Biology of the Phylum Nematomorpha

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ABSTRACT

Compared with most animal phyla, the Nematomorpha, also known as hair worms, is a relatively understudied metazoan phylum. Although nematomorphs make up only 1 of 3 animal phyla specializing solely on a parasitic life style, little attention has been focused on this enigmatic group scientifically. The phylum contains two main groups. The nectonematids are parasites of marine invertebrates such as hermit crabs. The gordiids are parasites of terrestrial arthropods, such as mantids, beetles, and crickets. Members of both of these groups are free-living as adults in marine and freshwaters respectively.

In recent years, large strides have been made to understand this group more fully. New information has come from collection efforts, new approaches in organismal biology, modern techniques in microscopy and molecular biology. This review will focus on the advances made in four main areas of research: (1) morphology, (2) taxonomy and systematics, (3) life cycle and ecology and (4) host behavioural alterations.

Recent research focus on the structure of both nectonematids and gordiids has added new insights on the morphology of adult worms and juveniles. The nervous system of gordiids is now well described, including the documentation of sensory cells. In addition, the availability of material from the juvenile of several species of gordiids has made it possible to document the development of the parasitic stage.

New collections and reinvestigations of museum specimens have allowed for a critical reevaluation of the validity of established genera and species. However, traditional taxonomic work on this group continues to be hampered by two impeding factors: first is the lack of

species-specific characters; and second is the problem of intraspecific variation, which has likely led to the description of numerous synonyms. Modern molecular techniques have been used recently to support independently the broad relationships among gordiids.

During the turn of the millennium, the study of the life cycle and general ecology of gordiids enjoyed a revival. The pivotal outcome of this research was the domestication of a common American gordiid species, *Paragordius varius*. This species was the first of this phylum to be laboratory-reared. Through this research, the life cycle of several distantly related gordiid species was investigated. Other work showed that gordiids persist in the environment in the cyst stage by moving through different hosts by paratenesis. These cysts have been shown to retain infectivity for up to a year. These factors have likely contributed to the finding that gordiid cysts are one of the most common metazoans in some aquatic environments.

Finally, recent work has focused on elucidating the mechanism of how gordiids make the transition from terrestrially based definitive hosts to a free-living aquatic environment. It has been shown that hosts are manipulated by the parasites to enter water. Using this study system, and using histology and proteomic tools, the method of manipulation used by these parasites is being further investigated. This manipulation, and the reaction of the cricket to this manipulation, has been postulated to benefit both the parasite and the host.

Although large strides have been made within the last 10 years in the understanding of nematomorphs, we make the case that a lot of basic information remains to be uncovered. Although seemingly a daunting task, the recent advances in information and techniques lay a solid foundation for the future study of this unique group of parasites.

1. MORPHOLOGY

When Bresciani (1991) summarized the knowledge on the microscopical and especially ultrastructural anatomy of nematomorphs, he could refer to only a handful of previous investigations. Since then, the number of ultrastructural studies has increased significantly, although the number of investigations is still scarce in comparison to several other animal taxa.

In general, few species within the phylum Nematomorpha have been thoroughly investigated, but it appears that the freshwater species (taxon Gordiida) hardly vary in their internal organization. This also applies to the marine genus *Nectonema*. Most data come from cross sections in the central region of the body, while detailed investigations of the anterior or posterior end are rare.

Very little is known about the development of the juvenile parasite inside the hosts. However, recent studies have investigated these developmental stages ultrastructurally (see Lanzavecchia *et al.* (1995) for *Gordius* sp. and Schmidt-Rhaesa (2005) for *P. varius*).

1.1. Morphology of Gordiida

The body of all nematomorphs is covered in the adult stage with a thick and rigid cuticle which is secreted by epidermal cells. This cuticle contains a thick layer of large fibres, which are organized in sheets with fibres of adjacent sheets being arranged in an angle of about 60°. The number of sheets varies between species, but also vary within a single animal with the highest number of sheets being present in the central body region (Protasoni *et al.*, 2003). Therefore, the number of sheets can only be used cautiously as a taxonomic character. The large fibres are proteinaceous, but not collageneous in nature (Brivio *et al.*, 2000; Protasoni *et al.*, 2003), and contain dityrosine compounds which indicate tyrosine cross-linking in a hardening process (Brivio *et al.*, 2000). The cuticles of *Pseudochordodes bedriagae* (de Villalobos and Restelli, 2001) and *P. varius* (Schmidt-Rhaesa, 2005; Zapotosky, 1971) include a further cuticular layer distal of the fibres. This layer is lacking in *Gordius aquaticus*.

During development, the cuticle described above, which is sometimes called adult cuticle, is formed in the last third of the intraparasitic phase under a thin larval cuticle (Schmidt-Rhaesa, 2005). This larval cuticle is moulted. It appears to be continuous with the cuticle covering the larva and therefore capable of enormous growth.

Epidermal cells under the larval cuticle of *P. varius* contain abundant endoplasmatic reticulum and mitochondria, which are signs of physiological activity. This is certainly due to the uptake of nutrients from the body cavity of the host. When the larval cuticle is replaced by the adult cuticle, the function of the cuticle appears to be protective, which is supported by its thickness, evidence for hardening processes and peroxidase activity within the cuticle (Brivio *et al*., 2000).

The epidermis is very thin in adults, but remains cellular in structure. The nervous system consists of a peripheral system of basiepidermal nerves, which appear to innervate the underlying musculature (Restelli *et al*., 2002; Schmidt-Rhaesa, 1996a). On the ventral side, the nervous system is organized in a single nerve strand that originates (at least in *P. varius*) from intraepidermal nerves in a ventral thickening of the epidermis and shifts into a subepidermal position during further development (Schmidt-Rhaesa, 2005). In the anterior end, there is a brain which is circumpharyngeal, but the region ventral of the pharynx is dominant and from this region small connectives run dorsally around the pharynx (Schmidt-Rhaesa, 1996a). Knowledge about sensory cells remains extremely scarce, and only Schmidt-Rhaesa (2004c) reported probable ciliary receptors passing through the cuticle in *P. varius* (Figure 1). The musculature is formed as a thick sheet of longitudinal muscle cells (see Restelli *et al*. (2002) for a detailed description; Schmidt-Rhaesa, 1998a). The sheet is a monolayer, but the abundant cells are distinctly flattened in the proximodistal direction. The intestine is comparatively small and joins the gonads in the posterior end in a cloacal opening. Intestinal cells appear to be active in nutrient uptake in early stages but not in later stages (Schmidt-Rhaesa, 2005). The structure of the intestinal system in the anterior end remains incompletely known.

In the centre of the nematomorph body is a primary body cavity (i.e. lined by extracellular matrix and not by an epithelium) in early developmental stages (Lanzavecchia *et al*., 1995; Schmidt-Rhaesa, 2005). This body cavity is progressively reduced during further development by growth of gonads and parenchyma. The exact development of the gonads remains incompletely known, but from investigations on *Gordius* sp. (Lanzavecchia *et al*., 1995) and

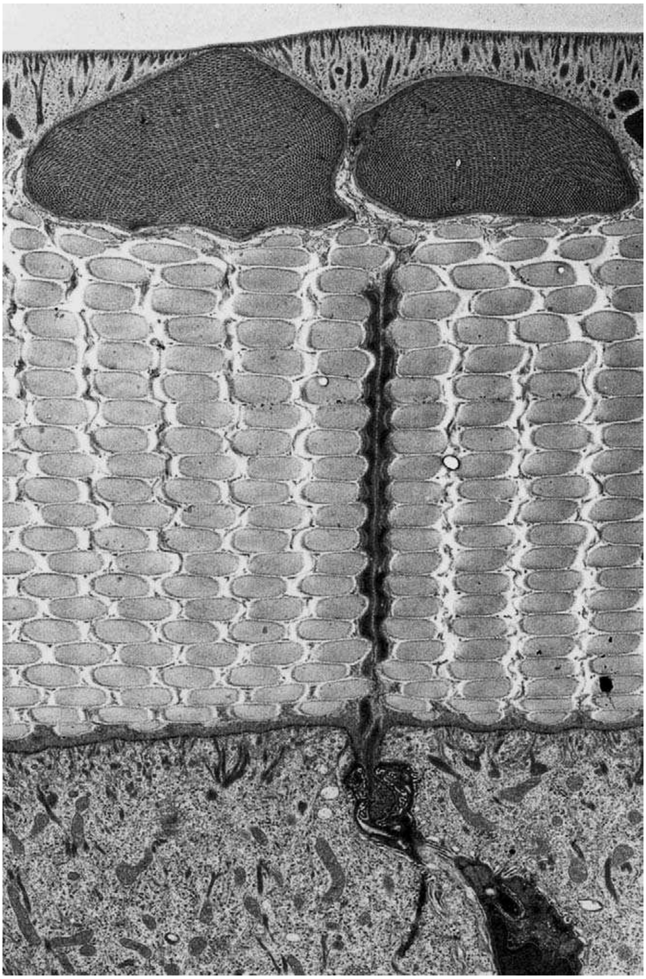


Figure 1 Transmission electron micrograph of the transcuticular structure of *Paragordius varius* with proposed sensory function from 25 days post-infection.

P. varius (Schmidt-Rhaesa, 2005) the following picture emerges. In both sexes, gonads originate as dorsolateral strands of solid tissue. These extend in males and become surrounded by abundant parenchyma cells. Cells within the testes differentiate into epithelial cells and gametes. The structure of spermatozoa (aflagellate, rod-shaped cells) is unique among metazoans, but appears to be uniform among freshwater species (Donin and Cotelli, 1977; Poinar, 2001; Schmidt-Rhaesa, 1997c). In females, fewer parenchyma cells are found. It appears that maturing oocytes proliferate from the dorsolateral strands until they almost completely fill the large body cavity.

1.2. Morphology of *Nectonema*

Besides few details from the Nearctic species *Nectonema agile* (Skaling and MacKinnon, 1988), all fine structural investigations dealt with the Norwegian species *Nectonema munidae* (Bresciani, 1991; Schmidt-Rhaesa, 1996b, 1997a,b, 1998a). The morphology of the cuticle and epidermis appears broadly comparable to that of freshwater Gordiida (Bresciani, 1991), but there are differences in the other organ systems. The muscle cells in males of *N. munidae* contain a large proximal portion which is free of myofilaments and which almost completely fills the body cavity of the animal (Schmidt-Rhaesa, 1998a). The ventral nerve cord remains within an epidermal thickening and an additional dorsal nerve cord is present (Schmidt-Rhaesa, 1996a). The intestine ends blindly; it is composed of two types of cells (Schmidt-Rhaesa, 1996b). The reproductive system differs remarkably between freshwater and marine nematomorphs. In males, there is an unpaired "sperm-sac" which is attached to the dorsal epidermal cord (Schmidt-Rhaesa, 1999). Gametes found within this sac were not mature and can therefore not be compared with the ones of freshwater species. In females, Feyel (1936) has reported a unique mode of proliferation of oocytes from a tissue proximal of the musculature. It could be confirmed by an ultrastructural reinvestigation (Schmidt-Rhaesa, 1997b) that there is a tissue consisting of cells extremely rich in vesicles and that circular parts, probably the oocytes, of this tissue are given off to the central body cavity.

In the anterior end of *N. agile* and *N. munidae* are four conspicuous giant cells. An ultrastructural investigation showed that these cells are connected to the nervous system and form a region rich in microvilli with the epidermis of the lateral body wall (Schmidt-Rhaesa, 1996b).

This suggests a sensory function, but the exact nature remains mysterious.

1.3. The Larva

The ultrastructural investigation of the larva of *P. varius* by Zapotosky (1974, 1975) has been complemented only by an ultrastructural and cytochemical investigation dealing with the larval musculature (Müller *et al.*, 2004) and two scanning electron microscopy (SEM) investigations (Adrianov *et al.*, 1998; Bohall *et al.*, 1997). Müller *et al.* (2004) confirm observations made by Zapotosky (1974, 1975) and add further details such as the presence of isolated muscles on the spines of the outermost ring of spines and differences in the arrangement of musculature in the posterior part of the body (postseptum) between the larvae of *P. varius* and *G. aquaticus*. The larva of *Nectonema* has not been investigated ultrastructurally.

1.4. Development within the Host

The whole morphogenesis from the microscopic larva to the macroscopic adult takes place within the host. Whereas in the laboratory system (Hanelt and Janovy, 1999, 2004b) the development of *P. varius* is rapid and takes about 30 days, while *Gordius robustus* needs about 3 months to be ready to leave the host. Ten days after experimental infection, *P. varius* had reached a length of about 1 mm; after about 25 days, the final length was reached (Figure 2). Young stages are white in colour, but late in development, beginning from the posterior end, a brown colouration proceeds. The rapid growth within the host is caused by epidermal and probably also intestinal uptake of nutrients (see Section 1.1). After the adult cuticle is formed (around day 20 after infection) and the larval cuticle is moulted, epidermis and intestine degenerate to a certain degree and decrease in size (Schmidt-Rhaesa, 2005). Other tissues, such as the musculature, develop continuously. Gonads are recognizable in their anlagen very

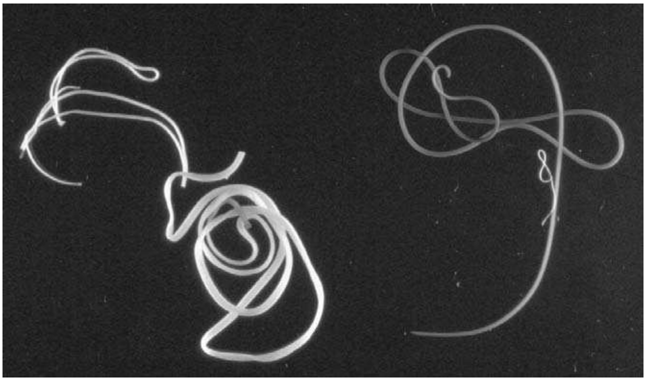


Figure 2 Developmental stages of *Paragordius varius* at various days post infection (DPI) of host *Gryllus firmus*. Adult (30 DPI), far right; note dark coloration. Juveniles: 10 DPI, immediately adjacent to adult; 15 DPI, top left; 25 DPI bottom left.

early, extend massively in size, and carry gametes during the last third of the development within the host (Figure 3 is Plate 4.3 in the Separate Color Plate section).

2. TAXONOMY AND SYSTEMATICS

2.1. Biodiversity of Nematomorpha

To date, approximately 300 species of horsehair worms have been described. The majority occur in freshwater and five species live in the sea. Since 1990, a total of 49 new species have been described (Table 1). Most new species were described from Argentina, the nematomorph fauna of which was already comparatively well studied (see de Miralles and de Villalobos, 1993b and references therein). New species have also been found in Europe and North America, which are densely sampled regions. This shows that even in the better sampled regions, the biodiversity of Nematomorpha is not completely known.

Between 1758 (genus *Gordius*) and 1965 (genus *Dacochordodes*), 20 genera of freshwater Nematomorpha (Gordiida) and one marine

Table 1 Species described since 1990

Collected from	Number of new species	Author
North America	4	de Miralles and de Villalobos (1995), Schmidt-Rhaesa (2004a), Schmidt-Rhaesa *et al.* (2003b)
South America	21	de Miralles and de Villalobos (1996a–e, 1997, 1998, 2000), de Villalobos (1995), de Villalobos and Camino (1999), de Villalobos and de Miralles (1997), de Villalobos *et al.* (2003), de Villalobos and Voglino (2000)
Central America	3	Schmidt-Rhaesa and Menzel (2005)
Europe	5	de Villalobos *et al.* (1999), Degrange and Martinot (1996), Schmidt-Rhaesa (2000), Spiridonov (1998)
Africa	3	Schmidt-Rhaesa and de Villalobos (2001), Spiridonov (2001)
Asia	8	Baek (1993), Baek and Noh (1992), Schmidt-Rhaesa *et al.* (2003a), Spiridonov (1993, 1998, 2000)
Australia	4	Schmidt-Rhaesa (2002), Schmidt-Rhaesa and Bryant (2004)
South Pacific Ocean	1	Poinar and Brockerhoff (2001)

genus, *Nectonema*, have been described. One further genus, *Noteochordodes*, was added in 2000 (de Miralles and de Villalobos, 2000). Besides the description of new species, several species were reinvestigated during the past years, in combination with a critical evaluation of the validity of genera. A reinvestigation of *Chordodiolus echinatus* revealed that this species, the only representative of the genus *Chordodiolus*, belongs to the genus *Beatogordius* (Schmidt-Rhaesa, 2001d; Schmidt-Rhaesa and Ehrmann, 2001). Schmidt-Rhaesa (2001a) suspected that several other genera including only one or very few species are not valid, but synonyms to other genera. Unpublished results confirm this for the genera *Pantacordodes* (with the only species *P. europaeus* (Heinze, 1952)) and *Dacochordodes* (with *D. bacescui* (Capuse, 1966)), which both belong to the genus *Spinochordodes* (Zanca and Schmidt-Rhaesa, unpublished results).

Crucial for the justification of genera is that they represent monophyletic taxa which can be recognized by autapomorphies (evolutionary novelties). From the 15 genera including more than one species, such autapomorphies could be found only for five genera

(*Chordodes*, *Nectonema*, *Beatogordius*, *Acutogordius* and *Noteochordodes*) (Schmidt-Rhaesa, 2001a). For some other genera, the evaluation for being monophyletic requires further investigation. For example, when introducing the genus *Gordionus*, Müller (1927) named adhesive warts ("Greifwarzen") as a characteristic feature. But since then, adhesive warts were documented only for a fraction of all known *Gordionus* species, which is better explained by lack of observation instead of confirmed absence. Additionally, such structures have also been recently found in species of *Beatogordius* (de Villalobos *et al*., 2003; Schmidt-Rhaesa and Bryant, 2004).

Further problems in taxonomy arise from difficulties in clear characterizations of genera. Interestingly, a comparable problem exists for the genus pair *Gordionus*/*Parachordodes* and for *Neochordodes*/*Pseudochordodes*. From each pair of genera, one genus (*Parachordodes* and *Pseudochordodes*) is characterized by the presence of two types of cuticular elevations, the so-called areoles (for examples of the diversity of cuticular structures see Figure 4). The other genus (*Gordionus*/*Neochordodes*) has only one type of areoles. For this typification, attention is only paid to the size and elevation of the areoles and not to their arrangement. The second type of areole, which is larger and more elevated, often arranged in characteristic clusters which may appear also when only one type of areole is present. Little attention has been focused on this type of arrangement of areoles, named the megareolar pattern (Schmidt-Rhaesa, 2001a).

Nematomorphs are relatively poor in characters which are important for species determination. Macroscopic characters are the shape of the posterior end (i.e. bilobed or round in males, trilobed or round in females) and the presence of cuticular structures such as a crescent-like fold close to the cloacal opening in the genera *Gordius* and *Acutogordius*. All other characters are found on the cuticle and many of them are so small that reliable detection with light microscopy is extremely difficult. Therefore, SEM has become the standard tool in recent species descriptions and reinvestigations (Schmidt-Rhaesa, 2001c) and has been included in the majority of recent species descriptions. One particular problem concerns the variability of the cuticle in different regions of the body. Especially in representatives

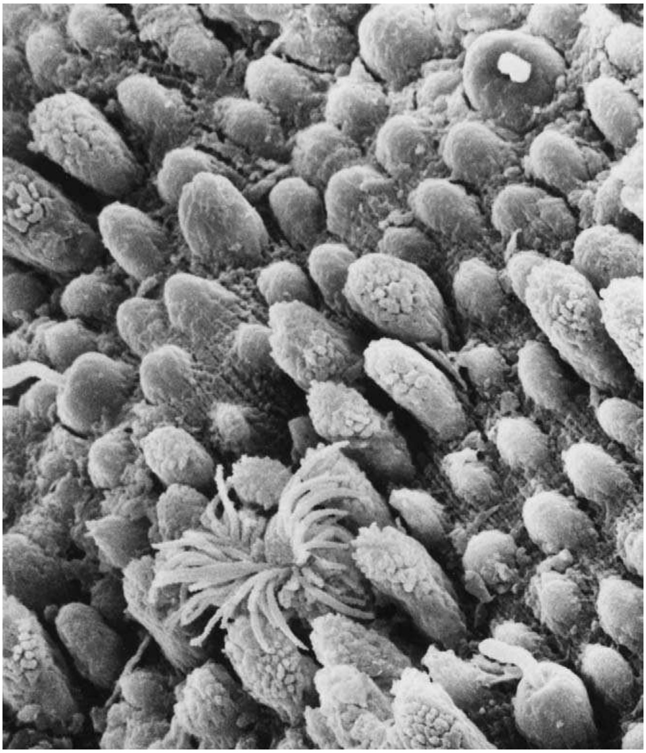


Figure 4 SEM image of the cuticle of *Chordodes queenslandi* from Australia. Note the diversity of cuticular structures.

of the genus *Chordodes*, it is essential to gain information not only on the types of cuticular structures, but also about their distribution (Schmidt-Rhaesa, 2002). For example, several species of *Chordodes* have two different types of the so-called crowned areoles. These are conspicuous elevated areoles with a basic “stem” and a “crown” of apical bristle-like filaments. In some species, crowned areoles with short apical filaments are present on the lateral sides of the body, while crowned areoles with very long filaments border both sides of the ventral and sometimes additionally the dorsal midline (see e.g. Schmidt-Rhaesa, 2002). Species with two types of crowned areoles appear to be sexually dimorphic because these differences in crowned areoles are distinctly elaborated in females but absent or insignificant in males (see Schmidt-Rhaesa (2002), for *Chordodes queenslandi* and further unpublished results).

2.2. Intraspecific Variation

Many reports on nematomorphs are based on findings of single or few species and a number of species are described on the basis of only one specimen. As some species are distinguished from others often by very delicate differences in shape or size of areoles, it is important to know whether these characters are specific for certain species. It seems, however, to be the case that several characters are quite variable. This has been stated previously by some authors whenever they could investigate larger numbers of specimens (see e.g. Montgomery (1898) for *Parachordodes tolosanus* and *Neochordodes occidentalis*; Vejdovsky (1888) for *P. tolosanus*) and was also confirmed in more recent investigations (see e.g. de Villalobos and Zanca (2001) for *Chordodes festae*; Schmidt-Rhaesa *et al.* (2003b) for *Chordodes morgani*). In investigating museum specimens of the genus *Gordionus* from Great Britain, Schmidt-Rhaesa (2001e) found that there is a continuous transition between the cuticular characters of *Gordionus violaceus* and of *G. wolterstorffii*. If further confirmed, this indicates one species with a wide range of morphological variability in cuticular characters. This in turn would have a great impact on taxonomy of the genus *Gordionus* because many species would fall within this range of morphological variation.

2.3. Biogeography

The recognition of biogeographical patterns of distribution rests on a representative sampling, and on a good quality of taxonomic assignments. Both are still in progress for nematomorphs, but first patterns have emerged.

North America appears to be a comparatively well-investigated region (summarized in Schmidt-Rhaesa *et al.*, 2003b), but with only

15 species the diversity appears to be low. In South America, nematomorph species are well known from Argentina (see Table 1 and de Miralles and de Villalobos, 1993b), but species from the remaining countries are much less known (de Miralles and de Villalobos, 1993a). The same applies to the Central American region, from which 22 species are known (Schmidt-Rhaesa and Menzel, 2005). With 99 species, which represent about one-third of all species described, Europe (including the Caucasian states Georgia, Armenia and Azerbeijan) appears to be a densely sampled region (Schmidt-Rhaesa, 1997a). This is, however, still a sketchy picture. From some countries, e.g. Denmark, Norway, Netherlands, Spain, Portugal, Greece, Albania and Turkey, there are no or very few reports. There are a number of species known from Africa, but no country has received special attention. African representatives of the genus *Beatogordius* were recently reviewed (Schmidt-Rhaesa and de Villalobos, 2001). However, nematomorph diversity in Africa and Asia has received comparatively little attention. Probably, the best known nematomorph fauna is found in Japan (see Inoue, 1994; Schmidt-Rhaesa, 2004b). From Australia, 11 species are known (Schmidt-Rhaesa, 2002; Schmidt-Rhaesa and Bryant, 2004) and from New Zealand, 5 (Poinar, 1991a; Schmidt-Rhaesa *et al.*, 2000).

The marine genus *Nectonema* is known from several locations worldwide (including both coasts of the Northern Atlantic, the Mediterranean, Indonesia, Japan and two questionable reports from the Southern Atlantic), with the latest report being from New Zealand (Poinar and Brockerhoff, 2001). With two exceptions, reports are single findings. The exceptions are the Bay of Fundy and some fjords close to Bergen in Norway. In most cases, *Nectonema* was found inside its hosts and rarely free-living.

Representatives of the genera *Gordius* and *Paragordius* occur worldwide while the distribution of other genera is more restricted. Among these, *Chordodes*, *Beatogordius*, *Neochordodes* and *Pseudochordodes* deserve further attention. With few exceptions, *Chordodes* species occur only in tropical and subtropical regions. This might be connected to the distribution of their main host group, praying mantids (Schmidt-Rhaesa and Ehrmann, 2001).

Species of the genus *Beatogordius* have been reported from Africa (Schmidt-Rhaesa and de Villalobos, 2001), South America (de Villalobos *et al.*, 2003) and recently also from Madagascar and Australia (Schmidt-Rhaesa and Bryant, 2004). This distribution can best be explained by the presence of the genus on the Gondwana continent before its break up and implies that *Beatogordius* is at least 180 million years old (MYO). This indirect measure of minimal age is of importance, because with the exception of two young reports (Dominican amber, see Poinar, 1999; Eocene brown coal, see Voigt, 1938), no fossils of nematomorphs have been found.

The genera *Neochordodes* and *Pseudochordodes* occur predominantly in South and Central America (de Miralles and de Villalobos, 1993b; Schmidt-Rhaesa and Menzel, 2005), but few species are found in the Southwest of the United States (Schmidt-Rhaesa *et al.*, 2003b). This might indicate that *Neochordodes* and *Pseudochordodes* originate in South America and spread to North America after the establishment of the Panama land bridge, which is about 45 MYO.

2.4. Phylogeny

Traditionally, nematomorphs have been classified into two taxonomic groups: the marine Nectonematoidea and the freshwater Gordiida (or Gordioidea). Among Gordiida, four families and 21 genera are recognized. Following the broad use of phylogenetic systematics during the past decades, this classification was translated into a tree, i.e. a hypothesis of phylogenetic relationships (Bleidorn *et al.*, 2002; Schmidt-Rhaesa, 2001a). The first step was to test if the taxa used in the traditional classification are monophyletic. This is evident only for five genera; 10 genera are either paraphyletic or their monophyly remains to be shown (Schmidt-Rhaesa, 2001a). A combination of morphological and molecular (18S rRNA gene) data supports a sister-group relationship between the marine genus *Nectonema* and the freshwater Gordiida (Bleidorn *et al.*, 2002). Among these, the Gordiidae (including the genera *Gordius* and *Acutogordius*) and Chordodidae (including all other genera) are sister taxa. It appears that the bifurcation of the male posterior end is an ancestral character in Gordiida and the posterior end became secondarily undivided in Chordodinae, a subtaxon of Chordodidae.

Three hypotheses of relationships of Nematomorpha have been tested, a closer relationship to Nematoda, to the nematode taxon Mermithida and to Scalidophora (Kinorhyncha, Priapulida and Loricifera). Among these, Schmidt-Rhaesa (1998b) assumed a sister–group relationship between Nematomorpha and Nematoda to be well supported, while both other hypotheses are based on fewer potential synapomorphies and are mainly due to convergences. This lach of work has been supported by 18S rRNA sequence analysis (see e.g. Bleidorn *et al.*, 2002; Garey and Schmidt-Rhaesa, 1998) and combined morphological and molecular analyses (Giribet *et al.*, 2000; Peterson and Eernisse, 2001; Sørensen *et al.*, 2000; Zrzavý *et al.*, 1998).

3. LIFE CYCLE AND ECOLOGY

In the last 10 years, large strides have been made in describing and experimentally determining the life cycle and ecology of freshwater nematomorphs or gordiids. Comparatively, virtually nothing is known about the general biology of marine nematomorphs or nectonematids. No life cycles of taxa within this group have been described, and to our knowledge, no laboratory work has been done on the life cycle of nectonematids. This lack of work has left large gaps in our knowledge of the basic biology of this group, such as the number of hosts involved in their life cycle, generation time, etc. Thus, this section will only deal with the advances made in the study of gordiids.

3.1. General Life Cycle

Gordiids are parasites as juveniles in terrestrial arthropods. Adults are free-living in aquatic environments such as rivers, streams and lakes. Here, worms mate and produce eggs. Eggs develop over 7–14 days into semi-sessile larvae (Figure 5), which are able to survive for a maximum of 2 weeks. Gordiids must thus make two critical transitions during their life cycle. The first transition is that from the terrestrial definitive host to water (see Section 4). The second transition is that from aquatic larvae to the definitive host. This latter transition has been the topic of much speculation, but has only recently been intensively studied.

Three transmission mechanisms have been proposed for gordiid larvae to reach their definitive hosts (see reviews by Hanelt and Janovy, 2003; Schmidt-Rhaesa, 2001b): (1) larvae are directly transmitted to definitive host without going through additional developmental stages or hosts; (2) larvae form cysts in the environment and infect definitive hosts when accidentally ingested with vegetation or water; (3) larvae enter and encyst within a paratenic host, which are preyed or scavenged upon by the definitive host.

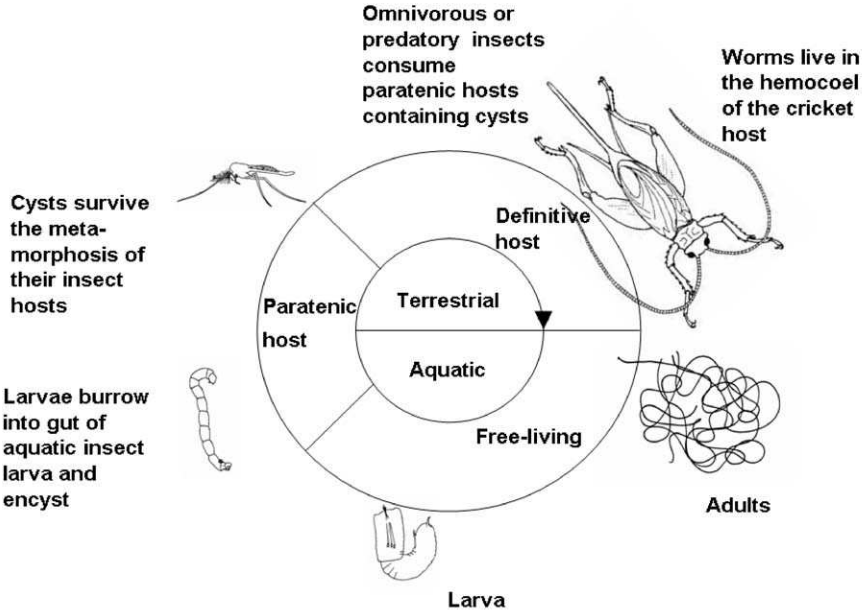


Figure 5 Natural life cycle of *Paragordius varius* (modified from Hanelt and Janovy, 2004b).

The first two transmission mechanisms are unlikely to figure prominently in the natural life cycle of gordiids. Dorier (1930, 1935a, b) reported that gordiid larvae were able to encyst freely in the absence of a host, for example on aquatic vegetation. Terrestrial arthropods could be infected by eating or drinking in the periphery of a water source. At that time, it was not elucidated whether these life cycle stages were actually infective. Since these reports by Dorier, free-living cysts have not been reported again. The second method of transmission has been supported by several studies, indicating the infection of definitive hosts by feeding of gordiids larvae (Hanelt and Janovy, 2004b; Inoue, 1962). Both of these transmission mechanisms rely on agile and highly mobile larvae to allow for their positioning in areas maximizing host encounters. However, gordiid larvae have often been described as being immobile, certainly not able to travel distances required to navigate from the bottom of a lake or stream to the edge's surface (Meissner, 1856; Poinar and Doelman, 1974; Villot, 1874a). Other hosts must therefore be responsible for bridging the gap between the free-living aquatic environment and parasitic terrestrial environment.

3.2. Paratenic Hosts

The paratenic host was originally described as one that bridges a trophic gap between the intermediate and the definitive host (Baer, 1951). Since the sole role of this type of host is transportation, the parasite does not undergo development while within this host. Paratenic hosts have been divided into different categories; spurious and true. Those able to become infected but unable to transmit this infection have been called "spurious" paratenic host (Schultz and Davtian, 1954). Only those animals able to become infected and able to transmit this infection are referred to as "true" paratenic hosts, or simply paratenic hosts.

The idea of the involvement of a paratenic host within the gordiid life cycle is an old one, starting with Meissner (1856). He suggested that aquatic insects could be a natural host able to carry gordiids from water to terrestrial hosts. The gordiid life cycle stage capable of surviving for an extended time in a paratenic host, the cyst (Figure 5), was first thoroughly researched about 20 years later (Villot, 1874a, b). This discovery, however, did not clear up the mystery of the life cycle since the cysts were first reported in hosts unlikely to play a role in the natural gordiid life cycle: annelids, snails and fish. Subsequent data did show cysts present in aquatic insects, such as mayflies and midges (see e.g. White, 1966, 1969). Although the life cycle of gordiids was able to be pieced together by these findings and subsequent infection trials (Hanelt and Janovy, 1999, 2004b; Inoue, 1960b, 1962), studies have only recently focused their attention on the host specificity and significance of gordiid cysts within spurious and true paratenic hosts.

3.2.1. The identity of paratenic hosts

The paratenic host specificity of the cyst stage of various species of gordiids has been difficult to quantify. Although studies have reported on naturally and laboratory-exposed paratenic hosts, the results of these studies remain difficult to interpret. Studies investigating the infection with cysts in natural populations (see e.g. White, 1969) have been able to provide data on the kinds of animals carrying gordiid cysts. However, these studies were not able to provide information on the host specificity of individual gordiid species since infection with multiple species of gordiids could not be ruled out. Only recently have morphometric methods been described allowing discrimination of species of gordiids based on non-adult characters (Hanelt and Janovy, 2002). Laboratory infection experiments have similar problems, in which most of these experiments did not involve laboratory-reared hosts and did not contain control groups. It was recently found in a survey of 1000 snails that almost 40% were naturally infected with gordiids (Hanelt *et al.*, 2001). Thus, infection experiments require carefully monitored control groups to avoid false-positive samples. Recently, a study of the host specificity of gordiid cysts was conducted considering these factors.

Hanelt and Janovy (2003) studied the paratenic host specificity of three Nearctic gordiid species. Freshly hatched gordiid larvae were used to expose laboratory-reared putative paratenic hosts. These host groups were chosen due to their likelihood of coming into contact naturally with gordiid larvae at the bottom of a river or stream. Putative paratenic hosts included a range of organisms from annelids, mollusks and arthropods to fish. Incredibly, of the 12 species exposed, all but two became infected with gordiid cysts (Table 2). All three gordiid species showed an identical infection pattern. Two putative paratenic hosts did not become infected, the water mite and the triclad flatworm. Failure of infection was most likely caused by behavioural traits rather than incompatibility. Both of these animals require the presence of prey or detritus material to initiate feeding behaviours. All other groups became infected with cysts within the gut lining or the haemocoel. Of all paratenic hosts able to become infected with cysts, only those that could act to bridge the gap between aquatic and

Table 2 Prevalence of infection of putative paratenic hosts exposed to cysts of three gordiid species

Putative host species	*Gordius robustus*	*Paragordius varius*	*Chordodes morgani*
Platyhelminthes: Turbellaria			
Dugesia tigrina	0	0	0
Oligochaeta: Tubificidae			
Limnodrilus hoffmeisteri	85	80	100
Mollusca: Gastropoda			
Physa sp.	75	95	95
Crustacea			
Hyalella azteca (Amphipoda)	60	70	95
Cypris sp. (Ostracoda)	50	60	65
Arachnoidea: Hydracarina			
Oxus sp.	0	0	0
Insecta			
Tenebrio molitor (Coleoptera)	60	55	85
Tanitarsis sp. (Diptera)	45	40	70
Caenis sp. (Ephemeroptera)	100	95	100
Neotrichia sp. (Trichoptera)	100	100	100
Culex tarsalis (Diptera)	[a]	[a]	[a]
Vertebrata: Osteichthyes			
Notropis ludibundus	100	100	100

Modified from Hanelt and Janovy (2003).

[a] Denotes destruction of cysts by immune reaction of host species.

terrestrial environments were considered a true paratenic host, while the others were considered spurious paratenic hosts.

3.2.2. Natural paratenic hosts

For Meissner's (1856) hypothesis (that aquatic insects that metamorphose into flying adults are the most likely natural paratenic hosts involved in the gordiid life cycle) to be correct, three conditions must be met by this host (Hanelt and Janovy, 2004a). First, the cysts must survive the metamorphosis of this host. Inoue (1960b, 1962) tested the survival of *Chordodes japonensis* cysts through the metamorphosis in midges, mayflies, and mosquitoes. Infection experiments of cysts from adult insects resulted in infections in the definitive hosts, mantids. Hanelt and Janovy (2004a) tested the survival of three common Nearctic species of gordiids, and found survival in laboratory-infected midges. This study also confirmed survival in mayflies infected naturally with *Gordius difficilis*.

Second, the natural paratenic host should not be able to mount an immune reaction efficient enough to kill cysts. Two different types of immune reactions to cysts have been described to cysts of *C. japonensis* (Inoue, 1960b). The reaction by mayflies was to surround the cyst in a thin spherical envelope of haemocytes. The more severe reaction was by midges, which not only consisted of a layer of haemocytes but also included a melanization reaction. This latter immune reaction caused a marked decrease in the viability of the cysts. This kind of melanization has also been reported in mosquito larvae leading to the destruction of cysts by *N. occidentalis* (Poinar and Doelman, 1974) and by *P. varius*, *G. robustus* and *C. morgani* (Hanelt and Janovy, 2003). However, experiments with the latter three gordiid species using midges, mayflies and trichoperans showed haemocyte encasement only in a few instances without melanization (Hanelt and Janovy, 2003). The viability of *P. varius* cysts was found to be unaffected by this mild type of host immune reaction (Hanelt and Janovy, 2004a).

The third condition necessary for aquatic insects to act naturally as gordiid paratenic hosts is that cysts must not alter the morphology or development of the paratenic host. White (1969), studying natural infection in aquatic insect larvae in Wisconsin, USA, found that individuals infected with cysts varied morphologically. The wing pads of mayflies were found to be smaller compared with uninfected individuals. More important, it was also reported that some species of Diptera and Trichoptera failed to pupate, making their role as true paratenic host impossible. However, multiple studies investigating metamorphosis of infected insects exposed in the laboratory did not report developmental irregularities of hosts (Hanelt and Janovy, 2004a; Inoue, 1960a). Additionally, Hanelt and Janovy (2004a), studying naturally infected mayflies, found no morphological differences between infected and uninfected hosts.

Ultimately, since the three conditions necessary to have aquatic insects act as natural paratenic hosts in the gordiid life cycle, it is clear that insects play an integral part in the life cycle of gordiids. However, further studies are necessary to study whether organisms raised and exposed in the laboratory act and develop differently than in nature. Furthermore, research needs to focus on whether changes in micro- and macrohabitat through the geographic range of gordiids may require local adaptations to different paratenic hosts.

3.2.3. Paratenesis

Paratenesis is defined as a parasite being transferred from one paratenic host to another (Beaver, 1969). Paratenesis has been documented in species of trematodes (Shoop, 1988), cestodes, (Halvorsen and Wissler, 1973) and nematodes (Daengsvang, 1968). Recent evidence appears to indicate that gordiid cysts can also be transmitted between paratenic hosts through paratenesis. Hanelt and Janovy (2003) found that although the free-living aquatic flatworm *Dugesia tigrina* did not become infected when exposed to larvae of *P. varius*, *G. robustus*, and *C. morgani*, flatworms could become infected by keeping them in tanks with infected snails. Thus, cysts were transferred from snails to the flatworms feeding on them. de Villalobos *et al.* (2003) found that *P. varius* cysts from snail tissues fed to fish established within the musculature and intestinal wall of the fish.

Although in the latter study it was assumed that some cysts may have been recruited from unencysted larvae within the snail tissue, it is clear that many cysts were transferred from the snail to the fish. Hanelt and Janovy (2004a) found that *P. varius* cysts could also be transferred from snails to species of beetles, and in one case to an unsuitable (one which could not serve as a definitive host) cricket host.

Since gordiid cysts appear to lack host specificity, mechanisms must exist allowing the recovery of cysts in spurious paratenic hosts, which are considered lost infective stages. Examples of these hosts include copepods, fish, amphipods, etc. However, through paratenesis these lost infected stages can be recovered and can therefore reenter the normal gordiid life cycle. Scavenger or predatory mayflies could easily pick up cysts from food items.

It appears that the transmission of gordiids from aquatic environments to the terrestrial environment is not as random as it first appears. It has often been assumed that parasites with high fecundity transmit their progeny by pure chance (or luck!), like randomly shooting a shotgun; eventually pellets will hit a target. Gordiids, through the mechanism of paratenesis, however, appear to get several attempts at getting to a paratenic host: this process is much more like a pinball game, in which cysts "bounce" around the environment until successfully transmitted.

Future studies are needed to investigate the impact of long-lasting cysts that are able to persist for extended periods and are able to move through multiple hosts during their life cycle. Due to the persistence of multiple generations of cysts at any point in time, this system is ideal for population and selection studies.

3.2.4. The role of the paratenic host

The evolution of parasite life cycle complexity continues to be a hot topic of discussion (Parker *et al.*, 2003). Although life cycle diagrams are usually drawn as static representations (Olsen, 1986), some have argued that life cycles are only fixed when perceived over the lifetime of human investigators (Combes, 2001). Over evolutionary time, most life cycles are probably astonishingly fluid. Many explanations have been given for the mechanisms leading to the change in complex life cycles. These can be broadly placed into two categories: accident and adaptation (Poulin, 1998b). Recent evidence has suggested that adaptation might be involved in some systems during the addition of hosts (Brown *et al.*, 2001; Choisy *et al.*, 2003; Morand *et al.*, 1996; Robert *et al.*, 1988), although the proximate mechanism for such an addition is unclear. Dogiel (1966) contended that the acquisition of intermediate hosts often began with the addition of a host as a paratenic host. Furthermore, Ryzhikov (Barus, 1964) believed that paratenic hosts were an intermediate step in the addition or deletion of an intermediate host. Although the involvement of a paratenic host in the evolution of complex life cycles could be critical, surprisingly few studies have been conducted to document the role of this type of host (but see Choisy *et al.*, 2003). Especially important are the benefits a parasite gains by using a paratenic host. These benefits could ultimately lead natural selection to include permanently this host in the parasite life cycle.

Gordiids can provide a good model with which to study the role of paratenic hosts, beyond the definition of this host as capable of bridging a trophic gap. Ryzhikov (Barus, 1964) provides an outline of additional roles that should be investigated. The summary of these roles provided below are presented in an expanded context of the suggestions provided by Rhzhikov.

(a) Increasing infectivity of parasites. Inoue (1962) found that infection of definitive hosts with *C. japonensis* cysts increased prevalence by up to 65% over infection with larvae. Hanelt and Janovy (2004b) showed that the infectivity of *P. varius* increased dramatically when exposed to cysts compared with larvae. For *G. robustus*, infection of the definitive host was found to be possible with cysts, not larvae (Hanelt and Janovy, 1999). These findings suggest that infection by larvae is much less effective than infection by cysts and has become impossible in some gordiid species. Although the mechanisms leading to higher infection ability of cysts is unknown, it could be caused by benefits from being surrounded by a cyst wall

(e.g. protection from mechanical damage) or physiological benefits of going through the cyst stage.

(b) Increasing parasite longevity. Gordiid larvae have been reported to be viable from 1 to 4 weeks (Meissner, 1856; Mühldorf, 1914). *P. varius* larvae remained active for only 10 days post-hatching. The rate of cyst formation of this species decreased to zero after 30 days, indicating complete loss of viability of the larvae (Hanelt and Janovy, 2004b). Similar rates of larval survival were found in *C. morgani* (Hanelt, personal observation). Cysts, however, can remain viable for much longer. Cysts of *P. varius* remained infective for at least 6 months (Hanelt and Janovy, 2003). In addition, *G. robustus* cysts, which were mechanically excysted 12 months post-infection were found to move in an identical manner to freshly hatched larvae (Hanelt, personal observation). Data of field-collected physid snails during the spring indicated that infections occurring during the late summer and early fall persist in snails through May, 8–10 months following the formation of the cysts (Hanelt *et al.*, 2001). It is clear that the cyst stage greatly increases the window of time gordiids have to be transmitted to their definitive hosts.

(c) Recovery of lost infective stages. Evidence suggests that cysts can be transferred between paratenic hosts (see Section 3.2.3). This passage may allow cysts being formed within spurious paratenic hosts to be transmitted to a definitive host. Thus, larvae encysting within hosts, which are incapable of transferring the parasite to the definitive host, could be transmitted to a paratenic host capable of such transition. In addition, this type of paratenesis could cause cysts to be transmitted following the death of a host to a scavenger animal. Transmission between paratenic hosts may thus allow cysts to remain viable for an extended period by being passed from one host to another.

(d) Dispersal/maintenance at a site. Aquatic insect paratenic hosts may also serve a role in the dispersal of cysts. Aquatic insects harbouring cysts that have metamorphosed into flying adults are

capable of distributing parasites over wide geographic areas. For example, using mitochondrial DNA markers, it has been established that trichoptera species can regularly disperse up to about 20 km (Myers *et al.*, 2001). This distance could likely increase based on weather factors such as temperature, relative humidity, wind and atmospheric pressure (McManus, 1988). Thus, the paratenic host could be an important factor in determining the genetic structure of populations of gordiids.

Furthermore, it has been suggested that the innate behaviour of insects might be responsible for maintaining gordiids at a particular site (Hanelt and Janovy, 2004a). Gordiids, as well as their paratenic hosts, commonly inhabit rivers and streams. Within this environment, inhabitants are constantly being displaced unidirectionally (i.e. downstream). Calculations of several macroinvertebrate taxa suggested that downstream displacement can be as high as 10 km during a single generation (Hemsworth and Brooker, 1979). Displacement can occur through two types of drifts (Brittain and Eikeland, 1988). Constant drift, which is the background drift, normally occurs in flowing waters; and catastrophic drift, which is rare and occurs when high discharge physically disturbs the substrate.

These drift events have been shown to affect most aquatic insects capable of serving as gordiid paratenic hosts, as well as gordiid adults (Thorne, 1940; Hanelt, personal observation). Drift also possibly affects other life cycle stages such as the egg strings, eggs, and larvae. Only *C. morgani* may mitigate drift of eggs by attaching them to the substrate (Hanelt and Janovy, 2002).

Despite the possibility of drift, a recent study showed that gordiids are found in 70% of isolated first- and second-order streams in Lancaster County, NE, USA (Hanelt *et al.*, 2001). This finding suggests that a mechanism exists to compensate for drift displacement. The persistence of upstream populations of invertebrates despite stream drift has been termed the “stream drift paradox” (Hershey *et al.*, 1993). One hypothesis to explain this paradox is by the “colonization cycle” (Mottram, 1932; Müller, 1982). This hypothesis states that the upstream-directed flight behaviour exhibited by many freshwater aquatic insects compensates for the gradual downstream movement of insect larvae (Müller, 1982). More recently, this same compensational effect has also been shown for active upstream movement under water by aquatic insect larvae (Söderström, 1987). Gordiids may ultimately benefit from the colonization cycle by being transported by their paratenic hosts upstream, compensating for drift and allowing gordiids to remain in the headwaters of many rivers and streams.

(e) The significance of the paratenic host in the evolution of the parasites. This is certainly the most important question raised by Ryzhikov (Barus, 1964). Regrettably, with the current paucity of data, this question cannot be addressed. Future studies on the life cycles of gordiids, especially nectonematids, must be conducted and combined with existing phylogenies to uncover the evolutionary history of the paratenic host within this phylum.

(f) Conclusion. Studies on the life cycle and ecology of gordiids over the last 10 years have allowed us a glimpse into the role of a paratenic host beyond the definition of a transport host. Ultimately, attempting to extend the above-described role of gordiid paratenic hosts to other groups of parasites must be made with caution. As Dogiel (1966) has pointed out for the role and origin of intermediate hosts, "…the hypotheses of the origin of the intermediate hosts…suffer from the attempt to place all existing types of host–parasite relationships in one scheme, true for all parasites… It is the task of investigators to study the origin of the case in hand individually".

3.3. Experimental Life Cycles

The domestication of gordiid has proven extremely difficult. Many attempts made over the last 100 years at completing the life cycle experimentally have failed. These studies usually ended prematurely yielding adult or juvenile worms from experimentally infected hosts (Hanelt and Janovy, 1999; Inoue, 1962; Svábeník, 1925), but failed at successfully mating worms. The main reason for making culturing difficult is the long developmental time within the definitive hosts.

For example, Hanelt and Janovy (1999) isolated *G. robustus* using Mormon crickets as definitive hosts naturally. To make this system more amenable to laboratory culture, *Gryllus firmus* was used as a host. Maturation time for *G. robustus* within the definitive host is up to 3 months, although the average adult life span of adult *G. firmus* is about 30–40 days.

Several years later, Hanelt and Janovy (2004b) reported the successful domestication of *P. varius* (Figure 6). This species was found to be much more amenable to lab rearing. The most important feature is that the worms complete development within definitive hosts in as few as 27 days. Thus, crickets with short life spans can be used as hosts. This includes the easily available "feeder cricket", *Acheta domestica*, which are available at most pet stores for feeding pet reptiles. In this laboratory-maintained life cycle, *Physa* sp. is used as a paratenic host. This aquatic snail is long-lived, can maintain cysts for more than a year, and can get infected with hundreds of cysts. In

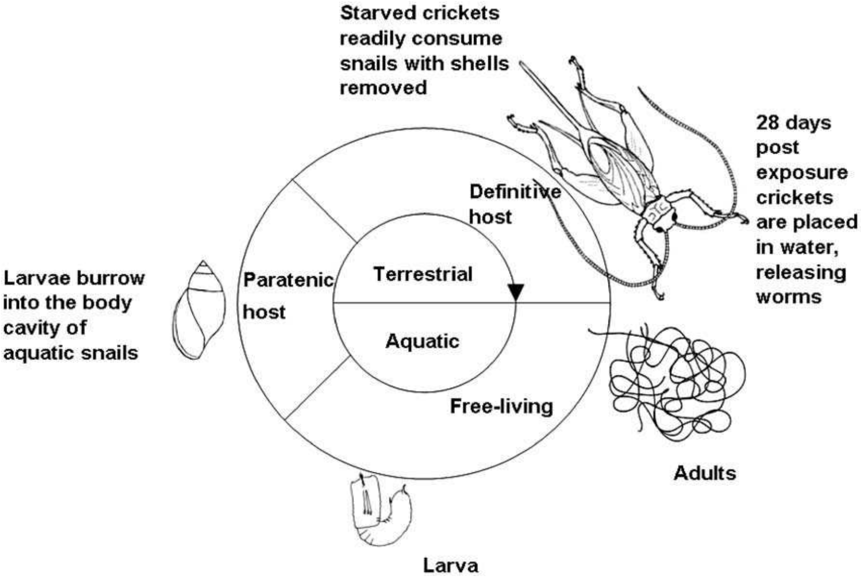


Figure 6 Laboratory life cycle of *Paragordius varius* (modified from Hanelt and Janovy, 2004b). Note the use of snails as paratenic hosts rather than insects.

addition, *Physa* sp. can easily be reared in the laboratory. The *P. varius* life cycle can be completed in the laboratory in as few as 45 days with minimal effort at rearing hosts.

This experimental life cycle can now be used to investigate novel facets of gordiid biology (such as their behaviour), or to investigate field observations in a very controlled environment (see e.g. Section 3.5). In addition, since material of various life cycle stages is available from this worm species on a consistent basis and in a controlled manner, the study of nematomorphs has been made more tractable, especially using molecular techniques. Ultimately, we hope that the *P. varius* life cycle will become a model system to investigate gordiid biology as well as parasite–host interactions.

3.4. Sex Ratio

The literature is full of reports indicating that the sex ratio of gordiid adults collected in nature is skewed. However, the direction of the skew is not consistent. In some collections, females are predominant. Watermolen and Haen (1994) report collecting 67 *G. robustus* in Wisconsin, 66 of which were females. More often, however, a predominance of males is found. Cochran *et al.* (2004), enumerating *G. difficilis* worms within Gordian knots collected during a 32-year period in six Midwestern states of the United States, reported that of the 1391 worms, 1205 were males. Similarly, Thomas *et al.* (1999), studying *Euchordodes nigromaculatus* in New Zealand, collected only 61 males and no females. Other studies have shown that males and females were collected in equal ratios (de Villalobos and Camino, 1999; Valvassori *et al.*, 1988).

The discrepancies in these studies have recently been clarified by two investigations involving multiple samples from a site at different times of the season. Bolek and Coggins (2002) studied the seasonal occurrence of *G. difficilis* in Wisconsin by conducting a worm removal experiment over 3 years. The samples revealed that the same population of worms can change from being female bias early in the mating season, to being male bias late in the season. In total, all years of data showed an overall male bias. Poulin (1996) collected *Gordius dimorphus* from New Zealand's South Island at two time points during the same year. During the spring, the sex ratio was female-biased; whereas during the summer, the sex ratio was highly male-biased.

These two studies suggest that rather than a real difference in sexes being produced during meiosis, observed differences are instead due to ecological factors. Hanelt and Janovy (2004b), studying a laboratory maintained isolate of *P. varius*, supported this contention. Of worms produced during several generations, there was no statistically significant difference between the number of males and females produced.

Ecological causes that may be contributing to the often-reported skew in nature have never been empirically investigated. However, the literature offers several suggested explanations. Poulin (1996) proposes that variation in life spans causes the accumulation of males towards the end of the mating season. In addition, he suggests that males and females may have different developmental times, leading to insects hosting the larger worms (in this case males) to release these parasites later in the season.

In order to accept these explanations, additional field and laboratory experiments will be necessary. Ultimately, a genetic test able to discriminate sexes should be used to determine sex ratio of various life cycle stages, such as eggs, larvae, cysts, and juvenile worms. Rapid molecular methods have, for example, been employed to identify the sex of nonadult life cycle stages of schistosomes (Walker *et al.*, 1989).

3.5. Distribution

3.5.1. Introduction

Historically, the manner in which gordiid worms is distributed in space and time has been difficult to quantify. Data of their spatial distribution have largely come from random or semi-random collections of adult worms. These collections either have been chance encounters or were made in areas where worms happen to overlap with human activity (see Section 3.7). Distribution data have been further biased by limiting collection to a single time point. Recently, several studies have been conducted on gordiids to address their distribution both at small scales and temporally.

3.5.2. Distribution at fine scale

Recently, new methods of quantifying gordiids have led to very different perceptions of how these worms are distributed in limited geographic areas. Several studies have used meta-analysis to determine distribution within individual states in the United States. These studies used collection data of adults to identify sites. Chandler (1985) compiled 18 years worth of data for the state of Tennessee; 18 sites containing gordiids were identified. Watermolen and Haen (1994) compile 69 years of data for the state of Wisconsin; 15 sites were identified as containing worms. However, four of these sites were unsubstantiated verbal reports of sightings. These datasets give the impression that gordiids are geographically widespread within defined areas, although their distribution is spotty.

Hanelt *et al.* (2001) found that basing distribution data on the presence of adults is problematic. First, the detection and collection of adults are extremely difficult. Gordiid adults are easily missed due to their seasonality, short life span (4–6 weeks), cryptic nature, hiding behaviours during mating, and the lack of effective sampling technique. A single collection locality, such as a small stream, can contain worms entwined in vegetation at the periphery, between rocks on the bottom, buried in the substrate, free swimming, wound around submerged sticks, or entangled in a knot near the substrate. Thus, thorough gordiid collection entails an extensive search of possible localities. Second, the finding of an adult gordiid does not necessarily indicate that the life cycle is occurring at that site. Thus, as mentioned above, finding a gordiid in a toilet bowl only serves to indicate that the definitive host carried the parasite into an unnatural environment.

Thus, the use of gordiid cysts was suggested as an indicator for the presence of gordiids (Hanelt *et al.*, 2001). This technique is preferable since many of the problems with using adults as indicators are eliminated. One female gordiid can produce several million cysts. Cysts are also long-lived, and are able to persist in snail paratenic hosts for more than a year (Hanelt, unpublished). Furthermore, the sampling of gordiids in this manner can be standardized by quantifying number of cysts per specific paratenic host.

Using gordiid cysts within *Physa* sp. (Mollusca: Pulmonata), this technique was capable of identifying 35 out of 50 sites positive for gordiid cysts within Lancaster County, Nebraska (2193 km^2) (Hanelt *et al.*, 2001). Three years of intensive sampling had resulted in the detection of gordiid adults at only a single site within the same area. Of 1000 snails tested (20 from each of the 50 sites), 8073 cysts were found in 395 snails. Similarly, this study was repeated in Keith County, Nebraska (2874 km^2), separated by 300 miles from Lancaster County. (Grother and Hanelt, unpublished data). In this area, 4 years of intensive sampling yielded adult gordiids from 3 sites. Testing for cysts in snails, of 15 sites tested, 10 proved positive for cysts. Of 251 snails examined, 2674 cysts were found in 75 snails.

The results of these studies clearly indicate that the perception of how gordiids are distributed is greatly influenced by sampling methodology. Based on adult collections, gordiids appear to be rare and unevenly distributed. However, based on cysts, gordiids appear to be common and widespread (Hanelt *et al.*, 2001).

At the time these studies were made, the main drawback of this method was that species could not be determined from cyst morphology (Hanelt *et al.*, 2001). Therefore, it was impossible to determine the biodiversity of gordiids by studying cysts. Subsequent morphometric techniques were developed allowing for the identification of species-specific characters and sets of characters making species-level identification of gordiid cysts possible (Hanelt and Janovy, 2002). In addition, the technique of sampling for cysts will allow investigators to identify sites that are worthwhile for intensive sampling of adults. This will make the study of gordiids more tractable, since lack of study material has been cited as a reason for making its study difficult (de Villalobos and Voglino, 2000; Inoue, 1958; Reutter, 1972).

3.5.3. Temporal distribution

Bolek and Coggins (2002) is the only study which seasonally tracks a population of gordiids. This study included 3 sample years (1996–1999) and involved the bimonthly collection and enumeration of specimens of *G. difficilis*. The combined data show that worms appear in June or July; their numbers peak in late July and early August, and disappear by September (Figure 7). When the data are considered by year, variation in number and timing is apparent. Population fluctuations were also noticed by year. In 1996, very few worms were collected, compared with the other years. Population peaks were in July in 1998, but not until August in 1997. Much more work, similar to this, on many more species is needed to understand fully the seasonality of gordiids.

3.5.4. Conclusion and future study

The last decade has provided several important studies producing a framework for future work in the temporal and geographic distribution of gordiids. Large-scale collection efforts of adult worms in understudied areas will undoubtedly lead to the description of countless

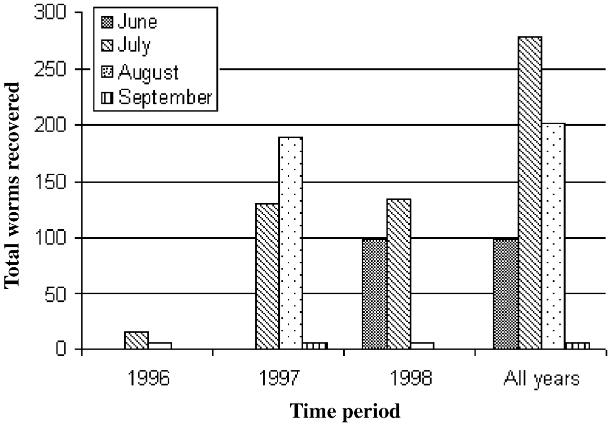


Figure 7 Number of *Gordius difficilis* adults recorded and removed from man-made ponds. Based on data from Bolek and Coggins (2002).

new species. In addition, since most gordiids have only been described from single localities, it is possible that many species have a very limited distribution. Large-scale sampling efforts would possibly lead to the discovery of new species in even the most well-sampled areas of the world. The use of cysts in paratenic hosts as indicators of gordiid distribution appears as a viable alternative to adults. Although morphological characters of cysts can be used to determine species-level identification, molecular markers could be established allowing individual or pooled paratenic hosts to be tested for the presence of cysts.

3.6. Humans and Gordiids

3.6.1. Gordiids in sources of potable water

Numerous reports exist of nematomorphs "contaminating" city water systems. Cappucci and Schultz (1982) provides a detailed review of reported cases since 1870. These cases occurred in countries within a wide spectrum of economic development, from Zimbabwe and Malaysia to England and United States. More recently, such cases have been reported from areas such as Bosnia-Herzegovina (Pikula *et al.*, 1996), Turkey (Aydemir *et al.*, 1996) and Australia. Often these reports associate the presence of gordiids in water systems with water quality. For example, in a leading Australian farming magazine, an author wrote that gordiid adults (which do not feed nor have a working mouth) wreak havoc on water systems by eating out water filters (Walker *et al.*, 1989). Gordiid worms within water supplies do not pose danger to humans, but are simply an indication that insect-definitive hosts are capable of getting into the water source.

3.6.2. Gordiids around the home

Gordiids are also frequently noted in and around the home. Worms often appear in the bathroom, where standing water is typical; examples are toilets, showers (see e.g. collection locality of *P. varius* in

Hanelt and Janovy, 2002), bathtubs (Spiridonov *et al.*, 1992) and hot tubs. Often, the worms are carried by their insect-definitive hosts. In other cases, crickets are disposed of in the toilet after being killed, only to have the worms within the cricket wiggle back up the pipes. After subsequent use of the facility, by either adults or especially children, people can become unduly alarmed at the sight of undulating worms (Herter and Neese, 1989). In another case brought to the attention of one of the authors (BH), a man recently returned home to Washington State from travel in Australia. Three days after his return, he was aghasted to find a gordiid in his bathtub. The worm was brought into the local health clinic where it was processed. The serial section revealed a male gordiid worm (Figure 8 is Plate 4.8 in the Separate Color Plate section). The worm was likely carried into the tub by the insect host, either before or during use, or brought in through the pipes.

Pet owners also have reported finding worms within their pet's water dish, leading to unnecessary trips to the veterinarian. In addition, worms are often reported from temporary standing water in the yard or the driveways after heavy rains.

3.6.3. Pseudoparasitism

Since many of the reports of worms associated with humans are simply due to the spurious presence of worms in or near places of human habitation, reports of gordiids "infecting" humans must thus be interpreted with great caution. In one unpublished case, a worm was recovered from the underwear of a woman in Lincoln, Nebraska (Figure 9). The assumption was that the worm came from inside the patient, but no evidence was given for this assumption. Although exactly how the worm came to be inside the woman's clothing is unknown, it is most likely that the worm was carried in by the host.

However, it is clear that in some cases gordiids are resident within the digestive systems of humans. However, these cases do not represent real parasitism, but rather pseudoparasitism. Pseudoparasitism is defined as a parasite present in a host due to accidental circumstances. This host organism is not a natural host and usually the parasite does

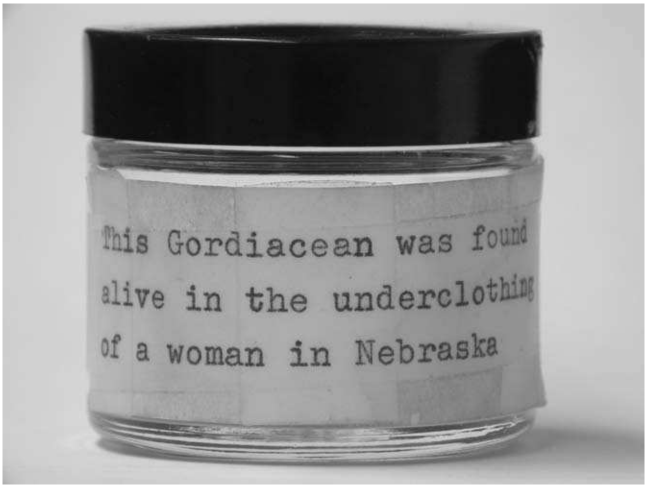


Figure 9 Sample of *Paragordius varius* female adult worm recovered from the "underclothing" of an adult female. Although the specifics of this case are unknown, this worm did not likely infect the woman but rather entered the clothing by being carried in by the insect host. This sample was brought to the attention of the authors by John Janovy Jr., University of Nebraska-Lincoln, USA.

not thrive (and often does not survive for extended periods) in this foreign environment. Most, if not all, cases reporting "infection" of humans with gordiids are instances of pseudoparasitism.

For example, in a recent case, a girl in Korea was found to have vomited two *Gordius* sp. worms (Lee *et al.*, 2003). It is clear from this report that the girl ate an insect (which looked like a cricket) shortly before she vomited. The most likely scenario for this event is that the cricket eaten by the patient was itself hosting the gordiids, which were subsequently released in the gastric juices of her stomach. Similar reports exist for worms vomited by a domestic cat (Saito *et al.*, 1987) and by a domestic dog (Horton, 1986). Numerous reports exist of patients passing worms per rectum and per urethra (Beaver *et al.*, 1984). In several instances, the infection of the urinary tract was reported. In one case, an adult male was admitted to a hospital in Brazil after having passed worms per urethram. While in the hospital, the patient expelled an additional adult worm, which was found in his chamber pot (de Lucena and Campelo, 1975).

These reports are plausible, but since the documentation of most of these cases was less than thorough, it is unknown whether these patients were even suffering from pseudoparasitism. Furthermore, no diagnosis of an active case of infection with gordiids has ever been reported. It is clear that the sole method of infection of humans by gordiids is through the ingestion of the adult form. This could occur through the ingestion of adults in untreated water, or through the ingestion of infected insect-definitive hosts. Herter and Neese (1989) urged doctors to distinguish between pseudoparasitism from true helminthic infection. Misdiagnosis and ignorance of the benign nature of these worms has caused patients to undergo undue stress and financial burden of unnecessary regiments of antihelminthics (see e.g. Smith *et al.*, 1990).

4. HOST BEHAVIOURAL ALTERATIONS

4.1. Introduction

As seen before, hairworms are parasitic in arthropods when juveniles but they are free-living in aquatic environments (shallow puddles, marshes, streams and ponds) as adults. Because most host species are terrestrial arthropods, returning to water is a challenging task for the large majority of hairworm species. How these parasitic worms overcome such a problem is undoubtedly one of the most fascinating aspects of their ecology. It has been first argued that hairworms would rely on chance: larva, when mature, just waits until the hosts are close to water on their own and take this opportunity to emerge into water. Also this is possible in some cases; it has also been hypothesized that hairworms would manipulate the behaviour of their host to enhance their probability of reaching an aquatic habitat. Placed in a general context, this hypothesis is not an odd idea as, indeed, parasite-induced alteration of host phenotype is now documented for a wide range of parasites (for a review, see Barnard and

Behnke, 1990; Combes, 1991; Moore, 2002; Poulin, 1998b). Numerous host phenotypic traits can be altered by parasites (e.g. behaviour, morphology and physiology), and these alterations can vary greatly in their magnitude, from slight shifts in the percentage of time spent in performing a given activity to the production of complex and spectacular behaviours (Moore, 2002; Poulin and Thomas, 1999).

In the case of hairworms, hosts harbouring a mature worm have been hypothesized to display a behaviour originally not present in their repertoire: they seek water and enter it (Begon *et al.*, 1996; Blunk, 1922; Dawkins, 1990; Jolivet, 1946; Poinar, 1991b; Schmidt-Rhaesa, 1997a; Thorne, 1940). From the parasite's perspective, such a behavioural change is likely to be adaptive as it ensures the adult worm to be released in an appropriate location for reproduction. However, until recently the water-seeking behaviour of insects parasitized by hairworms was only supported from anecdotal observations. Most popular ones included, for instance, insects flinging themselves into toilets and dog watering dishes.

4.2. Study System

Recently, Thomas *et al.* (2002) provided additional information concerning the behavioural changes of insects harbouring hairworms. Observations were made in southern France in a particular place; it was a private swimming pool located near a forest largely crisscrossed by several streams in which adult nematomorphs are commonly found during the summer. Every summer night, several insects (at least among the nine following species: *Nemobius sylvestris* infected by *Paragordius tricupidatus*; *Meconema thalassinum*, *Pholidoptera griseoptera*, *Uromenus rugosicollis*, *Ephippiger cunii*, *Barbitistes serricauda*, *Leptophyes punctatissima*, *Antaxius pedestris* and *Yersinella raymondi* infected by *Spinochordodes telinii*) "commit a mistake", instead of releasing their worm(s) into the streams, they jump into the water of the swimming pool (Thomas *et al.*, 2002). A behavioural sequence of this phenomenon is, for instance, illustrated on the most abundant cricket species, *N. sylvestris* (Figure 10 is Plate 4.10 in the

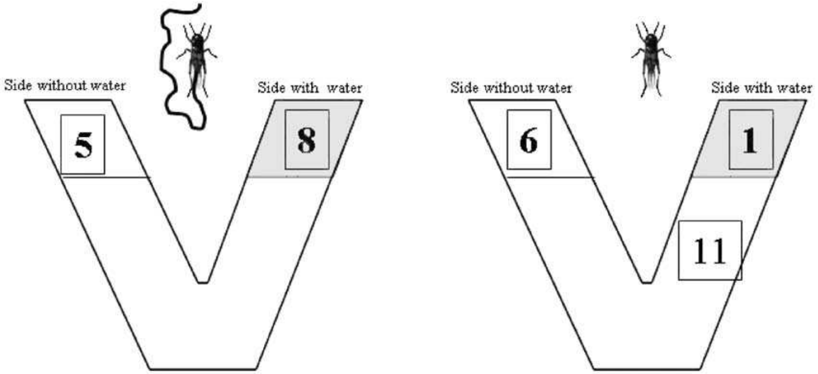
Separate Color Plate section). The majority of host species entered water during the first part of the night (i.e. before 1–2 a.m.). Except for the species *A. pedestris* and *M. thalassinum*, for which uninfected individuals could sometimes be found on the concrete area of the swimming pool, all the insect species mentioned before, when found around the swimming pool, were infected by a hairworm and sooner or later entered the water of the swimming pool. Insects entered the water by jumping into it or by entering gradually. After the host had entered the water, the emergence of the worm could be immediate (e.g. *S. telinii* emerging from *M. thalassinum*) or could take several minutes, i.e. after the host had drowned (frequently the case for *P. tricuspidatus* emerging from *N. sylvestris*). However, in the latter case, Thomas *et al.* (2002) noted that just after the host had jumped into the water, and was thus in contact with a liquid medium, the worm emerged 1–2 cm and returned inside the host, presumably because the end of the cricket's abdomen was not directly in contact with water (the worm was always seen to emerge fully within 2–5 min). A few seconds after complete emergence from the host, the worm actively swims away and leaves its host.

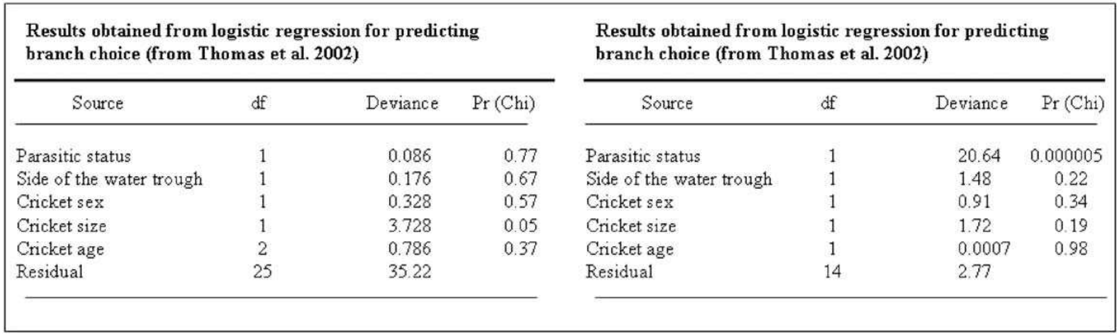
In accordance with the idea that the water-seeking behaviour in infected arthropods is a parasitic adaptation aimed at reaching a reproductive habitat, it is interesting to mention that the same behaviour was observed in two spider species (*Pistius truncatus* and *Olios argelasius*) harbouring mermithid nematodes (see also Maeyama *et al.*, 1994; Thomas *et al.*, 2002). Mermithids are phylogenetically distantly related to hairworms but they have similar biology (i.e. they also develop inside a terrestrial arthropod and are free-living in aquatic habitats when adults). This suggests the existence of an evolutionary convergence between nematomorphs and mermithids in their effect on host behaviour.

4.3. Host Behaviour

In addition to these observations, field and laboratory experiments in an Y-maze clearly indicate that crickets (*N. sylvestris*) infected by the nematomorph *P. triscupidatus* are more likely to jump into water than uninfected ones (Figure 11). Despite the significance of this result, the idea that the manipulation involved water detection from long distances by infected insects was not fully supported. Indeed, the results obtained by Thomas *et al.* (2002) were also in accordance with another, and maybe more realistic, hypothesis given the ecological context. For instance, we must remember that the necessity of water detection in this manipulation becomes questionable when we consider the ecological conditions in which this host–parasite system evolved. A behavioural alteration induced by nematomorphs could be the induction of an erratic behaviour: infected crickets would leave their microhabitat but in no particular direction. Given the abundance of streams in these native forests, this would undoubtedly bring the cricket close to a stream. Alternatively, if insects routinely encounter water during a time scale appropriate to worm development, there may be no need to induce erratic or water-seeking behaviour. In accordance with the former idea, all the crickets that were found in atypical habitats (car park, inside a hotel) harboured a worm. Thomas and colleagues even noticed that about 80% of the infected crickets collected in a summer season come from these atypical habitats, not from the edges of the swimming pool (F. Thomas, unpublished data). Along the same idea, it can be mentioned that even when the swimming pool was protected with a plastic cover at night (i.e. strong reduction of the humidity gradient), the mean number of infected crickets captured around the swimming pool remained unchanged (F. Thomas, unpublished data). Thus, all these results and observations support the idea that water detection from a long distance following a humidity gradient is not involved, at least in *N. sylvestris*.

Once infected crickets encounter water, there is however another important behavioural difference with uninfected individuals. While crickets harbouring a worm often jumped into the water, uninfected ones most of the time were reluctant to enter it. This behavioural difference is undoubtedly a key step in the manipulative process as it allows the hairworm to emerge immediately after its host enters water. Whether infected crickets are attracted by the liquid, or they





Results obtained from logistic regression for predicting branch choice (from Thomas et al. 2002)

Source	df	Deviance	Pr (Chi)
Parasitic status	1	0.086	0.77
Side of the water trough	1	0.176	0.67
Cricket sex	1	0.328	0.57
Cricket size	1	3.728	0.05
Cricket age	2	0.786	0.37
Residual	25	35.22	

Results obtained from logistic regression for predicting branch choice (from Thomas et al. 2002)

Source	df	Deviance	Pr (Chi)
Parasitic status	1	20.64	0.000005
Side of the water trough	1	1.48	0.22
Cricket sex	1	0.91	0.34
Cricket size	1	1.72	0.19
Cricket age	1	0.0007	0.98
Residual	14	2.77	

Figure 11 Results of the choice experiment in an Y-maze (from Thomas *et al.*, 2002).

simply do not perceive the danger linked to the presence of water is unclear. We cannot exclude that infected crickets do not react to a number of outside cues, including water, and therefore end up falling into it rather than avoiding it.

4.4. Host Changes Caused by Manipulation

4.4.1. Physiology and neurology

Recent attempts to explore the physiological and neuronal basis of these behavioural changes revealed substantial changes in the brain of insects infected by hairworms (Thomas *et al.*, 2003). Interestingly, the sampling procedure used by Thomas *et al.* (2003) allowed to disentangle nonspecific disorders by induced parasite from changes directly correlated with the manipulative process. The first category of crickets (*N. sylvestris*) was called "night-parasitized" crickets (NP) and corresponded to manipulated crickets, i.e. infected individuals captured between 10 p.m. and 1 a.m. near the edge of the swimming pool just before they jumped into water. As a control for this category, uninfected crickets at night were also collected in the nearby forest ("night-uninfected" crickets, NU). Third, in order to obtain crickets harbouring a mature worm without being manipulated, manipulated crickets (i.e. NP category) were dissected only the day after their capture, between 1 and 3 p.m. ("day-parasitized" crickets, DP), that is to say at a period of the day for which no behavioural change is observed under natural conditions (at least for *N. sylvestris*; F. Thomas, unpublished data). As a control for this category, uninfected crickets were also captured at night and kept until the day before being dissected between 1 and 3 p.m. ("day-uninfected" crickets, DU). Finally, a last category of crickets corresponded to individuals that have released their worm into water ("suicided" crickets, S). To obtain this category, infected insects arriving at the swimming pool were visually followed on the concrete area without disturbing them until they entered the swimming pool. Just after the emergence of the worm, the cricket was placed in a dry opaque plastic tumbler for 1 h. After this delay, most crickets were vigorous and were then dissected. This sampling design is interesting as it permits several sources of variation to be considered (e.g. infected versus uninfected, manipulated versus non-manipulated, etc.) when interpreting differences in the amount of neuroactive compounds among categories of crickets. Results obtained for polyamines, monoamines and amino acids revealed that the presence or absence of the parasite *per se* explained the largest part of the variation in compound concentrations: infected individuals display on average lower concentrations than uninfected ones (Figure 12a and b). The existence of several non-specific disorders may appear not really surprising given the relatively large size of the parasite relative to the host. This could

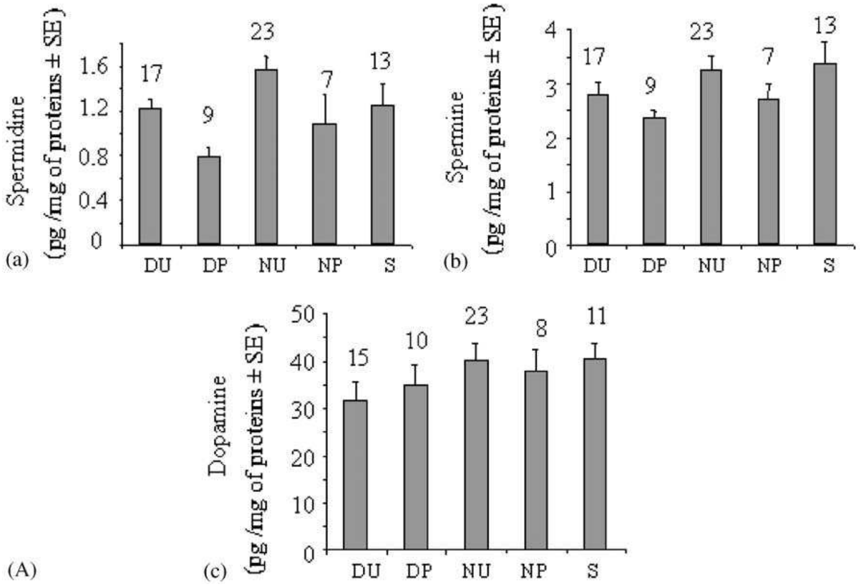


Figure 12 (A) Mean quantities of spermidine, spermine and dopamine among the five categories of crickets. Sample sizes are indicated above each bar (from Thomas *et al.*, 2003). (B) Mean quantities (pmol/µg of proteins ± SE) of amino acids among the five categories of crickets. Sample sizes: DU (day uninfected, $n = 15$), DP (day parasitized, $n = 10$), NU (night uninfected, $n = 24$), NP (night parasitized, $n = 8$) and S (suicided, $n = 13$) (from Thomas *et al.*, 2003).

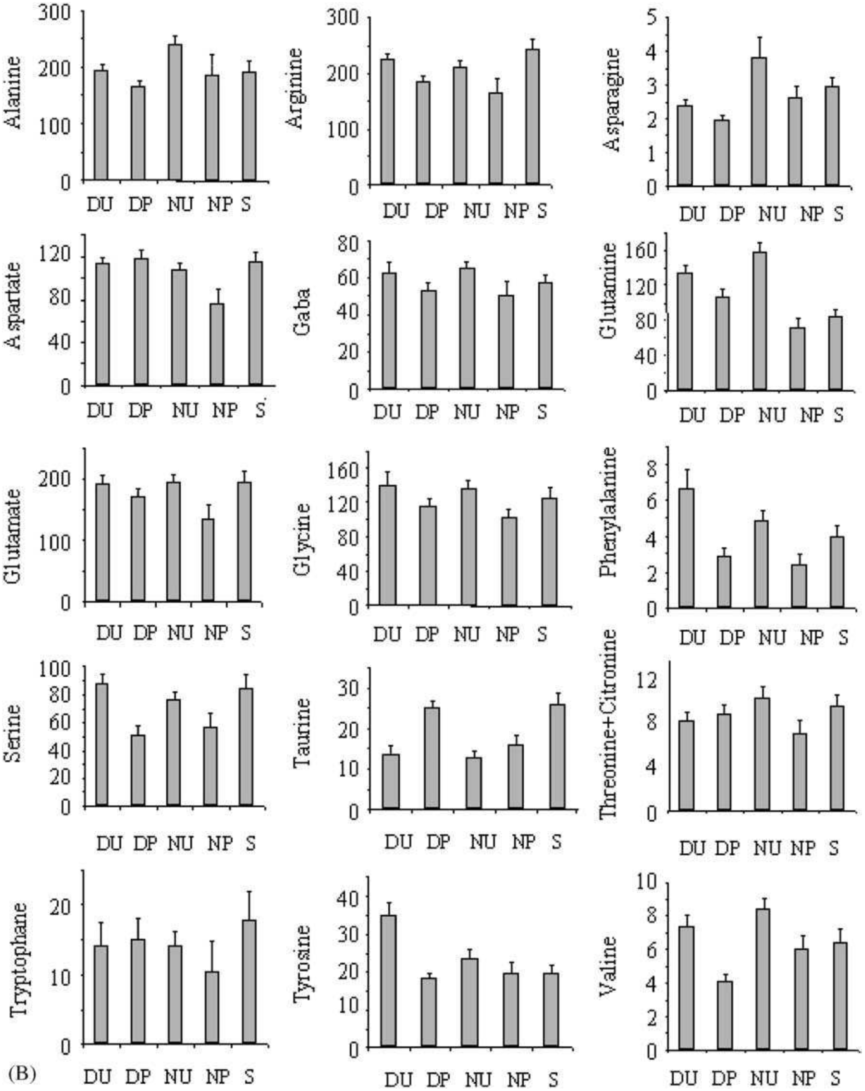


Figure 12 continued.

first result from a competition between the host and the parasite for nutrients *sensu lato*. Thomas *et al*. (2003) also did not exclude the hypothesis according to which this global depletion in infected individuals results from infected individuals spending more time searching for water than searching for food. In addition to these non-specific effects, a significant part of the variation for several amino acids however correlated with the manipulative process. This concerns especially taurine, valine and tyrosine. Infected crickets, during the day, displayed the highest concentration of taurine (Figure 12b). Interestingly, taurine is considered as an important neurotransmitter in insects (one of the most abundant free amino acids in insect brains) and it participates in neurotransmission of mushroom bodies (Sinakevitch *et al*., 2001). Furthermore, taurine also regulates many biological phenomena including brain osmoprotection (Schaffer *et al*., 2000). In this context, we might be tempted to speculate that, as suggested long ago (see e.g. Blunk, 1922), hairworms cause thirst in its host during the day in order to motivate it to seek water at night. However, further studies are needed to confirm all these results as, for instance, we cannot determine from this study whether these changes are causes or consequences of the manipulative process.

Thomas *et al*. (2003) also performed a histological study on the mushroom bodies of the brain in order to compare neurogenesis between infected and uninfected crickets. Interestingly, the mitotic index measured by counting all the cells in the M phase in the crickets mushroom bodies exhibited a two-fold increase in infected crickets as compared with uninfected ones (Figure 13 is Plate 4.13 in the Separate Color Plate section). Thus, neurogenesis was doubled in the brain of infected crickets. This strange result, as well as its precise role in the manipulative process, remains enigmatic at the moment. Knowing that taurine (at least in mammals) has been shown to stimulate neurogenesis (Chen *et al*., 1998), we might be tempted to establish a link between this enhanced neurogenesis and the fact that infected crickets displayed a higher concentration of taurine. Knowing that the mushroom bodies (where neurogenesis takes place) are the main sensory integrative centres of the insect brain, we can also speculate that this abnormal neurogenesis interferes with neural circuitry, perturbing the analysis of the environmental cues by the cricket. It is, for instance, known in rodents that uncontrolled addition of new neurons into existing circuits may potentially disrupt the function of the central nervous system (Feng *et al*., 2001).

Alternatively, we could argue that the increased neurogenesis in infected crickets reflects a normal host adaptation when exposed to adverse conditions: a higher neurogenesis could be host-induced to improve environmental perception when this is urgently necessary, for instance in case of severe dehydration. Under this scenario, we could also speculate that the parasite dehydrates its host, thus simulating a dry environment so as to induce in return a cascade of adaptive host processes aimed at finding water rapidly. Preliminary results do not however support this hypothesis as, indeed, we even observe the reverse result: when crickets (*A. domesticus*) are placed 2 days without water or food, the mitotic index did not increase; instead it was significantly reduced compared to the one observed in normally fed controls (Cayre and Thomas, unpublished data).

4.4.2. Proteomics

In a recent study, Biron and Thomas (unpublished data) used proteomic tools to identify the biochemical alterations that occur in the brain of the cricket *N. sylvestris* when it is driven to water by the hairworm *P. tricuspidatus* (Nematomorpha). In this study, simultaneously the host and the parasite proteomes were characterized at three strategic stages of the manipulative process, i.e. before, during, and after the expression of the water-seeking behaviour by the host. It was found that the parasitic worm produces effective molecules from the family WNT acting directly on the development of the central nervous system of the host. Interestingly, these WNT proteins display important similarities with those known in insects, suggesting a molecular mimicry. In the brain of manipulated crickets, there were also differential expressions of proteins specifically linked to the neurogenesis, the visual cycle, (circadian rhythm) and neurotransmitter activities. Finally, four proteins were detected in the brain of manipulated crickets for which the function(s) are still unknown (Biron and Thomas, unpublished data). These results support the hypothesis according to which the behavioural changes induced by hairworms in arthropod hosts rely on chemicals, some of them directly produced by the parasite.

4.5. Manipulation or Collaboration?

Until now, the water-seeking behaviour of insects harbouring hairworms has been interpreted as an adaptive parasite-induced behavioural change (i.e. a true manipulation) aimed at reaching a suitable place for reproduction. Although this is possible, other outcomes are theoretically possible. For instance, Biron *et al.* (2005) recently explored the idea that infected arthropods that would be collaborative, bringing mature hairworms into water, could achieve a higher fitness than those that do not collaborate (or are unable to find water). Conditions for host collaboration rather than host manipulation could be met if the host (i) does not always die after the emergence of the worm, (ii) is able to reproduce, at least partially, when the worm has been released into water (i.e. the host has not been totally castrated by the parasite and it can recover the damage caused by the parasite) and (iii) has no reproductive potential (e.g. complete castration) if it fails to bring the worm into water. To explore this hypothesis, Biron *et al.* (2005) quantified the life expectancy and the gonad development of infected crickets when allowed to release their parasite into water (i.e. collaborative behaviour) versus infected individuals prevented from bringing the parasite into a suitable aquatic environment (i.e. non-collaborative behaviour). Biron *et al.* (2005) first showed that hairworm emergence is not lethal *per se* for the host, instead the cricket can even live several months after having released the parasite. Interestingly, this work also revealed that a substantial proportion of the females (23%) that liberated the worm into water produced eggs or had developed ovaries several weeks post emergence, while such a phenomenon is absent when females were prevented from releasing the parasite. Thus, it seems that for infected females, bringing the parasite into a suitable aquatic environment is indeed a necessary condition before any gonad development becomes possible. The inability of non-collaborative females to produce eggs is observed even after the death of the parasite inside the host, indicating that failing to bring the parasite into water, or waiting for its death, is clearly not a profitable option for infected females. What remains to be elucidated beyond this study, is the quality of the eggs produced by collaborative females, as well as, the fitness of their offspring if these eggs are fertilized. Indeed, at this moment, preliminary tests suggest that collaborative females, even when they are recognized as sexual partners by males and are able to take the spermatophore like uninfected females, have serious problems ovipositing. Thus, until this aspect is clarified, we cannot exclude the hypothesis that egg production by certain collaborative females has no evolutionary role (e.g. eggs are not fertile, females are unable to oviposit, etc.), being just a non-adaptive consequence (a "by-product") of the worm emergence. In addition, all these results would also deserve to be confirmed under natural conditions. Indeed, in the field, the collaborative behaviour is likely to come with costs for infected crickets as jumping into water is a risky behaviour, increasing exposure to predators (fish, frogs) and drowning. Results obtained with male crickets were substantially different. From an evolutionary point of view the most important difference is the complete castration of infected individuals regardless of their behaviour (collaborative or not). Hence, it is difficult to argue that males bringing the parasite into water obtain fitness compensation for such collaborative acts.

4.6. Conclusion and Future Studies

In conclusion, it is clear that hairworms do not only rely on chance to reach water, instead they alter the behaviour of their hosts in a way that increases their probability to reach an aquatic environment. How? Despite considerable progress, underlying reasons for why infected insects "capitulate" and act in ways that benefit the parasite remain clearly enigmatic. Hairworm–insect interactions remains more than ever a fascinating model of research, full of challenging questions for anyone interested in parasitic strategies based on host manipulation. Further research needs to be integrative with specific efforts made to develop collaborations between parasitologists and researchers from other subdisciplines, especially physiology, neurobiology, and biochemistry. The answer to many questions on the evolution of insect manipulation by hairworms might come from this starting convergence between these disciplines.

5. GENERAL CONCLUSION

A few years ago, Poulin wondered why there was such a paucity of biologists investigating the phylum Nematomorpha (Poulin, 1998a). Reasons that have been cited as cause for this lack of study include the difficulty in finding reliable and consistent sources of study material, the unknown phylogenetic relationships within this group, the lack of a domesticated model system, and the lack of specific information on life cycles. Missing this most basic information, seasoned biologists as well as students have often overlooked this group in favour of more well-known and reliable systems. However, over the last 10 years, a small but dedicated group of investigators has made a sustained effort in bringing the knowledge of nematomorphs out of the dark ages and into the third millennium.

The most immediate need is to understand the basic gordiid biology better. Using a mixture of very traditional and modern techniques, we now have a greater understanding of their taxonomy, structure, ecology, and evolution. Detailed descriptions and redescriptions now exist for dozens of nematomorphs species. The structural features of several nematomorph species and developmental stages have been described, allowing us a glimpse into how these parasites grow from free-living larvae (50 μm long) to adults (some as long as 2 m) within relatively small insect hosts. The distribution of gordiids has finally been documented at several unique levels. Broad scale collections of adults, combined with extensive literature and voucher specimen reviews, has provided us with new information on the extent of individual gordiid species distribution. Focused collections of nematomorphs at much more local scales appear to indicate that gordiids are much more common than previously suggested. The tracking of a gordiid population over several years has revealed fluctuations of numbers and in seasonal timing. Our understanding of their life cycle has improved. Much of the speculation of how gordiids transition between their free-living and parasitic life cycle stages has been tested, supporting the involvement of a paratenic host. This work on the life cycle has culminated in the domestication of a gordiid species, which has been used to test field observations and further understand parasite development.

Our knowledge of nematomorphs has also benefited from recent studies employing molecular and proteomic techniques. Taxonomy based on ribosomal sequences supports the broad relationships suggested by character-based methods. Finally, studies of the host–parasite interaction at the end of the parasitic phase have begun to provide a framework for understanding how the parasite ensures transmission to aquatic environments. Experiments using proteomic tools suggest that rather than relying on chance, the parasite manipulates the chemical composition of the host's brain to ensure favourable transmission.

Although we do not suggest that the studies undertaken within the last 10 years have allowed us to get a complete understanding of this phylum, we do think that a solid foundation now exists for fruitful continued study. As usual, this research has led to more questions than answers. So, throughout this review, we have provided various avenues of future research that can now be taken, by building on existing data. In addition, many facets of gordiid biology are still completely unknown.

One of the main areas within this group in desperate need of further research is the general biology of the nectonematids (marine hairworms). Life cycle, hosts, distribution, and reproduction of nectonematids are completely unknown. Although getting at this information sounds like a daunting task, basing these kinds of studies on what is already known about gordiids (freshwater hairworms) and combining this with modern techniques, the risk in undertaking this kind of study is greatly reduced. For example, the documentation of the nectonematid life cycle has proven extremely difficult due to their rarity and habitat. It is unknown whether there are one or two hosts in the life cycle. Knowing that gordiids use a paratenic hosts, a fair assumption is that the same could be true for nectonematids. Using molecular techniques and nectonematid-specific primers, possible paratenic hosts could be screened in large batches to determine infection status. These primers can now be made since a nectonematid ribosomal DNA sequence has recently become available. This kind of method and insight will enable a relatively fast determination of who is involved in the life cycle, and will allow for a targeted study of the hosts.

Interestingly, Poulin's comments on the lack of researchers working on nematomorphs appear to have come at a time when the study of nematomorphs is enjoying a Renaissance. Ultimately, we hope that the information now available on this unique phylum will not only make its study more tractable but also more attractive to others.

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REFERENCES

Adrianov, A.V., Malakhov, V.V. and Spiridonov, S.E. (1998). Fine morphology of the larvae of hairworm *Gordius* sp. (Nematomorpha). *Doklady Akademii Nauk* **361**, 558–561.

Aydemir, M., Yorgancigil, B., Demirci, M. and Akkaya, H. (1996). A report of *Gordius aquaticus* Linnaeus, 1758 isolated from Isparta city drinking water. *Turkiye Parazitoloji Dergisi* **20**, 3–4.

Baek, K.M. (1993). Two species of genus Chordodes (Gordioidea, Nematomorpha) from Korea. *Korean Journal of Systematic Zoology* **9**, 221–228.

Baek, K.M. and Noh, Y.T. (1992). Two species of genus Gordius (Gordioidea, Nematomorpha) from Korea. *Korean Journal of Systematic Zoology* **8**, 223–230.

Baer, J.G. (1951). *Ecology of Animal Parasites*. Urbana, IL: Illinois Press.

Barnard, C.J. and Behnke, J.M. (1990). *Parasitism and Host Behaviour*. London: Taylor & Francis.

Barus, V. (1964). Freshwater snails as reservoir hosts of invasive larvae of the nematode *Syngamus trachea* (Montagu 1811). In: *Parasitic Worms and Aquatic Conditions* (R. Ergens and B. Rysavý, eds), pp. 83–90. Prague: House of the Czechoslovak Academy of Sciences.

Beaver, P.C. (1969). The nature of visceral larva migrans. *Journal of Parasitology* **55**, 3–12.

Beaver, P.C., Jung, R.C., Cupp, E.W. and Craig, C.F. (1984). *Clinical Parasitology*. Philadelphia, PA: Lea & Febiger.

Begon, M., Harper, J.L. and Townsend, C.R. (1996). *Ecology: Individuals, Populations, and Communities*. Oxford: Blackwell Science.

Biron, D.G., Ponton, F., Menigoz, A., Hanelt, B. and Thomas, F. (2005). Water seeking behavior in insects harboring hairworms: should the host collaborate? *Behavioral Ecology* **16**, 656–660.

Bleidorn, C., Schmidt-Rhaesa, A. and Garey, J.R. (2002). Systematic relationships of Nematomorpha based on molecular and morphological data. *Invertebrate Biology* **121**, 357–364.

Blunk, H. (1922). Die Lebensgeschichte der im Gelbrand schmarotzenden Saitenwürmer I. *Zoologischer Anzeiger* **54**, 110–132.

Bohall, P.J., Wells, M.R. and Chandler, C.M. (1997). External morphology of larvae of *Chordodes morgani* (Nematomorpha). *Invertebrate Biology* **116**, 26–29.

Bolek, M.G. and Coggins, J.R. (2002). Seasonal occurrence, morphology, and observation on the life history of *Gordius difficilis* (Nematomorpha: Gordioidea) from southeastern Wisconsin, United States. *Journal of Parasitology* **88**, 287–294.

Bresciani, J. (1991). Nematomorpha. In: *Microscopic Anatomy of Invertebrates* (F.W. Harrison, ed.), pp. 197–218. New York: Wiley-Liss, Inc.

Brittain, J.E. and Eikeland, T.J. (1988). Invertebrate drift—a review. *Hydrobiologia* **166**, 77–93.

Brivio, M.F., de Eguileor, M., Grimaldi, A., Vigetti, D., Valvassori, R. and Lanzavecchia, G. (2000). Structural and biochemical analysis of the parasite *Gordius villoti* (Nematomorpha, Gordiacea) cuticle. *Tissue and Cell* **32**, 366–376.

Brown, S.P., Renaud, F., Guegan, J.F. and Thomas, F. (2001). Evolution of trophic transmission in parasites: the need to reach a mating place? *Journal of Evolutionary Biology* **14**, 815–820.

Cappucci, D.T., Jr. and Schultz, M.G. (1982). Gordian worms (hairworms): biological and public health aspects. *CRC Handbook Series in Zoonoses. Section C: Parasitic Zoonoses.* Boca Raton, FL: CRC Press.

Capuse, I. (1966). Sur le *Dacochordodes bacescui* Capuse (ord. Chordodida, fam. Chordodidae). *Travers du Museum da Histoire Naturale "Grigore Antipa"* **6**, 17–23.

Chandler, C.M. (1985). Horsehair worms (Nematomorpha, Gordioidea) from Tennessee, with a review of taxonomy and distribution in the United States. *Journal of the Tennessee Academy of Sciences* **60**, 59–62.

Chen, X.C., Pan, Z.L., Liu, D.S. and Han, X. (1998). Effect of taurine on human fetal neuron cells: proliferation and differentiation. *Advances in Experimental Medicine and Biology* **442**, 397–403.

Choisy, M., Brown, S.P., Lafferty, K.D. and Thomas, F. (2003). Evolution of trophic transmission in parasites: why add intermediate hosts? *American Naturalist* **162**, 172–181.

Cochran, P.A., Newton, A.K. and Korte, C. (2004). Great Gordian knots: sex ratio and sexual size dimorphism in aggregations of horsehair worms (*Gordius difficilis*). *Invertebrate Biology* **123**, 78–82.

Combes, C. (1991). Ethological aspect of parasite transmission. *American Naturalist* **138**, 866–880.

Combes, C. (2001). *Parasitism: the Ecology and Evolution of Intimate Interactions*. Chicago: University of Chicago Press.

Daengsvang, S. (1968). Further observations on the experimental transmission of *Gnathostoma spinigerum. Annals of Tropical Medicine and Parasitology* **62**, 88–94.

Dawkins, R. (1990). Parasites, desiderata lists and the paradox of the organism. *Parasitology* **100**, S63–S73.

de Lucena, D.T. and Campelo, M. (1975). Accidental parasitism of man with two gordiids. *Revista do Instituto de Medicina Tropical de São Paulo* **17**, 390–393.

de Miralles, D.A.B. and de Villalobos, L.C. (1993a). Distribucion geografica de los Gordiaceos en la region neotropical. In: *Fauna de agua dulce de la Repulica Argentina* (Z.A. de Castellanos, ed.), pp. 17–32. La Plata: Profadu (Conicet).

de Miralles, D.A.B. and de Villalobos, L.C. (1993b). Distribucion geografica de los Gordiaceos en la republica Argentina. In: *Fauna de agua dulce de la Repulica Argentina* (Z.A. de Castellanos, ed.), pp. 1–16. La Plata: Profadu (Conicet).

de Miralles, D.A.B. and de Villalobos, L.C. (1995). A review of the Gordiacea (Nematomorpha) in the collection of the California Academy of Sciences with the description of a new species. *Graellsia* **51**, 17–22.

de Miralles, D.A.B. and de Villalobos, L.C. (1996a). Dos nuevas especies de Gordiáceos para Ventania, Argentina (Nematomorpha). *Comunicaciones do Museo de Ciencias y Tecnologia, PUCRS, Série Zoologia* **9**, 3–9.

de Miralles, D.A.B. and de Villalobos, L.C. (1996b). New Gordius for Argentina (Gordiacea Nematomorpha). *Acta Zoologica Mexicana Nueva Serie* **68**, 67–73.

de Miralles, D.A.B. and de Villalobos, L.C. (1996c). Nueva especie de *Neochordodes* (Gordiacea Nematomorpha) parasita de *Scapteriscus* sp. (Grillotalpidae). *Revista de la Academia de Ciencias de Zaragoza* **51**, 237–246.

de Miralles, D.A.B. and de Villalobos, L.C. (1996d). Species of *Neochordodes* in Argentina. *Iheringia Serie Zoologia* **81**, 145–150.

de Miralles, D.A.B. and de Villalobos, L.C. (1996e). Una nueva especie de *Gordius* (Linne, 1758) (Gordiaceae, Nematomorpha) de la Patagonia. *Naturalia Patagónica, Ciencias Biológicas* **4**, 41–45.

de Miralles, D.A.B. and de Villalobos, L.C. (1997). Ultraestructura cuticular de una nueva especie de *Chordodes* Creplin, 1874, para la Argentina (Nematomorpha—Gordioidea—Chordodidae). *Estudos de Biologia* **42**, 41–48.

de Miralles, D.A.B. and de Villalobos, L.C. (1998). Dos nuevos especies y una nueva combinacion para el genero Acutogordius (Nematomorpha: Gordiidae). *Neotropica* **44**, 75–79.

de Miralles, D.A.B. and de Villalobos, L.C. (2000). *Noteochordodes*, un nuevo género para la republica Argentina (Nematomorpha, Gordiaceo). *Revista del Museo de La Plata (NS) Zoologia* **14**, 279–283.

de Villalobos, L.C. (1995). A new species of the genus Chordodes Creplin, 1874, in Argentina. *Neotropica (La Plata)* **41**, 63–65.

de Villalobos, L.C. and Camino, N. (1999). Two new species of Gordiacea (Nematomorpha) parasites of *Stagmatoptera hyaloptera* (Mantidae) from Argentina. *Iheringia, Serie Zoologia* **86**, 71–76.

de Villalobos, L.C. and de Miralles, D.A.B. (1997). Una nueva especie de *Chordodes* (Gordiaceo Nematomorpha) parasita de *Blaptica* sp. (Blaberidae, Blataria). *Comunicaciones do Museo de Ciencias y Tecnologia, PUCRS, Série Zoologia* **10**, 45–51.

de Villalobos, L.C. and Restelli, M.A. (2001). Ultrastructural study of the cuticle and epidermis in *Pseudochordodes bedriagae*. *Cell and Tissue Research* **305**, 129–134.

de Villalobos, L.C., Ribera, I. and Downie, I.S. (1999). Hairworms found in Scottish agricultural land, with descriptions of two new species of

Gordionus Müller (Nematomorpha: Gordiidae). *Journal of Natural History* **33**, 1767–1780.

de Villalobos, L.C., Schmidt-Rhaesa, A. and Zanca, F. (2003). Revision of the genus *Beatogordius* (Gordiida, Nematomorpha) II. South American species with description of two new species. *Memórias do Instituto Oswaldo Cruz* **98**, 115–128.

de Villalobos, L.C. and Voglino, D. (2000). Description of *Gordionus porosus* n. sp. and redescription of *Gordionus ondulatus* (Nematomorpha, Parachordodinae) with scanning electron microscopy. *Journal of Parasitology* **86**, 358–365.

de Villalobos, L.C. and Zanca, F. (2001). Scanning electron microscopy and intraspecific variation of *Chordodes festae* Camerano, 1897 and *C. peraccae* (Camerano, 1894) (Nematomorpha: Gordioidea). *Systematic Parasitology* **50**, 117–125.

Degrange, C. and Martinot, J.P. (1996). Gordiacea, parasites of Orthoptera in Vanoise National Park. *Travaux Scientifiques du Parc National de la Vanoise* **19**, 161–182.

Dogiel, V.A. (1966). *General Parasitology*. New York: Academic Press.

Donin, C.L.L. and Cotelli, F. (1977). The rod-shaped sperm of Gordioidea (Aschelminthes, Nematomorpha). *Journal of Ultrastructural Research* **61**, 193–200.

Dorier, A. (1930). Recherches biologiques et systématiques sur les Gordiacés. *Annales Université de Grenoble* **7**, 1–183.

Dorier, A. (1935a). Etude biologique et morphologique de la larva de *Parachordodes gemmatus* (Villot). *Travaux du Laboratoire d'Hydrobiologie et de Pisciculture de Grenoble* **25**, 147–161.

Dorier, A. (1935b). Sur le passage a la vie latente des larves des Gordiacés. *Comptes Rendus de l'Académie des Sciences, Paris* **200**, 491–494.

Feng, R., Rampon, C., Tang, Y.P., Shrom, D., Jin, J., Kyin, M., Sopher, B., Miller, M.W., Ware, C.B., Martin, G.M., Kim, S.H., Langdon, R.B., Sisodia, S.S. and Tsien, J.Z. (2001). Deficient neurogenesis in forebrain-specific presenilin-1 knockout mice is associated with reduced clearance of hippocampal memory traces. *Neuron* **32**, 911–926.

Feyel, T. (1936). Researches histologiques sur *Nectonema agile* Verr. étude de la forme parasitaire. *Archives d'Anatomie Microscopique* **32**, 197–234.

Garey, J.R. and Schmidt-Rhaesa, A. (1998). The essential role of "minor" phyla in molecular studies of animal evolution. *American Zoologist* **38**, 907–917.

Giribet, G., Distel, D.L., Polz, M., Sterrer, W. and Wheeler, W.C. (2000). Triploblastic relationships with emphasis on the acoelomates and the position of Gnathostomulida, Cycliophora Plathelminthes and

Chaetognatha: a combined approach of 18S rDNA sequences and morphology. *Systematic Biology* **49**, 539–562.

Halvorsen, O. and Wissler, K. (1973). Studies on the helminth fauna of Norway XXVIII: an experimental study of the ability of *Dypyllobothrium latum* (L.), *D. dendriticum* (Nitzsch), and *D. ditremum* (Creplin) (Cestodes Pseudophyllidea) to infect paratenic hosts. *Norwegian Journal of Zoology* **21**, 201–210.

Hanelt, B. and Janovy, J., Jr. (1999). The life cycle of a horsehair worm, *Gordius robustus* (Gordioidea: Nematomorpha). *Journal of Parasitology* **85**, 139–141.

Hanelt, B. and Janovy, J., Jr. (2002). Morphometric analysis of non-adult characters of common species of American gordiids (Nematomorpha: Gordioidea). *Journal of Parasitology* **88**, 557–562.

Hanelt, B. and Janovy, J., Jr. (2003). Spanning the gap: identification of natural paratenic hosts of horsehair worms (Nematomorpha: Gordioidea) by experimental determination of paratenic host specificity. *Invertebrate Biology* **122**, 12–18.

Hanelt, B. and Janovy, J., Jr. (2004a). Life cycle and paratenesis of American gordiids (Nematomorpha: Gordiida). *Journal of Parasitology* **90**, 240–244.

Hanelt, B. and Janovy, J., Jr. (2004b). Untying a Gordian knot: the domestication and laboratory maintenance of a Gordian worm, *Paragordius varius* (Nematomorpha: Gordiida). *Journal of Natural History* **39**, 939–950.

Hanelt, B., Grother, L.E. and Janovy, J., Jr. (2001). Physid snails as sentinels of freshwater nematomorphs. *Journal of Parasitology* **87**, 1049–1053.

Heinze, K. (1952). Über Gordioidea. Eine systematische Studie über Insektenparasiten aus der Gruppe der Nematomorpha. *Zeitschrift für Parasitenkunde* **15**, 183–202.

Hemsworth, R.J. and Brooker, M.P. (1979). The rate of downstream displacement of macro invertebrates in Upper Wye Wales UK. *Holarctic Ecology* **2**, 130–136.

Hershey, A.E., Pastor, J., Peterson, B.J. and Kling, G.W. (1993). Stable isotopes resolve the drift paradox for *Baetis* mayflies in an Arctic River. *Ecology* **74**, 2315–2325.

Herter, C.D. and Neese, R.E. (1989). Pseudoparasitism with *Gordius robustus*. *American Family Physician* **39**, 139–142.

Horton, M.L. (1986). Nonparasitic worm vomited by a dog. *Modern Veterinary Practice* **67**, 905.

Inoue, I. (1958). Studies on the life history of *Chordodes japonensis*, a species of Gordiacea. I. The development and structure of the larva. *Japanese Journal of Zoology* **12**, 203–218.

Inoue, I. (1960a). Studies on the ecology of the larvae of the hairworm, *Chordodes japonensis*. *Bulletin of the Tokyo Gakugei University* **11**, 21–25.

Inoue, I. (1960b). Studies on the life history of *Chordodes japonensis*, a species of Gordiacia. II. On the manner of entry into the aquatic insect-larvae of *Chordodes* larvae. *Annotationes Zoologicae Japonenses* **33**, 132–141.

Inoue, I. (1962). Studies on the life history of *Chordodes japonensis*, a species of Gordiacea. III. The modes of infection. *Annotationes Zoologicae Japonenses* **35**, 12–19.

Inoue, I. (1994). Gordiod specimens deposited at the Toyama Science Museum. *Bulletin of the Toyama Science Museum* **17**, 101–103.

Jolivet, P. (1946). Nouvelle station française et nouvel hôte du ver *Gordius aquaticus*. *Miscellanea Entomologica* **42**, 139–140.

Lanzavecchia, G., De Eguileor, M., Valvassori, R. and Scari, G. (1995). Body cavities of Nematomorpha. In: *Body Cavities: function and phylogeny* (G. Lanzavecchia, R. Valvassori and M.D. Candia Carnevali, eds), pp. 45–60. Modena: Mucchi.

Lee, K.J., Bae, Y.T., Kin, D.H., Deung, Y.K., Ryang, Y.S., Im, I.K. and Young, T.S. (2003). A Gordius worm found in a three year old girl's vomitus. *Yonsei Medical Journal* **44**, 557–560.

Maeyama, T., Terayama, M. and Matsumoto, T. (1994). The abnormal behaviour of *Colobopsis* sp. (Hymenoptera, Formicidae) parasitized by *Mermis* (Nematoda) in Papua New Guinea. *Sociobiology* **24**, 115–119.

McManus, M.L. (1988). Weather, behaviour and insect dispersal. *Memoirs of the Entomological Society of Canada* **146**, 71–94.

Meissner, G. (1856). Beiträge zur Antonomie und Physiologie der Gordiaceen. *Zeitschrift für wissenschaftliche Zoologie* **7**, 1–140.

Montgomery, T.H., Jr. (1898). The Gordiacea of certain American collections with particular reference to the North American fauna II. *Proceedings of the California Academy of Sciences. 3rd ser: Zoology* **1**, 333–344.

Moore, J. (2002). *Parasites and the Behaviour of Animals*. Oxford: Oxford University Press.

Morand, S., Robert, F. and Connors, V.A. (1996). Complexity in parasite life cycles: population biology of cestodes in fish. *Journal of Animal Ecology* **64**, 256–264.

Mottram, J.C. (1932). The living drift of rivers. *Transactions of the Newbury District Field Club* **6**, 195–198.

Mühldorf, A. (1914). Beiträge zur Erwicklungsgeschichte und zu den phylogenetischen Beziehungen der *Gordius* Larve. *Zeitschrift für wissenschaftliche Zoologie* **111**, 1–75.

Müller, G.W. (1927). Über Gordiaceen. *Zeitschrift für die Morphologie und Ökologie der Tiere* **7**, 134–270.

Müller, K. (1982). The colonization cycle of fresh water insects. *Oecologia* **52**, 202–207.

Müller, M.C.M., Jochmann, R. and Schmidt-Rhaesa, A. (2004). The musculature of horsehair worm larvae (*Gordius aquaticus*, *Paragordius varius*, Nematomorpha): F-actin staining and reconstruction by cLSM and TEM. *Zoomorphology* **123**, 45–54.

Myers, M.J., Sperling, F.A.H. and Resh, V.H. (2001). Dispersal of two species of Trichoptera from desert springs: conservation implications for isolated vs connected populations. *Journal of Insect Conservation* **5**, 207–215.

Olsen, O.W. (1986). *Animal Parasites: Their Life Cycles and Ecology*. New York: Dover.

Parker, G.A., Chubb, J.C., Ball, M.A. and Roberts, G.N. (2003). Evolution of complex life cycles in helminth parasites. *Nature* **425**, 480–484.

Peterson, K.J. and Eernisse, D.J. (2001). Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. *Evolutionary Development* **3**, 170–205.

Pikula, Z.P., Spiridonov, S.E. and Drljevic, E.T. (1996). *Dacochordodes bacescui* Capuse, 1966: report of the first two isolations from humans. *Canadian Journal of Infectious Diseases* **7**, 144–146.

Poinar, G.O., Jr. (1991a). Hairworm (Nematomorpha: Gordioidea) parasites of New Zealand wetas (Orthoptera: Stenopelmatidae). *Canadian Journal of Zoology* **69**, 1592–1599.

Poinar, G.O., Jr. (1991b). Nematomorpha. In: *Ecology and Classification of North American Freshwater Invertebrates* (J.H. Thorp and A.P. Covich, eds), pp. 273–282. New York: Academic Press, Inc.

Poinar, G.O., Jr. (2001). Nematoda and Nematomorpha. In: *Ecology and Classification of North American Freshwater Invertebrates* (J.H. Thorp and A.P. Covich, eds), pp. 255–295. San Diego: Academic Press.

Poinar, G.O., Jr. and Brockerhoff, A.M. (2001). *Nectonema zealandica* n. sp. (Nematomorpha: Nectonematoidea) parasitizing the purple rock crab *Hemigrapsus edwardsi* (Brachyura: Decapoda) in New Zealand, with notes on the prevalence of infection and host defense reactions. *Systematic Parasitology* **50**, 149–157.

Poinar, G.O., Jr. and Doelman, J.J. (1974). A reexamination of *Neochordodes occidentalis* (Montg.) comb. n. (Chordodidae: Gordioidea): larval penetration and defense reaction in *Culex pipens* L. *Journal of Parasitology* **60**, 327–335.

Poinar, G.P. (1999). *Paleochordodes protus* n.g., n.sp. (Nematomorpha, Chordodidae) parasites of a fossil cockroach, with a critical examination of other fossil hairworms and helminths of extant cockroaches (Insecta: Blattaria). *Invertebrate Biology* **118**, 109–115.

Poulin, R. (1996). Observations on the free-living adult stage of *Gordius dimorphus* (Nematomorpha: Gordioidea). *Journal of Parasitology* **82**, 845–846.

Poulin, R. (1998a). Book review: Nematomorpha. *Journal of Parasitology* **84**, 35.

Poulin, R. (1998b). *Evolutionary Ecology of Parasites: From Individuals to Communities*. London: Chapman and Hall.

Poulin, R. and Thomas, F. (1999). Phenotypic variability induced by parasites. *Parasitology Today* **15**, 28–32.

Protasoni, M., de Eguileor, M., Congiu, T., Grimaldi, A. and Reguzzoni, M. (2003). The extracellular matrix of the cuticle of *Gordius panigettensis* (Gordioiidae, Nematomorpha): observations by TEM, SEM and AFM. *Tissue Cell* **35**, 306–311.

Restelli, M.A., de Villalobos, L.C. and Zanca, F. (2002). Ultrastructural description of the musculature, the intraepidermal nervous system, and their interrelation in *Pseudochordodes bedriagae* (Nematomorpha). *Cell and Tissue Research* **308**, 299–306.

Reutter, K. (1972). *Gordius* das Wasserkalb. *Mikroskosmos* **61**, 198–204.

Robert, F., Renaud, F., Mathieu, E. and Gabrion, C. (1988). Importance of the paratenic host in the biology of *Bothriocephalus gregarius* (Cestoda, Pseudophyllidea), a parasite of the turbot. *International Journal for Parasitology* **18**, 611–621.

Saito, Y., Inoue, I., Hayashi, F. and Itagaki, H. (1987). A hairworm, *Gordius* sp., vomited by a domestic cat. *Nippon Juigaku Zasshi* **49**, 1035–1037.

Schaffer, S., Takahashi, K. and Azuma, J. (2000). Role of osmoregulation in the actions of taurine. *Amino Acids* **19**, 527–546.

Schmidt-Rhaesa, A. (1996a). The nervous system of *Nectonema munidae* and *Gordius aquaticus*, with implications for the ground pattern of the Nematomorpha. *Zoomorphology* **116**, 133–142.

Schmidt-Rhaesa, A. (1996b). Ultrastructure of the anterior end in three ontogenetic stages of *Nectonema mundiae* (Nematomorpha). *Acta Zoologica* **77**, 267–278.

Schmidt-Rhaesa, A. (1997a). Nematomorpha. In: *Süßwasserfauna Mitteleuropas* (J. Scwoerbel and P. Zwick, eds), pp. 1–124. Stuttgart: Gustav Fischer.

Schmidt-Rhaesa, A. (1997b). Ultrastructural features of the female reproductive system and female gametes of *Nectonema munidae* Brinkmann 1930 (Nematomorpha). *Parasitology Research* **83**, 77–81.

Schmidt-Rhaesa, A. (1997c). Ultrastructural observations of the male reproductive system and spermatozoa of *Gordius aquaticus* L., 1758. *Invertebrate Reproduction and Development* **32**, 31–40.

Schmidt-Rhaesa, A. (1998a). Muscular ultrastructure in *Nectonema munidae* and *Gordius aquaticus* (Nematomorpha). *Invertebrate Biology* **117**, 37–44.

Schmidt-Rhaesa, A. (1998b). Phylogenetic relationships of the Nematomorpha—a discussion of current hypothesis. *Zoologischer Anzeiger* **236**, 203–216.

Schmidt-Rhaesa, A. (1999). Nematomorpha. In: *Encyclopedia of Reproduction* (E. Knobil and J.D. Neill, eds), pp. 333–341. San Diego: Academic Press.

Schmidt-Rhaesa, A. (2000). Rasterelektronenmikroskopische Untersuchungen an *Gordionus violaceus* Baird 1853 und *Gordius elongiporus* spec. nova (Nematomorpha) aus Niederosterreich. *Linzer Biologische Beitraege* **32**, 455–461.

Schmidt-Rhaesa, A. (2001a). Are the genera of Nematomorpha monophyletic taxa? *Zoologica Scripta* **31**, 185–200.

Schmidt-Rhaesa, A. (2001b). The life cycle of horsehair worms (Nematomorpha). *Acta Parasitologica* **46**, 151–158.

Schmidt-Rhaesa, A. (2001c). Problems and perspectives in the systematics of Nematomorpha. *Organisms, Diversity and Evolution* **1**, 161–163.

Schmidt-Rhaesa, A. (2001d). Reinvestigation and reinterpretation of *Chordodiolus echinatus* Linstow, 1901, a peculiar nematomorph species. *Mitteilungen des Museums für Naturkunde, Berlin, Zoologische Reihe* **77**, 91–94.

Schmidt-Rhaesa, A. (2001e). Variation of cuticular characters in the Nematomorpha: studies on *Gordionus violaceus* (Baird, 1853) and *G. wolterstorffii* (Camerano, 1888) from Britain and Ireland. *Systematic Parasitology* **49**, 41–57.

Schmidt-Rhaesa, A. (2002). Australian species of *Chordodes* (Nematomorpha) with remarks on the genus and its life history. *Journal of Natural History* **36**, 1569–1588.

Schmidt-Rhaesa, A. (2004a). *Gordius longitriatus* a new species or Nematomorph from Hawaii. *Records of the Hawaii Biological Survey for 2003 Bishop Museum Occasional Papers* **78**, 53–55.

Schmidt-Rhaesa, A. (2004b). Japanese horsehair worms (Nematomorpha) from the Lake Biwa Museum. *Species Diversity* **9**, 97–107.

Schmidt-Rhaesa, A. (2004c). Ultrastructure of an integumental organ with probable sensory function in *Paragordius varius* (Nematomorpha). *Acta Zoologica* **85**, 15–19.

Schmidt-Rhaesa, A. (2005). Morphogenesis of *Paragordius varius* (Nematomorpha) during the parasitic phase. *Zoomorphology* **124**, 33–46.

Schmidt-Rhaesa, A. and Bryant, M.S. (2004). Filling the Gondwana gaps: new species and new reports of *Beatogordius* Heinze, 1934 (Nematomorpha) from Australia and Madagascar. *Systematic Parasitology* **57**, 173–181.

Schmidt-Rhaesa, A., Chung, P.R. and Sohn, W.M. (2003a). *Parachordodes megareolatus*, a new species of horsehair worm (Nematomorpha) from Korea. *Korean Journal of Systematic Zoology* **19**, 161–166.

Schmidt-Rhaesa, A. and de Villalobos, L.R. (2001). Revision of the genus *Beatogordius* (Gordiida, Nematomorpha). I. African species with inclusion of *Chordodiolus echinatus* (Linstow, 1901). *Annales du Musée royal de l'Afrique Centrale* **206**, 1–25.

Schmidt-Rhaesa, A. and Ehrmann, R. (2001). Horsehair worms (Nematomorpha) as parasites of praying mantids with a discussion of their life cycle. *Zoologischer Anzeiger* **240**, 167–179.

Schmidt-Rhaesa, A., Hanelt, B. and Reeves, W. (2003b). Redescription and compilation of Nearctic freshwater Nematomorpha (Gordiida), with the description of two new species. *Proceedings of the Academy of Natural Sciences of Philadelphia* **153**, 77–117.

Schmidt-Rhaesa, A. and Menzel, L. (2005). Central American and Caribbean species of horsehair worms (Nematomorpha), with the description of three new species. *Journal of Natural History* **15**, 515–529.

Schmidt-Rhaesa, A., Thomas, F. and Poulin, R. (2000). Redescription of *Gordius paranensis* Camerano, 1892 (Nematomorpha), a species new for New Zealand. *Journal of Natural History* **34**, 333–340.

Schultz, R.S. and Davtian, E.A. (1954). The forms of host-parasite relationships in helminthology. *Zoologicheskii Zhurnal* **33**, 1201–1205.

Shoop, W.L. (1988). Trematode transmission patterns. *Journal of Parasitology* **74**, 46–59.

Sinakevitch, I., Farris, S.M. and Strausfeld, N.J. (2001). Taurine-, aspartate- and glutamate-like immunoreactivity identifies chemically distinct subdivisions of Kenyon cells in the cockroach mushroom body. *Journal of Comparative Neurology* **439**, 352–367.

Skaling, B. and MacKinnon, B.M. (1988). The absorptive surfaces of *Nectonema* sp. (Nematomorpha: Nectonematoidea) from *Pandalus montagui*: histology, ultrastructure, and absorptive capabilities of the body wall and intestine. *Canadian Journal of Zoology* **66**, 289–295.

Smith, D.R., Watson, D.D., Clevenger, R.R. and McBride, C. (1990). *Paragordius varius, Archives of Pathology and Laboratory Medicine* **114**, 981–983.

Söderström, O. (1987). Upstream movements of invertebrates in running waters—a review. *Archiv für Hydrobiologie* **111**, 197–208.

Sørensen, M.V., Funch, P., Willerslev, E., Hansen, A.J. and Olesen, J. (2000). On the phylogeny of the Metazoa in the light of Cycliophora and Micrognathozoa. *Zoologischer Anzeiger* **239**, 297–318.

Spiridonov, S.E. (1993). *Gordius annamensis* sp. n. (Nematomorpha: Parachordodadidae) a new species of gordian worm from central Vietnam. *Parazitologiya* **27**, 429–432.

Spiridonov, S.E. (1998). Horse hairworms of the genus *Gordius* Linne, 1776 (Gordiacea, Nematomorpha) in the collection of Scientific Zoological Museum M.V. Lomonosov Moscow State University. *Byulleten' Moskovskogo Obshchestva Ispytatelei Prirody Otdel Biologicheskii* **103**, 19–26.

Spiridonov, S.E. (2000). A new hairworm in the genus *Chordodes* (Nematomorpha: Chordodidae) from the Minusinsk depression. *Parazitologiia* **34**, 437–441.

Spiridonov, S.E. (2001). *Chordodes guineensis* sp.n., a new species of horse-hair worms from West Africa (Nematomorpha: Chordodidae). *Zoosystematica Rossica* **10**, 275–279.

Spiridonov, S.E., Pikula, Z.P. and Drljevic, E.T. (1992). Redecription of *Dacochordodes bacescui* Capuse, 1966 (Nematomorpha: Chordodidae). *Helminthologica* **29**, 193–196.

Svábeník, J. (1925). Parasitismus a metamorfosa druhu *Gordius tolosanus* Dujardin. *Publications de la Faculté des Sciences de l'Université Masaryk* **58**, 1–48.

Thomas, F., Schmidt-Rhaesa, A., Martin, G., Manu, C., Durand, P. and Renaud, F. (2002). Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? *Journal of Evolutionary Biology* **15**, 356–361.

Thomas, F., Schmidt-Rhaesa, A. and Poulin, R. (1999). Microhabitat characteristics and reproductive status of male *Euchordodes nigromaculatus* (Nematomorpha). *Journal of Helminthology* **73**, 91–93.

Thomas, F., Ulitsky, P., Augier, R., Dusticier, N., Samuel, D., Strambi, C., Biron, D.G. and Cayre, M. (2003). Biochemical and histological changes in the brain of the cricket *Nemobius sylvestris* infected by the manipulative parasite *Paragordius tricuspidatus* (Nematomorpha). *International Journal of Parasitology* **33**, 435–443.

Thorne, G. (1940). The hairworm, *Gordius robustus* Leidy, as a parasite of the Mormon cricket, *Anabrus simplex* Haldeman. *Journal of the Washington Academy of Sciences* **30**, 219–231.

Valvassori, R., Scarì, G., De-Eguileor, M., Lernia, L.D., Magnetti, P. and Melone, G. (1988). *Gordius villoti* (Nematomorpha) life cycle in relation with caddis fly larvae. *Bollettino di Zoologia* **55**, 269–278.

Vejdovsky, F. (1888). Studien über Gordiiden. *Zeitschrift für wissenschaftliche Zoologie* **46**, 188–216.

Villot, A. (1874a). Monographie des dragonneaux (Genre *Gordius* Dujardin). *Archives de Zoologie expérimentale* **3**, 39–72.

Villot, A. (1874b). Monographie des dragonneaux (Genre *Gordius* Dujardin). Deuxiéme partie. Anatomie et Physiologie. *Archives de Zoologie expérimentale* **3**, 181–238.

Voigt, E. (1938). Ein fossiler Saitenwurm (*Gordius tenuifibrosus* n. sp.) aus der eozäen Braunkohle des Geiseltales. *Nova acta Leopoldina new series* **5**, 351–360.

Walker, T.K., Rollinson, D. and Simpson, A.J. (1989). A DNA probe from *Schistosoma mansoni* allows rapid determination of the sex of larval parasites. *Molecular Biochemistry and Parasitology* **33**, 93–100.

Watermolen, D.J. and Haen, G.L. (1994). Horsehair worms (phylum Nematomorpha) in Wisconsin, with notes on their occurrence in the Great Lakes. *Journal of Great Lakes Research* **9**, 7–11.

White, D.A. (1966). A new host record for *Paragordius varius* (Nematomorpha). *Transactions of the American Microscopical Society* **85**, 579.

White, D.A. (1969). The infection of immature aquatic insects by larval *Paragordius* (Nematomorpha). *Great Basin Naturalist* **29**, 44.

Zapotosky, J.E. (1971). The cuticular ultrastructure of *Paragordius varius* (Leidy, 1851)(Gordioidea: Chordodidae). *Proceedings of the Helminthological Society of Washington* **38**, 228–236.

Zapotosky, J.E. (1974). Fine structure of the larval stage of *Paragordius varius* (Leidy, 1851) (Gordioidea: Paragordidae). I. The preseptum. *Proceedings of the Helminthological Society of Washington* **41**, 209–221.

Zapotosky, J.E. (1975). Fine structure of the larval stage of *Paragordius varius* (Leidy 1851) (Gordioidea: Paragordidae). II. The postseptum. *Proceedings of the Helminthological Society of Washington* **42**, 103–111.

Zrzavý, J., Mihulka, S., Kepka, P., Bezdek, A. and Tietz, D. (1998). Phylogeny of the metazoa based on morphological and 18S ribosomal DNA evidence. *Cladistics* **14**, 249–285.

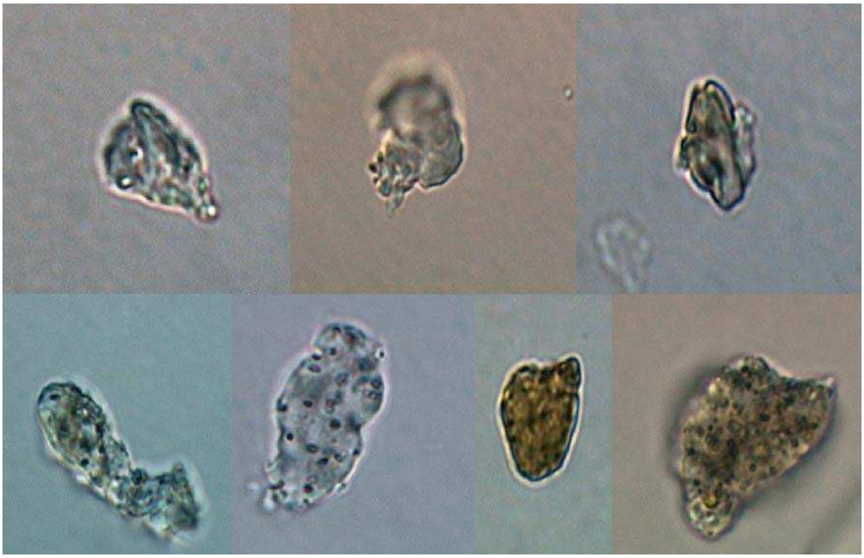

Plate 2.10 Novel, multinucleated structures formed upon the fusion of sporozoites of *C. parvum.*

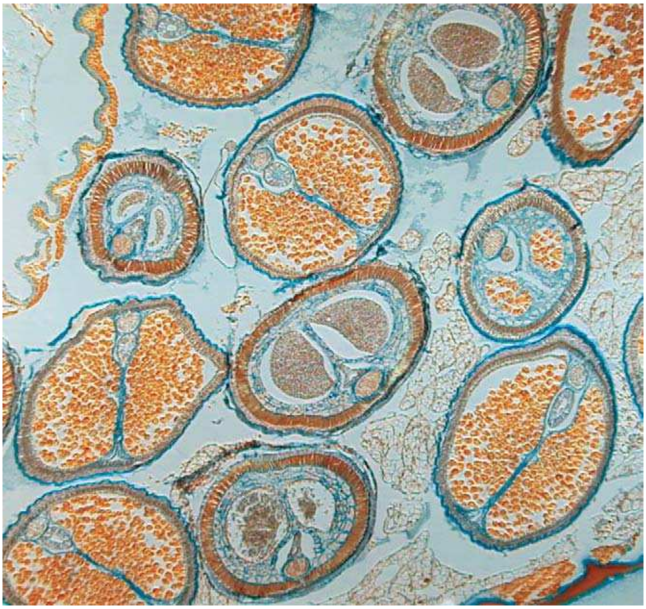

Plate 4.3 Histological section of developing *Paragordius varius* males and females within the definitive host *Gryllus firmus*, 25 days post-exposure.

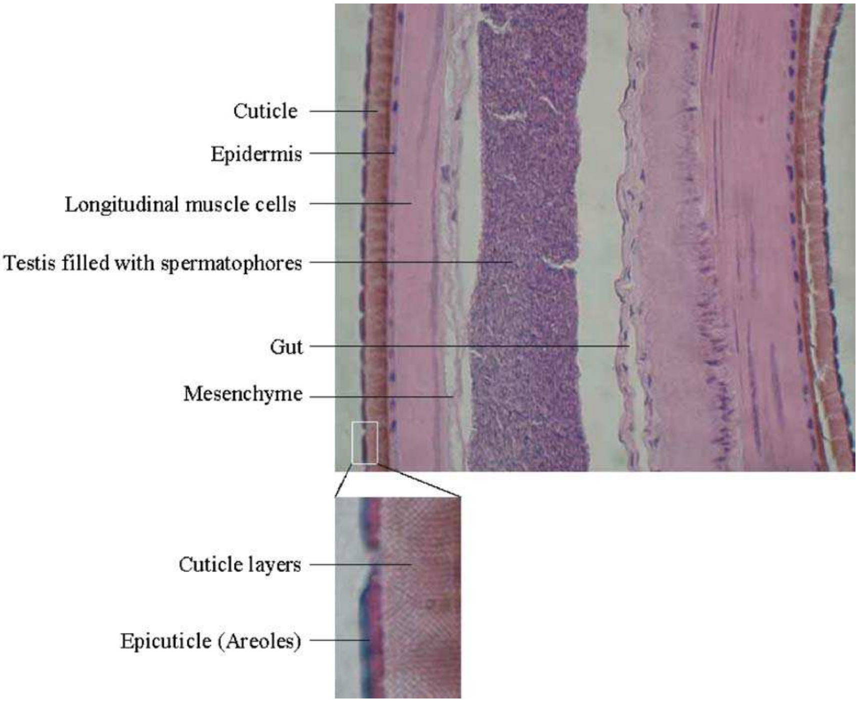

Plate 4.8 Longitudinal section of male worm recovered from the bath tub of a man presumed to be infected. The worm was recovered from Oregon State, USA; the man was presumed to have become infected during travel to Australia. After looking at this section, it was determined to be a gordiid species commonly found in North America, and likely entered the bathtub by being carried in by the insect-definitive host. Top: complete section, bottom: close up of cuticle layer. This sample was brought to the attention of the authors by Hector C. Aldape.

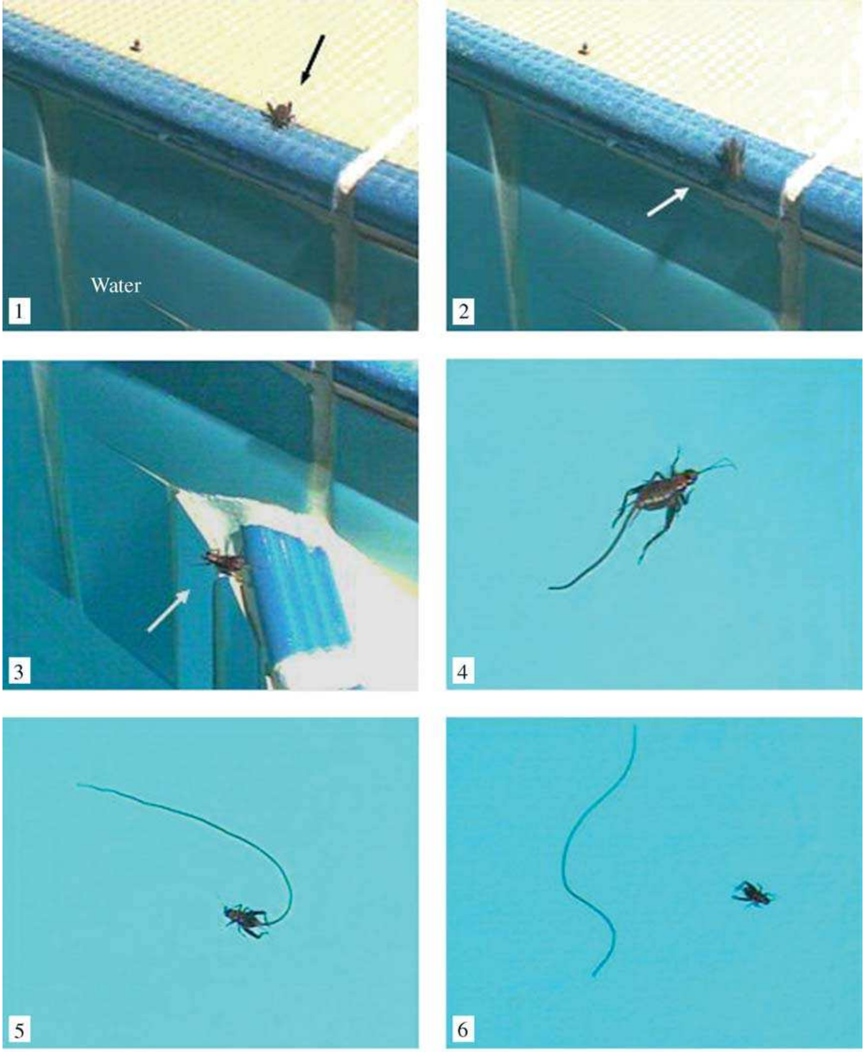

Plate 4.10 Illustration of the water-seeking behaviour of *Nemobius sylvestris* in an artificial water area followed by the emergence of the hairworm *Paragordius tricupidatus*.

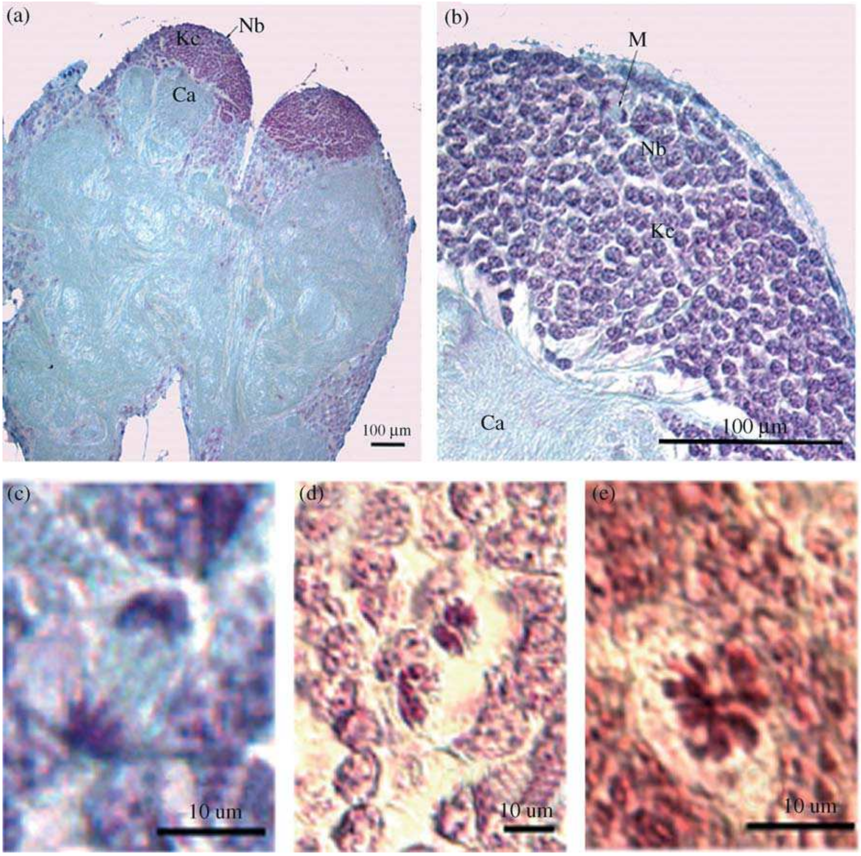

Plate 4.13 Histological sections of *Nemobius sylvestris* mushroom bodies treated by the nuclear coloration of Feulgen-Rossenbeck. (a) frontal section of the whole brain showing the position and structure of the mushroom bodies. (b) The mushroom body cortex is shown enlarged, allowing the visualization of the group of neuroblasts at the apex of the structure. A figure of mitosis is indicated by the arrow. (c–e) Pictures of anaphase and metaphases among the neuroblasts. Nb, neuroblasts; Ca, calyx; Kc, Kenyon cells; M, mitosis (from Thomas *et al*., 2003).