J Mol Evol (1997) 44:521–527

JOURNAL OF MOLECULAR EVOLUTION

Analysis of Ribosomal RNA Genes Suggests That Trypanosomes Are Monophyletic

Julius Lukeš,[1,2] Milan Jirků,[1,2] David Doležel,[2] Ivica Kral'ová,[1*] Laura Hollar,[3] Dmitri A. Maslov[3]

[1] Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budějovice, Czech Republic
[2] Department of Parasitology, Faculty of Biology, University of South Bohemia, 370 05 České Budějovice, Czech Republic
[3] Department of Biology, University of California, Riverside, 3401 Watkins Drive, CA 92521, USA

Received: 5 July 1996 / Accepted: 26 September 1996

Abstract. To further investigate the phylogeny of protozoa from the order Kinetoplastida we have sequenced the small subunit (SSU) and a portion of the large subunit (LSU) nuclear rRNA genes. The SSU and LSU sequences were determined from a lizard trypanosome, *Trypanosoma scelopori* and a bodonid, *Rhynchobodo* sp., and the LSU sequences were determined from an insect trypanosomatid, *Crithidia oncopelti*, and a bodonid, *Dimastigella trypaniformis.* Contrary to previous results, in which trypanosomes were found to be paraphyletic, with *Trypanosoma brucei* representing the earliest-diverging lineage, we have now found evidence for the monophyly of trypanosomes. Addition of new taxa which subdivide long branches (such as that of *T. brucei*) have helped to identify homoplasies responsible for the paraphyletic trees in previous studies. Although the monophyly of the trypanosome clade is supported in the bootstrap analyses for maximum likelihood at 97% and maximum parsimony at 92%, there is only a small difference in ln-likelihood value or tree length between the most optimal monophyletic tree and the best suboptimal paraphyletic tree. Within the trypanosomatid subtree, the clade of trypanosomes is a sister group to the monophyletic clade of the nontrypanosome genera. Different groups of trypanosomes group on the tree according to their mode of transmission. This suggests that the adaptation to invertebrate vectors plays a more important role in the trypanosome evolution than the adaptation to vertebrate hosts.

Key words: Kinetoplastida — rRNA — Phylogenetic tree — Monophyly — Homoplasy — *Trypanosoma* — *Rhynchobodo* — *Dimastigella* — *Crithidia oncopelti*

Introduction

Protozoa from the order Kinetoplastida are unique among the protozoa in having a kinetoplast, a DNA-containing compartment of their single mitochondrion (Vickerman and Preston 1976). RNA editing, an unusual form of the post-transcriptional RNA processing by insertions/deletions of uridylate residues, occurs in this organelle (for recent reviews see references Hajduk et al. 1993; Stuart 1993; Benne 1994; Simpson and Maslov 1994; Simpson and Thiemann 1995). Based on morphological differences, kinetoplastids are subdivided into the suborder Bodonina with two families, Bodonidae and Cryptobiidae, and the suborder Trypanosomatina with a single family Trypanosomatidae (Vickerman 1976). While bodonids/cryptobiids include free-living species, as well as ectocommensals and parasites, trypanosomatids are obligate parasites.

Molecular phylogenetic trees of kinetoplastid protozoa have been used to investigate the origin and evolution of parasitism and evolution of RNA editing (Lake et al. 1988; Fernandes et al. 1993; Maslov et al. 1994, 1996;

Permanent address: Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia
Correspondence to: D. Maslov; e-mail: dmitri.maslov@ucr.edu.

Landweber and Gilbert 1994). The initial tree of Trypanosomatidae was constructed using kinetoplast ribosomal 9S and 12S rRNA sequences (Lake et al. 1988). This tree was rooted at the lineage of a monogenetic (single host) insect parasite *Crithidia*, since no outgroup data was available at that time. On that tree, the digenetic (two hosts) parasites *Trypanosoma* and *Leishmania* represented the derived species. An evolutionary scenario deduced from this topology was compatible with the previously proposed notion that monogeneity in insects is a primitive state and digeneity in insects and mammals is a derived state (reviewed by Lainson and Shaw 1987).

Subsequently, nuclear small and large subunit (SSU and LSU, respectively) rRNA gene sequences were used to infer the phylogeny of kinetoplastids (Gomez et al. 1991; Fernandes et al. 1993; Landweber and Gilbert 1994; Maslov et al. 1994, 1996; Du et al. 1994). These trees were rooted with outgroup sequences from *Euglena gracilis;* a bodonid, *Bodo caudatus;* and a cryptobiid, *Trypanoplasma borreli.* Surprisingly, on these trees the separation of the lineage of an advanced mammalian parasite, *T. brucei*, represented the earliest divergence within Trypanosomatidae, followed by divergence of all the other trypanosomes and remaining genera of digenetic (*Leishmania, Endotrypanum, Phytomonas*) and monogenetic (*Leptomonas, Crithidia, Herpetomonas, Blastocrithidia*) parasites. The entire genus *Trypanosoma* was thus rendered paraphyletic. To reconcile this topology with the late appearance of hematophagous vectors, an independent recent acquisition of digeneity was proposed for different clades of trypanosomes and leishmanias (Fernandes et al. 1993; Maslov and Simpson 1995). An alternative hypothesis was also suggested such that the digeneity was primitive, and hence multiple losses of parasitism in vertebrates accounted for the origin of monogenetic insect parasites (Landweber and Gilbert 1994).

Recently, the kinetoplast 12S rRNA and protein gene sequences from a cryptobiid, *Trypanoplasma borreli*, have also been used to root the trypanosomatid tree (Lukeš et al. 1994). The root was found to be attached at the *Trypanosoma brucei* lineage, similar to the phylogenies based on nuclear rRNA genes.

However, with the addition of a bodonid, *Dimastigella trypaniformis*, to the outgroup, lineages of *Trypanosoma brucei* and another mammalian parasite, *Trypanosoma cruzi*, formed a monophyletic clade on the nuclear rRNA tree (Berchtold et al. 1994; Marche et al. 1995). This result was further supported by phylogenetic trees inferred from the glyceraldehyde-3-phosphate dehydrogenase (Wiemer et al. 1995) and other proteins (Alvarez et al. 1996). Since the *T. brucei* lineage and the outgroup lineage form two long branches on the rRNA phylogenetic trees (Gomez et al. 1991; Fernandes et al. 1993; Landweber and Gilbert 1994; Maslov et al. 1994, 1996; Du et al. 1994) the paraphyletic tree topology may be due to the high level of homoplasy in these fast-evolving lineages, thus representing another example of the ''branch attraction'' artifact (Felsenstein 1978; Hendy and Penny 1989).

To investigate this problem, we studied the effect of adding more taxa to the paraphyletic tree. We investigated whether subdividing the longest branches of the tree would change its topology, a method that may correct the ''branch attraction'' artifact (Hendy and Penny 1989). We have found with this approach that the support for paraphyly diminishes, and the support for monophyly increases. We concluded that trypanosomes are most likely monophyletic and that the paraphyly reported previously may be the result of a high rate of nucleotide substitutions in the *T. brucei* and outgroup lineages.

Materials and Methods

Origin of Strains. Trypanosoma scelopori sensu latum (Ayala 1970) was isolated from a desert lizard, *Sceloporus jarrovi* (Sceloporinae, Iguanidae), captured in 1995 in the Southfork Canyon, Chiricahua Mountains, Cochise County, Arizona. Out of 200 specimens examined, two adults were found to be infected. The flagellates were introduced into modified SNB-9 medium (Diamond and Herman 1954) and cultivated at 26°C. *Rhynchobodo* sp. (ATCC 50359) was obtained from the American Type Culture Collection and cultivated with feeder bacteria in ATCC medium 802 at 24°C. The Ulm strain of *Dimastigella trypaniformis* was obtained from H. Koenig (University of Ulm, Germany) and cultivated as described previously (Breunig et al. 1993). Total cell DNA of the endosymbiont-free strain of *Crithidia oncopelti* (ATCC 30264) was provided by K.-P. Chang (Finch University of Health Sciences, Chicago).

Nucleotide Sequences. Isolation of total cell DNA, PCR amplification, and sequencing with a set of conserved sequence primers were performed as described previously (Maslov et al. 1996). Nucleotide sequences were deposited to the GenBank database under the following accession numbers: U67182 (SSU of *T. scelopori*), U67181 (LSU of *T. scelopori*), U67183 (SSU of *Rhynchobodo* sp.), U67184 (LSU *Rhynchobodo* sp.), U67180 (LSU of *D. trypaniformis*), and U67436 (LSU of *C. oncopelti*). Nucleotide sequences of rRNA genes of *Trypanosoma vivax* (U22316), *Trypanosoma simiae* (U22320), and *Trypanosoma congolense* (U22319) were retrieved from the GenBank database.

Phylogenetic Analysis. Alignment of the combined SSU and LSU sequences was generated manually using the program SeqEdit (29), version 3.1, provided by G. Olsen (Olsen 1990). In order to reduce effect of alignment ambiguities, we have compared our previous alignment (I) (Maslov et al. 1996) with a de novo created alignment (II) and used only positions shared by the alignments I and II to generate a more conservative alignment (III). For the set of 20 taxa the alignment contains 2,591 positions. The alignment is available on request.

Maximum parsimony analysis was performed using the program package PAUP for Macintosh, version 3.1.1 (Swofford 1993). Shortest trees were searched for using the branch-and-bound option. The topology-dependent cladistic permutation tail probability (T-PTP) test (Faith 1991) and Kishino-Hasegawa test (Kishino and Hasegawa 1989) were performed using the test version PAUP 4.0*. Maximum likelihood analysis was done using the program fastDNAml, version 1.0.8. (Olsen et al. 1994) on the DEC Alpha/VMS computer. The search was performed using the empirical base frequencies, global rearrangements,

Table 1. Effect of adding taxa on the topology of the trypanosome clade

Taxon set[a]	Monophyletic tree	Parsimony[b] paraphyletic tree	Consensus tree	Monophyletic tree	Likelihood[c] paraphyletic tree	Consensus tree
10 species[d]	642	634 *1 tree*	Paraphyletic 82%	−7,305.14687	−7,301.47567	Paraphyletic 60%
10 species	552	547 *1 tree*	Paraphyletic 75%	−6,725.83863	−6,728.57549	Monophyletic 57%
11 species	595	594 *1 tree*	Monophyletic 56%	−7,010.02182	−7,026.03169	Monophyletic 81%
14 species	922 *1 tree*	926	Monophyletic 70%	−8,778.47559	−8,805.52686	Monophyletic 85%
16 species	1,288 *2 trees*	1,296	Monophyletic 92%	−10,620.70814	−10,660.99673	Monophyletic 97%
20 species	1,560 *1 tree*	1,564	Monophyletic 70%	−12,210.57383	−12,243.14898	Monophyletic 87%

[a] The core set of ten species includes *Leptomonas* sp., *L. donovani, T. avium, T. cruzi, T. brucei, T. rotatorium, T. boissoni, T. triglae, T. carassii,* and *T. borreli.* In the 11-taxon set, *T. scelopori* was added. In the 14-taxon set, *T. congolense, T. simiae,* and *T. vivax* were added to the 11 taxa. In the 16-taxon set, *D. trypaniformis* and *Rhynchobodo* sp. were added to the 14 taxa, and in the 20-taxon set, *C. fasciculata, C. oncopelti, B. culicis,* and *P. serpens* were added to the 16 taxa

[b] Length (in the number of steps, underlined) and the number of the most parsimonious trees (italicized), length of the user-defined alternative tree, and topology of the trypanosome clade on a consensus tree. Alternative trees represent the trees constructed under constraints of monophyly for ten- and 11-taxon sets and paraphyly for 14-, 16-, and 20-taxon sets. Bootstrap analysis was done using 100 pseudoreplicates. Indicated is the bootstrap value for the monophyly of the trypanosomatid clade which excludes the lineage of Salivarian trypanosomes (in case of paraphyly) or the clade which includes all lineages of trypanosomes (in case of monophyly) on the majority consensus tree

[c] Ln-likelihood of the maximum likelihood tree (underlined) and the user-defined alternative tree and topology of the trypanosome clade on a consensus tree. Bootstrap analysis was done using 100 pseudoreplicates

[d] Alignment I was used

and randomized taxon addition. Bootstrap values for the maximum likelihood trees inferred from bootstrapped alignments were determined using the CONSENS program of the PHYLIP 3.5 program package (Felsenstein 1995).

Results

Analysis of the Data with Parsimony

We began the analysis with the subset containing nine species of Trypanosomatina (including seven representatives of the genus *Trypanosoma*) and an outgroup, *T. borreli* (Table 1). This subset is equivalent to the group of species used in the previous analysis (Maslov et al. 1996); however, to reduce the homoplasy in the nontrypanosome clade, we included only slowly evolving *Leptomonas* and *Leishmania.*

When alignment I was used, maximum parsimony yielded a ten-taxon tree on which trypanosomes were paraphyletic (Table 1) similar to our previously published tree (Maslov et al. 1996). On this tree *T. brucei,* which represented one of the longest branches, grouped together with a cryptobiid, *Trypanoplasma borreli* (another long branch). On the maximum parsimony consensus tree, the bootstrap support for monophyly of the trypanosomatid clade which excluded *T. brucei* was 82% (regarded thereafter as a support for paraphyly).

When the more conservative alignment III was used, the support for paraphyly decreased to 75%. In order to further reduce the effect of homoplasy, we subdivided the long branches of *T. brucei* and *T. borreli* by the addition of new taxa. The incorporation of a new species, a lizard trypanosome, *T. scelopori,* had a profound effect: an 11-taxon monophyletic tree was produced, albeit with a low bootstrap support (56%). Incorporation of three additional species of Salivarian trypanosomes into the alignment increased the support for monophyly to 70%. Combined addition of four trypanosomes and two species of Bodonina resulted in the 16-taxon monophyletic tree with bootstrap support at the level of 92%. Since all the added taxa subdivide the long lineages of *T. brucei* and *T. borreli* on the most parsimonious tree, the observed effect indicates that the paraphyly of trypanosomes may represent a ‘‘long branch attraction’’ artifact (Felsenstein 1978; Hendy and Penny 1989).

Incorporation of four nontrypanosome species *Crithidia fasciculata*, *Phytomonas serpens*, *Blastocrithidia culicis,* and *Crithidia oncopelti*—the last two being fast evolving—reduces the bootstrap support to the level of 70%. It should be noted that unlike the previous additions, incorporation of these taxa does not result in a subdivision of the *T. brucei* and *T. borreli* branches. The observed reduction of support for monophyly may be caused by additional homoplasy introduced with the fast-evolving lineages. We have found previously that the position of *B. culicis* on a combined (LSU+SSU) maximum parsimony rRNA tree is particularly unstable (Maslov et al. 1996).

In order to determine the degree of support for the shortest monophyletic and paraphyletic trees, a PAUP

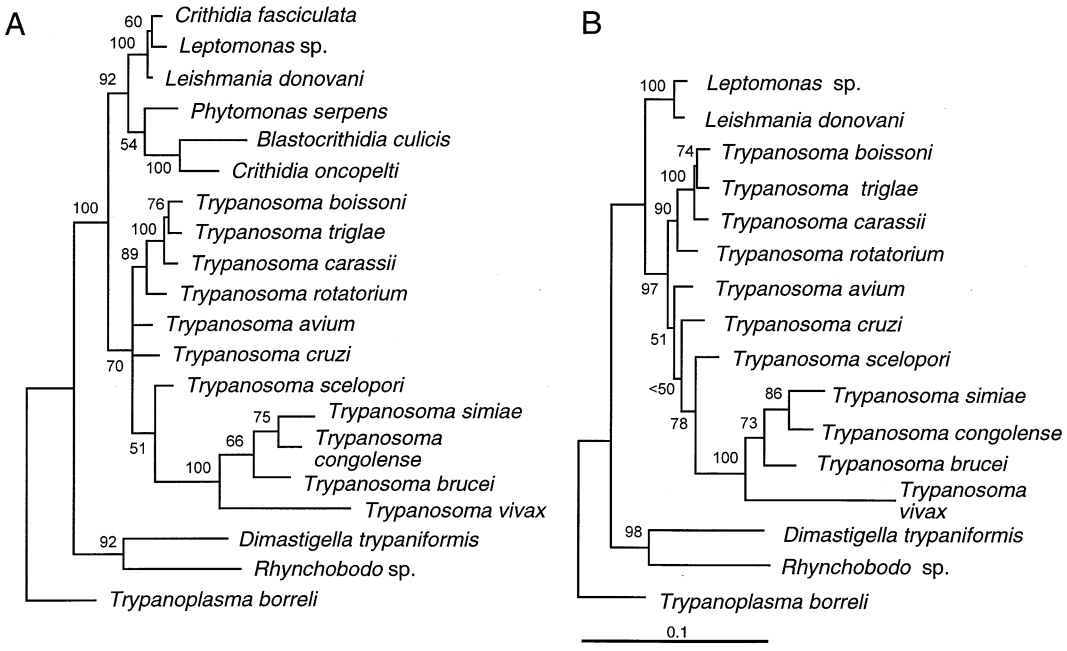

Fig. 1. **A** Majority consensus (50%) parsimony tree constructed for 20 taxa. Bootstrap analysis was performed with 100 replicates. The frequency of occurrence of each clade is indicated. **B** Maximum likelihood tree constructed for 16 taxa. A distance scale (corresponding to 0.1 substitutions per site) is shown under the tree. Ln-likelihood of the tree is −10,620.70814 for a transition/transversion parameter of 1.474510. Bootstrap values determined for 100 pseudoreplicate trees are indicated for each clade on the maximum likelihood tree.

constraints option was utilized to force monophyly in the case of ten and 11 taxa, and to force paraphyly in the case of 14, 16, and 20 taxa (Table 1). The results show that paraphyletic trees are several steps shorter for ten- and 11-taxon sets, and monophyletic trees are several steps shorter for 14-, 16-, and 20-taxon sets.

Significance of the observed differences between the shortest monophyletic and paraphyletic trees was evaluated using the T-PTP (topology-dependent cladistic permutation tail probability) test (Faith 1991). The test determines how often equal or fewer steps can be observed with randomized data sets in which the characters are permuted among the ingroup taxa. The results (not shown) indicate that in all cases the observed differences are not statistically significant.

Analysis of the Data with Likelihood

The maximum parsimony analysis suggests that the data set contains high levels of homoplasy from the fast-evolving lineages. Under these conditions, the marginal discrimination between the monophyletic model and paraphyletic model with parsimony may be due to the well-known limitations of this method (Felsenstein 1978; Hendy and Penny 1989). To obtain an additional estimate of the support for the monophyletic and paraphyletic models, we have analyzed the data with maximum likelihood (Felsenstein 1981).

A paraphyletic tree similar to previous results (Maslov et al. 1994, 1996; Landweber and Gilbert 1994) was produced with the ten-taxon alignment I (Table 1). A monophyletic tree was produced for the more conserved ten-taxon alignment III. However, the likelihood of this tree was still close to the likelihood of the paraphyletic tree (Table 1). On the consensus tree, monophyly was only supported at 57%.

Subdividing the branches of *T. brucei* by addition of *T. scelopori* (11-taxon set), and further by addition of three Salivarian species (14-taxon set), increased the bootstrap support to the level of 85%, which was substantially higher than the corresponding level of support with parsimony (70%). Incorporation of these taxa also increased the difference between the ln-likelihood of the monophyletic tree and ln-likelihood of the paraphyletic tree (Table 1). Additional subdividing of the outgroup branch with *D. trypaniformis* and *Rhynchobodo* sp. (16-taxon set) increased the bootstrap value of the trypanosome clade to 97%, as well as the difference in ln-likelihoods. As with parsimony, incorporation of the fast-evolving nontrypanosome species decreased the bootstrap value, but only to the level of 87%. Figure 1A and B shows the most parsimonious 20-taxon tree and the maximum likelihood 16-taxon tree, respectively.

We conclude that the data as a whole support the monophyletic model over the paraphyletic model, although the margin appears to be narrow.

Internal Topology of the Trypanosome Clade

Assuming the monophyletic model, two subgroups were found within the trypanosome clade with a 100% bootstrap support on all trees. The first subgroup included

leech-transmitted aquatic trypanosomes (*T. boissoni, T. carassii,* and *T. triglae*) and the second subgroup included Salivarian trypanosomes (*T. brucei, T. congolense, T. vivax,* and *T. simiae*). Branching order within both subgroups was resolved with low bootstrap support. An amphibian trypanosome, *T. rotatorium,* was found to be related to the aquatic subgroup with a bootstrap support of 90%. A lizard trypanosome, *T. scelopori,* was found to be related to the Salivarian subgroup, but the bootstrap support for this affinity was significantly lower. The position of *T. cruzi* and *T. avium* is less certain. On the maximum likelihood tree (Fig. 1B), these trypanosomes were found affiliated with the clade of terrestrial trypanosomes, although with low bootstrap support. On the consensus parsimony tree (Fig. 1A) the position of these lineages was not resolved. The insufficient resolution within the trypanosome clade may be due to elimination of informative characters which cannot be unambiguously aligned for all the taxa.

Discussion

In this paper we investigated the phylogeny of trypanosomes using the SSU and partial LSU rRNA sequences. In contrast to our previous results (Maslov et al. 1994, 1996; Du et al. 1994) and the results of others (Gomez et al. 1991; Fernandes et al. 1993; Landweber and Gilbert 1994), we obtained evidence for the monophyly of trypanosomes. This result agrees with other rooted trees inferred from the SSU rRNA sequences (Berchtold et al. 1994; Marche et al. 1995), and glyceraldehyde-3-phosphate dehydrogenase and α-tubulin sequences (Wiemer et al. 1995; Alvarez et al. 1996). It is noteworthy that Fernandes et al. (1993) also obtained a monophyletic tree for one of their alignments.

We demonstrated that subdividing the long branch of *T. brucei* combined with subdividing the long branch of *T. borreli* increases support for the monophyly of the trypanosomes. This effect was particularly pronounced with the method of maximum likelihood, which supported the monophyly of trypanosomes with the 97% bootstrap value for the 16-taxon data set. These results indicate that the paraphyletic tree topology may be an artifact caused by the high level of homoplasy in the lineages of *T. brucei* and the outgroup species. Nevertheless, given the low bootstrap values for other taxon sets and the results of the T-PTP test, our analysis does not completely rule out the paraphyletic model. In addition, the Kishino-Hasegawa randomization test (Kishino and Hasegawa 1989) performed for the 16-taxon maximum likelihood trees also indicated that the difference between them is not significant (L.H. and D.A.M., unpublished results). We conclude that the data support the monophyletic model rather than the paraphyletic model, although the difference in support is small. Obviously, additional data should be used to address this problem.

It is remarkable that a particularly strong increase of the support for monophyly was produced by the incorporation of *Rhynchobodo* sp. and *D. trypaniformis* in the analysis (Table 1, 16-taxon alignment). Since these two species formed long branches on the tree, one could anticipate that the observed effect is related to another ''long branch attraction'' artifact which involves the clade of *Rhynchobodo* sp. and *D. trypaniformis* and a nontrypanosome clade (*Leptomonas* sp. and *L. donovani*), thus rendering trypanosomes monophyletic. However, the nontrypanosome clade is slowly evolving; therefore, this mechanism could not have played a significant role.

Cladistic parsimony results have been used to investigate the evolution of RNA editing in trypanosomatids (Maslov et al. 1994; Landweber and Gilbert 1994). The most parsimonious explanation for the occurrence of pan-editing and 5′-editing on a paraphyletic tree of trypanosomatids was that pan-editing is primitive and 5′-editing is derived. However, because the new monophyletic tree contains only two major clades of trypanosomatids (trypanosomes with pan-editing and other genera with 5′-editing), the parsimony argument can no longer be used as the basis for this view. Nevertheless, the conclusion that pan-editing is ancestral is supported by the finding of pan-edited cryptogenes in a cryptobiid *T. borreli* (Maslov and Simpson 1994; Lukeš et al. 1994). In addition, this theory is indirectly supported by the observation that the loss of minicircles can occur during the prolonged cultivation (Thiemann et al. 1994). This loss has been proposed to drive selection for the retroposition of a partially edited mRNA that replaces the pan-edited cryptogene (Simpson and Maslov 1994b).

The new tree topology is consistent with both aforementioned views on the evolution of parasitism (Fernandes et al. 1993; Landweber and Gilbert 1994), although the number of independent acquisitions of the digenetic life cycle in the ''monogeneity first'' scenario (Fernandes et al. 1993) decreased by one. With the exact branching order within the trypanosome clade being poorly resolved and many important species of trypanosomes not yet investigated, the exact order of the host acquisitions and/or losses cannot be reconstructed. Nevertheless, the Salivarian trypanosomes (*T. vivax, T. brucei, T. congolense,* and *T. simiae*) form a well-supported derived clade. In this group, the developmental cycle terminates in salivary glands of the tsetse fly vector, while in Stercorarian trypanosomes (represented by *T. cruzi* and *T. avium*), and monogenetic parasites of insects, the cycle of development terminates in the hindgut of the vector. Thus, the topology of the tree indicates that development in the anterior station of a vector is a derived trait. This conclusion is consistent with the view that the Stercorarian cycle is more ancient than the Salivarian cycle (Hoare 1972).

The early separation of the lineage of *T. vivax* within

the Salivaria is also consistent with the view that this organism represents an early stage of adaptation to transmission by tsetse flies (Hoare 1972). The close relatedness of *T. congolense* and *T. simiae* is in agreement with the morphological taxonomy, according to which these species belong to the same subgenus, *Nannomonas* (Hoare 1964).

Leech-transmitted trypanosomes represented by *T. rotatorium, T. carassii, T. triglae,* and *T. boissoni* constitute another group where development into the infectious stage occurs in the anterior station (proboscis) of a leech vector. These trypanosomes also form a derived clade on our tree. Although a similar developmental pattern is seen in the outgroup, *Trypanoplasma borreli* (Lom 1976), given the present topology of the tree, anterior station development in leeches is also derived.

Phylogenetic analysis of a reptilian trypanosome, *T. scelopori,* extends our previous work on the phylogeny of trypanosomes isolated from the various classes of vertebrates (Maslov et al. 1996). We have found that *T. scelopori* forms a separate, early diverging lineage. Although the actual vector is unknown and the life cycle has not been described, it has been noticed that these trypanosomes can develop in phlebotomine mosquitoes (Ayala 1970) and that these insects represent the most likely vector of *T. scelopori.* In this case, the mode of transmission may be similar to that of the leishmanias, where the terminal stage of the life cycle takes place in the anterior midgut and the foregut of the vector. It still remains to be shown whether other mosquito-transmitted amphibian and reptilian trypanosomes will add to this clade. The finding that trypanosomes group on the tree according to their mode of transmission suggests that the adaptation to invertebrate vectors plays a more important role in the trypanosome evolution than the adaptation to vertebrate hosts.

Acknowledgments. We would like to thank A. Polák for technical assistance, G. Olsen for providing the source code of the SeqEdit program, C. McDaniels for help with its installation, D. Swofford for permission to use PAUP 4.0* for this publication, and Marian Peris and Mark Springer for the critical reading of the manuscript. The strain of *D. trypaniformis* was kindly provided by H. Koenig, and the lizards infected with *T. scelopori* were obtained from J. Volf. We acknowledge the help of D. Modrý and E. Nohýnková with establishing the original culture of *T. scelopori.* This work was supported in part by grants from the Grant Agency of the Czech Republic and the Grant Agency of the Czech Academy of Sciences to J.L. D.A.M. is a Burroughs Wellcome Fund New Investigator in Molecular Parasitology.

References

Alvarez F, Cortinas MN, Musto H (1996) The analysis of protein coding genes suggests monophyly of *Trypanosoma.* Mol Phylogenet Evol 5:333–343

Ayala SC (1970) Two new trypanosomes from California toads and lizards. J Protozool 17:370–373

Benne R (1994) RNA editing in trypanosomes. Eur J Biochem 221:9–23

Berchtold M, Philippe H, Breunig A, Brugerolle G, Koenig H (1994) The phylogenetic position of *Dimastigella trypaniformis* within the parasitic kinetoplastids. Parasitol Res 80:672–679

Breunig A, Brugerolle G, Vickerman K, Hertel H, Koenig H (1993) Isolation and ultrastructural features of a new strain of *Dimastigella trypaniformis* Sandon 1928 (Bodonina, Kinetoplastida) and comparison with a previously isolated strain. Eur J Protistol 29:416–424

Diamond LS, Herman CM (1954) Incidence of trypanosomes in the Canada goose as revealed by bone marrow culture. J Parasitol 40:195–202

Du Y, Maslov DA, Chang K-P (1994) Monophyletic origin of β-division proteobacterial endosymbionts and their coevolution with insect trypanosomatid protozoa *Blastocrithidia culicis* and *Crithidia* spp. Proc Natl Acad Sci USA 91:8437–8441

Faith DP (1991) Cladistic permutation tests for monophyly and nonmonophyly. Syst Zool 40:366–375

Felsenstein J (1978) Cases in which parsimony or compatibility methods will be positively misleading. Syst Zool 27:401–410

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1995) PHYLIP—Phylogeny Inference Package, version 3.5. University of Washington, Seattle, computer program distributed by the author

Fernandes AP, Nelson K, Beverley SM (1993) Evolution of nuclear ribosomal RNAs in kinetoplastid protozoa: perspectives on the age and origins of parasitism. Proc Natl Acad Sci USA 90:11608–11612

Gomez E, Valdes AM, Pinero D, Hernandez R (1991) What is a genus in the Trypanosomatidae family? Phylogenetic analysis of two small rRNA sequences. Mol Biol Evol 8:254–259

Hajduk SL, Harris ME, Pollard VW (1993) RNA editing in kinetoplastid mitochondria. FASEB J 7:54–63

Hendy MD, Penny D (1989) A framework for quantitative study of evolutionary trees. Syst Zool 38:297–309

Hoare CL (1964) Morphological and taxonomical studies on mammalian trypanosomes. X. Revision of the systematics. J Protozool 11:200–210

Hoare CA (1972) The trypanosomes of mammals. Blackwell Scientific, Oxford

Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J Mol Evol 29:170–179

Lainson R, Shaw JJ (1987) Evolution, classification and geographical distribution. In: Peters W, Killick-Kendrick R (eds) The Leishmaniases in biology and medicine. Academic Press, London, pp 1–120

Lake J, de la Cruz V, Ferreira P, Morel C, Simpson L (1988) Evolution of parasitism: Kinetoplastid protozoan history reconstructed from mitochondrial rRNA gene sequences. Proc Natl Acad Sci USA 85:4779–4783

Landweber LF, Gilbert W (1994) Phylogenetic analysis of RNA editing: a primitive genetic phenomenon. Proc Natl Acad Sci USA 91:918–921

Lom J (1976) Biology of the trypanosomes and trypanoplasms of fish. In: Lumsden WHR, Evans DA (eds) Biology of the Kinetoplastida. Academic Press, London, pp 267–337

Lukeš J, Arts GJ, Van den Burg J, De Haan A, Opperdoes F, Sloof P, Benne R (1994) Novel pattern of editing regions in mitochondrial transcripts of the cryptobiid *Trypanoplasma borreli.* EMBO J 13: 5086–5098

Marche S, Roth C, Philippe H, Dollet M, Baltz T (1995) Characterization and detection of plant trypanosomatids by sequence analysis of the small subunit ribosomal RNA gene. Mol Biochem Parasitol 71:15–26

Maslov DA, Simpson L (1994) RNA editing and mitochondrial genomic organization in the cryptobiid kinetoplastid protozoan, *Trypanoplasma borreli.* Mol Cell Biol 14:8174–8182

Maslov DA, Simpson L (1995) Evolution of parasitism in kinetoplastid protozoa. Parasitol Today 11:30–32

Maslov DA, Avila HA, Lake JA, Simpson L (1994) Evolution of RNA editing in kinetoplastid protozoa. Nature 365:345–348

Maslov DA, Lukeš J, Jirků M, Simpson L (1996) Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for the evolution of parasitism in the trypanosomatid protozoa. Mol Biochem Parasitol 75:197–205

Olsen GJ (1990) Sequence Editor and Analysis Program. University of Illinois, Urbana, computer program distributed by the author

Olsen GJ, Matsuda H, Hagstrom R, Overbeek R (1994) fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Comput Appl Biosci 10:41–48

Simpson L, Maslov DA (1994a) Ancient origin of RNA editing in kinetoplastid protozoa. Curr Opin Genet Dev 4:887–894

Simpson L, Maslov DA (1994b) RNA editing and the evolution of parasites. Science 264:1870–1871

Simpson L, Thiemann OH (1995) Sense from nonsense: RNA editing in mitochondria of kinetoplastid protozoa and slime molds. Cell 81:837–840

Stuart K (1993) RNA editing in mitochondria of African trypanosomes. In: Benne R (ed) RNA editing—the alteration of protein coding sequences of RNA. Ellis Horwood, New York, pp 225–252

Swofford DL (1993) PAUP: Phylogenetic Analysis Using Parsimony, version 3.1. Champaign, IL, computer program distributed by the Illinois Natural History Survey

Thiemann OH, Maslov DA, Simpson L (1994) Disruption of RNA editing in *Leishmania tarentolae* by the loss of minicircle-encoded guide RNA genes. EMBO J 13:5689–5700

Vickerman K (1976) The diversity of the kinetoplastid flagellates. In: Lumsden WHR, Evans DA (eds) Biology of the Kinetoplastida. Academic Press, London, pp 11–34

Vickerman K, Preston TM (1976) Comparative cell biology of the kinetoplastid flagellates. In: Lumsden WHR, Evans DA (eds) Biology of the Kinetoplastida. Academic Press, London, pp 35–130

Wiemer EAC, Hannaert V, Van den IJssel PRLA, Van Roy J, Opperdoes FR, Michels PAM (1995) Molecular analysis of glyceraldehyde-3-phosphate dehydrogenase in *Trypanoplasma borelli:* an evolutionary scenario of subcellular compartmentation in Kinetoplastida. J Mol Evol 40:443–454