Parasites within the new phylogeny of eukaryotes

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In the past few years, molecular phylogenetic and cladistic analyses of the interrelationships of the living phyla have resulted in a radical reorganization of eukaryote groups. This reorganization has significance for parasitologists, in that it places as sister taxa some of the more speciose and highly parasitic phyla (nematodes and insects), reorganizes what is now recognized as paraphyletic sets of 'wormy taxa' as the Aschelmintha, and draws numerous bridges between different realms (plants, fungi and animals). This review attempts to explore the role of parasites within the phylogeny of eukaryotes. Extant described parasitic organisms are less common among the eukaryotes than is commonly admitted in the literature.

Recent advances in molecular phylogenetics and morphological cladistics have considerably changed the picture we had on the tree of life (e.g. see Refs [1–16]). In particular, the alteration of the evolutionary relationships between eukaryotic organisms have split some traditional clades (e.g. Protozoa, Protophyta, Acoelomata, Aschelmintha) into highly divergent new branches, and have gathered formerly strongly distant organisms into new groups (e.g. Sporozoa with Dinoflagellata, Nematozoa with Arthropoda, Platyhelmintha with Mollusca and Annelida). Although the place that parasites occupy in this new phylogeny has already been discussed for Metazoa [14,15], a global view for eukaryotes, and the importance of parasitism itself, has not been analysed previously. The relationships of all eukaryotes and what proportion are parasitic are examined below; the discussion is based on two major references [11,13], which review the position and/or number of described species.

Defining the group under discussion

We have considered as parasites all organisms that at one stage of their life cycle live at the expense of, and are dependent on, a host; true mutualism is excluded from this (e.g. photosynthetic algae associated with fungi, cnidarians, molluscs). Phytophagous organisms (e.g. aphids, lepidopterans, mistletoe) and parasitoids (e.g. entomophagous insects) are thus included in this study because at one stage each individual depends on one host (or very few for certain insects) (Box 1). By contrast, neither herbivores nor carnivores were included because they depend on several (and sometimes numerous) 'preys'; in addition, mosquitoes were not included because only females depend on a host and they exploit several different individual hosts in one lifetime.

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In the phylogenies presented here, we have tried to derive a consensus of the different proposed topologies found in the literature [1–16]. Species numbers have been derived from the literature cited [13,15,17–30] and from several websites (Box 2). In some cases relevant to parasites, discrepancies found in the literature will be discussed. Species counts only consider named species and not estimates of species diversity.

Role of parasites within the phylogeny of eukaryotes

From Fig. 1, it can be seen that Nematozoa (Nematoda and Nematomorpha) belongs to the same great clade as Athropoda and is separated from the Acanthocephala (Lophotrochozoa) and Platyhelmintha, itself grouped with Mollusca and Annelida. Consequently, Acoelomata and Aschelmintha clades have vanished. It can also be seen that the majority of parasites are in Protostoma (i.e. 92% of named parasitic species) and 87% are in Ecdysozoa. The number of parasitic species within the great clade containing Platyhelmintha and Acanthocephala represent only 5% of described parasitic species. Thus, other clades, including protists, fungi and plants, contain only 7% of the named eukaryotic parasitic species. The contribution of Deuterostoma (Echinoderms and Chordates) to parasitism is negligible (0.05%).

Only 0.8% of species of the green lineage (green and red algae, and plants) are parasites (Fig. 2). The clade Oomyceta (e.g. *Saprolegna*), formerly included within Myceta, and the Opalina, formerly classified in Sarcomastigophora (an obsolete Protozoa phylum), are now grouped within the Chromista (i.e. brown algae). A new clade named Alveolata brings together Ciliata (e.g. *Balantidium*), Sporozoa (e.g. *Plasmodium*, *Eimeiria*, *Babesia*) and Dinoflagellata (formerly considered as brown algae). The Kinetoplastida (e.g. *Trypanosoma*, *Leishmania*) are now classified in Euglenozoa, where they are associated with Euglenophyta (formerly in Protophyta). Microsporidia are in the Myceta. The Myxozoa (e.g. *Myxobolus*) has been included in the parasitic Cnidaria clade Endocnydozoa [31].

Within the new clade Lophotrochozoa (Fig. 3), 96% of the parasitic species are encountered among the Platyhelmintha (63%), Mollusca (28%) and Acanthocephala (5%). Acanthocephala (formerly in the Aschelminth obsolete clade) is now associated with Rotifera. Monogenea and Trematoda are no longer sister groups [14,15].

The majority of members of Ecdysozoa (Fig. 4) are Nematozoa and Arthropoda species. Among the large phylum Nematoda, the majority of species are parasites (60%), but an overwhelming number of parasites are found among the insects (Hexapoda) (91% of Ecdysozoa parasites). The Pentastomida (formerly *incertae sedis* Pararthropoda) are now classified within the Maxillipedia crustaceans (with Copepoda, Branchioura, Ostracoda and Cirripedia).

The position of several clades containing parasites presented in the preceding section is under discussion among phylogeneticists. This is the case for the

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Box 1. Definitions and description of some unusual taxa

Parasite: We expect no general agreement on the following definition. In the present paper, a parasite is an organism that requires, at one stage of its life cycle, to live at the expense of one (or a few) individual host(s), which is necessarily bigger than the parasite, and with no reference to a measurable (and most of the time not measurable) cost inflicted to the host. Such a cost may be variable in space and time and even reverse into a benefit [a]. True mutualists, such as algae in fungi or animals (probably issued from predation), were not considered as parasites.
Acanthocephala: Thorny-headed worms.
Achaeta: Leeches.
Acoela: Flatworms with no pharynx or true gut.
Actinopoda: Ray feet single-celled organisms (Radiolaria, Heliozoa and Acanthara).
Brachiopoda: Clam-like marine animals.
Bryozoa: Aquatic colonial 'moss' animals.
Chaetognatha: Arrow-worms, predominantly pelagic.
Chlorarachniophyta: Reticulate plasmodium of net-like mass of amoeboid cells found in warmer marine waters worldwide.
Ctenophora: Comb jellies.
Echiura: Marine nonsegmented worms.
Entoprocta: Small marine organisms living in shallow coastal waters.
Foraminifera: Protists with shells.
Gastrotricha: Lobed head, transparent worm-like aquatic animals.
Glaucophyta: Rare freshwater unicellular algae, with two unequal flagella.
Gnathostomulida: Jawed-mouth interstitial animals living in fine, anoxic marine sediments.
Haptophyta: Coccolithoporids are the major component of the marine phytoplankton (and of chalk).
Hexapoda: Insects in the wide sense (includes Collembola, Diplura and Protura).
Kinorhyncha: Segmented, marine invertebrates found in subtidal mud.
Loricifera: Small animals (200–400 μm) found in the interstitial space between marine gravel.
Monoblastozoa: Animals consisting of a single layer of cells only (doubtful existence).
Myxomyceta: Slime molds.
Nematomorpha: Long (up to 1 m), but very slender animals (1–3 mm), parasitic in arthropods as juveniles (e.g. gordian worms).
Nemertea: Ribbon worms.
Oligochaeta: Earth worms.
Onychophora: Velvet worms.
Percolozoa: Single-celled organisms alternating between an amoeboid and a flagellated form (e.g. *Naegleria*).
Phoronida: Horseshoe worms.
Placozoa: Marine animals with cells arranged in a double-layer plate.
Pogonophora and Vestimentifera: Deep-sea unsegmented tube-worms.
Polychaeta: Segmented worms with many bristles (e.g. *Nereis*).
Porifera: Sponges, consists of three distant phyla.
Priapulida: Short, plump, exclusively marine worms.
Pycnogonida: Sea spiders.
Rhizopoda: Amoebae and allied.
Rhodophyta: Red algae.
Rotifera: Microscopic animals found in freshwater environments and in moist soil.
Sipuncula: Peanut worms.
Streptophyta: Green plants.
Tardigrada: Small animals called water bears, living in mosses, soil, fresh water and the sea.
Temnocephala: Aquatic ectoparasitic flatworms with finger-like projections on the head.
Testaceafilosea: Testate amoebae lacking flagellated stages with a coating of siliceous scales.

Reference

a Thomas, F. *et al.* (2000) Are there pros as well as cons to being parasitized? *Parasitol. Today* 16, 533–536

Rhombozoa and Orthonectida (Mesozoa) and the Acoela (Platyhelmintha), which all could be placed at the base of the Bilateria [14]. According to Lecointre and Le Guyader [13], the Myzostomida (parasites of echinoderms) are Polychaeta annelids; however, this taxon has also been placed among the Platyhelmintha by others [14,32].

Concluding remarks

Parasitism is a character that has appeared independently in different lineages. The proportion of named species that are parasitic is ~30% and appears to be largely inferior to what has been proposed previously (50% [33,34] to 71% [35]). However, this may be a huge underestimate arising from differential efforts in studying the taxonomy of parasitic organisms as compared with free living ones. For example, it is probable that many Nematozoa and Arthropoda species, which contain most of all described parasitic species (87%), are still to be described. Furthermore, some groups are almost totally unknown, such as the

Box 2. Websites concerning species and parasite diversity counts

The Pseudocoelomate Phyla:
http://biosci238.bsd.uchicago.edu/pseudocoel.outl.html

Kingdoms of eukaryotic protists:
http://comenius.susqu.edu/bi/202/protists/

Parasitic red algae:
http://dana.ucc.nau.edu/~lmf2/algae97/reds/parasite.html

Classification of the Earth's biosphere:
http://geology.csusb.edu/360/biotax31.htm

The protists:
http://infusion.allconet.org/webquest/theprotists.html

The Tree of Life web project:
http://tolweb.org/tree/phylogeny.html

The microbiology of different kingdoms:
http://server.nicholls.edu/atemplet/bio108homepage/chap28.html

The five kingdoms:
http://www.abdn.ac.uk/~nhi708/classify/kingdoms.html

Phyla of the animal kingdom:
http://www.earthlife.net/inverts/an-phyla.html

Fish database:
http://www.fishbase.org/search.cfm

***Cryptosporidium* and the coccidians:**
http://www.ksu.edu/parasitology/

The Parasitic Plant Connection:
http://www.science.siu.edu/parasitic-plants

The Fungi kingdom:
http://www.sidwell.edu/us/science/vlb5/labs/classification_lab/eukarya/fungi/

The biosphere: life on earth:
http://www.ucmp.berkeley.edu/alllife/threedomains.html

The insects (in German):
http://zoologie.forst.tu-muenchen.de/heitland/detinvert/_orders/insect_orders.html

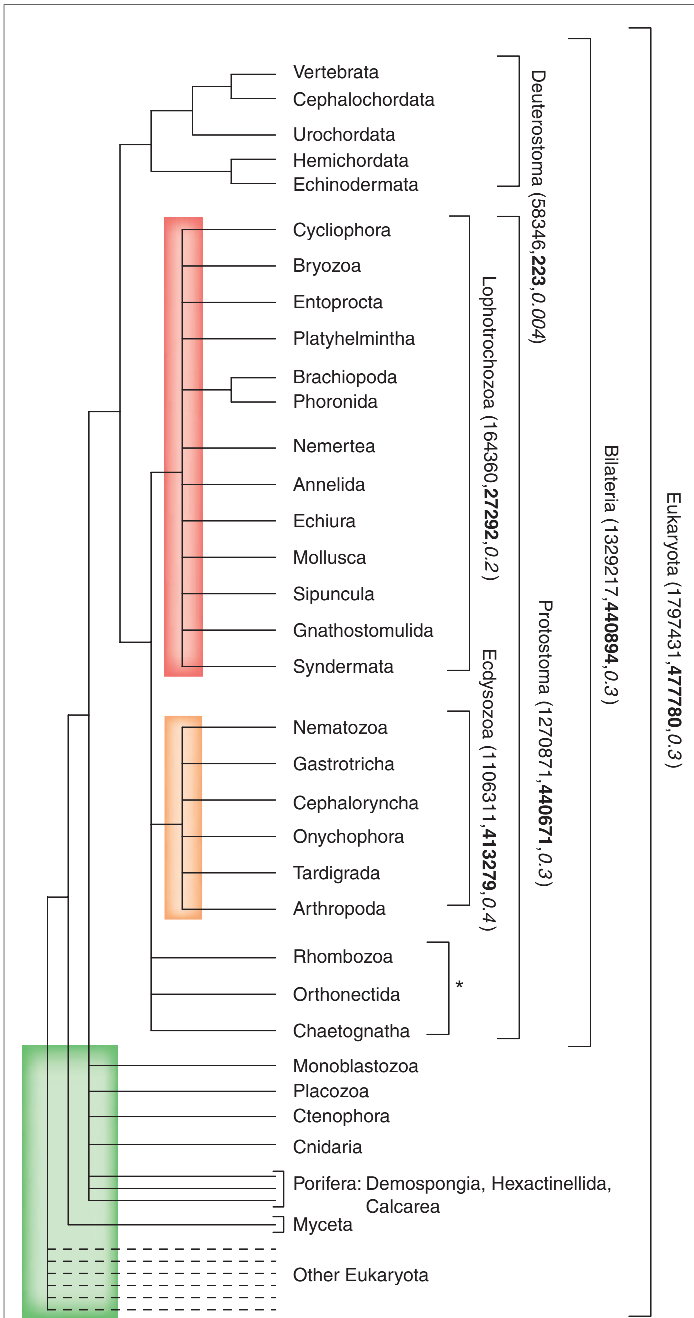

Fig. 1.

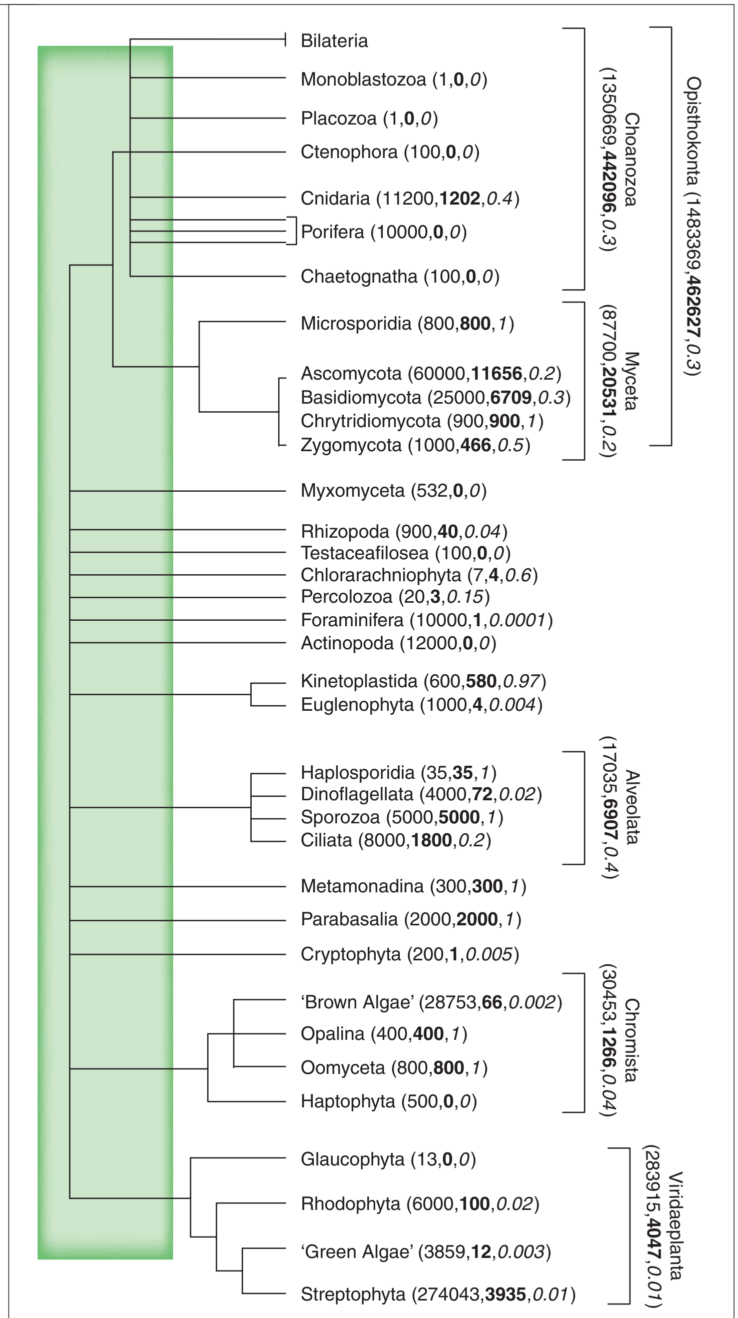

Fig. 2.

Fig. 1. Phylogenetic relationships of parasites within Eukaryota. Coloured boxes correspond to the following groups: green, all Eukaryota except Bilateria; orange, Ecdysozoa; red, Lophotrochozoa. Numbers given in parentheses refer to the total number of species (plain text), the number of parasitic species (bold text) and the ratio of parasitic species (italic text). Within Deuterostoma, parasitic species are only represented by Vertebrata: Petromyzonidae (lampreys) (38 species) and fishes of the Carapidae (e.g. *Fierasfer*) (30 species) and Trichomycteridae (e.g. *Vandelia*) (155 species). *Desmodus rotundus* (the vampire bat) could also be included. The asterisk indicates the Chaetognatha, which are a small group of 100 species with no parasites; Rhombozoa (75 species) and Orthonectida (25 species), all parasitic, were previously gathered within the clade Mesozoa. See Box 1 for further information on nomenclature.

Fig. 2. Phylogenetic relationships of parasites within the non-bilaterian Eukaryota, indicated by the green box. Numbers given in parentheses refer to the total number of species (plain text), the number of parasitic species (bold text) and the ratio of parasitic species (italic text). See Box 1 for further information on nomenclature.

new phylum Cycliophora, where the single species *Symbion pandora* was recently described from *Nephrops norvegicus* (Norway lobster) [23]. On the basis of host specificity, this taxon may contain as many species as available host species (say Malacostraca, i.e. 22 671 species [13]). Monogenea species are known for their strict host specificity, but only 5000 species have been described from >20 000 host species (actinopterygian fishes and anouran amphibians) [13]. If the same argument applies to gregarines (around 2000 named species) and their hosts (Arthropoda), the number of parasites would increase by more than one million.

Within the eukaryotes, the majority of parasitic species are encountered in higher taxa (i.e. at the top of the tree), especially the plant parasitic insects, which represent 64% of named parasite species in eukaryotes. This highlights how biased our knowledge on biodiversity may be towards

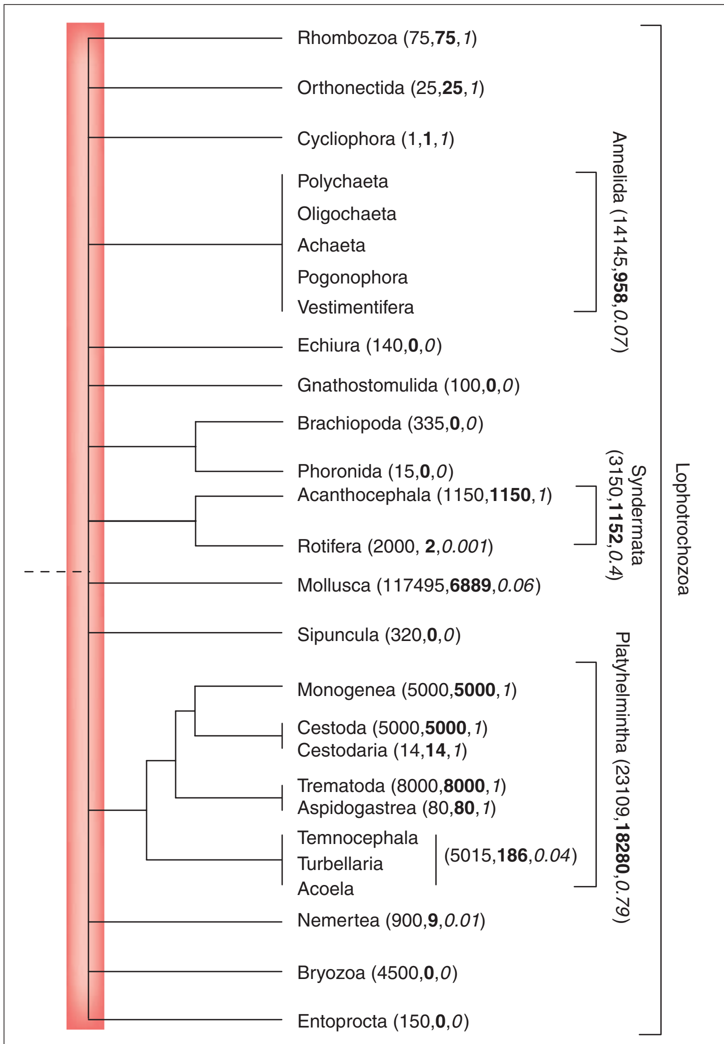

Fig. 3.

TRENDS in Parasitology

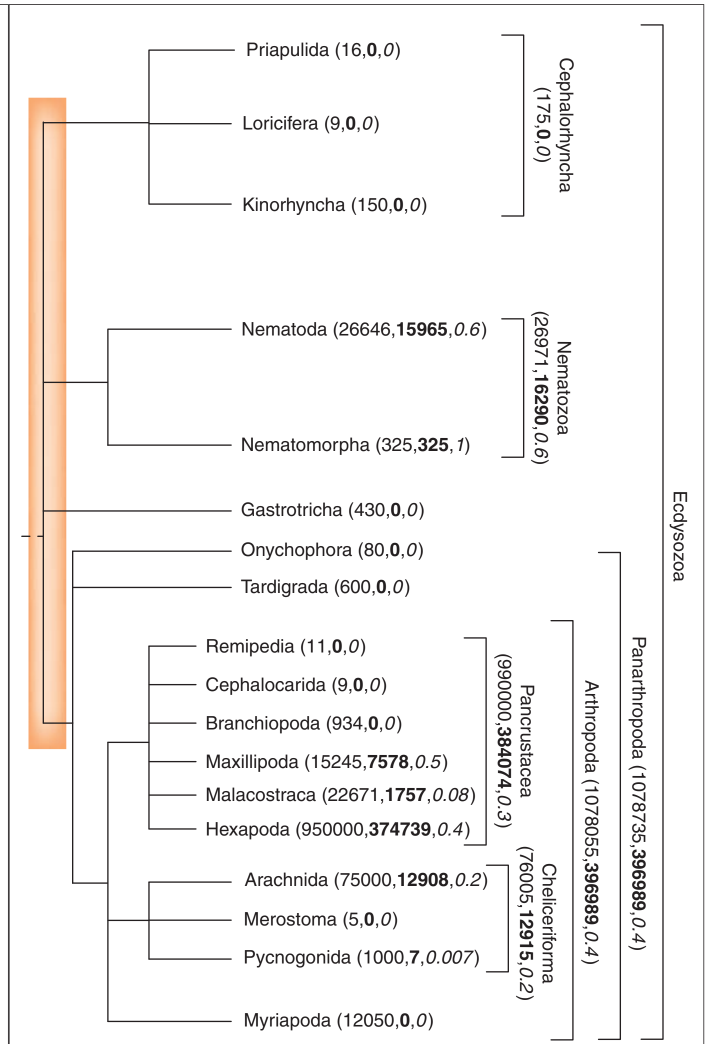

Fig. 4.

TRENDS in Parasitology

Fig. 3. Phylogenetic relationships of parasites within Lophotrochozoa, indicated by the red box. Numbers given in parentheses refer to the total number of species (plain text), the number of parasitic species (bold text) and the ratio of parasitic species (italic text). See Box 1 for further information on nomenclature.

Fig. 4. Phylogenetic relationships of parasites within Ecdysozoa, indicated by the orange box. Numbers given in parentheses refer to the total number of species (plain text), the number of parasitic species (bold text) and the ratio of parasitic species (italic text). See Box 1 for further information on nomenclature.

economically relevant species, at the expense of ecologically and even medically or veterinary relevant species. Our perception of biodiversity should thus be reconsidered. To date, the picture we have on life diversity is clearly not correct.

Parasitology, from this new perspective of eukaryote phylogeny, now encompasses a much wider diversity of organisms than was classically included in the disciplines of zoology (Protozoa, Metazoa) and mycology. This is obvious when considering Euglenozoa and Alveolata, which group together 'protophytes' and 'protozoans'. Other examples include Oomyceta (i.e. *Saprolegna*) and Opalina, now placed with brown algae, and Microsporidia classified in the Myceta.

To illustrate the changes in phylogenetic perspectives, it is worth insisting on groups of major concern in human health. The Kinetoplastida and the Sporozoa both display important affinities with groups that were previously classified as algae; the Aschelmintha clade (i.e. Nematoda, Nematomorpha, Acanthocephala) is no longer valid and spectacularly splits into deeply divergent phyla. Such considerations open new perspectives for a better understanding of the biology of these medically relevant parasites. As already written [36], parasitologists 'can use this information to look for free living relatives of important parasites that may be difficult to culture, or for ways to combat pests'. Indeed, patterns are essential to understand processes.

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Is *Toxoplasma* egress the first step in invasion?

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The protozoan parasite *Toxoplasma gondii* maintains an intracellular lifestyle that requires careful timing and coordination when exiting one cell (egress) and entering another (invasion). Here it is argued that *T. gondii* uses similar molecular mechanisms for egress and invasion, based on common morphology, dependence on motility, and regulation by a calcium-dependent signal transduction pathway. In our view, this strategy is highly advantageous because it allows the parasite to egress rapidly from one cell and immediately invade an adjacent cell, thereby minimizing exposure to the extracellular environment where it could be destroyed by host immune mediators.

Obligate intracellular parasites are unwelcome guests in eukaryotic cells, often killing these hosts in the course of completing their life cycles. *Toxoplasma gondii* is a protozoan parasite that infects virtually all nucleated cells in its many vertebrate hosts. In humans, it can cause serious disease both in neonates (e.g. blindness, mental retardation) and in immunocompromised individuals (e.g. encephalitis). *Toxoplasma gondii* must enter a host cell in order to replicate (Fig. 1 and see http://archive.bmn.com/supp/part/ani1.html). During invasion, the parasite sets up a protective non-fusogenic vacuole in the cytoplasm [1–4], in which it replicates asexually by endodyogeny to produce 2^n parasites per parasitophorous vacuole (PV). When the fully developed parasites exit (egress), they invade a fresh batch of neighboring cells and the initial host cell is left to die. This necrotic cell death directly or indirectly (through the ensuing inflammatory response) leads to the observed pathology in especially vulnerable target tissues such as the brain.

Invasion and egress are both rapid events (<60 seconds) that are crucial to *T. gondii* survival. In examining these processes, the important questions have been: (1) how does the parasite cross the host plasma membrane, in one case (invasion) forming a PV and in the other case (egress) not?; (2) how does the parasite respond to environmental cues to initiate invasion or egress? and (3) what are the cues themselves? Several recent studies support the notion that the parasite uses the same or similar molecular mechanisms for invasion and egress.

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