Phylogeny of the Kinetoplastida: Taxonomic Problems and Insights into the Evolution of Parasitism

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To further investigate phylogeny of kinetoplastid protozoa, the sequences of small subunit (18S) ribosomal RNA of nine bodonid isolates and ten isolates of insect trypanosomatids have been determined. The root of the kinetoplastid tree was attached to the branch of Bodo designis *and/or* Cruzella marina. *The suborder Trypanosomatina appeared as a monophyletic group, while the suborder Bodonina was paraphyletic. Among bodonid lineages, parasitic organisms were intermingled with free-living ones, implying multiple transitions to parasitism and supporting the 'vertebrate-first hypothesis'. The tree indicated that the genera* Cryptobia *and* Bodo *are artificial taxa. Separation of fish cryptobias and* Trypanoplasma borreli *as different genera was not supported. In trypanosomatids, the genera* Leptomonas *and* Blastocrithidia *were polyphyletic, similar to the genera* Herpetomonas *and* Crithidia *and in contrast to the monophyletic genera* Trypanosoma *and* Phytomonas. *This analysis has shown that the morphological classification of kinetoplastids does not in general reflect their genetic affinities and needs a revision.*

Key words: Kinetoplastida - Bodonina - Trypanosomatina - parasitism - phylogeny

Kinetoplastids are a group of protozoa characterized by the presence of a characteristic organelle, the kinetoplast (Vickerman 1976). This structure was observed by early researchers in light microscope as basophilic granules located near the basal body of a flagellum. This association was thought to meaningful, and the term 'kinetoplast' was coined by Alexeieff in 1917 (Alexeieff 1917, Vickerman & Preston 1976). Only in the 60s, after the first electron microscopic studies of this organelle conducted by Vickerman and Steinert, had it become clear that the kinetoplast represents a portion of a single branched mitochondrion of the cell containing a large mass of mitochondrial DNA. The presence of the kinetoplast is easy to determine by fluorescence with a DNA binding dye DAPI or by Giemsa staining.

This group of organisms is usually assigned the rank of an order – Kinetoplastida, which, together with two other groups: Euglenida and Diplonemida – belongs to the phylum Euglenozoa (Cavalier-Smith 1993, Corliss 1994). This assemblage has been confirmed by a number of works, mainly based on the ribosomal SSU RNA phylogenetic analysis, which showed Euglenozoa as a monophyletic group, well separated from the eukaryotic crown (Sogin & Silberman 1998, Maslov et al. 1999; see Philippe & Adoute 1998 for a review of protein based phylogenies).

The existing taxonomy of kinetoplastids is based on morphology and life cycles. Traditionally the group is subdivided into two suborders: Bodonina and Trypanosomatina (Vickerman 1976, Lom 1976). The first one includes two families: Bodonidae and Cryptobiidae, members of which have a relatively large kinetoplast and two flagella: anterior (always remains free) and recurrent (free in bodonids and attached in cryptobiids). Bodonids show a variety of life styles, ranging from free living, such as *Bodo, Parabodo, Dimastigella, Rhynchobodo, Cruzella*, to parasitic. The latter are represented by ectoparasites of the fish skin and gills such as *Ichtyobodo*, endoparasites of the reproductive tract of snails (some *Cryptobia* species) and gastro-intestinal tract of fish (some other *Cryptobia*) and also by digenetic parasites of fish blood (*Trypanoplasma*) transmitted by leeches. The second group of kinetoplastids, the trypano-

somatids, forms a single family, Trypanosomatidae, which includes obligatory parasitic organisms with a single flagellum and a small kinetoplast. Trypanosomatids parasitize all classes of vertebrates, as well as some invertebrates, preferentially insects from the orders Diptera and Hemiptera, and also plants. The invertebrates can either represent hosts, as in monogenetic parasites, such as *Crithidia, Leptomonas, Herpetomonas* and *Blastocrithidia*, or serve as vectors, as in digenetic genera *Trypanosoma, Leishmania* and in bug-transmitted parasites of plants, *Phytomonas*.

The system of the trypanosomatid genus assignment was largely developed by Hoare and Wallace (1966) on the basis of the observed morphotypes (Molyneux & Ashford 1983). The morphotypes are defined by the relative position of the nucleus and the kinetoplast and the overall body shape. At least two morphotypes are observed in each genus, depending on a life cycle stage and physiological conditions of the cells. Some morphotypes are unique to a certain genus, while others, such as promastigotes, are shared by many genera. This system has been criticized in the past as potentially misleading.

A novel genus, *Wallaceina* has recently been proposed to include monogenetic insect parasites with an 'endomastigote' morphology, characterized by a long curved flagellar pocket (Podlipaev et al. 1990).

With the progress of molecular biological studies of kinetoplastids it has become increasingly clear that existing taxonomy does not reflect the true genetic affinities in these organisms, calling for the application of molecular phylogenetic methods (McGhee & Cosgrove 1980, Camargo et al. 1982, Wallace et al. 1983, Kolesnikov et al. 1990, Podlipaev et al. 1991, Dollet 1994, Vickerman 1994, Podlipaev & Lobanov 1996, Hollar et al. 1998, Wright et al. 1999). The phylogenetic tree can also be used as a framework to study the origin and evolution of parasitism and a number of unique molecular and biochemical mechanisms seen in this group, such as the kinetoplast DNA networks, uridylate-insertional/deletional type of RNA editing, tRNA importation in the mitochondrion, antigenic variation and the others (recently reviewed in Donelson et al. 1999, Simpson et al. 2000).

In the first phylogenetic reconstructions, a relatively small number of available trypanosomatid and only two bodonid SSU sequences were employed (Fernandes et al. 1993, Du et al. 1994, Landweber & Gilbert 1994, Maslov et al. 1994, 1996, Maslov & Simpson 1995). The root of the tree was established using *Euglena gracilis* as the outgroup and was found to be attached in bodonids.

The trypanosomatids formed a so-called paraphyletic tree, according to the topology of trypanosomes. The lineage of *Trypanosoma brucei* and other trypanosomes were not monophyletic, with *T. brucei* representing the earliest divergence in the family. Other trypanosomes diverged later as a sister-clade to all non-trypanosome genera. The paraphyletic tree was conflicting with the protein-derived trees which all showed trypanosomes as a monophyletic assembly. However, in 1997 it was shown that the paraphyletic topology was a tree reconstruction artefact caused by unequal rate effects (Lukeš et al. 1997). Due to a high rate of sequence divergence in *T. brucei* and the outgroup, the level of accidental similarities (homoplasies) became very high resulting in a placement of these lineages together on a tree. After subdividing the lineages of *T. brucei* and outgroups by addition of the new trypanosome and bodonid species, the support for paraphyly of trypanosomes disappeared and support for their monophyly became very strong. This result was later confirmed by other works which utilized a larger number of trypanosome sequences (Haag et al. 1998, Stevens et al. 1999).

The updated trypanosomatid tree published in 1998 was composed of five major clades (Hollar et al. 1998). Only two of them, the clade of *Trypanosoma* and the clade of *Phytomonas,* represented examples of consistency between phylogeny and taxonomy. The genera *Herpetomonas* and *Crithidia* were polyphyletic: the endosymbiont-containing members of these genera formed a separate monophyletic clade, while the endosymbiont-free members were found elsewhere on the tree.

The question concerning the status of remaining trypanosomatid genera has been addressed in a more recent work (Merzlyak et al. 2001). The emphasis has been made on isolates from insects identified as *Leptomonas, Blastocrithidia* and *Wallaceina* according to their morphology in insect host. In this reconstruction, as in the previous work, the root of the tree was attached between the clade of trypanosomes and the clade of all non-trypanosomes (Fig. 1). Interestingly, among the latter, *Blastocrithidia triatoma*, was the earliest diverging lineage. Additional analysis has shown that the tree topologically constrained for an earlier divergence of *B. triatoma* is not significantly different from the best unconstrained tree. This indicates that a blastocrithidia-like organism might have been a progenitor of all trypanosomatids, including trypanosomes. This finding is intriguing, because earlier Hoar and Baker hypothesized that such an organism, which they called an "epimastigote" stock, was an ancestral form to most trypanosomes (Baker 1963, Hoare 1972).

The tree showed that the genus *Blastocrithidia* is polyphyletic. Two *bona fide* blastocrithidias, *B. culicis* and *B. triatoma*, were unrelated. The situation with *B. gerricola* is not clear – this isolate was represented by epimastigotes in the host while only pro- and choanomastigote were observed in culture. It is possible that a mixed infection occurred in the original host. The tree also clearly showed that the genus *Leptomonas* is polyphyletic as well. This finding was not completely unexpected, taken into account a large extent of genetic variability among the *Leptomonas* isolates (Camargo et al. 1992) and the lack of truly distinguishing characters for this genus.

With at least four genera of trypanosomatids being polyphyletic (*Crithidia, Herpetomonas, Leptomonas* and *Blastocrithidia*), it has become obvious that the morphological taxonomy of trypanosomatids does not reflect their genetic affinities and should be revised in the future. However, there
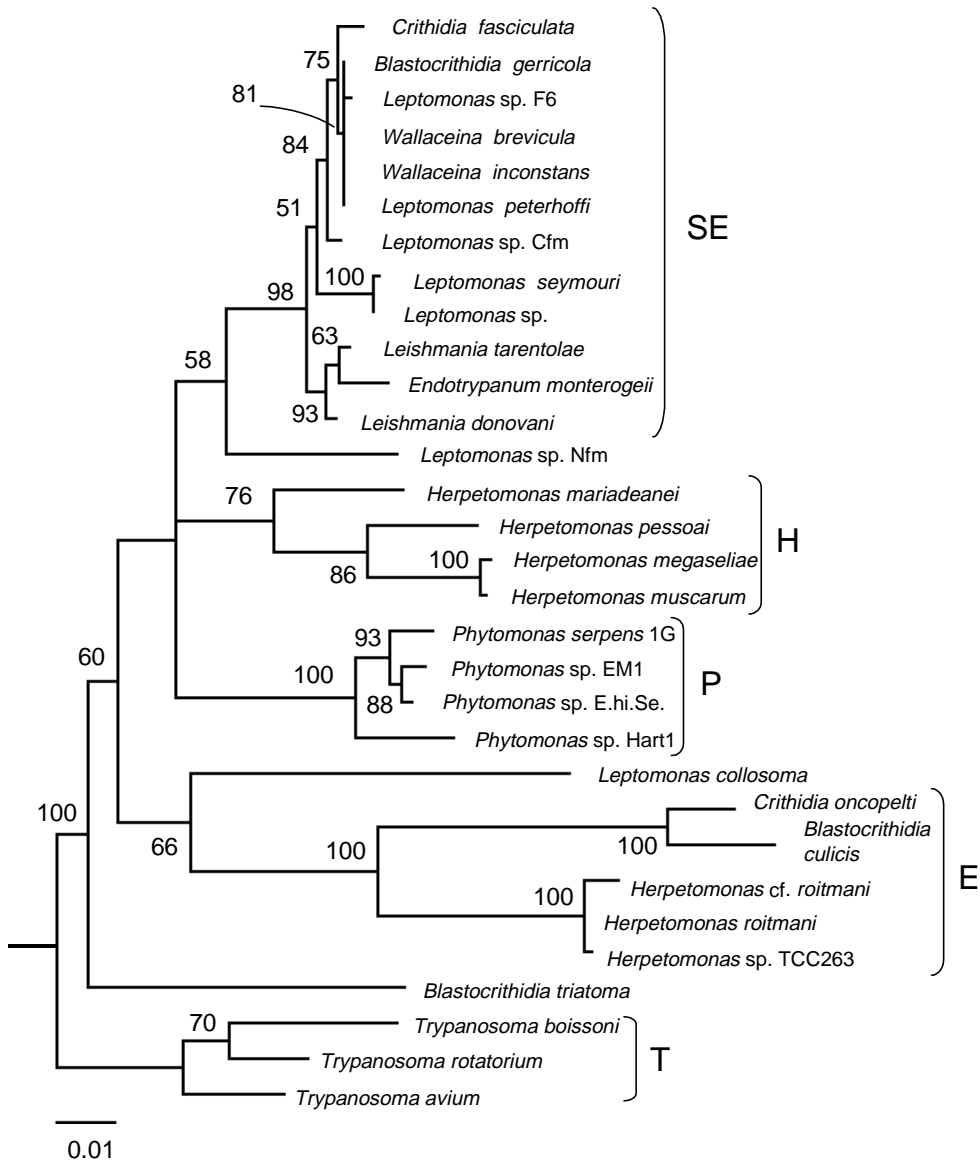

Fig. 1: the maximum likelihood consensus tree of insect trypanosomatids inferred from the small subunit ribosomal RNA sequences (Merzlyak et al. 2001). T- trypanosomes, E - endosymbiont-containing trypanosomatids, P - *Phytomonas* spp., H - endosymbiont-free *Herpetomonas* spp., SE - trypanosomatids with slowly-evolving SSU rRNA sequences. From Merzlyak et al. (2001) with permission

is no need to rush. It is clear even from this relatively modest expansion of the number of analyzed isolates that the natural diversity of trypanosomatid is much greater than currently expected. Only after completion of a more satisfactory survey of this group, will it be possible to propose a comprehensive taxonomic system for this group.

The phylogeny of bodonids was investigated in another recent work, which included nine additional bodonid species (Dole•el et al. 2000). The root of the tree (Fig. 2) was determined to be attached to the lineages of free-living organisms *Cruzella marina* and *Bodo designis*, thus making bodonids a paraphyletic assembly in contrast to trypanosomatids which separated from bodonids relatively late as a monophyletic group.

As in trypanosomatids, the tree indicated the conflict between taxonomy and phylogeny. It is best exemplified by the polyphyly of the genus *Bodo*, the members of which are found in the different parts of the tree. Another discrepancy is that the subdivision of the suborder Bodonina onto two families, Bodonidae and Cryptobiidae (Vickerman 1976, 1978), as both members mingle with each

other on the tree. A separation of the fish parasites onto the genera *Cryptobia* and *Trypanoplasma*, supported by some researchers (Lom 1976, Becker 1977, Brugerolle et al. 1979), and challenged by the others (Bower & Margolis 1983, Woo 1994), is also not supported.

An interesting feature of the bodonid tree is that free-living species often mingle with parasitic ones. In general, such topology suggests occurrence of the multiple transitions to parasitic life style in the kinetoplastids. Moreover, it seems that this process still continues and different steps of it can be observed in the existing clades. The examples of *C. marina* and *D. trypaniformis*, for which both endocommensal and free-living strains have been described, may reflect the very early stages of transition towards endoparasitism. A next step in this direction is illustrated by the endoparasitic *C. helicis* which is likely to have evolved from a free-living bodonid such as related species *B. caudatus, P. nitrophilus* and *B. sorokini*. An independent transition to parasitism occurred in the lineage of *T. borreli* – fish *Cryptobia* spp. In this case, the evolution reached the stage of transition from intesti-
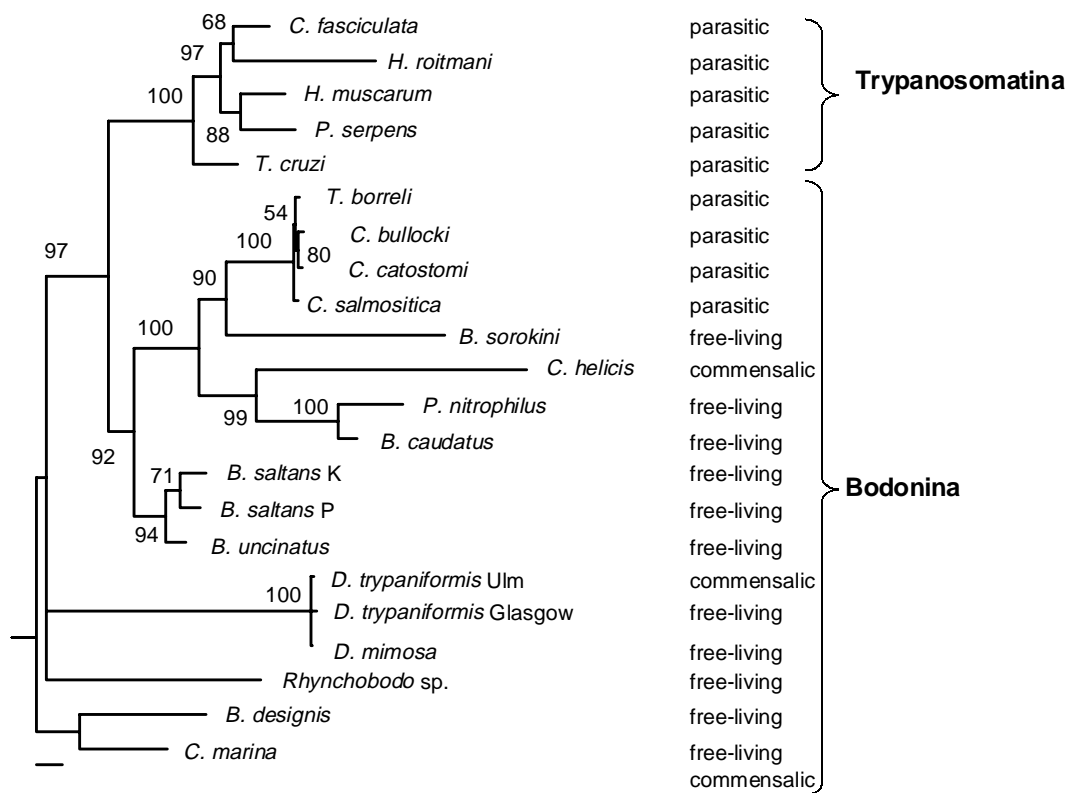

Fig. 2: majority consensus maximum likelihood ribosomal RNA tree of bodonids (Dole•el et al. 2000). Indication of the lifestyle and the hosts is shown on the right. From Dole•el et al. (2000) with permission

nal parasites to hemoparasites, as this compact group contains both types of organisms. The fact that none of the bodonid groups illustrates the entire evolutionary process should not be surprising. The intermediate stages might have become extinct, in addition the present survey of extant kinetoplastids is incomplete.

Thus the tree topology provides a support to the 'vertebrate first' hypothesis of Minchin (1908) who postulated that the hemoparasites of vertebrates evolved from their endoparasites, which in turn were derived from the free-living species. The results also indicate an independent origin and evolution of parasitism in trypanosomatids. If the evolution in this case had also followed Minchin's scenario, then organisms representing the intermediate stages, such as endoparasitic trypanosomatids of vertebrates, might remain unknown or have become extinct. Alternatively the evolution here might have followed the 'invertebrate first' scenario of Leger (1904) who outlined that original parasites were those infecting the gut of invertebrates (insects) while hemoparasites of vertebrates evolved later.

Finding a monogenetic trypanosomatid, such as *B. triatoma*, branching off early would support the 'invertebrate first' hypothesis for this case. Therefore, the century-old conflict between the 'vertebrate-first' and 'invertebrate-first' hypotheses might end in a tie, with different routes towards parasitism taken in different evolutionary lineages.

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