTI B. & AFZELIUS B. A. 1976. *The Biology of the Sperm Cell*. Karger, Basel.
- BAER J. G. & EUZET L. 1961. Classe des Monogènes. In: *Traité de Zoologie*, Vol. 4 (Edited by GRASSÉ P. P.), pp. 243–325. Masson, Paris.
- BAVERSTOCK P. R., FIELKE R., JOHNSON A. M., BRAY R. A. & BEVERIDGE I. 1991. Conflicting phylogenetic hypotheses for the parasitic platyhelminths tested by partial sequencing of 18S ribosomal RNA. *International Journal for Parasitology* **21**: 329–339.
- BEVERLEY-BURTON M. 1984. Monogenea and Turbellaria. In: *Guide to the Parasites of Fishes in Canada* (Edited by MARGOLIS L. & KABATA Z.). *Canadian Special Publication of Fisheries and Aquatic Sciences* **74**, Part 1: 5–209.
- BEVERLEY-BURTON M. & KLASSEN G. J. 1990. New approaches to the systematics of the ancyrocephalid Monogenea from nearctic freshwater fishes. *Journal of Parasitology* **76**: 1–21.
- BOEGER W. A. & KRITSKY D. C. 1989. Phylogeny, co-evolution, and revision of the Hexabothriidae Price, 1942 (Monogenea). *International Journal for Parasitology* **19**: 425–440.
- BROOKS D. R. 1982. Higher level classification of parasitic Platyhelminthes and fundamentals of cestode classification. In: *Parasites—Their World and Ours* (Edited by METTRICK D. F. & DESSER S. S.), pp. 189–193. Elsevier Biomedical Press, Amsterdam.
- BROOKS D. R., O'GRADY R. T. & GLEN D. R. 1985. The phylogeny of the Cercomeria Brooks, 1982 (Platyhelminthes). *Proceedings of the Helminthological Society of Washington* **52**: 1–20.
- BROOKS D. R. 1989a. A summary of the database pertaining to the phylogeny of the major groups of parasitic platyhelminths, with a revised classification. *Canadian Journal of Zoology* **67**: 714–720.
- BROOKS D. R. 1989b. Erratum: a summary of the database pertaining to the phylogeny of the major groups of parasitic platyhelminths, with a revised classification. *Canadian Journal of Zoology* **67**: 2607–2608.
- BROOKS D. R. 1989c. The phylogeny of the Cercomeria (Platyhelminthes: Rhabdocoela) and general evolutionary principles. *Journal of Parasitology* **75**: 606–616.
- BURTON P. R. 1972. Fine structure of the reproductive system of a frog lung-fluke. III. The spermatozoon and its differentiation. *Journal of Parasitology* **58**: 68–83.
- BYCHOWSKY B. E. 1957. [Monogenetic Trematodes, Their Systematics and Phylogeny] (in Russian). Izdatel'stvo Akad. Nauk SSSR, Moskva-Leningrad. English Edition Edited by HARGIS W. H., A.I.B.S., Washington, 1961.
- EHLERS U. 1984. Phylogenetisches System der Platyhelminthes. *Verhandlungen des Naturwissenschaftlichen Vereins Hamburg (NF)* **27**: 291–294.
- EHLERS U. 1985a. Phylogenetic relationships within the Platyhelminthes. In: *The Origins and Relationships of Lower Invertebrates* (Edited by CONWAY MORRIS S., GEORGE J. D., GIBSON R. & PLATT H. M.), pp. 143–158. Oxford University Press, Oxford.
- EHLERS U. 1985b. *Das Phylogenetische System der Platyhelminthes*. G. Fischer, Stuttgart.
- EHLERS U. 1986. Comments on a phylogenetic system of the Platyhelminthes. *Hydrobiologia* **132**: 1–12.
- EHLERS U. 1988. The Prolethophora—a monophyletic taxon of the Platyhelminthes? *Fortschritte der Zoologie* **36**: 359–365.
- EL-NAGGAR M. M., KHIDR A. A. & KEARN G. C. 1990. Ultrastructural observations on the oviduct, Mehli's glands and ootype of the monogenean *Cichlidogyrus halli typicus* (Price & Kirk, 1967) Paperna, 1979. *International Journal for Parasitology* **20**: 203–209.
- EUZET L., SWIDERSKI Z. & MOKHTAR-MAAMOURI F. 1981. Ultrastructure comparée du spermatozoïde des cestodes. Relations avec la phylogénèse. *Annales de Parasitologie Humaine et Comparée* **56**: 247–259.
- FELSENSTEIN J. 1988. PHYLIP (Phylogeny Inference Package), version 3.1. A software distributed in its Macintosh version by W. Ellis.
- FINK W. L. 1986. Microcomputers and phylogenetic analysis. *Science* **234**: 1135–1139.
- FRANZÉN Å. 1956. On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates. *Zoologiska Bidrag från Uppsala* **31**: 355–482.
- HALTON D. W. & HARDCASTLE A. 1976. Spermatogenesis in a monogenean, *Diclidophora merlangi*. *International Journal for Parasitology* **6**: 43–53.
- HALTON D. W. & HARDCASTLE A. 1977. Ultrastructure of the male accessory ducts and prostate gland of *Diclidophora merlangi*. (Monogenoidea). *International Journal for Parasitology* **7**: 393–401.
- HOLLAND L. Z. 1990. Spermatogenesis in *Pyrosoma atlanticum* (Tunicata: Thaliacea: Pyrosomatida): implications for tunicate phylogeny. *Marine Biology* **105**: 451–470.
- JAMIESON B. G. M. 1987. A biological classification of sperm types, with special reference to annelids and molluscs, and an example of spermiocladistics. In: *New Horizons in Sperm Cell Research* (Edited by MOHRI H.), pp. 311–332. Japan Science Societies Press, Tokyo, and Gordon & Breach Science Publishers, New York.

- JUSTINE J.-L. & MATTEI X. 1982a. Réinvestigation de l'ultrastructure du spermatozoïde d'*Haematoloechus* (Trematoda: Haematoloechidae). *Journal of Ultrastructure Research* **81**: 322-332.
- JUSTINE J.-L. & MATTEI X. 1982b. Présence de spermatozoïdes à un seul axonème dans trois familles de Monogènes Monopisthocotylea: Ancyrocephalidae, Diplectanidae et Monocotylidae. *Annales de Parasitologie Humaine et Comparée* **57**: 419-420.
- JUSTINE J.-L. 1983. A new look at Monogenea and Digenea spermatozoa. In: *The Sperm Cell* (Edited by ANDRÉ J.), pp. 454-457. Martinus Nijhoff, The Hague.
- JUSTINE J.-L. & MATTEI X. 1983a. Étude ultrastructurale comparée de la spermiogenèse des Monogènes. 1. *Megalocotyle* (Monopisthocotylea Capsalidae). *Journal of Ultrastructure Research* **82**: 296-308.
- JUSTINE J.-L. & MATTEI X. 1983b. Comparative ultrastructural study of spermiogenesis in monogeneans (flatworms). 2. *Heterocotyle* (Monopisthocotylea, Monocotylidae). *Journal of Ultrastructure Research* **84**: 213-223.
- JUSTINE J.-L. & MATTEI X. 1983c. Comparative ultrastructural study of spermiogenesis in monogeneans (flatworms). 3. Two species of *Amphibdelloides* (Monopisthocotylea, Amphibdellatidae). *Journal of Ultrastructure Research* **84**: 224-237.
- JUSTINE J.-L. & MATTEI X. 1984a. Ultrastructure du spermatozoïde du Monogène *Hexostoma* (Polyopisthocotylea, Hexostomatidae). *Annales de Parasitologie Humaine et Comparée* **59**: 227-229.
- JUSTINE J.-L. & MATTEI X. 1984b. Comparative ultrastructural study of spermiogenesis in monogeneans (flatworms). 4. *Diplectanum* (Monopisthocotylea Diplectanidae). *Journal of Ultrastructure Research* **88**: 77-91.
- JUSTINE J.-L. & MATTEI X. 1984c. Ultrastructural observations on the spermatozoon, oocyte and fertilization process in *Gonapodasmius*, a gonochoristic Trematode (Trematoda: Digenea: Didymozoidae). *Acta Zoologica* (Stockholm) **65**: 171-177.
- JUSTINE J.-L., LAMBERT A. & MATTEI X. 1985. Spermatozoon ultrastructure and phylogenetic relationships in the monogeneans (Platyhelminthes). *International Journal for Parasitology* **15**: 601-608.
- JUSTINE J.-L., LE BRUN N. & MATTEI X. 1985a. First report of aflagellate spermatozoon in a parasitic platyhelminth, found in the monogenean *Diplozoon gracile* (Polyopisthocotylea, Diplozoidae). *Annales de Parasitologie Humaine et Comparée* **60**: 761-762.
- JUSTINE J.-L., LE BRUN N. & MATTEI X. 1985b. The aflagellate spermatozoon of *Diplozoon* (Platyhelminthes: Monogenea: Polyopisthocotylea). A demonstrative case of relationship between sperm ultrastructure and biology of reproduction. *Journal of Ultrastructure Research* **92**: 47-54.
- JUSTINE J.-L. & MATTEI X. 1985a. A spermatozoon with undulating membrane in a parasitic flatworm, *Gotocotyla* (Monogenea, Polyopisthocotylea, Gotocotylidae). *Journal of Ultrastructure Research* **90**: 163-171.
- JUSTINE J.-L. & MATTEI X. 1985b. Particularités ultrastructurales des spermatozoïdes de quelques Monogènes Polyopisthocotylea. *Annales des Sciences Naturelles, Zoologie, 13ème Série* **7**: 143-152.
- JUSTINE J.-L. & MATTEI X. 1985c. Ultrastructure du spermatozoïde de trois Monogènes Polyopisthocotylea: *Cemocotyle*, *Heteraxine* et *Heteraxinoides*. *Annales de Parasitologie Humaine et Comparée* **60**: 569-663.
- JUSTINE J.-L. & MATTEI X. 1985d. Ultrastructure de la spermiogenèse et du spermatozoïde de *Loimosina wilsoni* et affinités phylétiques des Loimoidae (Plathelminthes, Monogenea, Monopisthocotylea). *Zoologica Scripta* **14**: 169-175.
- JUSTINE J.-L. & MATTEI X. 1986a. Ultrastructural observations on fertilization in *Dionchus remorae* (Platyhelminthes, Monogenea, Dionchidae). *Acta Zoologica* (Stockholm) **67**: 97-101.
- JUSTINE J.-L. & MATTEI X. 1986b. Comparative ultrastructural study of spermiogenesis in monogeneans (flatworms). 5. *Calceostoma* (Monopisthocotylea, Calceostomatidae). *Journal of Ultrastructure and Molecular Structure Research* **96**: 54-63.
- JUSTINE J.-L. & MATTEI X. 1987. Phylogenetic relationships between the families Capsalidae and Dionchidae (Platyhelminthes, Monogenea, Monopisthocotylea) indicated by the comparative ultrastructural study of spermiogenesis. *Zoologica Scripta* **16**: 111-116.
- JUSTINE J.-L. & MATTEI X. 1988. Bending of 9 + '1' axonemes of flatworm spermatozoa in hypotonic media: an experimental study. *Journal of Ultrastructure and Molecular Structure Research* **100**: 31-38.
- JUSTINE J.-L. (in press a) Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. *Canadian Journal of Zoology*.
- JUSTINE J.-L. (in press b) Spermatozoa as a tool for taxonomy of species and supraspecific taxa in the platyhelminthes (Edited by BACCETTI B.). Raven Press.
- KLASSEN G. J. & BEVERLEY-BURTON M. 1987. Phylogenetic relationships of *Ligictaluridus* spp. (Monogenea: Ancyrocephalidae) and their ictalurid (siluriformes) hosts: an hypothesis. *Proceedings of the Helminthological Society of Washington* **54**: 84-90.
- KLASSEN G. J. & BEVERLEY-BURTON M. 1988. North American freshwater ancyrocephalids (Monogenea) with articulating haptor bars: phylogeny reconstruction. *Systematic Parasitology* **11**: 49-57.
- KORNAKOVA E. E. 1987. [The structure of the reproductive system of *Udonella murmanica* (Turbellaria, Udonellida).] In: [Systematics, Faunistics and Morphology of Parasitic Organisms] (Edited by PUGACHEV O. N. & RYSS A. Yu.). *Trudy Zoologicheskii Institut Akademii Nauk SSSR, Leningrad* **161**: 24-50 (in Russian).
- KORNAKOVA E. E. 1988. On morphology and phylogeny of Udonellida. *Fortschritte der Zoologie* **36**: 45-49.
- KRITSKY D. C. 1976. [Observations on the ultrastructure of spermatozoa and spermiogenesis in the monogenean *Gyrodactylus eucaliae* Ikezaki et Hoffman, 1957.] *Trudy Dal'nevostochny Nauchny Tsentralbiologo Pochvennyi Institut, Vladivostok* **34** (N.S.): 70-74 (in Russian).
- KRITSKY D. C. & BOEGER W. A. 1989. The phylogenetic status of the Ancyrocephalidae Bychowsky, 1937 (Monogenea: Dactylogyroidea). *Journal of Parasitology* **75**: 207-211.
- LAMBERT A. 1980. Oncomiracidiums et phylogénèse des Monogenea (Plathelminthes). Deuxième partie: structure argyrophiles des oncomiracidiums et phylogénèse des Monogenea. *Annales de Parasitologie Humaine et Comparée* **55**: 281-325.
- LEBEDEV B. I. 1988. Monogenea in the light of new evidence and their position among platyhelminths. *Angewandte Parasitologie* **29**: 149-167.
- LEBEDEV B. I. 1989. [On the system of class Monogonoidea.] In: [Investigation in Parasitology, Collection of Papers] (Edited by LEBEDEV B. I.), pp. 16-23. Academy of Sciences of the U.S.S.R., Far East Branch, Vladivostok (in Russian).

- LE BRUN N., LAMBERT A. & JUSTINE J.-L. 1986. Oncomiracidium, morphogénèse du haptor et ultrastructure du spermatozoïde de *Pseudodactylogyrus anguillae* (Yin et Sproston, 1948) Gussev, 1965 (Monogenea, Monopisthocotylea, Pseudodactylogyridae n. fam.). *Annales de Parasitologie Humaine et Comparée* **61**: 273–284.
- LLEWELLYN J. 1970. Monogenea. *Journal of Parasitology* **56** (4, Sect. II, Part 3): 493–504.
- MACDONALD S. & CALEY J. 1975. Sexual reproduction in the monogenean *Diclidophora merlangi*: tissue penetration by sperms. *Zeitschrift für Parasitenkunde* **45**: 323–334.
- MADDISON W. P. & MADDISON D. R. 1987. MacClade, version 2.1. A phylogenetics computer program distributed by the authors.
- MALMBERG G. 1990. On the ontogeny of the haptor and the evolution of the Monogenea. *Systematic Parasitology* **17**: 1–65.
- MALMBERG G. & AFZELIUS B. A. 1990. Sperm ultrastructure in *Myxiniocotyle* and *Acanthocotyle* (Platyhelminthes, Monogenea, Acanthocotylidae). *Zoologica Scripta* **19**: 129–132.
- MEASURES L. N., BEVERLEY-BURTON M. & WILLIAMS A. 1990. Three new species of *Monocotyle* (Monogenea: Monocotylidae) from the stingray, *Himantura uarnak* (Rajiformes: Dasyatidae) from the Great Barrier Reef: phylogenetic reconstruction, systematics and emended diagnoses. *International Journal for Parasitology* **20**: 755–767.
- MEHLHORN H. 1988. Reproduction, Platyhelminthes. In: *Parasitology in Focus* (Edited by MEHLHORN H.), pp. 330–344. Springer, Berlin.
- PLATNICK N. I. 1987. An empirical comparison of micro-computer parsimony programs. *Cladistics* **3**: 121–144.
- PLATNICK N. I. 1989. An empirical comparison of micro-computer parsimony programs, II. *Cladistics* **5**: 145–161.
- ROHDE K. 1971. Phylogenetic origin of trematodes. *Parasitologische Schriftenreihe* **21**: 17–27.
- ROHDE K. 1975. Fine structure of the Monogenea, especially *Polystomoides* Ward. *Advances in Parasitology* **13**: 1–33.
- ROHDE K. 1980. Some aspects of the ultrastructure of *Gotocotyla secunda* and *Hexostoma euthynni*. *Angewandte Parasitologie* **21**: 32–48.
- ROHDE K. & WATSON N. 1986. Ultrastructure of spermatogenesis and sperm of *Austramphilina elongata* (Platyhelminthes, Amphilinidea). *Journal of Submicroscopic Cytology* **18**: 361–374.
- ROHDE K., JUSTINE J.-L. & WATSON N. 1989. Ultrastructure of the flame bulbs of the monopisthocotylean Monogenea *Loimosina wilsoni* (Loimoidae) and *Calceostoma herculanea* (Calceostomatidae). *Annales de Parasitologie Humaine et Comparée* **64**: 433–442.
- ROHDE K., WATSON N. & ROUBAL F. 1989. Ultrastructure of flame bulbs, sense receptors, tegument and sperm of *Udonella* (Platyhelminthes) and the phylogenetic position of the genus. *Zoologischer Anzeiger* **222**: 143–157.
- ROHDE K. 1990. Phylogeny of Platyhelminthes, with special reference to parasitic groups. *International Journal for Parasitology* **20**: 979–1007.
- SATO M., OH M. & SAKODA K. 1967. Electron microscopic study of spermatogenesis in the lung fluke (*Paragonimus miyazakii*). *Zeitschrift für Zellforschung* **77**: 232–243.
- SCHMAHL G. & OBIKEZIE A. 1991. Fine structure of spermatogenesis in polyopisthocotylid monogeneans (*Protomicrocotyle ivoriensis*, *Gastrocotyle* sp.). *Parasitology Research* **77**: 115–122.
- SHAPIRO J. E., HERSHENOV B. R. & TULLOCH G. S. 1961. The fine structure of *Haematoleechus* spermatozoon tail. *Journal of Biophysics and Biochemical Cytology* **9**: 211–217.
- SPROSTON N. G. 1946. A synopsis of the monogenetic trematodes. *Transactions of the Zoological Society* **25**: 185–600.
- SWOFFORD D. L. 1990. PAUP: Phylogenetic analysis using parsimony, Version 3.0k for the Apple Macintosh. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.
- TAPPENDEN T. & KEARN G. C. 1990. Spermiogenesis and sperm ultrastructure in the monogenean parasite *Acanthocotyle lobianchi*. *International Journal for Parasitology* **20**: 747–753.
- TAPPENDEN T. & KEARN G. C. 1991a. Spermiogenesis and sperm ultrastructure in the monocotylid monogenean *Calicotyle kroyeri*. *International Journal for Parasitology* **21**: 57–63.
- TAPPENDEN T. & KEARN G. C. 1991b. Crystalline bodies associated with spermatozoa in the vas deferens of the monogeneans *Entobdella soleae* and *E. hippoglossi*. *Parasitology Research* **77**: 421–424.
- TUZET O. & KTARI M.-H. 1971a. La spermiogénèse et la structure du spermatozoïde de *Microcotyle mormyri* Lorenz, 1878 (Monogenea). *Comptes Rendus de l'Académie des Sciences (Paris) Série D. Sciences Naturelles* **272**: 2702–2705.
- TUZET O. & KTARI M.-H. 1971b. Recherches sur l'ultrastructure du spermatozoïde de quelques Monogènes. *Bulletin de la Société Zoologique de France* **96**: 535–540.
- WATROUS L. E. & WHEELER Q. D. 1981. The out-group comparison method of character analysis. *Systematic Zoology* **30**: 1–11.
- WHEELER T. A. & BEVERLEY-BURTON M. 1989. Systematics of *Onchocleidus* Mueller, 1936 (Monogenea: Ancyrocephalidae): phylogenetic relationships, evolution, and host associations. *Canadian Journal of Zoology* **67**: 706–713.
- WILEY E. O. 1981. *Phylogenetics. The Theory and Practice of Phylogenetic Systematics*. Wiley, New York.
- XYLANDER W. E. R. 1988. Ultrastructural studies on Udonellidae: evidence for a position within the Neodermata. *Fortschritte der Zoologie* **36**: 51–57.
- XYLANDER W. E. R. 1989. Ultrastructural studies on the reproductive system of Gyrocotylidae and Amphilinidea (Cestoda): spermatogenesis, spermatozoa, testes and vas deferens of *Gyrocotyle*. *International Journal for Parasitology* **19**: 897–905.