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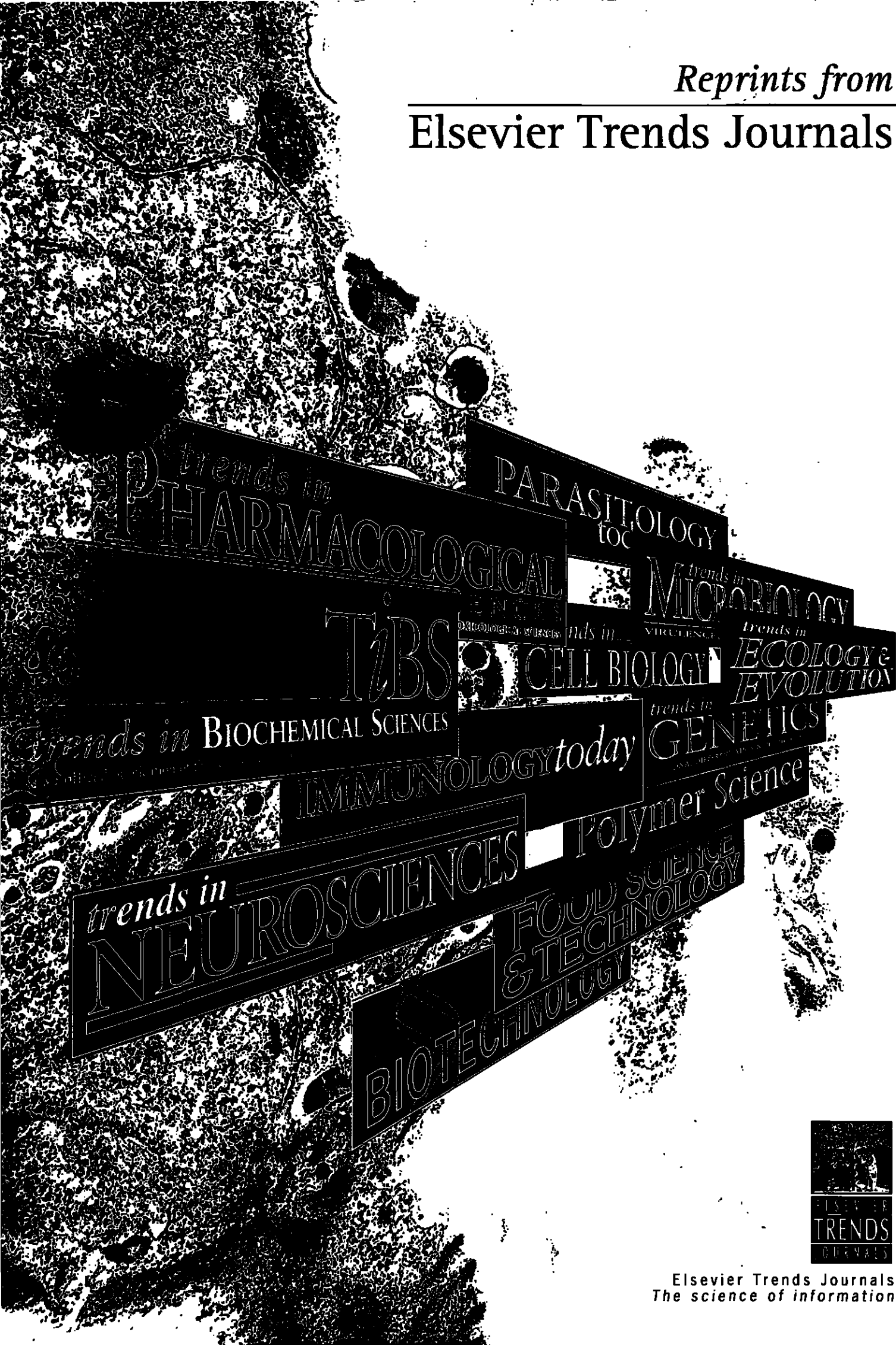


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# Evolution of Parasitism in Kinetoplastid Protozoa

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*Molecular phylogeny has provided a new insight on the almost century-old discussion on the origin of parasitism in kinetoplastid protozoa. Phylogenetic trees constructed on the basis of ribosomal RNA sequences show that digenetic parasites (which alternate between insect vector and vertebrate host) did not descend from the same common ancestor. Lineages of* Trypanosoma *appeared early in evolution and descended directly from an ancestral trypanosomatid, while lineages of* Leishmania *and* Endotrypanum *separated late from monogenetic parasites. Here, Dmitri Maslov and Larry Simpson discuss how these new results have changed our view of the evolution of parasitism.*

The kinetoplastid protozoa provide a unique opportunity for studying the evolution of parasitism. This order of protozoa is defined by the presence of the kinetoplast, an organelle which represents a specialized compartment of the single mitochondrion, which contains a large mass of DNA[1]. Together with the sister group of euglenoids, the kinetoplastids represent the earliest extant group of eukaryotic organisms containing mitochondria[2]. During their long evolutionary history, the kinetoplastids have developed an impressive variety of life styles and adaptations to parasitism. Two suborders are distinguished within this group: biflagellated Bodonina and uniflagellated Trypanosomatina[3]. Bodonids include free-living and commensalic organisms (*Bodo*), ectoparasites (*Ichtyobodo*) and endoparasites of the alimentary tract and reproductive organs of fish and of some invertebrates (*Cryptobia*), as well as some fish blood parasites (*Trypanoplasma*). Trypanosomatids are all obligate parasites. Many trypanosomatids are monogenetic. They are found in a single host from such widely diverse groups of invertebrates as ciliates, rotifers, nematodes, molluscs, annelids and arachnids, but are found predominantly in insects (Ref. 4 and references therein). Among the latter group, 35% occur in hemipteran hosts and 55% in dipteran hosts[5]. Digenetic life cycles occur in species from the genera *Trypanosoma*, *Leishmania* and *Endotrypanum*, and include both an insect vector and a vertebrate host. All classes of vertebrates are known to be parasitized by trypanosomes. A unique type of digeneity that involves insect and plant hosts occurs in *Phytomonas*.

## Old Hypotheses

Two major hypotheses have been proposed for the origin and evolution of parasitism in kinetoplastids[4]. Both theories agree that parasitic trypanosomatids originated from free-living bodo-like flagellates, but disagree on the nature of the primary host. The 'invertebrate first' point of view stems from Leger[6]. It was further developed by Hoare[7] and by Baker[8]. This hypothesis assumes that parasitism by bodo-like flagellates was first established in the alimentary tract of primitive Precambrian invertebrates. Evolution and speciation of the host invertebrates yielded the present-day wide distribution of kinetoplastids among many types of invertebrates. According to this theory, both insects and leeches inherited their parasites from their common annelid-like ancestors. For an unknown reason, intensive diversification of trypanosomatids took place in insects and produced the 'promastigote stock' (with the anterior position of the flagellum-kinetoplast) and the 'epimastigote stock' (with the posterior position of the flagellum-kinetoplast and the undulating membrane), the epimastigotes perhaps being more adapted for life in a viscous environment. Following the acquisition of haematophagy of vertebrates by some hemipterans and dipterans, digenetic life cycles were developed by their kinetoplastid parasites. *Leishmania* evolved from promastigote leptomonads, while *Trypanosoma* evolved independently from epimastigote *Blastocrithidia*-like organisms. The leech-transmitted trypanosomes of aquatic vertebrates would have evolved directly from parasites of ancestral leeches, for which case a modified scenario was also suggested in which trypanosomes were inoculated into amphibians by mosquitoes. The 'invertebrate first' hypothesis predicts that the monogenetic parasites of invertebrates would form the earliest diverging branches of the phylogenetic tree, while the digenetic parasites would be among the latest evolving lineages.

An alternative 'vertebrate first' hypothesis was pioneered by Minchin[9] and was later supported by Wallace[5]. It holds that monogenetic parasitism was first established in the gut of vertebrates, from where parasites gained entry into the blood system. This hypothesis was based on the presence of modern leptomonads in intestine and peripheral blood of some amphibians and reptiles. After the evolutionary appearance of leeches and hematophagous arthropods,

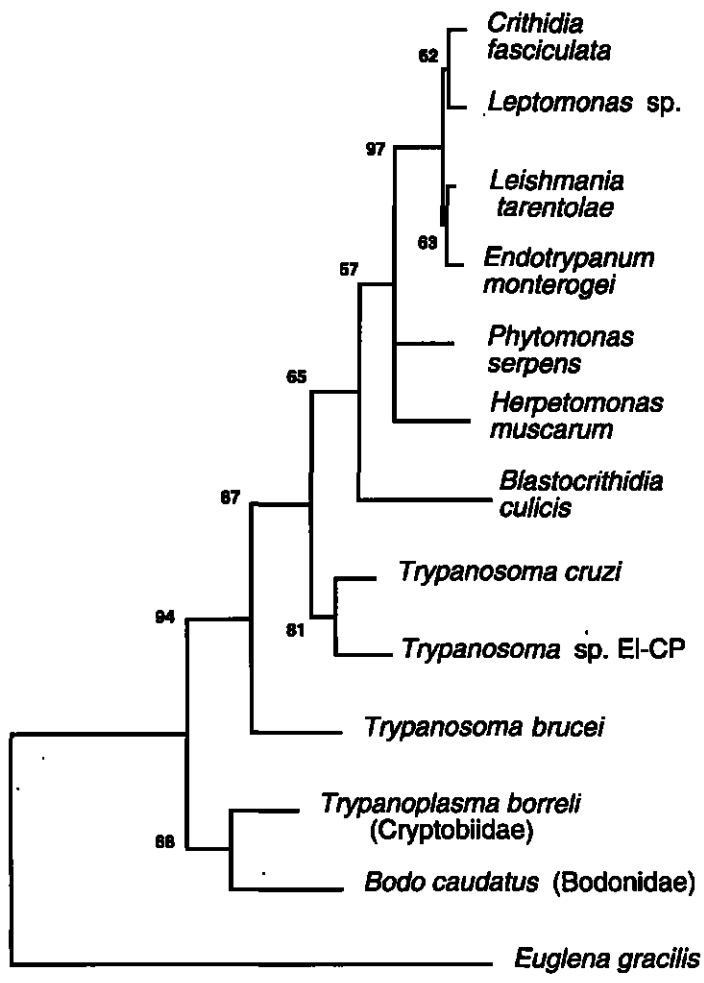


**Fig. 1. Majority consensus parsimony tree constructed from nuclear 18S rRNA of kinetoplastid protozoa. The maximum parsimony algorithm[19] was used to infer the phylogeny, and bootstrap analysis[20] (resampling the data by drawing with replacement) was used to evaluate statistically the level of support for a particular tree topology. A consensus tree was generated from 200 replicates. For each clade, the frequency of its occurrence in a bootstrapped pool is indicated on the consensus tree. The topology is considered strongly supported, if a clade appears in more than 95% of the bootstrapped trees. In the tree presented here, the topology in which *T. brucei* forms the most deeply diverging lineage is well supported. However, the branching order for the *Blastocrithidia*, *Herpetomonas* and *Phytomonas* lineages is supported by fewer sites in the alignment, with the two latter lineages being unresolved on the consensus tree (any of the alternative topologies occurred in less than 50% of cases). The late divergence and monophyly of *Crithidia*, *Leptomonas*, *Leishmania* and *Endotrypanum* is strongly supported, although there is little support for the indicated branching order within this clade. The maximum-likelihood algorithm[21,22] generated a tree with similar topology. However, *Blastocrithidia*, *Herpetomonas* and *Phytomonas* formed a monophyletic clade, representing a sister group to the *Crithidia–Endotrypanum* clade. The sequences included in this analysis are from Refs 11–15. The *Phytomonas serpens* 18S rRNA sequence is from D.A. Maslov and L. Simpson, unpublished.**

blood-to-blood transmission of parasites became possible and gut infections became rare. Monogenetic parasites of invertebrates originated from digenetic forms by the loss of infectivity to vertebrates. This theory explains why most invertebrates that harbor the trypanosomatids belong to groups with blood-sucking habits. It also finds some support in the series of gradual adaptations to parasitism in bodonids discussed above. This theory predicts that digenetic trypanosomes and leishmanias would be ancestral to insect trypanosomatids on a phylogenetic tree. Wallace also pointed out the possible significance of the kinetoplast for allowing the rapid metabolic switches which were required for adaptation of the parasites to a new environment.

## New Results

Since both hypotheses are supported by reasonable arguments, this century-long dispute persists. In the absence of a protozoan fossil record, only the methods of molecular phylogenetic reconstruction can provide an evolutionary framework which can be used to verify and negate the various hypotheses. Several years ago, an unrooted mitochondrial ribosomal RNA tree was constructed[10]. In the absence of an outgroup sequence, this tree was rooted at the monogenetic trypanosomatid lineage. Since digenetic trypanosomes and leishmanias formed a recently evolving monophyletic group, the authors concluded that it is fully consistent with Leger's hypothesis. Recently, however, a more complex picture has emerged from results obtained in several laboratories. Phylogenetic trees were obtained based on the sequences of nuclear small- and large-subunit ribosomal RNAs[11–15]. Rooting of the tree was accomplished using the *Euglena gracilis* rRNA sequence as an outgroup. Although this rooting may reflect unequal rate effects caused by the large divergence of the *Euglena* sequence, it is nevertheless supported by morphological and ultrastructural data[16]. A synthetic tree based on published and unpublished reconstructions is shown in Fig. 1. Consistent with the major subdivision of the two suborders, the earliest divergence event involved the separation of Bodonina from Trypanosomatina. The Salivarian trypanosome, *T. brucei*, and Stercorarian trypanosomes, *T. cruzi* and *Trypanosoma* sp. El-CP (a fish trypanosome), form the two earliest separating lineages of the trypanosomatids. These are followed by the separation of the lineages of the monogenetic insect parasites, *Blastocrithidia* and *Herpetomonas*, the digenetic *Phytomonas*, and the branches containing the monogenetic *Crithidia–Leptomonas* and the digenetic *Leishmania–Endotrypanum*. The branching orders of the *Blastocrithidia–Herpetomonas–Phytomonas* lineages are not reliably resolved by any reconstruction. The finding that trypanosomes form a paraphyletic assembly was unexpected and may eventually lead to redefining the trypanosomatid genera[11].

What can this tree tell us about the evolution of parasitism in the kinetoplastids? Surprisingly, this tree provides some support for both hypotheses. The finding that digenetic organisms are interspersed with monogenetic ones may imply that this character evolved more than once in accordance with Leger's line of reasoning. However, all 'primitive' insect trypanosomatids included in these studies evolved late, which is consistent with the 'vertebrate early' point of view. There seems to be no separate 'promastigote' or 'epimastigote' stocks, since the former (represented

by *Herpetomonas*, *Phytomonas*, *Leptomonas* and *Leishmania*) emerge from the lineage of epimastigote cells.

The depth of the branches suggests that divergence within the kinetoplastids is very ancient. The estimated divergence between a free-living *Bodo* and a parasitic *T. brucei* is only slightly less than divergence of vertebrates and invertebrates[13]. This means that, if the rate of evolutionary sequence change was not substantially different from what is seen in other eukaryotes, the trypanosomatid lineage appeared on the evolutionary scene around the time of the vertebrate appearance in the Ordovician, approximately 500 million years ago. This time for the origin of trypanosomatids opens up a formal possibility for the Minchin–Wallace scenario. Similar estimates show that the Stercorarian trypanosomes and the monogenetic parasites of insects might have separated before reptiles separated from amphibians in the Carboniferous, approximately 340 million years ago. The extent of divergence between the Old World and the New World *Leishmania* corresponds to the divergence between the mammalian orders that occurred approximately 85 million years ago, due to the separation of Africa and South America[13,17].

The distribution of digenetic and monogenetic organisms on the phylogenetic tree prompted Landweber and Gilbert[14] to conclude that the ancestral trypanosomatid was digenetic. They suggested that digeneity was lost after the separation of Stercorarian trypanosomes and was re-acquired in an ancestor of *Leishmania* and *Endotrypanum*. However, while a vertebrate host might have existed at that time, the invertebrate vectors did not. The first authentic Diptera are known from the Triassic–Jurassic boundary[18], about 210 million years ago, while the blood-sucking predecessors of *Glossina* flies first evolved from non-hematophagous flies only in the Cretaceous[18], which began 140 million years ago. Similarly, hemipteran bugs appeared in the early Triassic but hematophagy was developed much later. This indicates that acquisition of digeneity in *T. brucei* and *T. cruzi* must have occurred long after the separation of these lineages. Authentic fossils of psychodids (the dipteran group that includes mosquitoes) are known only from Tertiary amber, suggesting that the group appeared first in the Cretaceous[18]. This is consistent with a late and independent acquisition of digeneity in the *Leishmania* branch.

This sort of reasoning caused Fernandes *et al.*[13] to conclude, following Leger's original hypothesis, that digeneity evolved several times independently from monogeneity in invertebrates or even from the free-living state. This conclusion may be further supported by the striking dissimilarities in strategies that digenetic parasites presently utilize to avoid host defense mechanisms. While the bloodstream form of *T. brucei* uses a variable surface glycoprotein coat to avoid destruction by the host immune system, *T. cruzi* proliferates within the cytosol of host muscle cells and the levels of blood infection are usually low, and *Leishmania* reside within remodeled phagolysosomes of host macrophages.

## Perspectives

Since digeneity is also a derived character in the Minchin–Wallace hypothesis, a key to the final solution of the origin of parasitism in these cells has not yet been found. Such a key may lie in studying the organisms that were not yet included in this phylogenetic analysis, such as the trypanosomes of fish, amphibians and reptiles. If parasitism was first established in vertebrates, one would expect that these would form the most ancient lineages. However, the position of the only fish trypanosome yet studied[15] is more consistent with a secondary invasion of this niche rather than with a direct descent from ancient parasites. Another important group of organisms that should be studied includes the leptomonads which are found in the numerous phyla of invertebrates other than arthropods. Those are expected to form the most ancient lineages if parasitism was first established in early invertebrates and the parasites were later inherited by insects from which digeneity arose.

In conclusion, recent phylogenetic reconstructions of the various kinetoplastid lineages based on alignments of rRNA sequences have provided a firm foundation for eventual understanding of such important events as the origin of parasitism and digenetic life cycles.

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