Review

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Recent advances in trypanosomatid research: genome organization, expression, metabolism, taxonomy and evolution

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Abstract

Unicellular flagellates of the family Trypanosomatidae are obligatory parasites of invertebrates, vertebrates and plants. Dixenous species are aetiological agents of a number of diseases in humans, domestic animals and plants. Their monoxenous relatives are restricted to insects. Because of the high biological diversity, adaptability to dramatically different environmental conditions, and omnipresence, these protists have major impact on all biotic communities that still needs to be fully elucidated. In addition, as these organisms represent a highly divergent evolutionary lineage, they are strikingly different from the common 'model system' eukaryotes, such as some mammals, plants or fungi. A number of excellent reviews, published over the past decade, were dedicated to specialized topics from the areas of trypanosomatid molecular and cell biology, biochemistry, host–parasite relationships or other aspects of these fascinating organisms. However, there is a need for a more comprehensive review that summarizing recent advances in the studies of trypanosomatids in the last 30 years, a task, which we tried to accomplish with the current paper.

Introduction

The motivation behind writing this review was to summarize the current views on biology of Trypanosomatidae. While several recent reviews were focused on specific aspects of this field (Hajduk and Ochsenreiter, 2010; Jackson, 2015; Read *et al.*, 2016; Gibson, 2017; Kaufer *et al.*, 2017), a rather broad aim of our paper includes advances in taxonomy, genetics, molecular and cellular biology, and biochemistry of these fascinating organisms.

It is hard to overemphasize the significance of trypanosomatids for both basic and applied research. This group was first outlined by William Saville-Kent, who united genera *Herpetomonas* and *Trypanosoma* into the new order Trypanosomata (Saville-Kent, 1880). This order (now spelled as Trypanosomatida) encompasses a single family Trypanosomatidae (Hoare, 1966; Vickerman, 1976), the members of which are obligatory parasites of invertebrates, vertebrates and plants (Nussbaum *et al.*, 2010; Lukeš *et al.*, 2014). Dixenous (with two hosts in their life cycle) parasites employ an invertebrate (arthropod or leech) vector to shuttle between the vertebrate (genera *Endotrypanum*, *Leishmania*, *Paraleishmania*, *Trypanosoma*) or plant (genus *Phytomonas*) hosts. Most monoxenous (with a single host) trypanosomatids parasitize insects. The importance of dixenous species is incontestable as they cause severe diseases in humans, domestic animals and economically important plants (Simpson *et al.*, 2006). In contrast, until recently monoxenous species have been viewed as less relevant relatives of the all-important pathogens such as *Trypanosoma brucei*, *T. cruzi* or *Leishmania* spp. Only in the last decade had they attracted the due attention as the group of high diversity, ability to adapt to dramatically different environmental conditions, ubiquity and impact on insect hosts' communities (Maslov *et al.*, 2013; Hamilton *et al.*, 2015; Votýpka *et al.*, 2015; Ishemgulova *et al.*, 2017; Lukeš *et al.*, 2018). Besides, as the dixenous trypanosomatids evolved from their monoxenous kin (Fernandes *et al.*, 1993; Hughes and Piontkivska, 2003; Jirků *et al.*, 2012; Flegontov *et al.*, 2013), the study of insect parasites is imperative for understanding the evolutionary pathways in the family.

Classification system and evolution of dixenous lifestyle

The traditional classification system of this group relied on a very limited set of diagnostic traits and, in essence, was based on rough cell morphology and particularities of the life cycle, such as the monoxenous *vs* dixenous mode, as well as host specificity (Hoare and

Wallace, 1966; Vickerman, 1976). A modern system is phylogeny based, but even after more than two decades of molecular phylogenetic analyses some relationships among the trypanosomatid major clades remain unresolved (Votýpka *et al.*, 2015). Nowadays, nucleotide sequences with thousands of informative characters are routinely used for inferring evolutionary relationships between these protists (Borghesan *et al.*, 2013; d'Avila-Levy *et al.*, 2015). The main constraint of such a molecular phylogenetic approach is that it remains based on a limited number of genetic loci – usually 18S rRNA and gGAPDH (glycosomal glyceraldehyde 3-phosphate dehydrogenase). These molecular markers work well for resolving relationships between genera and higher taxa, but are not best-suited to delineate subgeneric ranks (Yurchenko *et al.*, 2006b; Votýpka *et al.*, 2010). In any case, phylogenies inferred from single or a few genes can be misleading and frequently are poorly resolved (Grybchuk-Ieremenko *et al.*, 2014; Yurchenko *et al.*, 2014; Frolov *et al.*, 2017). The foreseeable solution to this problem (facilitated by rapidly decreasing prices of the next-generation sequencing and development of increasingly powerful methods of data analysis) lies with phylogenomic analyses based on the whole-genome sequences (Flegontov *et al.*, 2016; Skalický *et al.*, 2017).

In the current classification system, six formally recognized subfamilies and 22 genera are included in the family Trypanosomatidae (Fig. 1). The subfamily Leishmaniinae (Jirků *et al.*, 2012; Kostygov and Yurchenko, 2017) unites monoxenous parasites of insects (genera *Borovskyia*, *Crithidia*, *Leptomonas*, *Lotmaria*, *Novymonas* and *Zelonia*) and dixenous parasites of insects and vertebrates (genera *Leishmania*, *Paraleishmania* and 'Endotrypanum'). The monogeneric subfamilies Blechomonadinae (Votýpka *et al.*, 2013) and Paratrypanosomatinae (Flegontov *et al.*, 2013) include monoxenous parasites of Siphonaptera (genus *Blechomonas*) and the early branching lineage of the genus *Paratrypanosoma*, respectively. The subfamily Strigomonadinae (Votýpka *et al.*, 2014) encompasses bacterial endosymbiont-harbouring genera *Angomonas*, *Kentomonas*, and *Strigomonas*, while the subfamily Phytomonadinae (Yurchenko *et al.*, 2016) contains genera *Phytomonas*, *Herpetomonas* and *Lafontella*. Previously, the genus *Trypanosoma* was not assigned to any subfamily. We deem, that in accordance with the International Code of Zoological Nomenclature principle of coordination, this genus, as a name-bearing type, should be included into the nominotypical subfamily, i.e. Trypanosomatinae. The genera *Blastocrithidia*, *Jaenimonas* (Hamilton *et al.*, 2015), *Sergeia* (Svobodová *et al.*, 2007) and *Wallacemonas* (Kostygov *et al.*, 2014) remain orphans and not united into higher-order groups for now. In addition, several clades have been revealed by the analyses of environmental samples (Týč *et al.*, 2013; Votýpka *et al.*, 2015), yet their formal description awaits the availability of respective trypanosomatids in culture.

The historical 'chicken-or-egg' debate over the origin of dixenous trypanosomatids (insect-first *vs* vertebrate-first scenarios) seems to be resolved. It is now generally accepted that the dixenous lifestyle has evolved from the monoxenous one several times in evolution leading to the emergence of the genera *Trypanosoma*, *Leishmania*, and *Phytomonas* (Fernandes *et al.*, 1993; Hamilton *et al.*, 2004; Lukeš *et al.*, 2014). The boundary between monoxenous and dixenous types of parasitism appears to be dynamic, as members of some typical monoxenous groups were found in warm-blooded (usually, immuno-compromised) hosts (Dedet and Pratlong, 2000; Chicharro and Alvar, 2003; Ghosh *et al.*, 2012), while descendants of some formerly dixenous species switched back to monoxeny (Lai *et al.*, 2008; Frolov *et al.*, 2016). The molecular mechanisms governing successful transitions between the monoxenous and dixenous life cycles remain to be investigated.
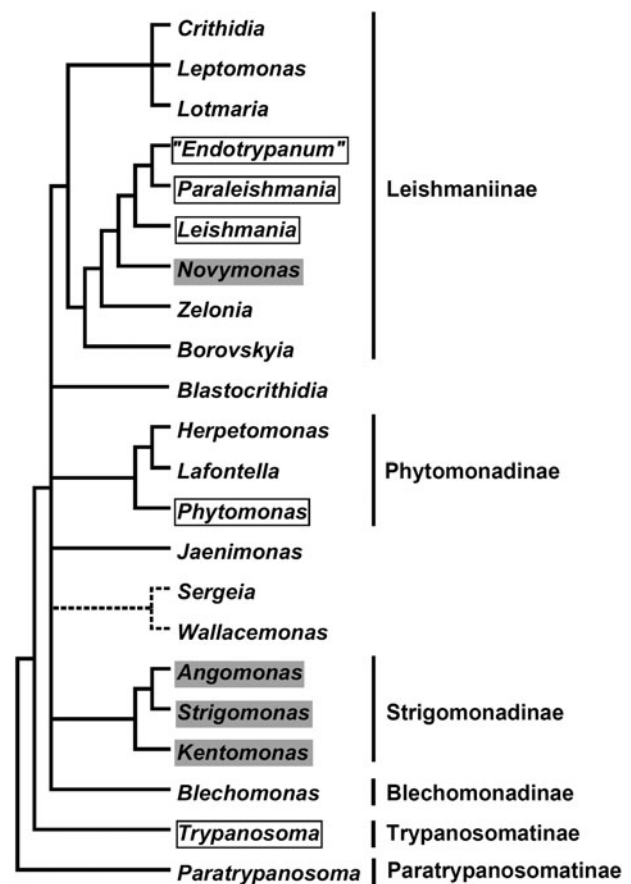

Fig. 1. A schematic phylogenetic tree demonstrating relationships between known Trypanosomatidae taxa. Dixenous and endosymbiont-containing genera are framed and shaded, respectively. The precise phylogenetic position of the *Sergeia/Wallacemonas* group is uncertain and indicated by broken lines. Formally recognized subfamilies are listed on the right.

Nuclear gene organization and expression

The Excavata, a protist superclade, which includes trypanosomatids, had separated very early from the rest of the eukaryotic tree (Cavalier-Smith *et al.*, 2014). This long independent evolution resulted in the development of a wide range of cellular and molecular features unique to this group. Thus, while staying within the general eukaryotic cell and molecular layout, trypanosomatids had evolved to become drastically different from the 'textbook' eukaryotes, such as Metazoa or Fungi, at every level of gene organization and expression (Lukeš *et al.*, 2014). Naturally, these differences, especially those present in pathogenic trypanosomatids, have been a subject of intense investigations ultimately aimed at finding potential targets for disease treatment and control, as well as expanding the knowledge base beyond boundaries of the common model organisms. Since the sheer volume of the accumulated information precludes its in-depth review within the limits of a single section or even an entire review, we kindly refer the reader to the numerous reviews dealing with specific aspects of this burgeoning field (Myler, 2008; Bindereif, 2012; Preusser *et al.*, 2012; Clayton, 2014; Horn, 2014). Below is a brief overview of the most striking features of trypanosomatid gene expression, which set these parasites very far apart from their metazoan hosts and vectors.

Genome organization

Trypanosomatid genomes are relatively compact, ranging from 18.0 Mb in *Phytomonas* sp. to 32.6 Mb in *Crithidia fasciculata*.

The number of nuclear-encoded genes varies from the reduced set of 6400 genes in *Phytomonas* to 16 900 genes estimated for *Angomonas* (Porcel *et al.*, 2014; Jackson, 2015). The chromosomal structure is by far best known for *T. brucei*. Its genome is divided among 11 large diploid chromosomes (1 to 6 Mb in size) (Melville *et al.*, 1998), ~5 intermediate-size chromosomes (200–900 kb) and approximately 100 mini-chromosomes (30–150 kb) (Wickstead *et al.*, 2004; Daniels *et al.*, 2010). The inheritance of intermediate-size- and mini-chromosomes is non-Mendelian, as they show mixed ploidy for analysed genetic markers (Alsford *et al.*, 2001). These chromosomes serve as depositories of genetic material used for the generation of novel variable surface glycoprotein (VSG) genes (Wickstead *et al.*, 2004), which are instrumental for parasite survival and propagation in the mammalian bloodstream. As for *Leishmania* spp., their similar size genomes are split over 35–36 chromosomal pairs of smaller lengths (Myler, 2008; Cantacessi *et al.*, 2015).

The high gene density in trypanosomatids is explained by the near complete lack of introns and the relatively short intergenic regions (Günzl, 2010). Individual genes are arranged as same-strand tandem arrays that may include up to hundreds of genes. This organization is particularly pronounced in *Leishmania*, in which a megabase-sized chromosome may contain just two such clusters (Myler *et al.*, 1999; Martínez-Calvillo *et al.*, 2004), whereas gene clusters in *T. brucei* are usually shorter (Bindereif, 2012). Out of 191 transcription initiation sites were mapped in *T. brucei*, the majority (129) were found at the 5′ ends of the tandem clusters with the remaining sites localized within the clusters (Kolev *et al.*, 2010). Unlike bacterial operons, trypanosomatid genes within the same cluster are not functionally related but seem to be arrayed randomly. However, the distance from the transcription initiation site within a cluster was crucial for the proper temporal expression of the heat shock and cell cycle-dependent genes. Moreover, such 'positional bias' was also observed for several functional gene groups, suggesting that temporal control by location within a cluster being an important principle of the *T. brucei* genome organization and expression (Kelly *et al.*, 2012).

Closely related species exhibit a remarkably high level of synteny in gene organization, and long regions of gene collinearity are observed even between distant relatives, such as between trypanosomes and leishmanias (Ghedin *et al.*, 2004; Peacock *et al.*, 2007; Flegontov *et al.*, 2016). This conservation of gene order can be explained if spatial gene organization is implicated in the temporal control of gene expression, as it is in *T. brucei*. Nevertheless, group-specific differences were also documented. For example, while in all *Trypanosoma* spp. the arrays of rRNA genes comprising 28S, 18S and 5.8S rRNAs are well conserved and are repeated throughout the genome extending across several chromosomes to facilitate their high expression, in *Leishmania* spp. they are arranged as a single tandem array. The snRNAs genes occur within tRNA clusters in all trypanosomatids, although the location of these clusters varies among the species (Ivens *et al.*, 2005).

RNA polymerases and transcription

The tight spacing of protein-coding genes within clusters indicates the lack of individual promoters and the ability for independent gene transcription. Instead, it appears that RNA polymerase II (Pol II) initiates transcription at the 'switch' regions between the clusters or even randomly transcribes an entire cluster with a constant rate as a single polycistronic unit (Puechberty *et al.*, 2007; Das *et al.*, 2008; Kolev *et al.*, 2010). The nascent polygenic RNA is processed co-transcriptionally. However, neither the promoters nor the transcription termination sites have been identified so far (Günzl *et al.*, 2007; Myler, 2008). The only well-characterized Pol II promoters are those for transcription of spliced leader (SL) RNA genes (Gilinger and Bellofatto, 2001; Dossin Fde and Schenkman, 2005). These small non-coding transcripts are used during mRNA maturation, hence hundreds of individual SL RNA genes are present in the trypanosomatid genome in order to sustain the necessary rate of mRNA processing (Liang *et al.*, 2003; Lee *et al.*, 2007b). These genes are arranged as clusters of tandem units, but each gene is individually transcribed by Pol II using a promoter and a transcription termination signal. Pol II itself is composed of 12 rather conservative subunits (Das *et al.*, 2006; Martinez-Calvillo *et al.*, 2007). Its unique property is the absence of the conserved heptad amino acid sequence in the carboxy-terminal domain (CTD) of the largest subunit RPB1. This difference apparently reflects the fact that a co-transcriptional capping of a monocistronic pre-mRNA (mediated by CTD in other organisms) does not take place in trypanosomatids (see below). The pre-initiation complex assembled at the SL RNA promoter includes recognizable homologues of metazoan basal transcription factors, such as TRF4 (TATA-box binding protein-related factor 4) and some subunits of TFIIH (Ivens *et al.*, 2005). Biochemical analyses revealed additional rather divergent subunits of TFIIH, as well as TFIIA, TFIIB and Mediator complex (Das and Bellofatto, 2003; Schimanski *et al.*, 2005; 2006; Lee *et al.*, 2009; 2010), to the total of more than 20 proteins.

The peculiar utilization of RNA polymerases in trypanosomatids is further illustrated by the participation of Pol I in the transcription of the protein-coding genes. This enzyme, canonically serving to transcribe ribosomal RNA genes, also performs that function in trypanosomatids (Hernandez and Cevallos, 2014). However, in *T. brucei* it also transcribes a special group of genes, which are located in the subtelomeric regions of large chromosomes, namely the expressed version of the VSG genes and a group of expression site-associated genes (ESAGs) (Vanhamme and Pays, 1995; Navarro and Gull, 2001; Günzl *et al.*, 2003). Another class of protein-coding genes transcribed by Pol I in *T. brucei* is procyclin, which constitutes the major surface component of the procyclic stage (Günzl *et al.*, 2003). The transcriptionally active Pol I complex contains at least 12 subunits, most of which are conserved but at least one is trypanosomatid-specific (Nguyen *et al.*, 2007). Each large chromosome has two VSG Pol I promoters in its subtelomeric regions. These promoters are composed of two short sequence element and are structurally different from the ribosomal RNA and procyclin promoters with a more complex architecture, however, their recognition depends on the same multi-subunit transcription factor CITFA (Brandenburg *et al.*, 2007; Kolev *et al.*, 2017). There is a total ∼ 20 subtelomeric expression sites (ES) for VSG genes (∼5 ES's for metacyclic VSG genes and ∼15 ES's for bloodstream VSG genes), but in a given cell only one ES is active at any time (Navarro *et al.*, 1999). A bloodstream ES is 45–60 kbp long and includes 9–10 ESAGs in addition to a single telomere-proximal VSG gene separated from the upstream ESAGs by a long block of 70 bp repeats. A metacyclic ES is short (up to 6 kb), lacks ESAGs and a repeat block. The ESAG and VSG genes in the active site are transcribed by in form of a polycistronic unit, with the RNA processing occurring co-transcriptionally. The choice of the single active ES is regulated epigenetically (Günzl *et al.*, 2015; Maree *et al.*, 2017). Transcription of all the inactive ESs also gets initiated, but terminates prematurely before reaching the promoter-distant VSG gene due to telomere-dependent epigenetic silencing (Batram *et al.*, 2014; Kassem *et al.*, 2014). This silencing is, at least in part, mediated by a telomeric protein RAP1, whose depletion results in de-repression of the silent ESs (Yang *et al.*, 2009). So does depletion of the histone H3 methylase

DOT1 indicating that chromatin structure also plays important role in this silencing (Figueiredo *et al.*, 2008). However, expression level at the derepressed ESs does not achieve that of the active ES, indicating that additional controls are at play, including transcription initiation (Nguyen *et al.*, 2014; Günzl *et al.*, 2015). A possible mechanism can be based sequestration of all necessary factors in a single transcription focus. This sequestration can be enforced by a recently discovered VEX1 (VSG exclusion) factor (Glover *et al.*, 2016). Localized in a single nuclear focus next to ESB, VEX1 is proposed to exert both negative regulation (on silent ESs) and positive regulation (on the active ES). Its molecular mechanism is unclear but it appears to be homology-based. Stage-specific transcription of the epimastigote coat protein BARP (brucei alanine-rich protein) may also be Pol I-dependent (Urwyler *et al.*, 2007; Savage *et al.*, 2016).

Finally, RNA polymerase III (Pol III) is the only polymerase in trypanosomes that retained its canonical functions, transcribing the tRNA and 5S rRNA genes (Das *et al.*, 2008).

mRNA processing by trans-splicing coupled with 3′-end cleavage and polyadenylation

All mature mRNAs in trypanosomatids contain a non-coding 39 nucleotide-long SL RNA (Parsons *et al.*, 1984). These add-on sequences are derived from the initial SL RNA gene transcripts, which in addition to the mini-exon on the 5′-end contain a variable species-dependent length mini-intron on the 3′-end (Goncharov *et al.*, 1998; Mandelboim *et al.*, 2002). There is a co-transcriptionally added hyper-methylated cap 4. This structure contains m⁷GpppG cap on the 5′-end and 2′-O methylations at nucleotides 1 and 2, commonly seen in other organisms (Perry *et al.*, 1987). Unique to trypanosomatids, it also contains 2′-O methyl groups at nucleotides 3 and 4, and methylated bases at nucleotides 1 (m_2^6A) and 4 (m^3U) (Ullu and Tschudi, 1993). The functional significance of the trypanosomatid-specific hyper-methylated cap remains unclear (Sturm *et al.*, 2012).

The capped SL is added to mRNA by *trans*-splicing and is essential for stability and translatability of the latter (Sturm *et al.*, 2012). Although *trans*-splicing is not unique to trypanosomatids, and occurs, along with conventional *cis*-splicing, in some Metazoa and protists (Lukeš *et al.*, 2009), in trypanosomatids it is a major and obligatory step in the maturation of each mRNA. In addition, due to continuous transcription of protein-coding gene clusters, the processes of *trans*-splicing and 3′-end cleavage/polyadenylation are tightly coupled (LeBowitz *et al.*, 1993; Matthews *et al.*, 1994). Intergenic regions included in the nascent RNA contain the cleavage/polyadenylation sites positioned 3′ to each coding region. Cleavage of the nascent RNA at this site not only enables the 3′ maturation of the upstream pre-mRNA but also liberates the 5′-end of the downstream pre-mRNA for participation in *trans*-splicing. Mechanistically, the process of *trans*-splicing includes two *trans*-esterification reactions as in conventional *cis*-splicing (Günzl, 2010). The SL RNA participates in the reaction as a specific snRNP particle (Goncharov *et al.*, 1999), apparently substituting the U1 snRNP in a *trans*-spliceosome. Other snRNPs, U2, U4–U6, have also been identified in trypanosomatids (Palfi *et al.*, 1994). Interestingly, the U1 snRNP, typically involved in 5′ splice site recognition is also present, because at least two *T. brucei* genes – poly(A)-polymerase and DEAD/H RNA helicase – contain a *cis*-splicing intron (Mair *et al.*, 2000; Siegel *et al.*, 2010).

The absence of individual promoters and the constant rate of transcription by Pol II of most protein-coding genes dictate that gene regulation does not operate at the level of transcription initiation, in contrast to most other eukaryotes. Instead, gene expression is mainly controlled post-transcriptionally, with the main level being mRNA stability (McNicoll *et al.*, 2005; Requena, 2011). The abundance of mRNA depends on its half-life, which averages around 20 min in this life stage (Fadda *et al.*, 2014; Kramer, 2017b). A mature mRNA is 5′-capped and 3′-polyadenylated and its exonucleolytic degradation by the exosome requires removal of either structural feature.

Stability of mRNA is mainly defined by the structure of its 3′ untranslated region (3′-UTR). Typically, it is long enough (~400 nt) to accommodate several RNA-binding proteins (Nozaki and Cross, 1995; De Gaudenzi *et al.*, 2005), of which there is a diverse population with varying functions, binding constants and copy numbers per cell (Erben *et al.*, 2014; Lueong *et al.*, 2016). Some of those proteins facilitate mRNA degradation by recruiting deadenylating or decapping factors (Kramer, 2017a), or exosomes (Fadda *et al.*, 2013), while others stabilize the mRNA either directly by protecting it from degradation or indirectly by competing with the degradation factors (Estevez, 2008). These proteins are engaged in dynamic interactions with the mRNA and define the longevity of a given transcript.

Multiplicity of translation factors

Additional regulation occurs at the translation level, as demonstrated by the abundance of a given protein frequently not correlating with levels of its encoding mRNA (McNicoll *et al.*, 2006; Leifso *et al.*, 2007). In other eukaryotes, translation is often regulated at the stage of initiation and this also seems to be the case in trypanosomatids (Rezende *et al.*, 2014). However, as indicated by the uniquely complex 5′-end structure of mRNA, in trypanosomatids this process has deviated from the canonical eukaryotic pattern. At least four paralogs of eIF4E and six of eIF4 G were identified by genome analysis (Zinoviev and Shapira, 2012). While their functions are not fully ascribed, the available evidence indicates that trypanosomatid eIF4E-1 and eIF4E-4 are the *bona fide* parts of the respective eIF4F complexes and may be involved in life cycle stage-specific (developmental) regulation of translation (Yoffe *et al.*, 2009; Zinoviev *et al.*, 2011), while eIF4E-2 may mediate mRNA–ribosome interactions during elongation (Yoffe *et al.*, 2004). Unlike in higher eukaryotes, where eIF4G mediates interactions between eIF4E and 3′-bound poly-A binding protein (PABP), in trypanosomatids this interaction is performed directly by eIF4E (−1 or −4), while the subunit eIF4G-3 links the former with eIF4A1, which is assumed to be involved in recognition of the initiation codon (Pestova *et al.*, 2001). Out of the three isoforms of PABP in trypanosomatids, only PABP-1 participates in the formation of the cap-dependent initiation complex (Kramer *et al.*, 2013).

Trypanosomatids lack the homologue of a small eIF4E-binding protein (4E-BP), which in higher eukaryotes can block the formation of the cap-bound initiation complex, and hence translation, by preventing the interaction between the eIF4E and eIF4G subunits. Instead, trypanosomatids possess a different type of 4E-BP, called 4E-IP, which interacts with eIF4E-1 (Zinoviev *et al.*, 2011). In *Leishmania*, this protein appears to participate in stage-specific phosphorylation-dependent translation control (Zinoviev and Shapira, 2012).

Unexpected codon reassignment

The already long list of oddities encountered in trypanosomatids has been recently extended by a unique codon reassignment found in several representatives of the genus *Blastocrithidia*. Here, all three stop codons are reassigned to code for amino acids (Záhonová *et al.*, 2016), a deviation paralleled only by two ciliate species (Swart *et al.*, 2016). Most changes of the genetic code involve reassignment of stop codon(s), in particular, UGA

to decode Trp in many mitochondria and bacteria, yet almost always at least one stop codon is retained for terminating translation (Keeling, 2016). Trypanosomatids are no exception and use UAG to specify Trp in their kinetoplast DNA, which does not encode any tRNA genes (de la Cruz *et al.*, 1984). Consequently, all tRNAs have to be imported into the mitochondrion from the cytosol (Simpson *et al.*, 1989). Since there is only a single tRNA^{Trp} in the trypanosomatid nuclear genome, the anticodon of which recognizes the standard Trp UGG codon, in order to decode UGA the tRNA^{Trp} undergoes C to U editing at the first position of its anticodon. However, to prevent read-through of the UGA stop codon in the cytoplasm, the deamination must happen only after import into the organelle (Alfonzo *et al.*, 1999). While the enzyme responsible for this single-site editing has yet to be identified, trypanosomatids were shown to possess two distinct tryptophanyl-tRNA synthetases to charge tRNA^{Trp} in the mitochondrion and the cytosol (Charriere *et al.*, 2006). In *Blastocrithidia* spp., however, codon reassignment happens also in the cytosol, as UAR and UGA code for Glu and Trp, respectively, with UAA being also used as a genuine stop codon. It remains to be established how these flagellates distinguish between in-frame and genuine stops so that proper translation termination can occur.

Kinetoplast, kinetoplast DNA and RNA editing

These aspects represent one of the major trypanosomatid 'oddities' justifying the attention, including a historical coverage, given to this subject.

Organization of the kinetoplast DNA

The defining feature of the class Kinetoplastea is the existence of the kinetoplast, a particular region of the cell's single mitochondrion, with the bulk of mitochondrial DNA. Due to its intense staining with the basophilic dyes, this structure could be easily detected using light microscopy, which facilitated its discovery more than a century ago (Laveran and Mesnil, 1901). The kinetoplast's location adjacent to the basal body of the flagellum led early researchers to believe that this organelle might be involved in the flagellar kinetic properties and named it accordingly (Alexeieff, 1917). With the advent of electron microscopy, it was found that kinetoplast contains highly compacted DNA (termed kinetoplast DNA or kDNA) and that purified kDNA represents a network composed of thousands of catenated heterogeneous minicircles (Steinert, 1960; Kleisen and Borst, 1975; Vickerman, 1976). The size of minicircles was species-specific and in most cases varies from 1 to 2.5 kb, although species with larger size minicircles were also found (Kidane *et al.*, 1984; Yurchenko *et al.*, 1999). Each minicircle molecule contains from one to four (depending on the species) conserved regions with the remaining sequence forming the variable region(s) (Ray, 1989; Simpson, 1997; Yurchenko and Kolesnikov, 2001). The number of sequence classes can differ greatly even among the related species, with some species (e.g. *T. equiperdum*) having almost homogeneous minicircles and others (e.g. *T. brucei*) displaying hundreds of classes of minicircles per network (Lai *et al.*, 2008; Koslowsky *et al.*, 2014). These properties of minicircles, as well as the localization of the protein-coding genes, remained enigmatic until the discovery of maxicircles and RNA editing. The former was detected as a minor component of kDNA with the size (varying from 20 kb in *T. brucei* to 40 kb in *T. cruzi*) comparable with that of other mitochondrial genomes (Borst and Fase-Fowler, 1979; Simpson, 1979). Maxicircles from all investigated species contained a 16 kb 'conserved' region with the colinear arrangement of the cross-hybridizing DNA

fragments (Muhich *et al.*, 1983; Maslov *et al.*, 1984). Maxicircle size differences were attributed to a 'divergent' region that is composed of repeats highly variable in size and sequence (Muhich *et al.*, 1985; Horváth *et al.*, 1990; Flegontov *et al.*, 2006). This region may contain the origins of maxicircle DNA replication (Myler *et al.*, 1993), but its exact function remains elusive even today.

Maxicircles and RNA editing

DNA sequencing of the conserved region in *L. tarentolae* and *T. brucei* revealed a set of protein-coding genes typical for mitochondria: 12S (large subunit) and 9S (small subunit) ribosomal RNA genes, three subunits of cytochrome *c* oxidase (COI, COII and COIII), a subunit (Cyb) of the cytochrome bc_1 complex, several subunits of NADH dehydrogenase (ND1, ND3, ND5, ND7) and one subunit of F_1F_o ATP synthase (A6) (Benne *et al.*, 1983; de la Cruz *et al.*, 1984). There were also six G-rich regions (G1–G6) and several reading frames with no recognizable function (MURF1, MURF2, MUR5) (Simpson *et al.*, 2015). Surprisingly, some of the identified genes appeared to be defective: thus, the proper initiator codons were absent in COIII and Cyb, and there was a −1 frameshift in COII. Analysis of the cDNA in *C. fasciculata* showed that the −1 frameshift in the COII DNA sequence is 'edited' by insertion of four U-residues in the mRNA (Benne *et al.*, 1986). Subsequently, it was shown that RNA editing is responsible for repairing the aforementioned defects present in the original (pre-edited) mRNAs thereby converting the pre-edited transcripts into translatable (fully edited) mRNAs (van der Spek *et al.*, 1988; Feagin *et al.*, 1988*b*). The amount of editing required for different transcripts varies drastically. Thus, a relatively modest editing by insertion of a dozen or so (and removal of a smaller number) of U-residues takes place in Cyb, MURF2, ND7 and COIII mRNAs in *L. tarentolae* (5′-editing and internal frameshift correction). On the other side of the spectrum are the A6, COIII and ND7 mRNAs of *T. brucei*, which emerge from pre-edited transcripts by incorporating hundreds of U-residues (and also deleting a small number of some of the maxicircle-encoded U-residues) (Feagin *et al.*, 1988*a*; Koslowsky *et al.*, 1990). Such cases of massive editing were termed 'pan-editing', while the respective genomic regions were termed 'cryptogenes'. In addition, six maxicircle G-rich regions turned out to represent pan-edited cryptogenes for five NADH dehydrogenase subunits and ribosomal protein S12 (RPS12) (Maslov *et al.*, 1992; Read *et al.*, 1992; Thiemann *et al.*, 1994). The editing of A6 transcript extends its reading frame by almost one third of its original length in *L. tarentolae* (Maslov and Simpson, 1992), and it is pan-edited in *T. brucei* (Bhat *et al.*, 1990). Thus, the maxicircles in both flagellates contain the same set of genes, but vary in the amount of editing for some of them. The other studied trypanosomatids have the same gene organization pattern, with the notable exception of the plant parasites *Phytomonas* spp., which lack cytochrome *c* oxidase and apocytochrome *b* complexes in its inner mitochondrial membrane (Maslov *et al.*, 1999; Nawathean and Maslov, 2000). Accordingly, genes for the respective subunits (COI–COIII and Cyb) are missing from the maxicircle conserved region, while the rest of its gene content remains intact.

The question regarding the source of the sequence information for guiding was resolved by the search for maxicircle sequences complementary (allowing G-U base pairing) to the fully edited sequences of COII, ND7, MURF2 and Cyb. This analysis led to the identification of small transcripts, termed guide (g) RNAs (Blum *et al.*, 1990). Soon thereafter, gRNA genes were discovered in the variable region of minicircles, solving the long-standing mystery of the functional role of these molecules (Pollard *et al.*,

1990; Sturm and Simpson, 1990a). The rationale for partitioning the gRNA genes between the maxicircles and the minicircles remains unclear, as in some cases both types participate in the editing of the same transcript. The mature gRNAs are 40–50 nt long and contain a post-transcriptionally added oligo(U)-tail on the 3′-end (Blum and Simpson, 1990). As the result of editing, a perfect sequence match is achieved between the mRNA and gRNA sequences. A single gRNA is sufficiently long to cover a stretch of the pre-edited sequence, which typically includes less than 20–30 insertions and a few deletions, termed 'editing block' (Simpson et al., 1993). Cryptogene-derived mRNAs are edited over the entire length and require editing by multiple gRNAs. The editing begins at the 3′-end of a pre-edited transcript and gradually spreads upstream so that the 5′-end of the mRNA is edited last (Sturm and Simpson, 1990b; Maslov et al., 1992).

When there is little or no redundancy in the gRNA content, a loss of a single gRNA would render completion of editing impossible. The stochastic nature of minicircle inheritance during the cell division makes such a loss a real possibility (Savill and Higgs, 1999). The ensuing disruption of the productive editing for even a single gene is likely to be lethal when each of the maxicircle products is required at least at some stage of the parasite's life cycle. Thus, the selection ensures the maintenance of a full editing capability in natural populations. However, mutants with editing loss for a dispensable product can survive in culture. This is the case of some strains of L. tarentolae, which display disrupted editing of several pan-edited mRNAs due to the loss of minicircle classes (Thiemann et al., 1994). A similar disruption of editing was observed for several strains of C. fasciculata, L. donovani and P. serpens (Sloof et al., 1994; Maslov et al., 1998; Neboháčová et al., 2009). So far, the only studied species, which maintain the full editing capacity in culture are L. mexicana (Maslov, 2010) and T. brucei, the latter case likely due to the high redundancy of its gRNA repertoire (Corell et al., 1993; Riley et al., 1994). It should be mentioned that in T. brucei, unlike other species, each minicircle encodes up to three different gRNAs and the number of minicircle classes is comparatively high, suggesting that this species is more refractory to an occasional loss of a minicircle class (Hong and Simpson, 2003).

Alternatively and partially edited RNA molecules may co-exist together with the fully edited mRNAs, contributing to the diversity of mitochondrial proteins (Ochsenreiter et al., 2008; Hajduk and Ochsenreiter, 2010; Gerasimov et al., 2018).

Evolution of editing

The evolutionary origin of editing and the rationale for its existence remain obscure (Simpson and Maslov, 1994a; Lukeš et al., 2005). So far, there is no satisfactory scenario explaining the origin of this process from the metabolic or gene regulation standpoint regardless of whether or not it was subsequently employed for any such purpose. An attractive hypothesis is the origin by constructive neutral evolution (CNE) (Lukeš et al., 2011; Gray, 2012), yet this remains a speculative scenario. CNE is a neutral evolutionary theory which aims to explain some aspects of cellular complexity by mechanisms that do not rely on positive selection (Stoltzfus, 1999). In this scenario, numerous T-deletions or insertions in kDNA would be tolerated due to the fortuitous interactions made possible by the pre-existence of enzymatic activities capable of restoring these mutations at the RNA levels ('presuppression'). Such activities, e.g. endo- and exonucleases, RNA ligase, would be derived from the cellular systems originally serving some other purpose(s). However, such interactions would lead to eventual formation of the dependence on such activities for kDNA gene expression, and thus, to preservation and further evolution of the editing machinery. A somewhat

extended version called 'irremediable complexity' postulates that when a given cellular component acquires mutations making it dependent on another component, such dependence will become complex and irreversible (Lukeš et al., 2011). Thus, CNE is evolutionary ratchet responsible for a steady increase of overall organismal complexity. However, selective advantages were also associated with the emergence of editing. One scenario postulates that as a consequence of pan-editing, information necessary for production of several proteins is spread over the kDNA, preventing loss of genes in parts of the life cycle when their products are not required (Speijer, 2006). In any case, since the U-insertion/ deletion type of editing is also found in various bodonids (Lukeš et al., 1994; Maslov and Simpson, 1994; Blom et al., 1998), while it is absent from their sister group Diplonemida (Faktorová et al., 2018), its origin likely coincided with that of the entire taxon Kinetoplastea, and for that matter with the origin of the kinetoplast itself (Lukeš et al., 2002; Simpson et al., 2002). The kDNA essentially represents the depository for the gRNA genes, so it is likely that its various forms emerged as different evolutionary answers to the problem of how to organize and maintain the extensive gRNA diversity in proximity to the editing itself (Lukeš et al., 2002). A minicircle-based concatenated network is the type that appeared in the ancestral trypanosomatids, and it is likely that pan-editing is also an ancestral trait for this taxon. If we assume, that editing per se does not play a significant or vital role, but is merely a product of the CNE, then it represents a substantial burden for the cells. However, the cells depend on it for mitochondrial mRNA production and, unlike in culture, a loss of productive editing in nature would be lethal due to the parasite's inability to complete its life cycle. The gRNA redundancy and diversity observed in T. brucei may have been developed in this phylogenetic lineage for preservation of the editing in spite of an occasional minicircle loss. A different evolutionary trend is observed in other trypanosomatid lineages, such as Leishmania and some monoxenous species, in which the ancestral cryptogenes appear to have been substituted by their less-edited counterparts (Maslov et al., 1994; Simpson and Maslov, 1994a). The replacement might have occurred via homologous recombination between a cDNA copy of the partially edited mRNA and the cryptogene (Simpson and Maslov, 1994b). This would in turn eliminate the essentiality of several gRNAs and the respective minicircle classes. As the copy numbers of the remaining minicircles would proportionally increase, the likelihood of their loss due to mis-segregation would decrease, thereby creating a more stable genetic system in the kinetoplast. The only cryptogene apparently unaffected by the replacement trend is RPS12, which is invariably pan-edited in all studied trypanosomatid and bodonid species. This may be related to the fact that this mRNA encodes an indispensable mitoribosomal protein and any change in its synthesis would impact translation of all mitochondrial transcripts, including those, which do not require editing, e.g. COI. Thus, preserving pan-editing of RPS12 mRNA might be important for coordination of the editing and translation systems (Aphasizheva et al., 2013).

Kinetoplast DNA replication

The problem of minicircle loss is alleviated to some degree by the evolution of a unique mechanism of kDNA replication, described here for T. brucei and other trypanosomatids, all of which have a catenated network composed of relaxed circles. Synthesis of kDNA occurs during the S phase of the cell cycle, while segregation of the daughter networks, along with the tightly coupled process of the flagellar duplication, is completed during the G_2 phase (Simpson and Kretzer, 1997). This strict timing is controlled by a cell cycle-dependent regulation of the key enzymes participating

in the process (Hines and Ray, 1997; Li *et al.*, 2007). The replication process has been described in a series of recent reviews (Klingbeil and Englund, 2004; Liu *et al.*, 2005; Jensen and Englund, 2012; Povelones, 2014), and is presented here only briefly. The minicircles are released from the covalently closed network and replicated in the kinetoflagellar zone (KFZ), which represents an intra-mitochondrial compartment between the kDNA and the basal body of the flagellum. The nicked or gapped daughter molecules are reattached to the network's periphery at the two antipodal sites, thereby slowly increasing the network's size. A yet unknown mechanism rotates the replicating network to ensure an even distribution of the reattached molecules. When all minicircles have been replicated, the kDNA network doubles in size and each minicircle contains nicks, which are closed before the network splits into two. This apparently highly complex mechanism serves to ascertain that each minicircle replicates only once. The antipodal attachment and network rotation likely serve to maximize the chances for the two daughter minicircles to segregate into the different networks during network division. Throughout the cell cycle, the kDNA network remains associated with the flagellar basal and parabasal bodies by a filamentous structure, termed TAC (tripartite attachment complex). This physical connection is thought to ascertain the coordinated duplication of the kDNA network and the flagellar apparatus.

During late stages of the kDNA replication, the two segregating sister kDNA networks remain for some time attached by a thin yet morphologically prominent connector, termed umbiliculum or nabelschnur, a filamentous structure which is cut at the final stage of the daughter network segregation (Gluenz *et al.*, 2007). It is likely composed of numerous dedicated proteins, with leucine aminopeptidase 1 being the only one identified so far (Pena-Diaz *et al.*, 2017).

Core catalytic activities of RNA editing

The recapitulation of the U-insertion or U-deletion at a single editing site (ES) *in vitro* using synthesized double-stranded (ds) RNA substrates and mitochondrial lysates supported the original hypothesis that trypanosome RNA editing requires a cascade of enzymatic activities (Blum *et al.*, 1990; Kable *et al.*, 1996; Seiwert *et al.*, 1996). These catalytic steps are orchestrated by the RNA editing core complex (RECC), also known as the 20S editosome or L-complex, the former alias reflecting the sedimentation coefficient of the enzymatically active complex (Simpson *et al.*, 2004; Stuart *et al.*, 2005; Read *et al.*, 2016). In *T. brucei*, RECC is made up of about 20 components abbreviated as kinetoplastid RNA editing (KRE) proteins (Stuart *et al.*, 2005). These subunits include two RNA ligases (KREL1 and 2) and U-specific exonucleases (KREX1 and 2), a terminal U transferase (KRET2) and three RNase III endonucleases (KREN1-3). Other subunits such as six OB-fold bearing proteins (KREPA1-6) serve a structural and/or RNA- binding role.

RECC catalyses the following steps. First, at the gRNA defined ES, the mRNA is cleaved by one of the KRENs to yield 5′ and 3′ fragments that are bridged by the bound gRNA. Next, depending on the gRNA information domain sequence, one or more Us are added by KRET2 or removed by KREX2 from the 5′-fragment. The catalytic activity of KREX1 is dispensable *in vivo* and the protein may play a more structural role (Rogers *et al.*, 2007; Carnes *et al.*, 2012). Finally, after the ES has been edited to be complementary to the gRNA information domain, the two mRNA fragments are sealed together by KREL1. The other ligase KREL2 plays a still undefined role in RNA editing that is expendable for this final step (Gao and Simpson, 2003).

The recognition that a given ES requires U-insertion, U-deletion or the editing of *cis*-gRNA-containing COX2 is

mediated by one of the three endonucleases, which specifically cleave only one type of dsRNA substrate (Carnes *et al.*, 2008). Typically, RNase III endonucleases form homodimers to cleave both dsRNA strands (MacRae and Doudna, 2007). Because RNA editing requires only the cleavage of the mRNA strand, each KREN protein dimerizes with its own unique catalytically inert partner (Carnes *et al.*, 2011). It remains unclear whether these KREN containing RECCs represent different, stable isoforms that specialize in processing a specific ES type, or if they represent discrete modules that are selectively added onto RECC depending on the bound ES. However, it is clear that editing of several ESs defined by one gRNA and pan-editing of mRNAs requiring multiple gRNAs requires a dynamic machinery involving more than RECC alone.

Multi-core processing: the MRB1 and other complexes

While the core editing activities encapsulated by RECC can be observed *in vitro*, the whole process of decrypting the ORFs of the pan-edited mRNAs that require a cascade of gRNAs cannot. Furthermore, RECC *in vitro* editing requires an already annealed dsRNA substrate, indicating that other factors are needed for the recruitment of one or both types of substrate RNAs to the complex for processing and/or other activities. The identification of molecules mediating such roles was started with the discovery of a dynamic collection of ~31 proteins with an association with RNA editing. These proteins were initially called either the mitochondrial RNA binding complex 1 (MRB1) (Hashimi *et al.*, 2008; Panigrahi *et al.*, 2008) or guide RNA binding complex (GRBC) (Weng *et al.*, 2008), the latter designation later ascribed to a sub-complex (see below) and replaced with RNA editing substrate binding complex (RESC) (Aphasizheva *et al.*, 2014). This complex will be referred to herein as MRB1 (Ammerman *et al.*, 2012).

Further refinement of MRB1 architecture has revealed that it is made up of two sub-complexes with different roles in RNA editing (Ammerman *et al.*, 2012; Aphasizheva *et al.*, 2014). Persistent in all reported MRB1 purifications are seven proteins that make up the MRB1 core (Read *et al.*, 2016). The paralogous gRNA associated proteins (GAPs) 1 and 2 form a heterotetramer that binds gRNAs, a requisite for their stability, are the only verified RNA-binding proteins of the MRB1 core (Weng *et al.*, 2008; Hashimi *et al.*, 2009). Thus, the editing of *cis*-gRNA-containing COX2 is not affected by their RNAi-silencing (Hashimi *et al.*, 2009). Knockdown of the other core proteins does not destabilize gRNAs but appears to affect RNA editing initiation (Acestor *et al.*, 2009; Ammerman *et al.*, 2013; Huang *et al.*, 2015). Thus, it has been proposed that the MRB1 core plays a role in editing initiation (Read *et al.*, 2016), although a general effect of MRB1 core ablation on gRNA utilization could be masked by an impaired gRNA phenotype. Since the GAP1/2 heterotetramer seems to have an extra-MRB1 localization, it may be involved in gRNA delivery to the editing reaction center, where these molecules pair with their cognate mRNAs.

The RNA editing mediator complex makes up the other major sub-complex of MRB1. It contains several RNA binding proteins, such as TbRGG2 (Ammerman *et al.*, 2010; Foda *et al.*, 2012), as well novel RNA binding proteins such as MRB8180 (Simpson *et al.*, 2017) or paralogues MRB8170 and MRB4160 (Kafková *et al.*, 2012; Dixit *et al.*, 2017). Ablation of these subunits preferentially leads to a stalling of pan-editing, which requires a cascade of gRNAs for its 3′–5′ progression (Fisk *et al.*, 2008; Kafková *et al.*, 2012), suggesting a role of this sub-complex in mediating this process.

It has been proposed that the two sub-complexes that make up MRB1 together with the core catalytic RECC represent the true

editosome holoenzyme (Aphasizheva et al., 2014; Aphasizheva and Aphasizhev, 2016). Certainly, the demonstrated roles of each of these modules can together account for the predicted machinery needed to decrypt a pan-edited mRNA. The reader can explore recent reviews dedicated to trypanosome RNA editing for more detailed discussions about the proteins and complexes involved in this fascinating phenomenon (Aphasizhev and Aphasizheva, 2011; Hashimi et al., 2013; Read et al., 2016).

The abundant mitochondrial RNA-binding proteins (MRP) 1 and 2 form a hetero-tetrameric complex with an electropositive surface that facilitates binding of the negatively charged sugar–phosphate backbone of RNA (Schumacher et al., 2006). Upon binding to the MRP1/2 complex, the RNA bases extrude out to be available for annealing complementary RNA, which is consistent with a proposed role in gRNA:mRNA annealing (Müller et al., 2001; Zíková et al., 2008a). A Nudix hydrolase was also pulled down with MRP1 that was later found to be part of the multiprotein mitochondrial edited RNA stability factor 1, MERS1 (Weng et al., 2008). A recently discovered complex containing the terminal uridylyl-transferase (Aphasizhev et al., 2003) and a homologue of a yeast 3′–5′ exonuclease (Mattiacio and Read, 2008) appears necessary for gRNA maturation (Suematsu et al., 2016).

Mitochondrial protein synthesis and the mRNA recognition problem

While the large body of evidence indicated that mitochondrial protein synthesis is responsible for the production of indispensable subunits of the respiratory complexes, its biochemical purification was problematic because kinetoplast-encoded polypeptides are extremely hydrophobic (Speijer et al., 1997). As of today, at least four proteins were confirmed as products of mitochondrial translation (Horváth et al., 2002; Škodová-Sveráková et al., 2015).

Pre-edited and partially edited transcripts are relatively abundant in the steady-state RNA population. Since they cannot be productively translated, it is likely that there is a mechanism allowing for an exclusive recognition of fully edited, translation-competent mRNAs. It was recognized early that this mechanism cannot be reduced to a simple creation of the initiation codon or a Shine-Dalgarno-like sequence. Yet, a reasonable possibility was that editing creates some form of a translatability hallmark on the mRNA's 5′-end, in particular, because the arrival of editing at the 5′-end indicates that the entire sequence downstream has been edited and is, therefore, translatable. Although the mechanisms involved have not yet been fully elucidated, there has been a significant progress in this direction over the last several years.

Early investigations showed that fully edited mRNAs possess two types of 3′-end poly(A)-tails: the short, ~20–30 nt and the long, ~200–300 nt, while pre-edited and partially edited mRNAs contain only a short tail (Bhat et al., 1990; Kao and Read, 2005). Subsequently, it was shown that conditional upon completion of editing, the initial short tail is extended to become a long A/U heteropolymer (Etheridge et al., 2008). This reaction is performed by a protein complex composed of two catalytic proteins (KPAP1, a poly(A) polymerase, and RET1, an uridylyl transferase) and two auxiliary proteins (KPAF1 and KPAF2) (Aphasizhev et al., 2011). The latter belong to the large family of PPR (pentatricopeptide repeat) proteins, which are relatively abundant in trypanosomatids. Discovered in plants, these proteins are involved in numerous aspects of mRNA maturation and translation in plants organelles, and they proved to play very important roles in the kinetoplasts as well (Aphasizhev and Aphasizheva, 2013). Inactivation of KPAF1 by RNAi resulted in a loss of the long (A/U)-tails, disruption of the mRNA interaction with the mitoribosomes and the cessation of the COI and Cyb synthesis. An attractive hypothesis that some of these PPR

proteins may act as mRNA-specific translational activators was supported by a differential effect on the (A/U)-tailing and translation of COI and Cyb polypeptides caused by inactivation of KRIPP1 and KRIPP8 ribosomal PPR proteins in T. brucei (Aphasizheva et al., 2016). These proteins are components of a 45S complex, which also contains the 9S SSU rRNA, a set of small subunit ribosomal proteins and several additional PPR proteins (Maslov et al., 2007). This complex, termed 45S SSU*, is abundant in procyclic T. brucei, but downregulated in its bloodstream stage (Ridlon et al., 2013). Its disruption in procyclics abolished the poly(A/U)-tailing and translation of several mRNAs, which are expressed during this stage of the life cycle, but did not affect constitutively expressed products such as RPS12 and A6 (Ridlon et al., 2013; Aphasizheva et al., 2016), suggesting that 45S SSU* complex is involved in the developmental regulation of mitochondrial translation in this species. Although details of this process remain unknown, the available data allow to speculate that a specific cis-signal is created upon completion of editing and the mRNA's 5′-end is recognized by an mRNA-specific PPR protein. This protein, acting as an mRNA specific translation activator, in turn mediates poly(A/U) tailing and recognition of the translation competent mRNA by the 45S SSU* complex. The steady-state level of kinetoplast-mitochondrial 50S ribosomes is low in comparison to ribosomal large subunits (Maslov et al., 2006; Ridlon et al., 2013), leading to a hypothesis that the active translation complex, which sediments at >80S, each time assembles de novo by association of the 40S large ribosomal subunit with the mRNA recognition complex.

The structure of the 50S Leishmania mitochondrial ribosomes has been investigated in detail by cryoelectron microscopy (Sharma et al., 2009). Surprisingly, the overall morphology of the 50S monosome appears remarkably eubacterial in spite of the drastic differences in the RNA and protein structure and composition. Indeed, the sizes of the 9S and 12S rRNAs are substantially smaller and their secondary structure lacks several stem-loop elements present in their eubacterial counterparts (Eperon et al., 1983; de la Cruz et al., 1985a, 1985b). The protein content represents a mixture of the conserved ribosomal and trypanosomatid-specific proteins (Maslov et al., 2006; 2007; Zíková et al., 2008b; Aphasizheva et al., 2011). In cryoelectron microscopy model, the missing RNA masses are only partially replaced by proteins resulting in an overall porous structure of the mitoribosome. A number of the functionally important regions, such as the mRNA and tRNA paths, and nascent polypeptide exit channel contain trypanosomatid-specific proteins or show other peculiarities, apparently reflecting the idiosyncratic modus operandi of these ribosomes, including its resistance to most inhibitors of protein synthesis (Sharma et al., 2009; Hashimi et al., 2016).

Mitoproteome

Mostly because of RNA editing and kDNA, the T. brucei mitochondrion belongs to the best studied organelles of unicellular eukaryotes. As a consequence, a high-quality mitoproteome became available (Panigrahi et al., 2009) and was used for identification of novel protein functions. The most prominent case is finding a protein responsible for the import of Ca^{2+} into the mitochondrion, an activity known for decades. Yet, the protein responsible for Ca^{2+} uptake, called the mitochondrial calcium uniporter (MCU), remained elusive until recently. It was the comparison of numerous mitochondrial profiles of organisms, known to either possess or lack this capacity, which facilitated discovery of the MCU (Baughman et al., 2011; Docampo and Lukeš, 2012).

Interestingly, this was not the only case. The prominent absence of several conserved proteins in the T. brucei mitoproteome was used in phylogenetic profiling, which resulted in

identification of several novel assembly factors of the human respiratory complex I (Pagliarini *et al.*, 2008). It is safe to predict that by virtue of being the only mitochondrion in the cell and by its significant functional and structural up- and downregulation throughout the life cycle (Zíková *et al.*, 2017), kinetoplast is particularly suitable for studies of processes that control mitochondrial functions and will provide important insight in this respect.

Organelles

Glycosomes

Virtually all eukaryotic cells have peroxisomes, i.e. microbodies involved in catabolism of long chain fatty acids, branched chain fatty acids, D-amino acids, polyamines, reduction of reactive oxygen species (ROS), specifically hydrogen peroxide, and biosynthesis of plasmalogen ether phospholipids. In trypanosomatids, glycolysis is associated with specialized peroxisomes called glycosomes, containing six enzymes involved in the early part of the glycolytic pathway, and two enzymes of glycerol metabolism (Opperdoes and Borst, 1977). In addition, trypanosomatid glycosomes are involved in gluconeogenesis, NADPH production *via* the glucose-6-phosphate dehydrogenase enzymes (Heise and Opperdoes, 1999), purine salvage and phosphate metabolism (Szöör *et al.*, 2014; Gabaldón *et al.*, 2016). None of the human-infective trypanosomatids (i.e. *Leishmania* spp., *T. brucei* or *T. cruzi*) possess a gene for the typical peroxisomal marker enzyme, catalase (Kraeva *et al.*, 2017). Only monoxenous *Crithidia* and *Leptomonas* spp. have a catalase gene (Opperdoes *et al.*, 2016), although the enzyme is not present in peroxisomes, but in the cytosol (Souto-Padron and de Souza, 1982). Interestingly, the related cryptobiids have peroxisomes/glycosomes with catalase activity (Opperdoes *et al.*, 1988; Ardelli *et al.*, 2000), while *B. saltans*, the closest known bodonid relative of trypanosomatids, lacks this gene.

The presence of an NADP-dependent isocitrate dehydrogenase and one of the four Fe-superoxide dismutase isoenzymes in glycosomes suggest that sufficient ROS protection mechanisms must be present in these organelles (Dufernez *et al.*, 2006). However, enzymes of the glyoxylate cycle, reported to be present in the peroxisomes of ciliates (Simon *et al.*, 1978) and two other typical peroxisomal marker enzymes, D-amino acid oxidase and 2-hydroxy acid oxidase, were not detected in any trypanosomatids, or *B. saltans* genomes (Opperdoes *et al.*, 2016).

Many orthologues of glycosomal proteins well-characterized in trypanosomatids were recently identified in *B. saltans* (Opperdoes *et al.*, 2016) suggesting their peroxisomes fulfill functions similar to those of trypanosomatid glycosomes. For a detailed account of the functions of glycosomes in trypanosomatid the reader is referred to recent papers (Opperdoes, 1987; Opperdoes and Szikora, 2006; Vertommen *et al.*, 2008; Haanstra *et al.*, 2016).

Traffic of solutes between cytosol and glycosomes

Solutes, such as small metabolites, cofactors, and acyl-CoAs, all seem to be translocated by specific transporter molecules, such as ATP-binding cassette (ABC) transporters and membrane channels. Three ABC transporters, called Glycosomal ABC transporters 1–3 (GAT1-3), have been identified in the glycosomal membrane of *T. brucei*, where they mediate ATP-dependent uptake of solutes from the cytosol into the glycosomal matrix. GAT1 was shown to transport primarily oleoyl-CoA (Igoillo-Esteve *et al.*, 2011). Smaller solutes, such as glycolytic intermediates, probably cross the membrane through several types of pore-forming channels (Gualdrón-López *et al.*, 2012).

The glycosome as an example of mathematical modelling

The long history of quantitative research and the detailed knowledge about the enzymes of carbohydrate metabolism, the reactions they catalyse, and their compartmentation within the glycosomes, has allowed one to construct a reliable kinetic computer model of trypanosome glycolysis (Bakker *et al.*, 1997; 2000; Haanstra *et al.*, 2008). Owing to this kinetic model, African trypanosomes have emerged as promising unicellular model organisms for the next generation of systems biology. The results are compiled in 'Silicon Trypanosome', a comprehensive, experiment-based, multi-scale mathematical model of trypanosome physiology (Bakker *et al.*, 2010). It is anticipated that quantitative modelling enabled by the 'Silicon Trypanosome' will play a key role in selecting the most suitable targets for developing new anti-parasite drugs.

Acidocalcisomes

Acidocalcisomes were first discovered in trypanosomes (Docampo *et al.*, 2005). They are 100 to and 200 nm in diameter electron-dense acidic organelles serving as the primary calcium (Ca^{2+}) reservoir, that is also rich in phosphate in the form of orthophosphate (Pi), pyrophosphate (PPi) and polyphosphate (polyP) (Lander *et al.*, 2016). Their internal acidity is maintained by proton pumps such as the vacuolar proton pyrophosphatase (V-H + -PPase, or VP1), the vacuolar proton ATPase (V-H + -ATPase), or both (Docampo, 2016). In addition to a number of protein pumps and antiporters, including aquaporins, the acidocalcisomal membranes contain various ATPases and Ca^{2+}/H^+ and Na^+/H^+ antiporters, suggesting a complex energetic requirement for their maintenance. The acidocalcisomes also play a role in autophagy and osmoregulation (Docampo, 2016; Docampo and Huang, 2016). When *T. cruzi* is exposed to an osmotic shock, these organelles located in the vicinity of the contractile vacuole fuse with it, thereby increasing its osmolarity. As a consequence, water from the cytoplasm enters the vacuole for expulsion (Rohloff *et al.*, 2004). The release of an important second messenger Ca^{2+} from intracellular stores is controlled by the inositol 1,4,5-trisphosphate receptor located inside the acidocalcisomes, while a plasma membrane Ca^{2+}-ATPase controls the cytosolic Ca^{2+} level. In trypanosomatids with an intracellular life stage, Ca^{2+}signalling is proposed to govern host cell invasion (Docampo and Huang, 2016).

Highly flexible flagellum

All trypanosomatids are equipped with a single flagellum (although, there is an 'amastigote' stage in some life cycles, characterized by an extremely short flagellum), which represents the most prominent morphological difference from their bodonid kins with two flagella (Adl *et al.*, 2012). The flagellum length is highly variable between and even within species, yet its structure is highly conserved and unique for this group of protists. It is also a highly flexible structure mostly involved in attachment, locomotion and environment sensing (Broadhead *et al.*, 2006; Hughes *et al.*, 2012). During the life cycle, the flagellum is subject to substantial restructuring to adapt to different functions (Ginger *et al.*, 2008). In the best-studied species, *T. brucei*, the flagellar motility is required for cell division, transmission *via* a vector, immune evasion (Engstler *et al.*, 2007) and is also intimately associated with the vital flagellar pocket structure (Field and Carrington, 2009). Recently, additional functions of this dexterous cellular component such as the production of the extracellular vesicles, which may mediate interaction with the vertebrate host, have been described (Szempruch *et al.*, 2016). Furthermore, protein

exchange between two trypanosomes seems to occur by flagellar membrane exchange, and both short and long-term fusions have been observed in cultured trypanosomes (Imhof et al., 2016).

The trypanosomatid flagellum, responsible for motility, contains the classical 9 + 2 axoneme (Ginger et al., 2008). The 9 + 0 axoneme has been observed in the amastigote stages of Leishmania spp., where the flagellum is likely to be more engaged in sensing and signalling (Wheeler et al., 2015). A characteristic feature of the trypanosomatid flagellum is the paraflagellar rod, an extra-axonemal structure. It is very prominent in some species (Yurchenko et al., 2006a; Maslov et al., 2010) and almost invisible in others (Yurchenko et al., 2014), with the arrangement of thin and thick filaments also being species-specific (Gadelha et al., 2005; Sant'Anna et al., 2005). So far, about 30 proteins have been identified as components of the T. brucei paraflagellar rod (Portman and Gull, 2010). Their ablation or deletion often, but not always results in a dramatic decrease in flagellar beating frequency. Interestingly, while in the procyclic stage of T. brucei RNAi-mediated downregulation of paraflagellar proteins occasionally causes cytokinesis defects (Farr and Gull, 2009), in the bloodstream stage flagellar motility seems to be invariably essential for viability (Broadhead et al., 2006), and hence is of medical relevance. It was proposed that the paraflagellar rod might be a site for integrating external signals detected by the flagellum (Portman and Gull, 2010).

During their life cycle, the cell shape of most trypanosomatids undergoes dramatic morphological changes. These are controlled by a specialized cytoskeletal structure termed the flagellum attachment zone. It laterally attaches the flagellum to the cytoskeleton and seems to play a key role in determining trypanosomatid morphology (Sunter et al., 2015). The flagellum attachment zone ranges from an extended form in trypomastigotes to a very short one in promastigotes (Wheeler et al., 2015). Recently, the early-branching Paratrypanosoma confusum was shown to restructure its flagellum during the life cycle from a promastigote with a long flagellum to an amastigote-like stage with no external flagellum, and then to a cell in which the flagellum is remodeled into a thin attachment pad (Skalický et al., 2017). Hence, the enormous flexibility of the flagellum and related structures seems to be an ancestral feature that might have predetermined trypanosomatids for their evolutionary expansion.

Gene exchange

Cellular mechanisms

The importance of the question of whether the binary fission is the only (or at least the predominant) reproduction mode in trypanosomatids goes far beyond being purely academic. The existence of meiosis and potential for gamete fusion or a similar type of sexual process would determine if trypanosomes are capable of gene exchange as opposed to strictly asexual (clonal) propagation in natural populations (Tait, 1980). This question is central to our understanding of the origin and spread of pathogenic traits with obvious implications for epidemiology and treatment.

A sexual process was first demonstrated in African trypanosomes in hybridization experiments during co-infection of tsetse flies with two parental clonal lines of T. brucei (Jenni et al., 1986). Selection of the hybrids for double drug resistance had greatly facilitated identification of the recombinant progeny as the mating, which occurs in salivary glands of infected tsetse flies, was found to be non-obligatory (Gibson and Whittington, 1993; Gibson and Bailey, 1994). Interestingly, while kDNA minicircles were inherited from both parents, the maxicircles initially appeared to be inherited uniparentally (Gibson and Garside, 1990). However, subsequently it was demonstrated that the maxicircle inheritance is biparental, but the initial heteroplasmic state is rapidly eliminated due to a stochastic segregation of maxicircles during mitotic divisions (Turner et al., 1995). The inheritance pattern of nuclear chromosomes was biparental and consistent with Mendelian segregation and independent assortment, providing further proof for the meiosis involvement (Turner et al., 1990; Gibson and Garside, 1991; MacLeod et al., 2005).

Further insights into details of the sexual process were obtained upon the development of green and red fluorescent parental lines, allowing the detection of individual hybrid trypanosomes by yellow fluorescence directly in the salivary glands of double-infected tsetse flies (Gibson et al., 2008). Being epimastigotes, the hybrids were observed exclusively in the salivary glands as soon as the parental cells have reached this compartment. Moreover, by using fluorescent tagging, the expression of three meiosis-specific genes was found to take place during a certain time window in all tested T. brucei subspecies, indicating that all are capable of gene exchange (Peacock et al., 2011, 2014b). With the aim to identify products of the meiotic cell division (gametes), the green and red fluorescent cells were recovered from the salivary glands of infected flies at the peak of meiosis-specific gene expression and mixed ex vivo for microscopic examination (Peacock et al., 2014a). Putative gametes were observed as haploid fluorescent red and green promastigote-like cells with a single or two kinetoplasts and a single long flagellum. These cells would interact by intertwining their flagella and apparently undergoing fusion as indicated by the appearance of yellow fluorescent cells shortly thereafter.

The question about the existence of mating types in trypanosomes remains open. They are able to undergo intraclonal mating (self-fertilization), although it is far less efficient compared to mating between different parental cells, indicating either the absence of mating types or a rather unconventional mating type system (Turner et al., 1990; Tait et al., 1996; Peacock et al., 2009). Recent analyses confirmed that T. brucei crosses are inconsistent with a 'two mating types' system (Peacock et al., 2014b). It was further hypothesized that these mating types may be controlled by multiple alleles of variable efficiency and there exists a potential for mating type switching during development in tsetse flies (Peacock et al., 2014a). These studies have established that trypanosomes have an intrinsic ability to undergo meiosis and to produce hybrids by gametic fusion, albeit actual gene exchange is not mandatory in the T. brucei life cycle (Gibson, 2015). Both selfing and interclonal mating are possible, and the sexual process is not limited to a particular subgroup of African trypanosomes but represents a general property of these parasites.

Less is known about meiosis and gene exchange in other trypanosomatids. Meiosis-specific genes are also present in the genomes of Leishmania species and, perhaps, most other trypanosomatids (Ramesh et al., 2005; Speijer et al., 2015), as they were recently identified in the genomes of two Leptomonas spp. (Kraeva et al., 2015; Flegontov et al., 2016). Experimental evidence for hybrid formation in the sand fly vector has originally been obtained for Leishmania major (Akopyants et al., 2009). Most biparental hybrid clones, selected by double drug resistance, were diploid, but some were triploid, and the inheritance of the kDNA maxicircles appeared to be uniparental. The frequency of hybridization was rather low, at the level of $\sim 10^{-5}$. Subsequently, using a double (red-green) fluorescence system in L. donovani (Sádlová et al., 2011) it was shown that the hybrid cells appear as procyclic promastigotes (but see below) in the midgut of infected sand flies as early as day 2 post-blood meal. The hybrid cell lines could not be recovered precluding their further characterization. More recently, numerous interclonal hybrids were obtained for L. major (Inbar et al., 2013) and two intraclonal

hybrids were obtained for *L. infantum* (Calvo-Alvarez *et al.*, 2014). While the *L. major* hybrids were mostly diploid with the frequent occurrence of triploid and even some tetraploid cell lines, the two *L. infantum* hybrid clones were triploid. Interestingly, these were able to infect mice. While diploid hybrids are consistent with the model involving meiosis and a haploid gametic fusion, the triploid cells would be produced by a fusion of a haploid and a diploid cell, as was suggested for triploid hybrids formed in some crosses of *T. brucei* (Gibson *et al.*, 1992). The timing of hybrid formation in *L. major* suggested that nectomonads, rather than procyclic promastigotes, represent the mating-competent developmental stage (Inbar *et al.*, 2013). Hybrid formation frequency suggested a lack of a strict mating type system in *Leishmania*. Overall, although many details of the sexual process in *Leishmania* still need to be elucidated and some of its aspects are likely to differ from their counterparts in *T. brucei*, it is clear that in both cases there is a solid evidence for sex based on meiosis and subsequent fusion of haploid gametes, which occurs in the insect vector.

Considering insect trypanosomatids, in *Crithidia bombi*, a parasite of bumblebees, there is evidence for a meiosis-related process with allele segregation and recombination, although the cellular mechanisms involved remain uncharacterized (Schmid-Hempel *et al.*, 2011; Cisarovsky and Schmid-Hempel, 2014). The recently sequenced genome of this species (Schmid-Hempel *et al.*, 2018) will help in this regard.

A similar sexual process may also exist in *T. cruzi* as suggested by the presence of the conserved meiosis-specific genes (Ramesh *et al.*, 2005), although the limited experimental evidence obtained so far supports a different scenario (Gaunt *et al.*, 2003). Hybrid *T. cruzi* were formed exclusively during coinfection of a mammalian cell culture, representing the vertebrate stage of the life cycle, and not during passage through a triatomine bug vector. The hybrids were characterized by the inheritance of all parental alleles at most loci and massive aneuploidy. To explain these observations, a parasexual process has been implied, according to which nuclear fusion creates a tetraploid intermediate, that undergoes homologous recombination and partial genome reduction (Messenger and Miles, 2015). Still, the existence of a meiosis-related process and its role in the formation of the naturally occurring *T. cruzi* hybrid lineages remain an open question (Lewis *et al.*, 2011; Messenger and Miles, 2015).

Implications for population structure

A demonstration of a genetic recombination in laboratory settings, especially if the process is found to be non-obligatory, does not automatically entail its recognition as an important factor shaping the natural populations of that organism. It is difficult to overestimate the importance of the mode(s) of propagation of a parasite in nature [e.g. clonal, epidemic or panmictic (Smith *et al.*, 1993)] has for understanding its evolutionary trends, as well as the origin and spread of the disease it causes (Heitman, 2006). The main advantage of the strictly clonal mode is the possibility of a rapid propagation of the most successful gene combinations (or MLGs, multilocus genotypes), which are optimal (the fittest) under given conditions. However, the inevitable accumulation of deleterious mutations would lead to a decrease in fitness and, eventually, extinction – a situation known as Muller's ratchet. Introduction and spread of favourable mutations in populations can be achieved by a sexual process, although this comes at the cost of potentially disrupting the fittest MLGs by genetic recombination (Barton and Charlesworth, 1998). The population genetics of pathogenic trypanosomatids has, therefore, attracted significant attention (Tibayrenc and Ayala, 2013; 2015; 2017;

Ramirez and Llewellyn, 2014; Messenger and Miles, 2015; Rougeron *et al.*, 2017).

Based on evidence against meiotic segregation of alleles (fixed heterozygocity, deviation from the Hardy–Weinberg expectation) and against genetic recombination (strong linkage disequilibrium, ubiquitous multilocus genotypes) observed in the natural populations of several parasitic protists, including trypanosomes and leishmanias, a 'clonal theory' was proposed (Tibayrenc *et al.*, 1990; Tibayrenc and Ayala, 1991). It postulates that in the absence of any consequential impact of gene exchange on a given population structure, 'uniparental reproduction is, at least for the cases herein surveyed, predominant enough in natural populations to generate clones that are stable in space and time, even on an evolutionary time scale' (Tibayrenc *et al.*, 1990). Stated this way, the clonal theory, while focusing on the importance of clonal reproduction for certain taxa or populations, does not necessarily exclude the occurrence of scenarios in which gene exchange, no matter how (in)frequent, would play a significant role. With time, the theory has evolved to become known as 'predominantly clonal evolution' (PCE), apparently to emphasize the long-term and large-scale implications of limited or absent genetic exchange.

In populations of *T. cruzi*, the showcase species for clonal theory, the predominantly clonal propagation mode was originally developed by analyses of isoenzyme electrophoretic patterns (MLEE) (Tibayrenc and Ayala, 1988), randomly amplified loci (Tibayrenc *et al.*, 1993) and microsatellites (Oliveira *et al.*, 1998). These analyses revealed the existence of a complex population structure of these parasites (Miles *et al.*, 1978; McDaniel and Dvorak, 1993; Barnabe *et al.*, 2000) with the existence of six major phylogenetic lineages (Brisse *et al.*, 2000; 2001). The scale of genetic separation among these lineages was comparable with that of African trypanosomes or *Leishmania* spp., yet in the absence of the formal taxonomic status, the major lineages of *T. cruzi* were termed Discrete Typing Units (Tibayrenc, 1998). Reflecting the evidence for genetic exchange in natural populations (Machado and Ayala, 2001; Brisse *et al.*, 2003), the term 'near-clones'/'near-clades' has been proposed for them recently (Tibayrenc and Ayala, 2012; 2015). Indeed, as four of these near-clades have a hybrid origin (Sturm *et al.*, 2003; Westenberger *et al.*, 2005; Lewis *et al.*, 2011), the strict clonality model is untenable. The PCE model postulates that although recombination in *T. cruzi* was important on a large evolutionary scale, it was unable to 'prevent evolutionary divergence of the near-clades' (Tibayrenc and Ayala, 2015).

Consistent with meiosis-based gene exchange being an inherent part of the life cycle in *T. brucei*, this process has been found to play a large role in shaping its natural populations. As two of its formal subspecies (*T. b. rhodesiense* and *T. b. gambiense*) are the causative agents of Human African Trypanosomiasis, gene exchange among those and non-infective subspecies (*T. b. brucei*) is important for understanding the origin and dynamics of disease foci (Gibson and Stevens, 1999; Hide and Tait, 2009). As described above, the relative importance of gene exchange *vs* clonality was not the same among different constituents of this species (MacLeod *et al.*, 2001a). By analysis of highly polymorphic minisatellite loci, it was demonstrated that East and South African human-infective *T. b. rhodesiense* is diverse and some isolates of this subspecies are genetically closer to local non-infective strains (regarded as the *T. b. brucei* subspecies) rather than to other infective strains (MacLeod *et al.*, 2001a, 2001c). This indicates that *T. b. rhodesiense* is just a host range variant of *T. b. brucei*, the populations of which are neither panmictic nor strictly clonal, but show evidence of limited gene exchange (epidemic structure) (MacLeod *et al.*, 2000; 2001b). Considering that human infectivity is defined by the presence of a single gene (serum resistance associated gene (SRA)) (De Greef and

Hamers, 1994; Xong *et al.*, 1998), the data strongly suggested that new strains of *T. b. rhodesiense* arise by genetic recombination spreading the SRA gene among local populations of *T. b. brucei* (Gibson *et al.*, 2002; Balmer *et al.*, 2011). Subsequently, the idea of *T. b. rhodesiense* evolving from diverse genetic backgrounds of *T. b. brucei* has been supported by population genomics (Sistrom *et al.*, 2014) and microsatellite studies, with the latter demonstrating genetic exchange occurring between some *T. b. rhodesiense* strains (Duffy *et al.*, 2013; Echodu *et al.*, 2015) and supporting the clonality of some others (Kato *et al.*, 2016).

The second pathogenic subspecies, the West African *T. b. gambiense*, has a different set of adaptations for human infectivity (Uzureau *et al.*, 2013) and was found to form groups 1 and 2 by MLEE (Gibson, 1986; Godfrey *et al.*, 1990). Microsatellite locus typing has shown that group 1 is distinct, shows clear signs of strict clonality and is composed of a set of clades that occupy distinct geographic locations (Koffi *et al.*, 2007, 2009; Morrison *et al.*, 2008). Clonal evolution in group 1 trypanosomes was recently corroborated by a population genomics study demonstrating the independent accumulation of mutations in individual members of each homologous pair of chromosomes due to a lack of recombination, known as the 'Meselson' effect (Weir *et al.*, 2016). On the contrary, *T. b. gambiense* group 2 was found to be indistinguishable from local *T. b. brucei*, demonstrating evidence for gene exchange within and between human infective and non-infective trypanosomes (Capewell *et al.*, 2013). The reason for such a drastic difference between *T. b. gambiense* groups 1 and 2 remains unclear, especially because both groups possess and express meiosis-specific genes (Peacock *et al.*, 2014*b*). However, future population genomics studies may shed some more light. Indeed, the analysis of two *T. b. rhodesiense* genomes showed that these East African strains share some alleles with *T. b. gambiense* group 1, suggesting a gene flow between these subspecies in the past (Goodhead *et al.*, 2013). It remains unclear if this was mediated by the local populations of *T. b. brucei* or occurred directly between the two pathogenic subspecies. In any case, the emerging picture presents a highly dynamic system, in which successful propagation is achieved by a combination of clonality and gene exchange.

Clonality was also proposed initially as the predominant propagation mode for *Leishmania* (Tibayrenc *et al.*, 1990; Banuls *et al.*, 1999). Subsequently, a more complex picture has emerged in which both clonality and gene exchange play significant roles, called a 'mixed-mating reproductive strategy' (Rougeron *et al.*, 2017). While significant inbreeding and clonality signatures were found in populations of *L. braziliensis* and *L. guyanensis* (Rougeron *et al.*, 2009, 2011*a*; Kuhls *et al.*, 2013), the preponderance of clonality was stronger in studied populations of *L. donovani* (Rougeron *et al.*, 2011*b*). A recent population genomics study of *L. donovani* from epidemic foci in India showed evidence for drug resistance having spread among populations by genetic recombination, as well as for clonal propagation of the major genetic groups under study (Imamura *et al.*, 2016). Thus in a way similar to *T. brucei*, *Leishmania* spp. illustrate how a successful parasite is able to utilize the advantages provided by each of the available propagation modes.

Adaptation of metabolism to parasitic lifestyle by gain and loss of genes

All Kinetoplastea share a number of unique metabolic characteristics. Most prominent are: (i) glycosomes (Opperdoes, 1987); (ii) a set of Pyr genes of the pyrimidine biosynthetic pathway with typical prokaryotic features (Opperdoes and Michels, 2007); (iii) ATP-dependent phosphofructokinase (PFK), strongly resembling bacterial pyrophosphate (PPi)-dependent PFKs, along with a

PPi-dependent pyruvate phosphodikinase (Michels *et al.*, 1997; Cosenza *et al.*, 2002); (iv) multiple phosphoglycerate kinases (Barros-Alvarez *et al.*, 2014) and two glyceraldehyde-phosphate dehydrogenases (Michels *et al.*, 1991); (v) pyruvate kinase, allosterically regulated by the metabolic activator fructose-2,6-bisphosphate (van Schaftingen *et al.*, 1985); (vi) trypanothione, rather than glutathione, as the major thiol involved in protection against the oxidative stress (Fairlamb *et al.*, 1985); (vii) synthesis of fatty acids *via* a unique set of elongases (Lee *et al.*, 2007*a*), and (viii) a mitochondrial pathway for the 'anaerobic' excretion of acetate with net synthesis of ATP (van Hellemond *et al.*, 1998). Thus, the last common ancestor of *B. saltans* and the trypanosomatids, which must have lived around 600 million years ago (Parfrey *et al.*, 2011; Lukeš *et al.*, 2014), had already acquired many genes of either bacterial or algal origin responsible for the aforementioned traits (Hannaert *et al.*, 2003; Opperdoes and Coombs, 2007; Opperdoes and Michels, 2007).

Comparison of the genome sequence of *B. saltans* (Jackson *et al.*, 2016; Opperdoes *et al.*, 2016) with those available for a large number of trypanosomatids (Berriman *et al.*, 2005; Ivens *et al.*, 2005; El-Sayed *et al.*, 2005*a*, 2005*b*; Porcel *et al.*, 2014; Kraeva *et al.*, 2015; Flegontov *et al.*, 2016) reveals that the adoption of the parasitic lifestyle has led to a reduction in gene number approximately by half. Despite this dramatic reduction in gene number, *B. saltans* and Trypanosomatidae still share about 2800 homologous protein-coding genes. In this section we concentrate only on a core subset of 581 house-keeping genes involved in metabolism. We followed their losses and gains throughout trypanosomatid evolution, always using *B. saltans* as an outgroup. An interactive phylogenetic tree showing these gains and losses can be accessed at http://big.icp.ucl.ac.be/~opperd/metabolism/kinetoplastida_LGT4.html

Emergence of a parasite: the first steps

Iron is an essential element for all living organisms. In order to survive inside their hosts, parasites must gain access to their host's iron stores. Similar to disease-causing bacteria that release iron-binding molecules such as siderophores or scavenge iron from host haemoglobin and transferrin, parasites have developed mechanisms that allow them to compete for the limited amounts of free iron in the insect or mammalian host. A recent identification of a ferric iron reductase [LFR1 (Flannery *et al.*, 2011)], a ferrous iron transporter [LIT1 (Jacques *et al.*, 2010)], a haem transporter [LHR1 (Miguel *et al.*, 2013)] and the haem scavenging protein [LABCG5 (Flannery *et al.*, 2013)] as virulence factors of *Leishmania* spp., has allowed us to identify the sequence of events involved in putting essential trypanosomatid iron-capture mechanisms in place. One of the primary adaptations required for a parasitic lifestyle must have been the acquisition of a high-affinity receptor/transporter for the capture and internalization of ferrous iron. This permits the effective competition for the limited amounts of free iron in the tissue fluids of the insect host. Although the free-living common ancestor of trypanosomatids was able to reduce insoluble ferric iron to soluble ferrous iron by a ferric reductase (present in most Kinetoplastea including *Bodo*), a ferrous transporter was likely lacking in this organism. *Bodo saltans*, which can be considered as a proxy of such an ancestor, does not have this transporter, apparently because of its bacteriotrophic lifestyle providing the flagellate with sufficient amount of reduced iron. The genome of the early branching *P. confusum*, or its direct ancestor, acquired a single copy gene of a plant-like ZIP-family ferrous iron transporter (Jacques *et al.*, 2010; Flannery *et al.*, 2013), and multicopy genes appeared subsequently in all other trypanosomatids. This must have been one of the first steps towards parasitism. A similar scenario

holds for the capture of haem. While all Kinetoplastea, including *B. saltans*, possess a LABCG5 homologue to compensate for the lack of haem biosynthesis, a dedicated haem transporter such as LHR1 was acquired by *P. confusum*, or its immediate ancestor, so permitting survival inside an insect host. This LHR1 gene was secondarily lost in one of the two plant-dwelling haem-lacking phytomonads and in the African trypanosome, *T. vivax* (Flannery *et al.*, 2013). *T. brucei*, a blood-dwelling parasite, captures iron and haem via, respectively, a transferrin receptor (ESAG6/ESAG7) (van Luenen *et al.*, 2005) and a haptoglobin–haemoglobin receptor (Vanhollebeke *et al.*, 2008). Both receptors seem to be specific adaptations to a life in the bloodstream, because in the procyclic insect stage of *T. brucei* these genes are not expressed, and haem is acquired only *via* the haem uptake protein TbHrg (Tb927.8.6010, (Horáková *et al.*, 2017)), an ortho-logue of the *Leishmania* LHR1 (LmjF24.2230) that shares only 24% identical residues.

Speciation by gene losses

The acquisition of the parasitic lifestyle by the common ancestor of all Trypanosomatidae was likely associated with a progressive loss of metabolic capacities. However, with no genomic information about an organism immediately ancestral to both *Bodo* and Trypanosomatidae available, loss of genes in the trypanosomatids and gene acquisition in *Bodo* are equally possible. Thus, one should err on the side of caution with a scenario that follows. It seems likely that almost immediately after the transition from the free-living kinetoplastid to the last common ancestor of all Trypanosomatidae, approximately 9500 genes were lost (Jackson *et al.*, 2016; Opperdoes *et al.*, 2016). Although most of these genes were members of some large multigene families (exemplified by the GP46-like surface antigen with 391 copies in the genome of *B. saltans*) or encoded enigmatic 'hypothetical proteins', a smaller number of them (35 from 581 analysed) encoded metabolic enzymes.

Several complete metabolic pathways became redundant because the corresponding products could be acquired from the host. Typical examples of such metabolic losses in trypanosomatids are Lys catabolism and aerobic degradation of the aromatic amino acids Phe and Tyr (Opperdoes *et al.*, 2016). Moreover, most of the genes for Trp degradation were lost when *P. confusum* branched off from the main trypanosomatid lineage (Skalický *et al.*, 2017). The His catabolism, still present in *B. saltans* and *P. confusum*, also disappeared from most of the trypanosomatids, with a single exception of *T. cruzi*.

An important evolutionary event was the loss of hydroxyl-methyl-glutaryl-CoA (HMG-CoA) lyase and β-hydroxy-butyrate dehydrogenase genes in Leishmaniinae. These enzymes are essential for the conversion of Leu into ketone bodies (acetoacetate and beta-hydroxybutyrate). Thus, all Leishmaniinae including *Leishmania* spp. use Leu over acetate for biosynthesis of their sterols (Ginger *et al.*, 2001). Members of the same clade also lost the gene for trypanosome alternative oxidase. Finally, *P. confusum*, the earliest-branching trypanosomatid, is the only species sharing a 'protist-type' arginase gene with *B. saltans*. This arginase was subsequently lost by all other trypanosomatids, while only the Leishmaniinae re-acquired an entirely different arginase gene from fungi (Gaur *et al.*, 2007; Opperdoes and Michels, 2007).

Bodo saltans, *P. confusum* and members of the genera *Leishmania*, *Crithidia* and *Leptomonas* are all able to metabolize the branched amino acids Ile and Val, as well as Met and Thr into the TCA-cycle intermediate succinate (Opperdoes *et al.*, 2016). In contrast, *Trypanosoma*, *Phytomonas* and *Blechomonas* spp. are unable to metabolize these amino acids, as they independently lost three genes of the methyl malonyl-CoA pathway:

propionyl-CoA carboxylase, methyl-malonyl-CoA mutase and methyl-malonyl-CoA epimerase. In addition, they lost xyluloki-nase, which is required for the utilization of the pentose sugar xylulose.

The African trypanosomes (*T. vivax*, *T. brucei* and *T. congo-lense*) and *Phytomonas* spp. have adapted to the life in glucose-rich fluids – mammalian blood and plant juices, respectively. Trypanosomes are able to reversibly suppress mitochondrial oxidative phosphorylation in favour of a metabolism exclusively geared towards the consumption of glucose, while phytomonads have irreversibly lost their cytochromes (Opperdoes, 1987; Sanchez-Moreno *et al.*, 1992). Such a high degree of specialization has led to convergent evolution between the African trypano-somes and *Phytomonas* spp., characterized by a parallel loss of numerous genes. The African trypanosomes have lost 35 meta-bolic genes, while phytomonads have lost more than twice as much (Porcel *et al.*, 2014). Of these, the two groups have 16 losses in common. Those are involved in the synthesis of phosphonoli-pids, Met and tetra-hydrofolate, long-chain polyunsaturated acids, as well as conversions of Glu into Pro, Asn into Asp, and Ser into Gly. In addition, genes for methyl-glyoxal detoxification, forma-tion of HMG-CoA from acetyl CoA, *trans*-hydrogenation *via* D-lactate dehydrogenase, tetrahydrofolate synthesis, Cys synthe-sis, β-oxidation of fatty acids, metabolism of ascorbate and pen-tose sugars, ribulokinase, quinonoid di-hydro-pteridine reductase, ascorbate peroxidase, and old yellow enzyme were all lost in these two highly specialized clades.

Speciation by gene gains

In the course of evolution, trypanosomatid genomes were reshaped not only by losses of genes but also by gene duplications and acquisitions *via* horizontal gene transfer. Early on in their evolution, more than 18 metabolic genes were acquired, possibly simultaneously. These include genes involved in the cyclopro-pane-fatty-acyl-phospholipid formation, bromodomain factor 1 permitting an additional level of enzyme regulation, and the fer-rous iron transporter allowing more efficient competition for the soluble iron within the host. A biopterin/folate/pteridin trans-porter was originally acquired by the common ancestor of *B. saltans* and trypanosomatids from one of three possibilities, a cyanobacterium, plant or algal organism (Klaus *et al.*, 2005; Opperdoes and Coombs, 2007). It is a single copy gene in *B. salt-ans*, but in all trypanosomatids it expanded into a multi-gene family. The number of its copies per haploid genome varies from 2 in *Blechomonas* and 4 in *P. confusum*, to over 50 copies in *C. fasciculata*. These folate transporter arrays, along with the acquisition of pteridine reductase, underline the importance of an efficient salvage of pteridines and their subsequent metabolism in the parasitic lifestyle.

In general, evolution of trypanosomatids featured significantly more losses than acquisitions of metabolic genes. An exception to this rule is the subfamily Leishmaniinae, which acquired consid-erably more metabolic genes (23) than were lost (4). Acquisitions include 3 genes of the haem biosynthetic pathway – protopor-phyrinogen oxidase, coprophyrinogen III oxidase and ferrochela-tase (Ivens *et al.*, 2005; Opperdoes and Coombs, 2007), three genes of the urea cycle – argininosuccinate synthase, argininosuc-cinate lyase and arginase and two more genes involved in glyco-sylation reactions.

Speciation of the genus *Trypanosoma* is characterized by the acquisition of phospholipase A1, GPI inositol deacylase 2 gene, and the loss of genes encoding chitinase, cyclopropane-fatty-acyl-phospholipid synthase, both the cytosolic and mitochondrial ser-ine hydroxyl-methyl-transferase isoenzymes, as well as xanthine phosphoribosyl transferase. The newly acquired phospholipase

A1, PLA(1) is clearly distinct from the lysosomal isoenzyme (Opperdoes and van Roy, 1982; Richmond and Smith, 2007b). The former lipase is an orthologue of a bacterial extracellular phospholipase A1 that was most likely acquired from a horizontal gene transfer from *Sodalis glossinidius*, a bacterial endosymbiont of tsetse flies (Richmond and Smith, 2007a). Interestingly, a BLAST search revealed that PLA1 is an orthologue of the *T. brucei* ESAG1, which encodes a transmembrane protein located in the flagellar pocket (Nolan *et al.*, 2002). In the bloodstream stage of the African trypanosomes, it probably functions as a phospholipase which captures fatty acids and phospholipids by scavenging the lysophosphatidylcholine present in a sub-millimolar concentration in the host plasma (Uttaro, 2014). In *T. cruzi*, a similar, but non-homologous PLPA1 isoenzyme was proposed as a putative virulence factor (Belaunzarán *et al.*, 2013). Trypanosomes have also acquired proline racemase gene, which was implicated in B-cell polyclonal activation, immunosuppression, and evasion of the host defense by *T. cruzi* (Reina-San-Martin *et al.*, 2000). This gene was also retained in *T. vivax*, but lost in *T. brucei* and *T. congolense* (Caballero *et al.*, 2015).

Blechomonas ayalai and *Phytomonas* spp. share the gene encoding isopropanol dehydrogenase (Molinas *et al.*, 2003). However, it seems to be functional only in *Phytomonas*, since the *Blechomonas* homologue appears pseudogenized.

Blechomonas ayalai and trypanosomes metabolize Thr *via* the Thr dehydrogenase pathway, which apparently became enabled after the acquisition of an additional Thr dehydratase gene by a common ancestor of all trypanosomatids except *Paratrypanosoma*. This event introduced the possibility of choice between two alternative pathways for Thr degradation (Opperdoes and Coombs, 2007) and eventually led to a differential loss of either the Thr dehydrogenase or the Thr dehydratase pathway. This has resulted in the dramatic differences in the way this amino acid is metabolized in *Trypanosoma* and *Leishmania*. (Opperdoes and Coombs, 2007).

The common ancestor of Leishmaniinae gained novel genes involved in sucrose and pentose sugar metabolism, as well as the catalase. The latter was then selectively lost in members of the genus *Leishmania*, likely due to their dixenous life cycle (Kraeva *et al.*, 2017). More recent acquisitions, shared only by *Crithidia* and *Leptomonas*, are genes encoding diaminopimelate metabolizing enzymes, β-glucosidase, nitroalkane oxidase, phenolic acid dehydrogenase and glycerol dehydrogenase. Genes involved in the conversion of the typical bacterial diaminopemelic acid into Lys are present only in *Crithidia* spp. and *L. pyrrhocoris*, but are absent in *Leishmania* spp. and *L. seymouri*.

Finally, *B. saltans* is not capable of ubiquinone biosynthesis, while all trypanosomatids encode proteins constituting this essential pathway. The most parsimonious scenario suggests that the phagotrophic lifestyle of B. *saltans*, which allows it to extract necessary ubiquinone from bacteria, facilitated the loss of these genes (Opperdoes *et al.*, 2016).

Endosymbionts of trypanosomatids

Intracellular bacteria of trypanosomatids were discovered at the beginning of the 20th century in the monoxenous fly parasite *Strigomonas culicis* (at that time *Blastocrithidia culicis*) (Novy *et al.*, 1907). With the advent of electron microscopy bacteria were also found in several other species of monoxenous trypanosomatids (Newton and Horne, 1957; Mundim *et al.*, 1974; Fiorini *et al.*, 1989; Motta *et al.*, 1991b). Their nature was confirmed by early analyses of DNA (Marmur *et al.*, 1963), 70S ribosomes (Zaitseva and Salikhov, 1972), as well as chloramphenicol sensitivity (Zaitseva and Salikhov, 1973).

The early-described trypanosomatids' intracellular bacteria are closely related to each other and so are the hosts harbouring them (Fig. 1). This suggests that bacterial acquisition was a single event in this group, which was followed by the subsequent long-term coevolution between the partners (Faria e Silva *et al.*, 1991; Du and Chang, 1994; Du *et al.*, 1994a, 1994b; Hollar *et al.*, 1998). The bacteria were assigned to the new beta-proteobacterial genus *Kinetoplastibacterium* (formally, *Candidatus* Kinetoplastibacterium) within the family Alcaligenaceae (Du *et al.*, 1994b), whereas their hosts were eventually united in two related genera – *Angomonas* and *Strigomonas* (Teixeira *et al.*, 2011). *Kentomonas*, the third genus in this group, was discovered recently and all three genera were assigned to a new subfamily Strigomonadinae to emphasize their relationship and shared features associated with endosymbiosis (Votýpka *et al.*, 2014).

The bacterial endosymbionts were also recorded in aquatic leech-transmitted trypanosomes – *Trypanosoma cobitis* (Lewis and Ball, 1980) and *T. fallisi* (Martin and Desser, 1990; 1991). In contrast to Strigomonadinae possessing only one bacterium per cell, the trypanosomes bear multiple intracytoplasmic bacteria. Regretfully, these studies were restricted to electron microscopy, and neither the identity of the endosymbionts nor their relationships with the flagellate hosts were investigated further.

The last bacterium-trypanosomatid endosymbiosis documented to date, that of *Pandoraea novymonadis* (beta-proteobacteria: Burkholderiaceae) and *Novymonas esmeraldas* (Leishmaniinae), has been described recently (Kostygov *et al.*, 2016). As in the trypanosomes, there are multiple bacteria per flagellate cell. Because none of the partners in this endosymbiotic system has close relatives involved in such a relationship suggested its independent and relatively recent origin (Fig. 1). Nevertheless, analysis of the *P. novymonadis* genome indicated that these symbiotic relationships are already well established (Kostygov *et al.*, 2017). Compared to Strigomonadinae, this endosymbiotic system remains understudied. Unlike the former, the specific insect host of *N. esmeraldas* is not known, as it has been documented in South American true bugs and African biting midges (Kostygov *et al.*, 2016). Thus, at the moment it is not possible to study the endosymbiont influence on the flagellate fitness in the insect using experimental infections.

Different viruses can also infect trypanosomatids and play an important role in their biology (Ives *et al.*, 2011; Grybchuk *et al.*, 2018a). We refer readers to several recent reviews discussing this topic (Lukeš *et al.*, 2018; Grybchuk *et al.*, 2018b).

Interactions of trypanosomatids with their bacterial endosymbionts

The relationships of Strigomonadinae and *Novymonas* with their endosymbionts demonstrate many important differences, which are noticeable even on the morphological/ultrastructural level. While *P. novymonadis* cells are localized in vacuoles and preserve a well-developed peptidoglycan layer in the cell wall (Kostygov *et al.*, 2016), *Kinetoplastibacterium* spp. are situated directly in the cytoplasm of the host cell and their peptidoglycan layer is reduced (Chang, 1974; Soares and De Souza, 1988; Motta *et al.*, 1991b). The absence of a vacuolar membrane around bacteria and their thinner (and thereby more permeable) cell wall in the latter case apparently facilitate an intense metabolic exchange with the host enabling a mutually beneficial division of labour in metabolic pathways. The relationships between *P. novymonadis* and *N. esmeraldas* appear to be more primitive: the host keeps endosymbionts in vacuoles, likely to exercise more tight control over them. Occasionally, the trypanosomatid digests bacteria using lysosomes, probably in order to regulate their number and consume their products (Kostygov *et al.*, 2016).

Strigomonadinae do not need to use such a crude method to control the number of their endosymbionts. Instead, they evolved a fine-tuned mechanism ensuring precise coordination between the division of the trypanosomatid cell and its single intracellular bacterium (Motta *et al.*, 2010; Brum *et al.*, 2014; Catta-Preta *et al.*, 2015).

As mentioned above, the main role of the bacterial endosymbionts is to supply the trypanosomatid hosts with essential nutrients. One of these is haem, which trypanosomatids are unable to synthesize, although it is indispensable for the production of numerous important enzymes, such as the cytochromes (Gill and Vogel, 1963; Chang *et al.*, 1975; de Menezes and Roitman, 1991; Kořený *et al.*, 2013).

Typical trypanosomatids require many vitamins for their growth, such as riboflavin, pantothenic acid, pyridoxamine, folic acid, thiamine, nicotinic acid and biotin (Roitman *et al.*, 1972). However, Strigomonadinae require only the last three of them, since the others are supplied by the endosymbionts (Mundim *et al.*, 1974; Klein *et al.*, 2013). Interestingly, they perform all steps of the panthotenic acid synthesis, but the last one, which is completed by the flagellate host, demonstrating an intimate cooperation between the two partners (Klein *et al.*, 2013). *Pandoraea novymonadis* is able to synthesize all the above-mentioned vitamins, thereby making its host, *N. esmeraldas*, not dependent on their availability in the environment (Kostygov *et al.*, 2017). As for the amino acids, most trypanosomatids are unable to synthesize Arg, His, Ile, Leu, Phe, Trp and Tyr (Opperdoes *et al.*, 2016). The same holds true for aposymbiotic strains of Strigomonadinae, which additionally require Cys, Lys, Met and Thr (Mundim and Roitman, 1977; Freymuller and Camargo, 1981). Meanwhile, wild-type strains are auxotrophic only for Met and Tyr, which they apparently obtain from their insect hosts (Mundim *et al.*, 1974; Alves *et al.*, 2013a). Owing to multiple horizontal gene transfers, synthetic pathways for several amino acids are interlaced between *Kinetoplastibacterium* spp. and their hosts, so that the enzymes missing in the bacteria are present in the trypanosomatids and vice versa, providing another example of their deep metabolic integration (Alves *et al.*, 2013a; Alves, 2017). *Pandoraea novymonadis* is unable to synthesize Ala, Asn, Asp, Cys, Met and Pro; yet these can be synthesized by the flagellate. In return, the bacterium preserves the enzymes required for synthesis of nine amino acids, for which its host is auxotrophic (Kostygov *et al.*, 2017).

The endosymbiotic lifestyle led to a significant genomic reduction of the intracellular bacteria in question (Alves *et al.*, 2013a, 2013b; Kostygov *et al.*, 2017). Besides the biosynthesis of amino acids, this reduction affected enzymes involved in the production of polyamines, which are essential for many cellular processes. Both *Kinetoplastibacterium* spp. and *P. novymonadis* rely on their hosts in this respect (Kostygov *et al.*, 2017). It was demonstrated that the bacterial endosymbiont of *Angomonas deanei* enhances the activity of host's ornithine decarboxylase, which leads to an intensification of the polyamine synthesis and accelerated the proliferation of the trypanosomatid (Frossard *et al.*, 2006). Since *Kinetoplastibacterium* spp. lost their ability to synthesize important components of membranes such as cardiolipin, phosphatidylethanolamine, and phosphatidylserine, these phospholipids have to be supplied by the flagellate hosts. In contrast, *P. novymonadis* is self-dependent in this regard, consistently with its more secluded mode of life within the host cell.

The metabolism of Strigomonadinae (glycolysis, ATP production and hydrolysis, oxygen consumption and oxidation–reduction processes) was shown to be boosted in the presence of the endosymbiont (Penha *et al.*, 2016; Loyola-Machado *et al.*, 2017). While in the aposymbiotic Strigomonadinae the glycosomes are dispersed throughout the cytoplasm, in the symbiont-

containing cells they are closely associated with the bacteria providing direct access to ATP (Motta *et al.*, 1997; Faria-e-Silva *et al.*, 2000; Loyola-Machado *et al.*, 2017). The mitochondrion of Strigomonadinae demonstrates an extensive branching on the periphery of the cell, with the consequent reorganization of subpellicular microtubules (Freymuller and Camargo, 1981). In *N. esmeraldas*, the single mitochondrion also seems to be hypertrophied, although its peripheral projections do not distort the microtubular corset (Kostygov *et al.*, 2016). Combined, these findings suggest an enhanced energy consumption of endosymbiont-containing trypanosomatids.

The *Kinetoplastibacterium* spp. affect the surface charge and composition of glucoconjugates on the trypanosomatid plasma membrane (Dwyer and Chang, 1976; Esteves *et al.*, 1982; Motta *et al.*, 1991a; de Faria-e-Silva *et al.*, 1999), which are responsible for the different efficiency of infecting the insect host documented for wild-type and aposymbiotic strains (Fampa *et al.*, 2003; d'Avila-Levy *et al.*, 2005). Moreover, this also correlates with the activities of ecto-phosphatases and gp63-like proteases differing in the endosymbiont-bearing and bacteria-free trypanosomatids (d'Avila-Levy *et al.*, 2008; Catta-Preta *et al.*, 2013).

The intimate and complex interactions between the cellular processes of the bacterial endosymbionts and their trypanosomatid hosts require a well-developed signalling system. Indeed, it has been demonstrated that the outer membrane of Strigomonadinae contains phosphatidylcholine, a host-produced lipid participating in cell signalling, which is typical for eukaryotes and their symbionts (Palmié-Peixoto *et al.*, 2006; de Azevedo-Martins *et al.*, 2007; 2015).

It still remains puzzling why a few groups of trypanosomatids evolved to compensate for their deficiency in synthetic capabilities by acquiring endosymbionts, while the majority remain restricted to nutrients supplied by their insect hosts. This may be related to the differences in the life cycles, which are largely unknown for the majority of monoxenous trypanosomatids.

Conclusions and perspectives

The recent years were characterized by several significant advances in the field of trypanosomatid biology. Technologically, this progress was dynamically driven forward by a wide-scale application of genomics (and the related -omics) tools, as well as further improvements in biochemical, reverse genetics, and microscopy approaches. The recent advances include (but are not limited to) new insights in trypanosomatid genetics and sexual processes, biodiversity and population structure, virulence factors and other aspects of host–parasite interactions, transitions from monoxenous to dixenous lifestyle, epigenetics and its role in VSG switching, enzymology of RNA editing, and studies of associated microbiota. Yet, many unanswered and exciting questions still remain awaiting new ideas, unorthodox experimental approaches, and perhaps, a new generation of scientist to tackle them.

Ethical standards. Not applicable.

References

Acestor N, Panigrahi AK, Carnes J, Zíková A and Stuart KD (2009) The MRB1 complex functions in kinetoplastid RNA processing. *RNA* **15**, 277–286.

Adl SM, Simpson AG, Lane CE, Lukeš J, Bass D, Bowser SS, Brown MW, Burki F, Dunthorn M, Hampl V, Heiss A, Hoppenrath M, Lara E, Le Gall L, Lynn DH, McManus H, Mitchell EA, Mozley-Stanridge SE, Parfrey LW, Pawlowski J, Rueckert S, Shadwick RS, Schoch CL, Smirnov A and Spiegel FW (2012) The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology* **59**, 429–493.

Akopyants NS, Kimblin N, Secundino N, Patrick R, Peters N, Lawyer P, Dobson DE, Beverley SM and Sacks DL (2009) Demonstration of genetic exchange during cyclical development of *Leishmania* in the sand fly vector. *Science* **324**, 265–268.

Alexeieff A (1917) Sur la fonction glycoplastique du kinétoplaste (=kinétonucleus) chez les flagellés. *C.R. Séances Soc Biol Ses Fil* **80**, 512–514.

Alfonzo JD, Blanc V, Estevez AM, Rubio MA and Simpson L (1999) C to U editing of the anticodon of imported mitochondrial tRNA(Trp) allows decoding of the UGA stop codon in *Leishmania tarentolae*. *EMBO Journal* **18**, 7056–7062.

Alsford S, Wickstead B, Ersfeld K and Gull K (2001) Diversity and dynamics of the minichromosomal karyotype in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **113**, 79–88.

Alves JMP (2017) Working together: amino acid biosynthesis in endosymbiont-harbouring trypanosomatidae. In D'Mello JPF (ed.), *The Handbook of Microbial Metabolism of Amino Acids*. Wallingford, UK Boston, USA: CAB International, pp. 371–383.

Alves JM, Klein CC, da Silva FM, Costa-Martins AG, Serrano MG, Buck GA, Vasconcelos AT, Sagot MF, Teixeira MM, Motta MC and Camargo EP (2013*a*) Endosymbiosis in trypanosomatids: the genomic cooperation between bacterium and host in the synthesis of essential amino acids is heavily influenced by multiple horizontal gene transfers. *BMC Evolutionary Biology* **13**, 190.

Alves JM, Serrano MG, Maia da Silva F, Voegtly LJ, Matveyev AV, Teixeira MM, Camargo EP and Buck GA (2013*b*) Genome evolution and phylogenomic analysis of *Candidatus* kinetoplastibacterium, the beta-proteobacterial endosymbionts of *strigomonas* and *angomonas*. *Genome Biology and Evolution* **5**, 338–350.

Ammerman ML, Presnyak V, Fisk JC, Foda BM and Read LK (2010) TbRGG2 facilitates kinetoplastid RNA editing initiation and progression past intrinsic pause sites. *RNA* **16**, 2239–2251.

Ammerman ML, Downey KM, Hashimi H, Fisk JC, Tomasello DL, Faktorová D, Kafková L, King T, Lukeš J and Read LK (2012) Architecture of the trypanosome RNA editing accessory complex, MRB1. *Nucleic Acids Research* **40**, 5637–5650.

Ammerman ML, Tomasello DL, Faktorová D, Kafková L, Hashimi H, Lukeš J and Read LK (2013) A core MRB1 complex component is indispensable for RNA editing in insect and human infective stages of *Trypanosoma brucei*. *PLoS ONE* **8**, e78015.

Aphasizhev R and Aphasizheva I (2011) Mitochondrial RNA processing in trypanosomes. *Research in Microbiology* **162**, 655–663.

Aphasizhev R and Aphasizheva I (2013) Emerging roles of PPR proteins in trypanosomes: switches, blocks, and triggers. *RNA Biology* **10**, 1495–1500.

Aphasizhev R, Aphasizheva I and Simpson L (2003) A tale of two TUTases. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 10617–10622.

Aphasizheva I and Aphasizhev R (2016) U-insertion/deletion mRNA-editing holoenzyme: definition in sight. *Trends in Parasitology* **32**, 144–156.

Aphasizheva I, Maslov D, Wang X, Huang L and Aphasizhev R (2011) Pentatricopeptide repeat proteins stimulate mRNA adenylation/uridylation to activate mitochondrial translation in trypanosomes. *Molecular Cell* **42**, 106–117.

Aphasizheva I, Maslov DA and Aphasizhev R (2013) Kinetoplast DNA-encoded ribosomal protein S12: a possible functional link between mitochondrial RNA editing and translation in *Trypanosoma brucei*. *RNA Biology* **10**, 1679–1688.

Aphasizheva I, Zhang L, Wang X, Kaake RM, Huang L, Monti S and Aphasizhev R (2014) RNA binding and core complexes constitute the U-insertion/deletion editosome. *Molecular and Cellular Biology* **34**, 4329–4342.

Aphasizheva I, Maslov DA, Qian Y, Huang L, Wang Q, Costello CE and Aphasizhev R (2016) Ribosome-associated pentatricopeptide repeat proteins function as translational activators in mitochondria of trypanosomes. *Molecular Microbiology* **99**, 1043–1058.

Ardelli BF, Witt JD and Woo PT (2000) Identification of glycosomes and metabolic end products in pathogenic and nonpathogenic strains of *Cryptobia salmositica* (Kinetoplastida: Bodonidae). *Diseases of Aquatic Organisms* **42**, 41–51.

Bakker BM, Michels PA, Opperdoes FR and Westerhoff HV (1997) Glycolysis in bloodstream form *Trypanosoma brucei* can be understood in terms of the kinetics of the glycolytic enzymes. *Journal of Biological Chemistry* **272**, 3207–3215.

Bakker BM, Westerhoff HV, Opperdoes FR and Michels PA (2000) Metabolic control analysis of glycolysis in trypanosomes as an approach to improve selectivity and effectiveness of drugs. *Molecular and Biochemical Parasitology* **106**, 1–10.

Bakker BM, Krauth-Siegel RL, Clayton C, Matthews K, Girolami M, Westerhoff HV, Michels PA, Breitling R and Barrett MP (2010) The silicon trypanosome. *Parasitology* **137**, 1333–1341.

Balmer O, Beadell JS, Gibson W and Caccone A (2011) Phylogeography and taxonomy of *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases* **5**, e961.

Banuls AL, Hide M and Tibayrenc M (1999) Molecular epidemiology and evolutionary genetics of *Leishmania* parasites. *International Journal for Parasitology* **29**, 1137–1147.

Barnabe C, Brisse S and Tibayrenc M (2000) Population structure and genetic typing of *Trypanosoma cruzi*, the agent of chagas disease: a multilocus enzyme electrophoresis approach. *Parasitology*, **120**(Pt 5), 513–526.

Barros-Alvarez X, Caceres AJ, Michels PA, Concepcion JL and Quinones W (2014) The phosphoglycerate kinase isoenzymes have distinct roles in the regulation of carbohydrate metabolism in *trypanosoma cruzi*. *Experimental Parasitology* **143**, 39–47.

Barton NH and Charlesworth B (1998) Why sex and recombination? *Science* **281**, 1986–1990.

Batram C, Jones NG, Janzen CJ, Markert SM and Engstler M (2014) Expression site attenuation mechanistically links antigenic variation and development in *Trypanosoma brucei*. *eLife* **3**, e02324.

Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V and Mootha VK (2011) Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* **476**, 341–345.

Belaunzarán ML, Wilkowsky SE, Lammel EM, Gimenez G, Bott E, Barbieri MA and de Isola EL (2013) Phospholipase A1: a novel virulence factor in *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology* **187**, 77–86.

Benne R, De Vries BF, Van den Burg J and Klaver B (1983) The nucleotide sequence of a segment of *Trypanosoma brucei* mitochondrial maxi-circle DNA that contains the gene for apocytochrome b and some unusual unassigned reading frames. *Nucleic Acids Research* **11**, 6925–6941.

Benne R, Van den Burg J, Brakenhoff JP, Sloof P, Van Boom JH and Tromp MC (1986) Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. *Cell* **46**, 819–826.

Berriman M, Ghedin E, Hertz-Fowler C, Blandin G, Renauld H, Bartholomeu DC, Lennard NJ, Caler E, Hamlin NE, Haas B, Bohme U, Hannick L, Aslett MA, Shallom J, Marcello L, Hou L, Wickstead B, Alsmark UC, Arrowsmith C, Atkin RJ, Barron AJ, Bringaud F, Brooks K, Carrington M, Cherevach I, Chillingworth TJ, Churcher C, Clark LN, Corton CH, Cronin A, Davies RM, Doggett J, Djikeng A, Feldblyum T, Field MC, Fraser A, Goodhead I, Hance Z, Harper D, Harris BR, Hauser H, Hostetler J, Ivens A, Jagels K, Johnson D, Johnson J, Jones K, Kerhornou AX, Koo H, Larke N, Landfear S, Larkin C, Leech V, Line A, Lord A, Macleod A, Mooney PJ, Moule S, Martin DM, Morgan GW, Mungall K, Norbertczak H, Ormond D, Pai G, Peacock CS, Peterson J, Quail MA, Rabbinowitsch E, Rajandream MA, Reitter C, Salzberg SL, Sanders M, Schobel S, Sharp S, Simmonds M, Simpson AJ, Tallon L, Turner CM, Tait A, Tivey AR, Van Aken S, Walker D, Wanless D, Wang S, White B, White O, Whitehead S, Woodward J, Wortman J,

Adams MD, Embley TM, Gull K, Ullu E, Barry JD, Fairlamb AH, Opperdoes F, Barrell BG, Donelson JE, Hall N, Fraser CM, Melville SE and El-Sayed NM (2005) The genome of the African trypanosome *Trypanosoma brucei*. *Science* **309**, 416–422.

Bhat GJ, Koslowsky DJ, Feagin JE, Smiley BL and Stuart K (1990) An extensively edited mitochondrial transcript in kinetoplastids encodes a protein homologous to ATPase subunit 6. *Cell* **61**, 885–894.

Bindereif A (2012) *RNA Metabolism in Trypanosomes*. Berlin Heidelberg: Springer.

Blom D, de Haan A, van den Berg M, Sloof P, Jirku M, Lukeš J and Benne R (1998) RNA editing in the free-living bodonid *Bodo saltans*. *Nucleic Acids Research* **26**, 1205–1213.

Blum B and Simpson L (1990) Guide RNAs in kinetoplastid mitochondria have a nonencoded 3' oligo(U) tail involved in recognition of the preedited region. *Cell* **62**, 391–397.

Blum B, Bakalara N and Simpson L (1990) A model for RNA editing in kinetoplastid mitochondria: 'guide' RNA molecules transcribed from maxicircle DNA provide the edited information. *Cell* **60**, 189–198.

Borghesan TC, Ferreira RC, Takata CS, Campaner M, Borda CC, Paiva F, Milder RV, Teixeira MM and Camargo EP (2013) Molecular phylogenetic redefinition of *Herpetomonas* (Kinetoplastea, Trypanosomatidae), a genus of insect parasites associated with flies. *Protist* **164**, 129–152.

Borst P and Fase-Fowler F (1979) The maxi-circle of *Trypanosoma brucei* kinetoplast DNA. *Biochimica et Biophysica Acta* **565**, 1–12.

Brandenburg J, Schimanski B, Nogoceke E, Nguyen TN, Padovan JC, Chait BT, Cross GA and Gunzl A (2007) Multifunctional class I transcription in *Trypanosoma brucei* depends on a novel protein complex. *EMBO Journal* **26**, 4856–4866.

Brisse S, Dujardin JC and Tibayrenc M (2000) Identification of six *Trypanosoma cruzi* lineages by sequence-characterised amplified region markers. *Molecular and Biochemical Parasitology* **111**, 95–105.

Brisse S, Verhoef J and Tibayrenc M (2001) Characterisation of large and small subunit rRNA and mini-exon genes further supports the distinction of six *trypanosoma cruzi* lineages. *International Journal for Parasitology* **31**, 1218–1226.

Brisse S, Henriksson J, Barnabe C, Douzery EJ, Berkvens D, Serrano M, De Carvalho MR, Buck GA, Dujardin JC and Tibayrenc M (2003) Evidence for genetic exchange and hybridization in *Trypanosoma cruzi* based on nucleotide sequences and molecular karyotype. *Infection Genetics and Evolution* **2**, 173–183.

Broadhead R, Dawe HR, Farr H, Griffiths S, Hart SR, Portman N, Shaw MK, Ginger ML, Gaskell SJ, McKean PG and Gull K (2006) Flagellar motility is required for the viability of the bloodstream trypanosome. *Nature* **440**, 224–227.

Brum FL, Catta-Preta CM, de Souza W, Schenkman S, Elias MC and Motta MC (2014) Structural characterization of the cell division cycle in *Strigomonas culicis*, an endosymbiont-bearing trypanosomatid. *Microscopy and Microanalysis* **20**, 228–237.

Caballero ZC, Costa-Martins AG, Ferreira RC, JM PA, Serrano MG, Camargo EP, Buck GA, Minoprio P and MM GT (2015) Phylogenetic and syntenic data support a single horizontal transference to a *Trypanosoma* ancestor of a prokaryotic proline racemase implicated in parasite evasion from host defences. *Parasites & Vectors* **8**, 222.

Calvo-Alvarez E, Alvarez-Velilla R, Jimenez M, Molina R, Perez-Pertejo Y, Balana-Fouce R and Reguera RM (2014) First evidence of intraclonal genetic exchange in trypanosomatids using two *Leishmania infantum* fluorescent transgenic clones. *PLoS Neglected Tropical Diseases* **8**, e3075.

Cantacessi C, Dantas-Torres F, Nolan MJ and Otranto D (2015) The past, present, and future of *Leishmania* genomics and transcriptomics. *Trends in Parasitology* **31**, 100–108.

Capewell P, Cooper A, Duffy CW, Tait A, Turner CM, Gibson W, Mehlitz D and Macleod A (2013) Human and animal trypanosomes in Cote d'Ivoire form a single breeding population. *PLoS ONE* **8**, e67852.

Carnes J, Trotter JR, Peltan A, Fleck M and Stuart K (2008) RNA editing in *Trypanosoma brucei* requires three different editosomes. *Molecular and Cellular Biology* **28**, 122–130.

Carnes J, Soares CZ, Wickham C and Stuart K (2011) Endonuclease associations with three distinct editosomes in *Trypanosoma brucei*. *Journal of Biological Chemistry* **286**, 19320–19330.

Carnes J, Lewis Ernst N, Wickham C, Panicucci B and Stuart K (2012) KREX2 is not essential for either procyclic or bloodstream form *Trypanosoma brucei*. *PLoS ONE* **7**, e33405.

Catta-Preta CM, Nascimento MT, Garcia MC, Saraiva EM, Motta MC and Meyer-Fernandes JR (2013) The presence of a symbiotic bacterium in *Strigomonas culicis* is related to differential ecto-phosphatase activity and influences the mosquito-protozoa interaction. *International Journal for Parasitology* **43**, 571–577.

Catta-Preta CM, Brum FL, da Silva CC, Zuma AA, Elias MC, de Souza W, Schenkman S and Motta MC (2015) Endosymbiosis in trypanosomatid protozoa: the bacterium division is controlled during the host cell cycle. *Frontiers in Microbiology* **6**, 520.

Cavalier-Smith T, Chao EE, Snell EA, Berney C, Fiore-Donno AM and Lewis R (2014) Multigene eukaryote phylogeny reveals the likely protozoan ancestors of opisthokonts (animals, fungi, choanozoans) and Amoebozoa. *Molecular Phylogenetics and Evolution* **81**, 71–85.

Chang KP (1974) Ultrastructure of symbiotic bacteria in normal and antibiotic-treated *Blastocrithidia culicis* and *Crithidia oncopelti*. *The Journal of Protozoology* **21**, 699–707.

Chang KP, Chang CS and Sassa S (1975) Heme biosynthesis in bacterium-protozoon symbioses: enzymic defects in host hemoflagellates and complemental role of their intracellular symbiotes. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 2979–2983.

Charriere F, Helgadottir S, Horn EK, Soll D and Schneider A (2006) Dual targeting of a single tRNA(Trp) requires two different tryptophanyl-tRNA synthetases in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 6847–6852.

Chicharro C and Alvar J (2003) Lower trypanosomatids in HIV/AIDS patients. *Annals of Tropical Medicine and Parasitology* **97**(suppl. 1), 75–78.

Cisarovsky G and Schmid-Hempel P (2014) Few colonies of the host *Bombus terrestris* disproportionately affect the genetic diversity of its parasite, *Crithidia bombi*. *Infection Genetics and Evolution* **21**, 192–197.

Clayton CE (2014) Networks of gene expression regulation in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **195**, 96–106.

Corell RA, Feagin JE, Riley GR, Strickland T, Guderian JA, Myler PJ and Stuart K (1993) *Trypanosoma brucei* minicircles encode multiple guide RNAs which can direct editing of extensively overlapping sequences. *Nucleic Acids Research* **21**, 4313–4320.

Cosenza LW, Bringaud F, Baltz T and Vellieux FM (2002) The 3.0 A resolution crystal structure of glycosomal pyruvate phosphate dikinase from *Trypanosoma brucei*. *Journal of Molecular Biology* **318**, 1417–1432.

d'Avila-Levy CM, Silva BA, Hayashi EA, Vermelho AB, Alviano CS, Saraiva EM, Branquinha MH and Santos AL (2005) Influence of the endosymbiont of *Blastocrithidia culicis* and *Crithidia deanei* on the glycoconjugate expression and on *Aedes aegypti* interaction. *FEMS Microbiology Letters* **252**, 279–286.

d'Avila-Levy CM, Santos LO, Marinho FA, Matteoli FP, Lopes AH, Motta MC, Santos AL and Branquinha MH (2008) *Crithidia deanei*: influence of parasite gp63 homologue on the interaction of endosymbiont-harboring and aposymbiotic strains with *Aedes aegypti* midgut. *Experimental Parasitology* **118**, 345–353.

d'Avila-Levy CM, Boucinha C, Kostygov A, Santos HL, Morelli KA, Grybchuk-Ieremenko A, Duval L, Votýpka J, Yurchenko V, Grellier P and Lukeš J (2015) Exploring the environmental diversity of kinetoplastid flagellates in the high-throughput DNA sequencing era. *Memorias do Instituto Oswaldo Cruz* **110**, 956–965.

Daniels JP, Gull K and Wickstead B (2010) Cell biology of the trypanosome genome. *Microbiology and Molecular Biology Reviews* **74**, 552–569.

Das A and Bellofatto V (2003) RNA polymerase II-dependent transcription in trypanosomes is associated with a SNAP complex-like transcription factor. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 80–85.

Das A, Li H, Liu T and Bellofatto V (2006) Biochemical characterization of *Trypanosoma brucei* RNA polymerase II. *Molecular and Biochemical Parasitology* **150**, 201–210.

Das A, Banday M and Bellofatto V (2008) RNA polymerase transcription machinery in trypanosomes. *Eukaryotic Cell* **7**, 429–434.

de Azevedo-Martins AC, Frossard ML, de Souza W, Einicker-Lamas M and Motta MC (2007) Phosphatidylcholine synthesis in *Crithidia deanei*: the influence of the endosymbiont. *FEMS Microbiology Letters* **275**, 229–236.

de Azevedo-Martins AC, Alves JM, de Mello FG, Vasconcelos AT, de Souza W, Einicker-Lamas M and Motta MC (2015) Biochemical and phylogenetic analyses of phosphatidylinositol production in *Angomonas deanei*, an endosymbiont-harboring trypanosomatid. *Parasites & Vectors* **8**, 247.

de Faria-e-Silva PM, Costa e Silva-Filho F and de Souza W (1999) Cell surface composition of promastigote and opisthomorph forms of *Herpetomonas roitmani* (Kinetoplastida: Trypanosomatidae). *Parasitology Research* **85**, 719–725.

De Gaudenzi J, Frasch AC and Clayton C (2005) RNA-binding domain proteins in Kinetoplastids: a comparative analysis. *Eukaryotic Cell* **4**, 2106–2114.

De Greef C and Hamers R (1994) The serum resistance-associated (SRA) gene of *Trypanosoma brucei rhodesiense* encodes a variant surface glycoprotein-like protein. *Molecular and Biochemical Parasitology* **68**, 277–284.

de la Cruz VF, Neckelmann N and Simpson L (1984) Sequences of six genes and several open reading frames in the kinetoplast maxicircle DNA of *Leishmania tarentolae*. *Journal of Biological Chemistry* **259**, 15136–15147.

de la Cruz VF, Lake JA, Simpson AM and Simpson L (1985a) A minimal ribosomal RNA: sequence and secondary structure of the 9S kinetoplast ribosomal RNA from *Leishmania tarentolae*. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 1401–1405.

de la Cruz VF, Simpson AM, Lake JA and Simpson L (1985b) Primary sequence and partial secondary structure of the 12S kinetoplast (mitochondrial) ribosomal RNA from *Leishmania tarentolae*: conservation of peptidyl-transferase structural elements. *Nucleic Acids Research* **13**, 2337–2356.

de Menezes CB and Roitman I (1991) Nutritional requirements of *blastocrithidia culicis*, a trypanosomatid with an endosymbiont. *The Journal of Protozoology* **38**, 122–123.

Dedet JP and Pratlong F (2000) *Leishmania*, *Trypanosoma* and monoxenous trypanosomatids as emerging opportunistic agents. *Journal of Eukaryotic Microbiology* **47**, 37–39.

Dixit S, Muller-McNicoll M, David V, Zarnack K, Ule J, Hashimi H and Lukeš J (2017) Differential binding of mitochondrial transcripts by MRB8170 and MRB4160 regulates distinct editing fates of mitochondrial mRNA in trypanosomes. *MBio* **8**, e02288-16.

Docampo R (2016) The origin and evolution of the acidocalcisome and its interactions with other organelles. *Molecular and Biochemical Parasitology* **209**, 3–9.

Docampo R and Huang G (2016) Acidocalcisomes of eukaryotes. *Current Opinion in Cell Biology* **41**, 66–72.

Docampo R and Lukeš J (2012) Trypanosomes and the solution to a 50-year mitochondrial calcium mystery. *Trends in Parasitology* **28**, 31–37.

Docampo R, de Souza W, Miranda K, Rohloff P and Moreno SN (2005) Acidocalcisomes – conserved from bacteria to man. *Nature Reviews Microbiology* **3**, 251–261.

Dossin Fde M and Schenkman S (2005) Actively transcribing RNA polymerase II concentrates on spliced leader genes in the nucleus of *trypanosoma cruzi*. *Eukaryotic Cell* **4**, 960–970.

Du Y and Chang KP (1994) Phylogenetic heterogeneity of three *Crithidia* spp. vs. *Crithidia fasciculata*. *Molecular and Biochemical Parasitology* **66**, 171–174.

Du Y, Maslov DA and Chang KP (1994a) Monophyletic origin of beta-division proteobacterial endosymbionts and their coevolution with insect trypanosomatid protozoa *Blastocrithidia culicis* and *Crithidia* spp. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 8437–8441.

Du Y, McLaughlin G and Chang KP (1994b) 16S ribosomal DNA sequence identities of beta-proteobacterial endosymbionts in three *Crithidia* species. *Journal of Bacteriology* **176**, 3081–3084.

Dufernez F, Yernaux C, Gerbod D, Noël C, Chauvenet M, Wintjens R, Edgcomb VP, Capron M, Opperdoes FR and Viscogliosi E (2006) The presence of four iron-containing superoxide dismutase isozymes in trypanosomatidae: characterization, subcellular localization, and phylogenetic origin in *Trypanosoma brucei*. *Free Radical Biology & Medicine* **40**, 210–225.

Duffy CW, MacLean L, Sweeney L, Cooper A, Turner CM, Tait A, Sternberg J, Morrison LJ and MacLeod A (2013) Population genetics of *Trypanosoma brucei rhodesiense*: clonality and diversity within and between foci. *Plos Neglected Tropical Diseases* **7**, e2526.

Dwyer DM and Chang KP (1976) Surface membrane carbohydrate alterations of a flagellated protozoan mediated by bacterial endosymbiotes. *Proceedings of the National Academy of Sciences of the United States of America* **73**, 852–856.

Echodu R, Sistrom M, Bateta R, Murilla G, Okedi L, Aksoy S, Enyioha C, Enyaru J, Opiyo E, Gibson W and Caccone A (2015) Genetic diversity and population structure of *Trypanosoma brucei* in Uganda: implications for the epidemiology of sleeping sickness and Nagana. *PLoS Neglected Tropical Diseases* **9**, e0003353.

El-Sayed NM, Myler PJ, Bartholomeu DC, Nilsson D, Aggarwal G, Tran AN, Ghedin E, Worthey EA, Delcher AL, Blandin G, Westenberger SJ, Caler E, Cerqueira GC, Branche C, Haas B, Anupama A, Arner E, Aslund L, Attipoe P, Bontempi E, Bringaud F, Burton P, Cadag E, Campbell DA, Carrington M, Crabtree J, Darban H, da Silveira JF, de Jong P, Edwards K, Englund PT, Fazelina G, Feldblyum T, Ferella M, Frasch AC, Gull K, Horn D, Hou L, Huang Y, Kindlund E, Klingbeil M, Kluge S, Koo H, Lacerda D, Levin MJ, Lorenzi H, Louie T, Machado CR, McCulloch R, McKenna A, Mizuno Y, Mottram JC, Nelson S, Ochaya S, Osoegawa K, Pai G, Parsons M, Pentony M, Pettersson U, Pop M, Ramirez JL, Rinta J, Robertson L, Salzberg SL, Sanchez DO, Seyler A, Sharma R, Shetty J, Simpson AJ, Sisk E, Tammi MT, Tarleton R, Teixeira S, Van Aken S, Vogt C, Ward PN, Wickstead B, Wortman J, White O, Fraser CM, Stuart KD and Andersson B (2005a) The genome sequence of *Trypanosoma cruzi*, etiologic agent of chagas disease. *Science* **309**, 409–415.

El-Sayed NM, Myler PJ, Blandin G, Berriman M, Crabtree J, Aggarwal G, Caler E, Renauld H, Worthey EA, Hertz-Fowler C, Ghedin E, Peacock C, Bartholomeu DC, Haas BJ, Tran AN, Wortman JR, Alsmark UC, Angiuoli S, Anupama A, Badger J, Bringaud F, Cadag E, Carlton JM, Cerqueira GC, Creasy T, Delcher AL, Djikeng A, Embley TM, Hauser C, Ivens AC, Kummerfeld SK, Pereira-Leal JB, Nilsson D, Peterson J, Salzberg SL, Shallom J, Silva JC, Sundaram J, Westenberger S, White O, Melville SE, Donelson JE, Andersson B, Stuart KD and Hall N (2005b) Comparative genomics of trypanosomatid parasitic protozoa. *Science* **309**, 404–409.

Engstler M, Pfohl T, Herminghaus S, Boshart M, Wiegertjes G, Heddergott N and Overath P (2007) Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes. *Cell* **131**, 505–515.

Eperon IC, Janssen JW, Hoeijmakers JH and Borst P (1983) The major transcripts of the kinetoplast DNA of *Trypanosoma brucei* are very small ribosomal RNAs. *Nucleic Acids Research* **11**, 105–125.

Erben ED, Fadda A, Lueong S, Hoheisel JD and Clayton C (2014) A genome-wide tethering screen reveals novel potential post-transcriptional regulators in *Trypanosoma brucei*. *PLoS Pathogens* **10**, e1004178.

Esteves MJ, Andrade AF, Angluster J, de Souza W, Mundim MH, Roitman I and Perreira ME (1982) Cell surface carbohydrates in *Crithidia deanei*: influence of the endosymbiote. *European Journal of Cell Biology* **26**, 244–248.

Estevez AM (2008) The RNA-binding protein TbDRBD$_3$ regulates the stability of a specific subset of mRNAs in trypanosomes. *Nucleic Acids Research* **36**, 4573–4586.

Etheridge RD, Aphasizheva I, Gershon PD and Aphasizhev R (2008) 3' adenylation determines mRNA abundance and monitors completion of RNA editing in *T. brucei* mitochondria. *EMBO Journal* **27**, 1596–1608.

Fadda A, Farber V, Droll D and Clayton C (2013) The roles of 3'-exoribonucleases and the exosome in trypanosome mRNA degradation. *RNA* **19**, 937–947.

Fadda A, Ryten M, Droll D, Rojas F, Farber V, Haanstra JR, Merce C, Bakker BM, Matthews K and Clayton C (2014) Transcriptome-wide analysis of trypanosome mRNA decay reveals complex degradation kinetics and suggests a role for co-transcriptional degradation in determining mRNA levels. *Molecular Microbiology* **94**, 307–326.

Fairlamb AH, Blackburn P, Ulrich P, Chait BT and Cerami A (1985) Trypanothione: a novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids. *Science* **227**, 1485–1487.

Faktorová D, Valach M, Kaur B, Burger G and Lukeš J (2018) Mitochondrial RNA editing and processing in diplonemid protists. In Gray MW and Cruz-Reyes J (eds), *RNA Metabolism in Mitochondria*. Verlag, Berlin, Heidelberg: Springer.

Fampa P, Correa-da-Silva MS, Lima DC, Oliveira SM, Motta MC and Saraiva EM (2003) Interaction of insect trypanosomatids with mosquitoes, sand fly and the respective insect cell lines. *International Journal for Parasitology* **33**, 1019–1026.

Faria e Silva PM, Sole-Cava AM, Soares MJ, Motta MC, Fiorini JE, de Souza W (1991) *Herpetomonas roitmani* (Fiorini, *et al.* 1989) n. Comb.: a trypanosomatid with a bacterium-like endosymbiont in the cytoplasm. *The Journal of Protozoology* **38**, 489–494.

Faria-e-Silva PM, Attias M and de Souza W (2000) Biochemical and ultrastructural changes in *Herpetomonas roitmani* related to the energy metabolism. *Biology of the Cell* **92**, 39–47.

Farr H and Gull K (2009) Functional studies of an evolutionarily conserved, cytochrome b5 domain protein reveal a specific role in axonemal organisation and the general phenomenon of post-division axonemal growth in trypanosomes. *Cell Motility and the Cytoskeleton* **66**, 24–35.

Feagin JE, Abraham JM and Stuart K (1988a) Extensive editing of the cytochrome c oxidase III transcript in *Trypanosoma brucei*. *Cell* **53**, 413–422.

Feagin JE, Shaw JM, Simpson L and Stuart K (1988b) Creation of AUG initiation codons by addition of uridines within cytochrome b transcripts of kinetoplastids. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 539–543.

Fernandes AP, Nelson K and Beverley SM (1993) Evolution of nuclear ribosomal RNAs in kinetoplastid protozoa: perspectives on the age and origins of parasitism. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 11608–11612.

Field MC and Carrington M (2009) The trypanosome flagellar pocket. *Nature Reviews Microbiology* **7**, 775–786.

Figueiredo LM, Janzen CJ and Cross GA (2008) A histone methyltransferase modulates antigenic variation in African trypanosomes. *PLoS Biology* **6**, e161.

Fiorini JE, Faria e Silva PM, Soares MJ and Brazil RP (1989) Three new species of insect trypanosomatids isolated in alfenas, minas gerais, Brazil. *Memorias do Instituto Oswaldo Cruz* **84**, 69–74.

Fisk JC, Ammerman ML, Presnyak V and Read LK (2008) TbRGG2, an essential RNA editing accessory factor in two *Trypanosoma brucei* life cycle stages. *Journal of Biological Chemistry* **283**, 23016–23025.

Flannery AR, Huynh C, Mittra B, Mortara RA and Andrews NW (2011) LFR1 ferric iron reductase of *Leishmania amazonensis* is essential for the generation of infective parasite forms. *Journal of Biological Chemistry* **286**, 23266–23279.

Flannery AR, Renberg RL and Andrews NW (2013) Pathways of iron acquisition and utilization in *Leishmania*. *Current Opinion in Microbiology* **16**, 716–721.

Flegontov PN, Strelkova MV and Kolesnikov AA (2006) The *Leishmania major* maxicircle divergent region is variable in different isolates and cell types. *Molecular and Biochemical Parasitology* **146**, 173–179.

Flegontov P, Votýpka J, Skalický T, Logacheva MD, Penin AA, Tanifuji G, Onodera NT, Kondrashov AS, Volf P, Archibald JM and Lukeš J (2013) *Paratrypanosoma* is a novel early-branching trypanosomatid. *Current Biology* **23**, 1787–1793.

Flegontov P, Butenko A, Firsov S, Kraeva N, Eliáš M, Field MC, Filatov D, Flegontova O, Gerasimov ES, Hlaváčová J, Ishemgulova A, Jackson AP, Kelly S, Kostygov A, Logacheva MD, Maslov DA, Opperdoes FR, O'Reilly A, Sádlová J, Ševčíková T, Venkatesh D, Vlček Č, Volf P, Votýpka J, Záhonová K, Yurchenko V and Lukeš J (2016) Genome of *Leptomonas pyrrhocoris*: a high-quality reference for monoxenous trypanosomatids and new insights into evolution of *Leishmania*. *Scientific Reports* **6**, 23704.

Foda BM, Downey KM, Fisk JC and Read LK (2012) Multifunctional G-rich and RRM-containing domains of TbRGG2 perform separate yet essential functions in trypanosome RNA editing. *Eukaryotic Cell* **11**, 1119–1131.

Freymuller E and Camargo EP (1981) Ultrastructural differences between species of trypanosomatids with and without endosymbionts. *The Journal of Protozoology* **28**, 175–182.

Frolov AO, Malysheva MN and Kostygov AY (2016) Transformations of life cycles in the evolutionary history of trypanosomatidae: endotransformations and aberrations. *Parazitologiia* **50**, 97–113.

Frolov AO, Malysheva MN, Ganyukova AI, Yurchenko V and Kostygov AY (2017) Life cycle of *Blastocrithidia papi* sp. n. (Kinetoplastea, Trypanosomatidae) in *Pyrrhocoris apterus* (Hemiptera, Pyrrhocoridae). *European Journal of Protistology* **57**, 85–98.

Frossard ML, Seabra SH, DaMatta RA, de Souza W, de Mello FG and Motta MC (2006) An endosymbiont positively modulates ornithine decarboxylase in host trypanosomatids. *Biochemical and Biophysical Research Communications* **343**, 443–449.

Gabaldón T, Ginger ML and Michels PA (2016) Peroxisomes in parasitic protists. *Molecular and Biochemical Parasitology* **209**, 35–45.

Gadelha C, Wickstead B, de Souza W, Gull K and Cunha-e-Silva N (2005) Cryptic paraflagellar rod in endosymbiont-containing kinetoplastid protozoa. *Eukaryotic Cell* **4**, 516–525.

Gao G and Simpson L (2003) Is the *Trypanosoma brucei* REL1 RNA ligase specific for U-deletion RNA editing, and is the REL2 RNA ligase specific for U-insertion editing? *Journal of Biological Chemistry* **278**, 27570–27574.

Gaunt MW, Yeo M, Frame IA, Stothard JR, Carrasco HJ, Taylor MC, Mena SS, Veazey P, Miles GA, Acosta N, de Arias AR and Miles MA (2003) Mechanism of genetic exchange in American trypanosomes. *Nature* **421**, 936–939.

Gaur U, Roberts SC, Dalvi RP, Corraliza I, Ullman B and Wilson ME (2007) An effect of parasite-encoded arginase on the outcome of murine cutaneous leishmaniasis. *Journal of Immunology* **179**, 8446–8453.

Gerasimov ES, Gasparyan AA, Kaurov I, Tichy B, Logacheva MD, Kolesnikov AA, Lukeš J, Yurchenko V, Zimmer SL and Flegontov P (2018) Trypanosomatid mitochondrial RNA editing: dramatically complex transcript repertoires revealed with a dedicated mapping tool. *Nucleic Acids Research* **46**, 765–781.

Ghedin E, Bringaud F, Peterson J, Myler P, Berriman M, Ivens A, Andersson B, Bontempi E, Eisen J, Angiuoli S, Wanless D, Von Arx A, Murphy L, Lennard N, Salzberg S, Adams MD, White O, Hall N, Stuart K, Fraser CM and El-Sayed NM (2004) Gene synteny and evolution of genome architecture in trypanosomatids. *Molecular and Biochemical Parasitology* **134**, 183–191.

Ghosh S, Banerjee P, Sarkar A, Datta S and Chatterjee M (2012) Coinfection of *Leptomonas seymouri* and *Leishmania donovani* in Indian leishmaniasis. *Journal of Clinical Microbiology* **50**, 2774–2778.

Gibson WC (1986) Will the real *Trypanosoma b. gambiense* please stand up. *Parasitology Today* **2**, 255–257.

Gibson W (2015) Liaisons dangereuses: sexual recombination among pathogenic trypanosomes. *Research in Microbiology* **166**, 459–466.

Gibson W (2017) Kinetoplastea. In Archibald JM, Simpson AG and Slamovits CH (eds), *Handbook of the Protists*. Cham, Switzerland: Springer International Publishing, pp. 1–50.

Gibson W and Bailey M (1994) Genetic exchange in *Trypanosoma brucei*: evidence for meiosis from analysis of a cross between drug-resistant transformants. *Molecular and Biochemical Parasitology* **64**, 241–252.

Gibson W and Garside L (1990) Kinetoplast DNA minicircles are inherited from both parents in genetic hybrids of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **42**, 45–53.

Gibson W and Garside L (1991) Genetic exchange in *Trypanosoma brucei brucei*: variable chromosomal location of housekeeping genes in different trypanosome stocks. *Molecular and Biochemical Parasitology* **45**, 77–89.

Gibson W and Stevens J (1999) Genetic exchange in the Trypanosomatidae. *Advances in Parasitology* **43**, 1–46.

Gibson W and Whittington H (1993) Genetic exchange in *Trypanosoma brucei*: selection of hybrid trypanosomes by introduction of genes conferring drug resistance. *Molecular and Biochemical Parasitology* **60**, 19–26.

Gibson W, Garside L and Bailey M (1992) Trisomy and chromosome size changes in hybrid trypanosomes from a genetic cross between *Trypanosoma brucei rhodesiense* and *T. b. brucei*. *Molecular and Biochemical Parasitology* **51**, 189–199.

Gibson W, Backhouse T and Griffiths A (2002) The human serum resistance associated gene is ubiquitous and conserved in *Trypanosoma brucei rhodesiense* throughout East Africa. *Infection Genetics and Evolution* **1**, 207–214.

Gibson W, Peacock L, Ferris V, Williams K and Bailey M (2008) The use of yellow fluorescent hybrids to indicate mating in *Trypanosoma brucei*. *Parasites & Vectors* **1**, 4.

Gilinger G and Bellofatto V (2001) Trypanosome spliced leader RNA genes contain the first identified RNA polymerase II gene promoter in these organisms. *Nucleic Acids Research* **29**, 1556–1564.

Gill JW and Vogel HJ (1963) A bacterial endosymbiote in *Crithidia* (*Strigomonas*) *oncopelti*: biochemical and morphological aspects. *The Journal of Protozoology* **10**, 148–152.

Ginger ML, Chance ML, Sadler IH and Goad LJ (2001) The biosynthetic incorporation of the intact leucine skeleton into sterol by the trypanosomatid *Leishmania mexicana*. *Journal of Biological Chemistry* **276**, 11674–11682.

Ginger ML, Portman N and McKean PG (2008) Swimming with protists: perception, motility and flagellum assembly. *Nature Reviews Microbiology* **6**, 838–850.

Glover L, Hutchinson S, Alsford S and Horn D (2016) VEX1 controls the allelic exclusion required for antigenic variation in trypanosomes. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 7225–7230.

Gluenz E, Shaw MK and Gull K (2007) Structural asymmetry and discrete nucleic acid subdomains in the *Trypanosoma brucei* kinetoplast. *Molecular Microbiology* **64**, 1529–1539.

Godfrey DG, Baker RD, Rickman LR and Mehlitz D (1990) The distribution, relationships and identification of enzymic variants within the subgenus *Trypanozoon*. *Advances in Parasitology* **29**, 1–74.

Goncharov I, Xu YX, Zimmer Y, Sherman K and Michaeli S (1998) Structure-function analysis of the trypanosomatid spliced leader RNA. *Nucleic Acids Research* **26**, 2200–2207.

Goncharov I, Palfi Z, Bindereif A and Michaeli S (1999) Purification of the spliced leader ribonucleoprotein particle from *leptomonas collosoma* revealed the existence of an Sm protein in trypanosomes. Cloning the SmE homologue. *Journal of Biological Chemistry* **274**, 12217–12221.

Goodhead I, Capewell P, Bailey JW, Beament T, Chance M, Kay S, Forrester S, MacLeod A, Taylor M, Noyes H and Hall N (2013) Whole-genome sequencing of *Trypanosoma brucei* reveals introgression between subspecies that is associated with virulence. *MBio* **4**, e00197-13.

Gray MW (2012) Evolutionary origin of RNA editing. *Biochemistry* **51**, 5235–5242.

Grybchuk-Ieremenko A, Losev A, Kostygov AY, Lukeš J and Yurchenko V (2014) High prevalence of trypanosome co-infections in freshwater fishes. *Folia Parasitologica* **61**, 495–504.

Grybchuk D, Akopyants NS, Kostygov AY, Konovalovas A, Lye LF, Dobson DE, Zangger H, Fasel N, Butenko A, Frolov AO, Votýpka J, d'Avila-Levy CM, Kulich P, Moravcová J, Plevka P, Rogozin IB, Serva S, Lukeš J, Beverley SM and Yurchenko V (2018a) Viral discovery and diversity in trypanosomatid protozoa with a focus on relatives of the human parasite *Leishmania*. *Proceedings of the National Academy of Sciences of the United States of America* **115**, E506–E515.

Grybchuk D, Kostygov AY, Macedo DH, d'Avila-Levy CM and Yurchenko V (2018b) RNA viruses in trypanosomatid parasites: a historical overview. *Memorias do Instituto Oswaldo Cruz* **113**, e170487.

Gualdron-López M, Vapola MH, Miinalainen IJ, Hiltunen JK, Michels PA and Antonenkov VD (2012) Channel-forming activities in the glycosomal fraction from the bloodstream form of *Trypanosoma brucei*. *PLoS ONE* **7**, e34530.

Günzl A (2010) The pre-mRNA splicing machinery of trypanosomes: complex or simplified? *Eukaryotic Cell* **9**, 1159–1170.

Günzl A, Bruderer T, Laufer G, Schimanski B, Tu LC, Chung HM, Lee PT and Lee MG (2003) RNA polymerase I transcribes procyclin genes and variant surface glycoprotein gene expression sites in *Trypanosoma brucei*. *Eukaryotic Cell* **2**, 542–551.

Günzl A, Kirkham JK, Nguyen TN, Badjatia N and Park SH (2015) Mono-allelic VSG expression by RNA polymerase I in *Trypanosoma brucei*: expression site control from both ends? *Gene* **556**, 68–73.

Günzl A, Vanhamme L and Myler PJ (2007) Transcription in trypanosomes: a different means to the end. In Barry D, McCulloch R, Mottram JC and Acosta-Serrano A (eds), *Trypanosomes: After the Genome*. Norfolk, UK: Horizon Bioscience, pp. 177–208.

Haanstra JR, van Tuijl A, Kessler P, Reijnders W, Michels PA, Westerhoff HV, Parsons M and Bakker BM (2008) Compartmentation prevents a lethal turbo-explosion of glycolysis in trypanosomes. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 17718–17723.

Haanstra JR, González-Marcano EB, Gualdrón-López M and Michels PA (2016) Biogenesis, maintenance and dynamics of glycosomes in trypanosomatid parasites. *Biochimica et Biophysica Acta* **1863**, 1038–1048.

Hajduk S and Ochsenreiter T (2010) RNA editing in kinetoplastids. *RNA Biology* **7**, 229–236.

Hamilton PB, Stevens JR, Gaunt MW, Gidley J and Gibson WC (2004) Trypanosomes are monophyletic: evidence from genes for glyceraldehyde phosphate dehydrogenase and small subunit ribosomal RNA. *International Journal for Parasitology* **34**, 1393–1404.

Hamilton PT, Votýpka J, Dostalova A, Yurchenko V, Bird NH, Lukeš J, Lemaitre B and Perlman SJ (2015) Infection dynamics and immune response in a newly described *Drosophila*-trypanosomatid association. *MBio* **6**, e01356–e01315.

Hannaert V, Bringaud F, Opperdoes FR and Michels PA (2003) Evolution of energy metabolism and its compartmentation in Kinetoplastida. *Kinetoplastid Biology and Disease* **2**, 11.

Hashimi H, Zikova A, Panigrahi AK, Stuart KD and Lukeš J (2008) TbRGG1, an essential protein involved in kinetoplastid RNA metabolism that is associated with a novel multiprotein complex. *RNA* **14**, 970–980.

Hashimi H, Čičová Z, Novotná L, Wen YZ and Lukeš J (2009) Kinetoplastid guide RNA biogenesis is dependent on subunits of the mitochondrial RNA binding complex 1 and mitochondrial RNA polymerase. *RNA* **15**, 588–599.

Hashimi H, Zimmer SL, Ammerman ML, Read LK and Lukes J (2013) Dual core processing: mRB1 is an emerging kinetoplast RNA editing complex. *Trends in Parasitology* **29**, 91–99.

Hashimi H, Kaltenbrunner S, Zíková A and Lukeš J (2016) Trypanosome mitochondrial translation and tetracycline: no sweat about Tet. *PLoS Pathogens* **12**, e1005492.

Heise N and Opperdoes FR (1999) Purification, localisation and characterisation of glucose-6-phosphate dehydrogenase of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **99**, 21–32.

Heitman J (2006) Sexual reproduction and the evolution of microbial pathogens. *Current Biology* **16**, R711–R725.

Hernandez R and Cevallos AM (2014) Ribosomal RNA gene transcription in trypanosomes. *Parasitology Research* **113**, 2415–2424.

Hide G and Tait A (2009) Molecular epidemiology of African sleeping sickness. *Parasitology* **136**, 1491–1500.

Hines JC and Ray DS (1997) Periodic synthesis of kinetoplast DNA topo-isomerase II during the cell cycle. *Molecular and Biochemical Parasitology* **88**, 249–252.

Hoare CA (1966) The classification of mammalian trypanosomes. *Ergebnisse Der Mikrobiologie, Immunitatsforschung Und Experimentellen Therapie* **39**, 43–57.

Hoare CA and Wallace FG (1966) Developmental stages of trypanosomatid flagellates: a new terminology. *Nature* **212**, 1385–1386.

Hollar L, Lukeš J and Maslov DA (1998) Monophyly of endosymbiont containing trypanosomatids: phylogeny versus taxonomy. *Journal of Eukaryotic Microbiology* **45**, 293–297.

Hong M and Simpson L (2003) Genomic organization of *Trypanosoma brucei* kinetoplast DNA minicircles. *Protist* **154**, 265–279.

Horáková E, Changmai P, Vancová M, Sobotka R, Van Den Abbeele J, Vanhollebeke B and Lukeš J (2017) The *Trypanosoma brucei* TbHrg protein is a heme transporter involved in the regulation of stage-specific morphological transitions. *Journal of Biological Chemistry* **292**, 6998–7010.

Horn D (2014) Antigenic variation in African trypanosomes. *Molecular and Biochemical Parasitology* **195**, 123–129.

Horváth A, Maslov DA, Peters LS, Haviernik P, Wuestenhagen T and Kolesnikov AA (1990) Analysis of the sequence repeats in the divergent region of maxicircle DNA from kinetoplasts of *Crithidia oncopelti*. *Molecular Biology (Mosk)* **24**, 1539–1548.

Horváth A, Nebohacova M, Lukeš J and Maslov DA (2002) Unusual poly-peptide synthesis in the kinetoplast-mitochondria from *Leishmania tarentolae*. Identification of individual *de novo* translation products. *Journal of Biological Chemistry* **277**, 7222–7230.

Huang Z, Faktorová D, Křížová A, Kafková L, Read LK, Lukeš J and Hashimi H (2015) Integrity of the core mitochondrial RNA-binding complex 1 is vital for trypanosome RNA editing. *RNA* **21**, 2088–2102.

Hughes AL and Piontkivska H (2003) Phylogeny of trypanosomatidae and Bodonidae (Kinetoplastida) based on 18S rRNA: evidence for paraphyly of *Trypanosoma* and six other genera. *Molecular Biology and Evolution* **20**, 644–652.

Hughes LC, Ralston KS, Hill KL and Zhou ZH (2012) Three-dimensional structure of the trypanosome flagellum suggests that the paraflagellar rod functions as a biomechanical spring. *PLoS ONE* **7**, e25700.

Igoillo-Esteve M, Mazet M, Deumer G, Wallemacq P and Michels PA (2011) Glycosomal ABC transporters of *Trypanosoma brucei*: characterisation of their expression, topology and substrate specificity. *International Journal for Parasitology* **41**, 429–438.

Imamura H, Downing T, Van den Broeck F, Sanders MJ, Rijal S, Sundar S, Mannaert A, Vanaerschot M, Berg M, De Muylder G, Dumetz F, Cuypers B, Maes I, Domagalska M, Decuypere S, Rai K, Uranw S, Bhattarai NR, Khanal B, Prajapati VK, Sharma S, Stark O, Schonian G, De Koning HP, Settimo L, Vanhollebeke B, Roy S, Ostyn B, Boelaert M, Maes L, Berriman M, Dujardin JC and Cotton JA (2016) Evolutionary genomics of epidemic visceral leishmaniasis in the Indian subcontinent. *eLife* **5**, e12613.

Imhof S, Fragoso C, Hemphill A, von Schubert C, Li D, Legant W, Betzig E and Roditi I (2016) Flagellar membrane fusion and protein exchange in trypanosomes; a new form of cell-cell communication? *F1000Research* **5**, 682.

Inbar E, Akopyants NS, Charmoy M, Romano A, Lawyer P, Elnaiem DE, Kauffmann F, Barhoumi M, Grigg M, Owens K, Fay M, Dobson DE, Shaik J, Beverley SM and Sacks D (2013) The mating competence of geographically diverse *Leishmania major* strains in their natural and unnatural sand fly vectors. *PLoS Genetics* **9**, e1003672.

Ishemgulova A, Butenko A, Kortišová L, Boucinha C, Grybchuk-Ieremenko A, Morelli KA, Tesařová M, Kraeva N, Grybchuk D, Pánek T, Flegontov P, Lukeš J, Votýpka J, Pavan MG, Opperdoes FR, Spodareva V, d'Avila-Levy CM, Kostygov AY and Yurchenko V (2017) Molecular mechanisms of thermal resistance of the insect trypanosomatid *Crithidia thermophila. PLoS ONE* **12**, e0174165.

Ivens AC, Peacock CS, Worthey EA, Murphy L, Aggarwal G, Berriman M, Sisk E, Rajandream MA, Adlem E, Aert R, Anupama A, Apostolou Z, Attipoe P, Bason N, Bauser C, Beck A, Beverley SM, Bianchettin G, Borzym K, Bothe G, Bruschi CV, Collins M, Cadag E, Ciarloni L, Clayton C, Coulson RM, Cronin A, Cruz AK, Davies RM, De Gaudenzi J, Dobson DE, Duesterhoeft A, Fazelina G, Fosker N, Frasch AC, Fraser A, Fuchs M, Gabel C, Goble A, Goffeau A, Harris D, Hertz-Fowler C, Hilbert H, Horn D, Huang Y, Klages S, Knights A, Kube M, Larke N, Litvin L, Lord A, Louie T, Marra M, Masuy D, Matthews K, Michaeli S, Mottram JC, Muller-Auer S, Munden H, Nelson S, Norbertczak H, Oliver K, O'Neil S, Pentony M, Pohl TM, Price C, Purnelle B, Quail MA, Rabbinowitsch E, Reinhardt R, Rieger M, Rinta J, Robben J, Robertson L, Ruiz JC, Rutter S, Saunders D, Schafer M, Schein J, Schwartz DC, Seeger K, Seyler A, Sharp S, Shin H, Sivam D, Squares R, Squares S, Tosato V, Vogt C, Volckaert G, Wambutt R, Warren T, Wedler H, Woodward J, Zhou S, Zimmermann W, Smith DF, Blackwell JM, Stuart KD, Barrell B and Myler PJ (2005) The genome of the kinetoplastid parasite, *Leishmania major. Science* **309**, 436–442.

Ives A, Ronet C, Prevel F, Ruzzante G, Fuertes-Marraco S, Schutz F, Zangger H, Revaz-Breton M, Lye LF, Hickerson SM, Beverley SM, Acha-Orbea H, Launois P, Fasel N and Masina S (2011) *Leishmania* RNA virus controls the severity of mucocutaneous leishmaniasis. *Science* **331**, 775–778.

Jackson AP (2015) Genome evolution in trypanosomatid parasites. *Parasitology* **142**(suppl. 1), S40–S56.

Jackson AP, Otto TD, Aslett M, Armstrong SD, Bringaud F, Schlacht A, Hartley C, Sanders M, Wastling JM, Dacks JB, Acosta-Serrano A, Field MC, Ginger ML and Berriman M (2016) Kinetoplastid phylogenomics reveals the evolutionary innovations associated with the origins of parasitism. *Current Biology* **26**, 161–172.

Jacques I, Andrews NW and Huynh C (2010) Functional characterization of LIT1, the *Leishmania amazonensis* ferrous iron transporter. *Molecular and Biochemical Parasitology* **170**, 28–36.

Jenni L, Marti S, Schweizer J, Betschart B, Le Page RW, Wells JM, Tait A, Paindavoine P, Pays E and Steinert M (1986) Hybrid formation between African trypanosomes during cyclical transmission. *Nature* **322**, 173–175.

Jensen RE and Englund PT (2012) Network news: the replication of kinetoplast DNA. *Annual Review of Microbiology* **66**, 473–491.

Jirků M, Yurchenko V, Lukeš J and Maslov DA (2012) New species of insect trypanosomatids from Costa Rica and the proposal for a new subfamily within the trypanosomatidae. *Journal of Eukaryotic Microbiology* **59**, 537–547.

Kable ML, Seiwert SD, Heidmann S and Stuart K (1996) RNA editing: a mechanism for gRNA-specified uridylate insertion into precursor mRNA. *Science* **273**, 1189–1195.

Kafková L, Ammerman ML, Faktorová D, Fisk JC, Zimmer SL, Sobotka R, Read LK, Lukeš J and Hashimi H (2012) Functional characterization of two paralogs that are novel RNA binding proteins influencing mitochondrial transcripts of *Trypanosoma brucei. RNA* **18**, 1846–1861.

Kao CY and Read LK (2005) Opposing effects of polyadenylation on the stability of edited and unedited mitochondrial RNAs in *Trypanosoma brucei. Molecular and Cellular Biology* **25**, 1634–1644.

Kassem A, Pays E and Vanhamme L (2014) Transcription is initiated on silent variant surface glycoprotein expression sites despite monoallelic expression in *Trypanosoma brucei. Proceedings of the National Academy of Sciences of the United States of America* **111**, 8943–8948.

Kato CD, Alibu VP, Nanteza A, Mugasa CM and Matovu E (2016) Population genetic structure and temporal stability among *Trypanosoma brucei rhodesiense* isolates in Uganda. *Parasites & Vectors* **9**, 259.

Kaufer A, Ellis J, Stark D and Barratt J (2017) The evolution of trypanosomatid taxonomy. *Parasites & Vectors* **10**, 287.

Keeling PJ (2016) Genomics: evolution of the genetic code. *Current Biology* **26**, R851–R853.

Kelly S, Kramer S, Schwede A, Maini PK, Gull K and Carrington M (2012) Genome organization is a major component of gene expression control in response to stress and during the cell division cycle in trypanosomes. *Open Biology* **2**, 120033.

Kidane GZ, Hughes D and Simpson L (1984) Sequence heterogeneity and anomalous electrophoretic mobility of kinetoplast minicircle DNA from *Leishmania tarentolae. Gene* **27**, 265–277.

Klaus SM, Kunji ER, Bozzo GG, Noiriel A, de la Garza RD, Basset GJ, Ravanel S, Rebeille F, Gregory III JF and Hanson AD (2005) Higher plant plastids and cyanobacteria have folate carriers related to those of trypanosomatids. *Journal of Biological Chemistry* **280**, 38457–38463.

Klein CC, Alves JM, Serrano MG, Buck GA, Vasconcelos AT, Sagot MF, Teixeira MM, Camargo EP and Motta MC (2013) Biosynthesis of vitamins and cofactors in bacterium-harbouring trypanosomatids depends on the symbiotic association as revealed by genomic analyses. *PLoS ONE* **8**, e79786.

Kleisen CM and Borst P (1975) Sequence heterogeneity of the mini-circles of kinetoplast DNA of *Crithidia luciliae* and evidence for the presence of a component more complex than mini-circle DNA in the kinetoplast network. *Biochimica et Biophysica Acta* **407**, 473–478.

Klingbeil MM and Englund PT (2004) Closing the gaps in kinetoplast DNA network replication. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 4333–4334.

Koffi M, Solano P, Barnabe C, de Meeus T, Bucheton B, Cuny G and Jamonneau V (2007) Genetic characterisation of *Trypanosoma brucei* s.l. using microsatellite typing: new perspectives for the molecular epidemiology of human African trypanosomiasis. *Infection Genetics and Evolution* **7**, 675–684.

Koffi M, De Meeus T, Bucheton B, Solano P, Camara M, Kaba D, Cuny G, Ayala FJ and Jamonneau V (2009) Population genetics of *Trypanosoma brucei gambiense*, the agent of sleeping sickness in Western Africa. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 209–214.

Kolev NG, Franklin JB, Carmi S, Shi H, Michaeli S and Tschudi C (2010) The transcriptome of the human pathogen *Trypanosoma brucei* at single-nucleotide resolution. *PLoS Pathogens* **6**, e1001090.

Kolev NG, Gunzl A and Tschudi C (2017) Metacyclic VSG expression site promoters are recognized by the same general transcription factor that is required for RNA polymerase I transcription of bloodstream expression sites. *Molecular and Biochemical Parasitology* **216**, 52–55.

Kořený L, Oborník M and Lukeš J (2013) Make it, take it, or leave it: heme metabolism of parasites. *PLoS Pathogens* **9**, e1003088.

Koslowsky DJ, Bhat GJ, Perrollaz AL, Feagin JE and Stuart K (1990) The MURF3 gene of *T. brucei* contains multiple domains of extensive editing and is homologous to a subunit of NADH dehydrogenase. *Cell* **62**, 901–911.

Koslowsky D, Sun Y, Hindenach J, Theisen T and Lucas J (2014) The insect-phase gRNA transcriptome in *Trypanosoma brucei. Nucleic Acids Research* **42**, 1873–1886.

Kostygov AY and Yurchenko V (2017) Revised classification of the subfamily Leishmaniinae (Trypanosomatidae). *Folia Parasitologica* **64**, 020.

Kostygov AY, Grybchuk-Ieremenko A, Malysheva MN, Frolov AO and Yurchenko V (2014) Molecular revision of the genus *Wallaceina. Protist* **165**, 594–604.

Kostygov A, Dobáková E, Grybchuk-Ieremenko A, Váhala D, Maslov DA, Votýpka J, Lukeš J and Yurchenko V (2016) Novel trypanosomatid–bacterium association: evolution of endosymbiosis in action. *MBio* **7**, e01985–e01915.

Kostygov AY, Butenko A, Nenarokova A, Tashyreva D, Flegontov P, Lukeš J and Yurchenko V (2017) Genome of *Ca.* Pandoraea novymonadis, an endosymbiotic bacterium of the trypanosomatid *Novymonas esmeraldas. Frontiers in Microbiology* **8**, 1940.

Kraeva N, Butenko A, Hlaváčová J, Kostygov A, Myškova J, Grybchuk D, Leštinová T, Votýpka J, Volf P, Opperdoes F, Flegontov P, Lukeš J and Yurchenko V (2015) *Leptomonas seymouri*: adaptations to the dixenous life cycle analyzed by genome sequencing, transcriptome profiling and co-infection with *Leishmania donovani. PLoS Pathogens* **11**, e1005127.

Kraeva N, Horáková E, Kostygov A, Kořený L, Butenko A, Yurchenko V and Lukeš J (2017) Catalase in Leishmaniinae: with me or against me? *Infection Genetics and Evolution* **50**, 121–127.

Kramer S (2017a) The ApaH-like phosphatase TbALPH1 is the major mRNA decapping enzyme of trypanosomes. *PLoS Pathogens* **13**, e1006456.

Kramer S (2017*b*) Simultaneous detection of mRNA transcription and decay intermediates by dual colour single mRNA FISH on subcellular resolution. *Nucleic Acids Research* **45**, e49.

Kramer S, Bannerman-Chukualim B, Ellis L, Boulden EA, Kelly S, Field MC and Carrington M (2013) Differential localization of the two *T. brucei* poly(A) binding proteins to the nucleus and RNP granules suggests binding to distinct mRNA pools. *PLoS ONE* **8**, e54004.

Kuhls K, Cupolillo E, Silva SO, Schweynoch C, Boite MC, Mello MN, Mauricio I, Miles M, Wirth T and Schonian G (2013) Population structure and evidence for both clonality and recombination among Brazilian strains of the subgenus *Leishmania* (*Viannia*). *PLoS Neglected Tropical Diseases* **7**, e2490.

Lai DH, Hashimi H, Lun ZR, Ayala FJ and Lukeš J (2008) Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 1999–2004.

Lander N, Cordeiro C, Huang G and Docampo R (2016) Polyphosphate and acidocalcisomes. *Biochemical Society Transactions* **44**, 1–6.

Laveran A and Mesnil F (1901) Sur les flagelles a membrane ondulante des poissons (genres *Trypanosoma* gruby et *trypanoplasma* n. gen.). *S.C.R. Academy of Sciences, Paris* **133**, 670–675.

LeBowitz JH, Smith HQ, Rusche L and Beverley SM (1993) Coupling of poly (A) site selection and trans-splicing in *Leishmania*. *Genes & Development* **7**, 996–1007.

Lee SH, Stephens JL and Englund PT (2007*a*) A fatty-acid synthesis mechanism specialized for parasitism. *Nature Reviews Microbiology* **5**, 287–297.

Lee JH, Nguyen TN, Schimanski B and Gunzl A (2007*b*) Spliced leader RNA gene transcription in *Trypanosoma brucei* requires transcription factor TFIIH. *Eukaryotic Cell* **6**, 641–649.

Lee JH, Jung HS and Gunzl A (2009) Transcriptionally active TFIIH of the early-diverged eukaryote *Trypanosoma brucei* harbors two novel core subunits but not a cyclin-activating kinase complex. *Nucleic Acids Research* **37**, 3811–3820.

Lee JH, Cai G, Panigrahi AK, Dunham-Ems S, Nguyen TN, Radolf JD, Asturias FJ and Gunzl A (2010) A TFIIH-associated mediator head is a basal factor of small nuclear spliced leader RNA gene transcription in early-diverged trypanosomes. *Molecular and Cellular Biology* **30**, 5502–5513.

Leifso K, Cohen-Freue G, Dogra N, Murray A and McMaster WR (2007) Genomic and proteomic expression analysis of *Leishmania* promastigote and amastigote life stages: the *Leishmania* genome is constitutively expressed. *Molecular and Biochemical Parasitology* **152**, 35–46.

Lewis JW and Ball SJ (1980) Ultrastructure of the epimastigotes of the fish trypanosome *Trypanosoma cobitis* Mitrophanow 1883, in the crop of the leech vector, *Hemiclepsis marginata*. *Journal of Parasitology* **66**, 948–953.

Lewis MD, Llewellyn MS, Yeo M, Acosta N, Gaunt MW and Miles MA (2011) Recent, independent and anthropogenic origins of *Trypanosoma cruzi* hybrids. *PLoS Neglected Tropical Diseases* **5**, e1363.

Li Y, Sun Y, Hines JC and Ray DS (2007) Identification of new kinetoplast DNA replication proteins in trypanosomatids based on predicted S-phase expression and mitochondrial targeting. *Eukaryotic Cell* **6**, 2303–2310.

Liang XH, Haritan A, Uliel S and Michaeli S (2003) *Trans* and *cis* splicing in trypanosomatids: mechanism, factors, and regulation. *Eukaryotic Cell* **2**, 830–840.

Liu B, Liu Y, Motyka SA, Agbo EE and Englund PT (2005) Fellowship of the rings: the replication of kinetoplast DNA. *Trends in Parasitology* **21**, 363–369.

Loyola-Machado AC, Azevedo-Martins AC, Catta-Preta CMC, de Souza W, Galina A and Motta MCM (2017) The symbiotic bacterium fuels the energy metabolism of the host trypanosomatid *Strigomonas culicis*. *Protist* **168**, 253–269.

Lueong S, Merce C, Fischer B, Hoheisel JD and Erben ED (2016) Gene expression regulatory networks in *Trypanosoma brucei*: insights into the role of the mRNA-binding proteome. *Molecular Microbiology* **100**, 457–471.

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

Lukeš J, Guilbride DL, Votýpka J, Zíková A, Benne R and Englund PT (2002) Kinetoplast DNA network: evolution of an improbable structure. *Eukaryotic Cell* **1**, 495–502.

Lukeš J, Hashimi H and Zíková A (2005) Unexplained complexity of the mitochondrial genome and transcriptome in kinetoplastid flagellates. *Current Genetics* **48**, 277–299.

Lukeš J, Leander BS and Keeling PJ (2009) Cascades of convergent evolution: the corresponding evolutionary histories of euglenozoans and dinoflagellates. *Proceedings of the National Academy of Sciences of the United States of America* **106**(suppl. 1), 9963–9970.

Lukeš J, Archibald JM, Keeling PJ, Doolittle WF and Gray MW (2011) How a neutral evolutionary ratchet can build cellular complexity. *IUBMB Life* **63**, 528–537.

Lukeš J, Skalický T, Týč J, Votýpka J and Yurchenko V (2014) Evolution of parasitism in kinetoplastid flagellates. *Molecular and Biochemical Parasitology* **195**, 115–122.

Lukeš J, Butenko A, Hashimi H, Maslov DA, Votýpka J and Yurchenko V (2018) Trypanosomatids are much more than just trypanosomes: clues from the expanded family tree. *Trends in Parasitology* **34**, 466–480.

Machado CA and Ayala FJ (2001) Nucleotide sequences provide evidence of genetic exchange among distantly related lineages of *Trypanosoma cruzi*. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 7396–7401.

MacLeod A, Tweedie A, Welburn SC, Maudlin I, Turner CM and Tait A (2000) Minisatellite marker analysis of *Trypanosoma brucei*: reconciliation of clonal, panmictic, and epidemic population genetic structures. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 13442–13447.

MacLeod A, Tait A and Turner CM (2001*a*) The population genetics of *Trypanosoma brucei* and the origin of human infectivity. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **356**, 1035–1044.

MacLeod A, Turner CM and Tait A (2001*b*) The detection of geographical substructuring of *Trypanosoma brucei* populations by the analysis of minisatellite polymorphisms. *Parasitology* **123**, 475–482.

MacLeod A, Welburn S, Maudlin I, Turner CM and Tait A (2001*c*) Evidence for multiple origins of human infectivity in *Trypanosoma brucei* revealed by minisatellite variant repeat mapping. *Journal of Molecular Evolution* **52**, 290–301.

MacLeod A, Tweedie A, McLellan S, Hope M, Taylor S, Cooper A, Sweeney L, Turner CM and Tait A (2005) Allelic segregation and independent assortment in *T. brucei* crosses: proof that the genetic system is mendelian and involves meiosis. *Molecular and Biochemical Parasitology* **143**, 12–19.

MacRae IJ and Doudna JA (2007) Ribonuclease revisited: structural insights into ribonuclease III family enzymes. *Current Opinion in Structural Biology* **17**, 138–145.

Mair G, Shi H, Li H, Djikeng A, Aviles HO, Bishop JR, Falcone FH, Gavrilescu C, Montgomery JL, Santori MI, Stern LS, Wang Z, Ullu E and Tschudi C (2000) A new twist in trypanosome RNA metabolism: *cis*-splicing of pre-mRNA. *RNA* **6**, 163–169.

Mandelboim M, Estrano CL, Tschudi C, Ullu E and Michaeli S (2002) On the role of exon and intron sequences in *trans*-splicing utilization and cap 4 modification of the trypanosomatid *Leptomonas collosoma* SL RNA. *Journal of Biological Chemistry* **277**, 35210–35218.

Maree JP, Povelones ML, Clark DJ, Rudenko G and Patterton HG (2017) Well-positioned nucleosomes punctuate polycistronic pol II transcription units and flank silent VSG gene arrays in *Trypanosoma brucei*. *Epigenetics & Chromatin* **10**, 14.

Marmur J, Cahoon ME, Shimura Y and Vogel H (1963) DNA type attributable to a bacterial endosymbiote in the protozoon, *Crithidia* (*Strigomonas*) *oncopelti*. *Nature* **196**, 1228–1229.

Martin DS and Desser SS (1990) A light and electron microscopic study of *Trypanosoma fallisi* n. sp. in toads (*Bufo americanus*) from algonquin park, Ontario. *The Journal of Protozoology* **37**, 199–206.

Martin DS and Desser SS (1991) Development of *Trypanosoma fallisi* in the leech, *Desserobdella picta*, in toads (*Bufo americanus*), and *in vitro*. A light and electron microscopic study. *Parasitology Research* **77**, 18–26.

Martínez-Calvillo S, Nguyen D, Stuart K and Myler PJ (2004) Transcription initiation and termination on *Leishmania major* chromosome 3. *Eukaryotic Cell* **3**, 506–517.

Martinez-Calvillo S, Saxena A, Green A, Leland A and Myler PJ (2007) Characterization of the RNA polymerase II and III complexes in *Leishmania major*. *International Journal for Parasitology* **37**, 491–502.

Maslov DA (2010) Complete set of mitochondrial pan-edited mRNAs in *Leishmania mexicana amazonensis* LV78. *Molecular and Biochemical Parasitology* 173, 107–114.

Maslov DA and Simpson L (1992) The polarity of editing within a multiple gRNA-mediated domain is due to formation of anchors for upstream gRNAs by downstream editing. *Cell* 70, 459–467.

Maslov DA and Simpson L (1994) RNA editing and mitochondrial genomic organization in the cryptobiid kinetoplastid protozoan *trypanoplasma borreli*. *Molecular and Cellular Biology* 14, 8174–8182.

Maslov DA, Kolesnikov AA and Zaitseva GN (1984) Conservative and divergent base sequence regions in the maxicircle kinetoplast DNA of several trypanosomatid flagellates. *Molecular and Biochemical Parasitology* 12, 351–364.

Maslov DA, Sturm NR, Niner BM, Gruszynski ES, Peris M and Simpson L (1992) An intergenic G-rich region in *Leishmania tarentolae* kinetoplast maxicircle DNA is a pan-edited cryptogene encoding ribosomal protein S12. *Molecular and Cellular Biology* 12, 56–67.

Maslov DA, Avila HA, Lake JA and Simpson L (1994) Evolution of RNA editing in kinetoplastid protozoa. *Nature* 368, 345–348.

Maslov DA, Hollar L, Haghighat P and Nawathean P (1998) Demonstration of mRNA editing and localization of guide RNA genes in kinetoplast-mitochondria of the plant trypanosomatid *Phytomonas serpens*. *Molecular and Biochemical Parasitology* 93, 225–236.

Maslov DA, Nawathean P and Scheel J (1999) Partial kinetoplast-mitochondrial gene organization and expression in the respiratory deficient plant trypanosomatid *Phytomonas serpens*. *Molecular and Biochemical Parasitology* 99, 207–221.

Maslov DA, Sharma MR, Butler E, Falick AM, Gingery M, Agrawal RK, Spremulli LL and Simpson L (2006) Isolation and characterization of mitochondrial ribosomes and ribosomal subunits from *Leishmania tarentolae*. *Molecular and Biochemical Parasitology* 148, 69–78.

Maslov DA, Spremulli LL, Sharma MR, Bhargava K, Grasso D, Falick AM, Agrawal RK, Parker CE and Simpson L (2007) Proteomics and electron microscopic characterization of the unusual mitochondrial ribosome-related 45S complex in *Leishmania tarentolae*. *Molecular and Biochemical Parasitology* 152, 203–212.

Maslov DA, Yurchenko VY, Jirků M and Lukeš J (2010) Two new species of trypanosomatid parasites isolated from heteroptera in Costa Rica. *Journal of Eukaryotic Microbiology* 57, 177–188.

Maslov DA, Votýpka J, Yurchenko V and Lukeš J (2013) Diversity and phylogeny of insect trypanosomatids: all that is hidden shall be revealed. *Trends in Parasitology* 29, 43–52.

Matthews KR, Tschudi C and Ullu E (1994) A common pyrimidine-rich motif governs trans-splicing and polyadenylation of tubulin polycistronic pre-mRNA in trypanosomes. *Genes & Development* 8, 491–501.

Mattiacio JL and Read LK (2008) Roles for TbDSS-1 in RNA surveillance and decay of maturation by-products from the 12S rRNA locus. *Nucleic Acids Research* 36, 319–329.

McDaniel JP and Dvorak JA (1993) Identification, isolation, and characterization of naturally-occurring *Trypanosoma cruzi* variants. *Molecular and Biochemical Parasitology* 57, 213–222.

McNicoll F, Muller M, Cloutier S, Boilard N, Rochette A, Dube M and Papadopoulou B (2005) Distinct 3'-untranslated region elements regulate stage-specific mRNA accumulation and translation in *Leishmania*. *Journal of Biological Chemistry* 280, 35238–35246.

McNicoll F, Drummelsmith J, Muller M, Madore E, Boilard N, Ouellette M and Papadopoulou B (2006) A combined proteomic and transcriptomic approach to the study of stage differentiation in *Leishmania infantum*. *Proteomics* 6, 3567–3581.

Melville SE, Leech V, Gerrard CS, Tait A and Blackwell JM (1998) The molecular karyotype of the megabase chromosomes of *Trypanosoma brucei* and the assignment of chromosome markers. *Molecular and Biochemical Parasitology* 94, 155–173.

Messenger LA and Miles MA (2015) Evidence and importance of genetic exchange among field populations of *Trypanosoma cruzi*. *Acta Tropica* 151, 150–155.

Michels PA, Marchand M, Kohl L, Allert S, Wierenga RK and Opperdoes FR (1991) The cytosolic and glycosomal isoenzymes of glyceraldehyde-3-phosphate dehydrogenase in *Trypanosoma brucei* have a distant evolutionary relationship. *European Journal of Biochemistry* 198, 421–428.

Michels PA, Chevalier N, Opperdoes FR, Rider MH and Rigden DJ (1997) The glycosomal ATP-dependent phosphofructokinase of *Trypanosoma brucei* must have evolved from an ancestral pyrophosphate-dependent enzyme. *European Journal of Biochemistry* 250, 698–704.

Miguel DC, Flannery AR, Mittra B and Andrews NW (2013) Heme uptake mediated by LHR1 is essential for *Leishmania amazonensis* virulence. *Infection and Immunity* 81, 3620–3626.

Miles MA, Souza A, Povoa M, Shaw JJ, Lainson R and Toye PJ (1978) Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochthonous patients with chagas' disease in amazonian Brazil. *Nature* 272, 819–821.

Molinas SM, Altabe SG, Opperdoes FR, Rider MH, Michels PA and Uttaro AD (2003) The multifunctional isopropyl alcohol dehydrogenase of *phytomonas* sp. Could be the result of a horizontal gene transfer from a bacterium to the trypanosomatid lineage. *Journal of Biological Chemistry* 278, 36169–36175.

Morrison LJ, Tait A, McCormack G, Sweeney L, Black A, Truc P, Likeufack AC, Turner CM and MacLeod A (2008) *Trypanosoma brucei gambiense* Type 1 populations from human patients are clonal and display geographical genetic differentiation. *Infection Genetics and Evolution* 8, 847–854.

Motta MC, Saraiva EM, Costa e Silva Filho F and de Souza W (1991*a*) Cell surface charge and sugar residues of *Crithidia fasciculata* and *Crithidia luciliae*. *Microbios* 68, 87–96.

Motta MCM, Cava AMS, Silva PMF, Fiorini JE, Soares MJ and Desouza W (1991*b*) Morphological and biochemical characterization of the trypanosomatids *Crithidia desouzai* and *Herpetomonas anglusteri*. *Canadian Journal of Zoology* 69, 571–577.

Motta MC, Soares MJ, Attias M, Morgado J, Lemos AP, Saad-Nehme J, Meyer-Fernandes JR and De Souza W (1997) Ultrastructural and biochemical analysis of the relationship of *Crithidia deanei* with its endosymbiont. *European Journal of Cell Biology* 72, 370–377.

Motta MC, Catta-Preta CM, Schenkman S, de Azevedo Martins AC, Miranda K, de Souza W and Elias MC (2010) The bacterium endosymbiont of *Crithidia deanei* undergoes coordinated division with the host cell nucleus. *PLoS ONE* 5, e12415.

Muhich ML, Simpson L and Simpson AM (1983) Comparison of maxicircle DNAs of *Leishmania tarentolae* and *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences of the United States of America* 80, 4060–4064.

Muhich ML, Neckelmann N and Simpson L (1985) The divergent region of the *Leishmania tarentolae* kinetoplast maxicircle DNA contains a diverse set of repetitive sequences. *Nucleic Acids Research* 13, 3241–3260.

Müller UF, Lambert L and Göringer HU (2001) Annealing of RNA editing substrates facilitated by guide RNA-binding protein gBP21. *EMBO Journal* 20, 1394–1404.

Mundim MH and Roitman I (1977) Extra nutritional requirements of artificially aposymbiotic *Crithidia deanei*. *The Journal of Protozoology* 24, 329–331.

Mundim MH, Roitman I, Hermans MA and Kitajima EW (1974) Simple nutrition of *Crithidia deanei*, a reduviid trypanosomatid with an endosymbiont. *The Journal of Protozoology* 21, 518–521.

Myler PJ (2008) Genome structure and content. In Myler PJ and Fasel N (eds), *Leishmania: After the Genome*. Wymondham: Caister Academic Press, pp. 15–28.

Myler PJ, Glick D, Feagin JE, Morales TH and Stuart KD (1993) Structural organization of the maxicircle variable region of *Trypanosoma brucei*: identification of potential replication origins and topoisomerase II binding sites. *Nucleic Acids Research* 21, 687–694.

Myler PJ, Audleman L, de Vos T, Hixson G, Kiser P, Lemley C, Magness C, Rickel E, Sisk E, Sunkin S, Swartzell S, Westlake T, Bastien P, Fu G, Ivens A and Stuart K (1999) *Leishmania major* Friedlin chromosome 1 has an unusual distribution of protein-coding genes. *Proceedings of the National Academy of Sciences of the United States of America* 96, 2902–2906.

Navarro M and Gull K (2001) A pol I transcriptional body associated with VSG mono-allelic expression in *Trypanosoma brucei*. *Nature* 414, 759–763.

Navarro M, Cross GA and Wirtz E (1999) *Trypanosoma brucei* variant surface glycoprotein regulation involves coupled activation/inactivation and chromatin remodeling of expression sites. *EMBO Journal* 18, 2265–2272.

Nawathean P and Maslov DA (2000) The absence of genes for cytochrome c oxidase and reductase subunits in maxicircle kinetoplast DNA of the

respiration-deficient plant trypanosomatid *Phytomonas serpens*. *Current Genetics* **38**, 95–103.

Neboháčová M, Kim CE, Simpson L and Maslov DA (2009) RNA editing and mitochondrial activity in promastigotes and amastigotes of *Leishmania donovani*. *International Journal for Parasitology* **39**, 635–644.

Newton BA and Horne RW (1957) Intracellular structures in *Strigomonas oncopelti*. I. Cytoplasmic structures containing ribonucleoprotein. *Experimental Cell Research* **13**, 563–574.

Nguyen TN, Schimanski B and Gunzl A (2007) Active RNA polymerase I of *Trypanosoma brucei* harbors a novel subunit essential for transcription. *Molecular and Cellular Biology* **27**, 6254–6263.

Nguyen TN, Muller LS, Park SH, Siegel TN and Gunzl A (2014) Promoter occupancy of the basal class I transcription factor A differs strongly between active and silent VSG expression sites in *Trypanosoma brucei*. *Nucleic Acids Research* **42**, 3164–3176.

Nolan DP, Garcia-Salcedo CA, Geuskens M., Salmon D, Paturiaux-Hanocq F, Pays A, Terbadi P and Pays E (2002) Endocytosis in african trypanosomes. In Black SJ and Seed JR (eds), *The African Trypanosomes*, vol. **1**. New York, US: Springer, pp. 127–141.

Novy FG, MacNeal WJ and Torrey HN (1907) The trypanosomes of mosquitoes and other insects. *Journal of Infectious Diseases* **4**, 223–276.

Nozaki T and Cross GA (1995) Effects of 3' untranslated and intergenic regions on gene expression in *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology* **75**, 55–67.

Nussbaum K, Honek J, Cadmus CM and Efferth T (2010) Trypanosomatid parasites causing neglected diseases. *Current Medicinal Chemistry* **17**, 1594–1617.

Ochsenreiter T, Cipriano M and Hajduk SL (2008) Alternative mRNA editing in trypanosomes is extensive and may contribute to mitochondrial protein diversity. *PLoS ONE* **3**, e1566.

Oliveira RP, Broude NE, Macedo AM, Cantor CR, Smith CL and Pena SD (1998) Probing the genetic population structure of *Trypanosoma cruzi* with polymorphic microsatellites. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 3776–3780.

Opperdoes FR (1987) Compartmentation of carbohydrate metabolism in trypanosomes. *Annual Review of Microbiology* **41**, 127–151.

Opperdoes FR and Borst P (1977) Localization of nine glycolytic enzymes in a microbody-like organelle in *Trypanosoma brucei*: the glycosome. *FEBS Letters* **80**, 360–364.

Opperdoes FR and Coombs GH (2007) Metabolism of *Leishmania*: proven and predicted. *Trends in Parasitology* **23**, 149–158.

Opperdoes FR and Michels PA (2007) Horizontal gene transfer in trypanosomatids. *Trends in Parasitology* **23**, 470–476.

Opperdoes FR and Szikora JP (2006) *In silico* prediction of the glycosomal enzymes of *Leishmania major* and trypanosomes. *Molecular and Biochemical Parasitology* **147**, 193–206.

Opperdoes FR and van Roy J (1982) The phospholipases of *Trypanosoma brucei* bloodstream forms and cultured procyclics. *Molecular and Biochemical Parasitology* **5**, 309–319.

Opperdoes FR, Nohýnková E, Van Schaftingen E, Lambeir AM, Veenhuis M and Van Roy J (1988) Demonstration of glycosomes (microbodies) in the Bodonid flagellate *Trypanoplasma borelli* (Protozoa, Kinetoplastida). *Molecular and Biochemical Parasitology* **30**, 155–163.

Opperdoes FR, Butenko A, Flegontov P, Yurchenko V and Lukeš J (2016) Comparative metabolism of free-living *Bodo saltans* and parasitic trypanosomatids. *Journal of Eukaryotic Microbiology* **63**, 657–678.

Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, Hill DE, Vidal M, Evans JG, Thorburn DR, Carr SA and Mootha VK (2008) A mitochondrial protein compendium elucidates complex I disease biology. *Cell* **134**, 112–123.

Palfi Z, Xu GL and Bindereif A (1994) Spliced leader-associated RNA of trypanosomes. Sequence conservation and association with protein components common to *trans*-spliceosomal ribonucleoproteins. *Journal of Biological Chemistry* **269**, 30620–30625.

Palmié-Peixoto IV, Rocha MR, Urbina JA, de Souza W, Einicker-Lamas M and Motta MC (2006) Effects of sterol biosynthesis inhibitors on endosymbiont-bearing trypanosomatids. *FEMS Microbiology Letters* **255**, 33–42.

Panigrahi AK, Ziková A, Dalley RA, Acestor N, Ogata Y, Anupama A, Myler PJ and Stuart KD (2008) Mitochondrial complexes in *Trypanosoma brucei*: a novel complex and a unique oxidoreductase complex. *Molecular and Cellular Proteomics* **7**, 534–545.

Panigrahi AK, Ogata Y, Ziková A, Anupama A, Dalley RA, Acestor N, Myler PJ and Stuart KD (2009) A comprehensive analysis of *Trypanosoma brucei* mitochondrial proteome. *Proteomics* **9**, 434–450.

Parfrey LW, Lahr DJ, Knoll AH and Katz LA (2011) Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 13624–13629.

Parsons M, Nelson RG, Watkins KP and Agabian N (1984) Trypanosome mRNAs share a common 5' spliced leader sequence. *Cell* **38**, 309–316.

Peacock CS, Seeger K, Harris D, Murphy L, Ruiz JC, Quail MA, Peters N, Adlem E, Tivey A, Aslett M, Kerhornou A, Ivens A, Fraser A, Rajandream MA, Carver T, Norbertczak H, Chillingworth T, Hance Z, Jagels K, Moule S, Ormond D, Rutter S, Squares R, Whitehead S, Rabbinowitsch E, Arrowsmith C, White B, Thurston S, Bringaud F, Baldauf SL, Faulconbridge A, Jeffares D, Depledge DP, Oyola SO, Hilley JD, Brito LO, Tosi LR, Barrell B, Cruz AK, Mottram JC, Smith DF and Berriman M (2007) Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. *Nature Genetics* **39**, 839–847.

Peacock L, Ferris V, Bailey M and Gibson W (2009) Intraclonal mating occurs during tsetse transmission of *Trypanosoma brucei*. *Parasites & Vectors* **2**, 43.

Peacock L, Ferris V, Sharma R, Sunter J, Bailey M, Carrington M and Gibson W (2011) Identification of the meiotic life cycle stage of *Trypanosoma brucei* in the tsetse fly. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 3671–3676.

Peacock L, Bailey M, Carrington M and Gibson W (2014a) Meiosis and haploid gametes in the pathogen *Trypanosoma brucei*. *Current Biology* **24**, 181–186.

Peacock L, Ferris V, Bailey M and Gibson W (2014b) Mating compatibility in the parasitic protist *Trypanosoma brucei*. *Parasites & Vectors* **7**, 78.

Pena-Diaz P, Vancova M, Resl C, Field MC and Lukeš J (2017) A leucine aminopeptidase is involved in kinetoplast DNA segregation in *Trypanosoma brucei*. *PLoS Pathogens* **13**, e1006310.

Penha LL, Hoffmann L, Souza SS, Martins AC, Bottaro T, Prosdocimi F, Faffe DS, Motta MC, Urmenyi TP and Silva R (2016) Symbiont modulates expression of specific gene categories in *Angomonas deanei*. *Memorias do Instituto Oswaldo Cruz* **111**, 686–691.

Perry KL, Watkins KP and Agabian N (1987) Trypanosome mRNAs have unusual 'cap 4' structures acquired by addition of a spliced leader. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 8190–8194.

Pestova TV, Kolupaeva VG, Lomakin IB, Pilipenko EV, Shatsky IN, Agol VI and Hellen CU (2001) Molecular mechanisms of translation initiation in eukaryotes. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 7029–7036.

Pollard VW, Rohrer SP, Michelotti EF, Hancock K and Hajduk SL (1990) Organization of minicircle genes for guide RNAs in *Trypanosoma brucei*. *Cell* **63**, 783–790.

Porcel BM, Denoeud F, Opperdoes FR, Noel B, Madoui M-A, Hammarton TC, Field MC, Da Silva C, Couloux A, Poulain J, Katinka M, Jabbari K, Aury J-M, Campbell DA, Cintron R, Dickens NJ, Docampo R, Sturm NR, Koumandou VL, Fabre S, Flegontov P, Lukeš J, Michaeli S, Mottram JC, Szoor B, Zilberstein D, Bringaud F, Wincker P and Dollet M (2014) The streamlined genome of *phytomonas* spp. Relative to human pathogenic kinetoplastids reveals a parasite tailored for plants. *PLoS Genetics* **10**, e1004007.

Portman N and Gull K (2010) The paraflagellar rod of kinetoplastid parasites: from structure to components and function. *International Journal for Parasitology* **40**, 135–148.

Povelones ML (2014) Beyond replication: division and segregation of mitochondrial DNA in kinetoplastids. *Molecular and Biochemical Parasitology* **196**, 53–60.

Preusser C, Jae N and Bindereif A (2012) mRNA splicing in trypanosomes. *International Journal of Medical Microbiology* **302**, 221–224.

Puechberty J, Blaineau C, Meghamla S, Crobu L, Pages M and Bastien P (2007) Compared genomics of the strand switch region of *Leishmania* chromosome 1 reveal a novel genus-specific gene and conserved structural features and sequence motifs. *BMC Genomics* **8**, 57.

Ramesh MA, Malik SB and Logsdon Jr JM (2005) A phylogenomic inventory of meiotic genes; evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. *Current Biology* **15**, 185–191.

Ramirez JD and Llewellyn MS (2014) Reproductive clonality in protozoan pathogens--truth or artefact? *Molecular Ecology* **23**, 4195–4202.

Ray DS (1989) Conserved sequence blocks in kinetoplast minicircles from diverse species of trypanosomes. *Molecular and Cellular Biology* **9**, 1365–1367.

Read LK, Myler PJ and Stuart K (1992) Extensive editing of both processed and preprocessed maxicircle CR6 transcripts in *Trypanosoma brucei*. *Journal of Biological Chemistry* **267**, 1123–1128.

Read LK, Lukeš J and Hashimi H (2016) Trypanosome RNA editing: the complexity of getting U in and taking U out. *Wiley Interdisciplinary Reviews. RNA* **7**, 33–51.

Reina-San-Martin B, Degrave W, Rougeot C, Cosson A, Chamond N, Cordeiro-Da-Silva A, Arala-Chaves M, Coutinho A and Minoprio P (2000) A B-cell mitogen from a pathogenic trypanosome is a eukaryotic proline racemase. *Nature Medicine* **6**, 890–897.

Requena JM (2011) Lights and shadows on gene organization and regulation of gene expression in *Leishmania*. *Frontiers in Bioscience* **17**, 2069–2085.

Rezende AM, Assis LA, Nunes EC, da Costa Lima TD, Marchini FK, Freire ER, Reis CR and de Melo Neto OP (2014) The translation initiation complex eIF3 in trypanosomatids and other pathogenic excavates – identification of conserved and divergent features based on orthologue analysis. *BMC Genomics* **15**, 1175.

Richmond GS and Smith TK (2007a) A novel phospholipase from *Trypanosoma brucei*. *Molecular Microbiology* **63**, 1078–1095.

Richmond GS and Smith TK (2007b) The role and characterization of phospholipase A1 in mediating lysophosphatidylcholine synthesis in *Trypanosoma brucei*. *Biochemical Journal* **405**, 319–329.

Ridlon L, Škodová I, Pan S, Lukeš J and Maslov DA (2013) The importance of the 45 S ribosomal small subunit-related complex for mitochondrial translation in *Trypanosoma brucei*. *Journal of Biological Chemistry* **288**, 32963–32978.

Riley GR, Corell RA and Stuart K (1994) Multiple guide RNAs for identical editing of *Trypanosoma brucei* apocytochrome b mRNA have an unusual minicircle location and are developmentally regulated. *Journal of Biological Chemistry* **269**, 6101–6108.

Rogers K, Gao G and Simpson L (2007) Uridylate-specific 3' 5'-exoribonucleases involved in uridylate-deletion RNA editing in trypanosomatid mitochondria. *Journal of Biological Chemistry* **282**, 29073–29080.

Rohloff P, Montalvetti A and Docampo R (2004) Acidocalcisomes and the contractile vacuole complex are involved in osmoregulation in *Trypanosoma cruzi*. *Journal of Biological Chemistry* **279**, 52270–52281.

Roitman C, Roitman I and de Azevedo HP (1972) Growth of an insect trypanosomatid at 37 °C in a defined medium. *The Journal of Protozoology* **19**, 346–349.

Rougeron V, De Meeus T, Hide M, Waleckx E, Bermudez H, Arevalo J, Llanos-Cuentas A, Dujardin JC, De Doncker S, Le Ray D, Ayala FJ and Banuls AL (2009) Extreme inbreeding in *Leishmania braziliensis*. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 10224–10229.

Rougeron V, Banuls AL, Carme B, Simon S, Couppie P, Nacher M, Hide M and De Meeus T (2011a) Reproductive strategies and population structure in *Leishmania*: substantial amount of sex in *Leishmania Viannia guyanensis*. *Molecular Ecology* **20**, 3116–3127.

Rougeron V, De Meeus T, Hide M, Le Falher G, Bucheton B, Dereure J, El-Safi SH, Dessein A and Banuls AL (2011b) Multifaceted population structure and reproductive strategy in *Leishmania donovani* complex in one Sudanese village. *PLoS Neglected Tropical Diseases* **5**, e1448.

Rougeron V, De Meeus T and Banuls AL (2017) Reproduction in *Leishmania*: a focus on genetic exchange. *Infection Genetics and Evolution* **50**, 128–132.

Sádlová J, Yeo M, Seblová V, Lewis MD, Mauricio I, Volf P and Miles MA (2011) Visualisation of *Leishmania donovani* fluorescent hybrids during early stage development in the sand fly vector. *PLoS ONE* **6**, e19851.

Sanchez-Moreno M, Lasztity D, Coppens I and Opperdoes FR (1992) Characterization of carbohydrate metabolism and demonstration of glycosomes in a *Phytomonas* sp. isolated from *Euphorbia characias*. *Molecular and Biochemical Parasitology* **54**, 185–199.

Sant'Anna C, Campanati L, Gadelha C, Lourenco D, Labati-Terra L, Bittencourt-Silvestre J, Benchimol M, Cunha-e-Silva NL and De Souza W (2005) Improvement on the visualization of cytoskeletal structures of protozoan parasites using high-resolution field emission scanning electron microscopy (FESEM). *Histochemistry and Cell Biology* **124**, 87–95.

Savage AF, Kolev NG, Franklin JB, Vigneron A, Aksoy S and Tschudi C (2016) Transcriptome profiling of *Trypanosoma brucei* development in the tsetse fly vector *Glossina morsitans*. *PLoS ONE* **11**, e0168877.

Savill NJ and Higgs PG (1999) A theoretical study of random segregation of minicircles in trypanosomatids. *Proceedings of the Royal Society of London B: Biological Sciences* **266**, 611–620.

Saville-Kent W (1880) *A Manual of the Infusoria Including a Description of all Known Flagellate, Ciliate, and Tentaculiferous Protozoa, British and Foreign and an Account of the Organization and Affinities of the Sponges*, London: David Bogue.

Schimanski B, Nguyen TN and Gunzl A (2005) Characterization of a multi-subunit transcription factor complex essential for spliced-leader RNA gene transcription in *Trypanosoma brucei*. *Molecular and Cellular Biology* **25**, 7303–7313.

Schimanski B, Brandenburg J, Nguyen TN, Caimano MJ and Gunzl A (2006) A TFIIB-like protein is indispensable for spliced leader RNA gene transcription in *Trypanosoma brucei*. *Nucleic Acids Research* **34**, 1676–1684.

Schmid-Hempel R, Salathe R, Tognazzo M and Schmid-Hempel P (2011) Genetic exchange and emergence of novel strains in directly transmitted trypanosomatids. *Infection Genetics and Evolution* **11**, 564–571.

Schmid-Hempel P, Aebi M, Barribeau S, Kitajima T, du Plessis L, Schmid-Hempel R and Zoller S (2018) The genomes of *Crithidia bombi* and *C. expoeki*, common parasites of bumblebees. *PLoS ONE* **13**, e0189738.

Schumacher MA, Karamooz E, Zíková A, Trantirek L and Lukeš J (2006) Crystal structures of *T. brucei* MRP1/MRP2 guide-RNA binding complex reveal RNA matchmaking mechanism. *Cell* **126**, 701–711.

Seiwert SD, Heidmann S and Stuart K (1996) Direct visualization of uridylate deletion *in vitro* suggests a mechanism for kinetoplastid RNA editing. *Cell* **84**, 831–841.

Sharma MR, Booth TM, Simpson L, Maslov DA and Agrawal RK (2009) Structure of a mitochondrial ribosome with minimal RNA. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 9637–9642.

Siegel TN, Hekstra DR, Wang X, Dewell S and Cross GA (2010) Genome-wide analysis of mRNA abundance in two life-cycle stages of *Trypanosoma brucei* and identification of splicing and polyadenylation sites. *Nucleic Acids Research* **38**, 4946–4957.

Simon MW, Martin E and Mukkada AJ (1978) Evidence for a functional glyoxylate cycle in the leishmaniae. *Journal of Bacteriology* **135**, 895–899.

Simpson L (1979) Isolation of maxicircle component of kinetoplast DNA from hemoflagellate protozoa. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 1585–1588.

Simpson L (1997) The genomic organization of guide RNA genes in kinetoplastid protozoa: several conundrums and their solutions. *Molecular and Biochemical Parasitology* **86**, 133–141.

Simpson L and Kretzer F (1997) The mitochondrion in dividing *Leishmania tarentolae* cells is symmetric and circular and becomes a single asymmetric tubule in non-dividing cells due to division of the kinetoplast portion. *Molecular and Biochemical Parasitology* **87**, 71–78.

Simpson L and Maslov DA (1994a) Ancient origin of RNA editing in kinetoplastid protozoa. *Current Opinion in Genetics & Development* **4**, 887–894.

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

Simpson AM, Suyama Y, Dewes H, Campbell DA and Simpson L (1989) Kinetoplastid mitochondria contain functional tRNAs which are encoded in nuclear DNA and also contain small minicircle and maxicircle transcripts of unknown function. *Nucleic Acids Research* **17**, 5427–5445.

Simpson L, Maslov DA and Blum B (1993) RNA editing in *Leishmania* mitochondria. In Benne R (ed.), *RNA Editing – the Alteration of Protein Coding Sequences of RNA*. New York: Ellis Horwood, pp. 53–85.

Simpson AG, Lukeš J and Roger AJ (2002) The evolutionary history of kinetoplastids and their kinetoplasts. *Molecular Biology and Evolution* **19**, 2071–2083.

Simpson L, Aphasizhev R, Gao G and Kang X (2004) Mitochondrial proteins and complexes in *Leishmania* and *Trypanosoma* involved in U-insertion/deletion RNA editing. *RNA* **10**, 159–170.

Simpson AG, Stevens JR and Lukeš J (2006) The evolution and diversity of kinetoplastid flagellates. *Trends in Parasitology* **22**, 168–174.

Simpson L, Douglass SM, Lake JA, Pellegrini M and Li F (2015) Comparison of the mitochondrial genomes and steady state transcriptomes of two strains of the trypanosomatid parasite, *Leishmania tarentolae*. *PLoS Neglected Tropical Diseases* 9, e0003841.

Simpson RM, Bruno AE, Chen R, Lott K, Tylec BL, Bard JE, Sun Y, Buck MJ and Read LK (2017) Trypanosome RNA editing mediator complex proteins have distinct functions in gRNA utilization. *Nucleic Acids Research* 45, 7965–7983.

Sistrom M, Evans B, Bjornson R, Gibson W, Balmer O, Maser P, Aksoy S and Caccone A (2014) Comparative genomics reveals multiple genetic backgrounds of human pathogenicity in the *Trypanosoma brucei* complex. *Genome Biology and Evolution* 6, 2811–2819.

Skalický T, Dobáková E, Wheeler RJ, Tesařová M, Flegontov P, Jirsová D, Votýpka J, Yurchenko V, Ayala FJ and Lukeš J (2017) Extensive flagellar remodeling during the complex life cycle of *Paratrypanosoma*, an early-branching trypanosomatid. *Proceedings of the National Academy of Sciences of the United States of America* 114, 11757–11762.

Škodová-Sveráková I, Horváth A and Maslov DA (2015) Identification of the mitochondrially encoded subunit 6 of F1FO ATPase in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* 201, 135–138.

Sloof P, Arts GJ, van den Burg J, van der Spek H and Benne R (1994) RNA editing in mitochondria of cultured trypanosomatids: translatable mRNAs for NADH-dehydrogenase subunits are missing. *Journal of Bioenergetics and Biomembranes* 26, 193–203.

Smith JM, Smith NH, O'Rourke M and Spratt BG (1993) How clonal are bacteria? *Proceedings of the National Academy of Sciences of the United States of America* 90, 4384–4388.

Soares MJ and De Souza W (1988) Cytoplasmic organelles of trypanosomatids: a cytochemical and stereological study. *Journal of Submicroscopic Cytology and Pathology* 20, 349–361.

Souto-Padron T and de Souza W (1982) Fine structure and cytochemistry of peroxisomes (microbodies) *Leptomonas samueli*. *Cell and Tissue Research* 222, 153–158.

Speijer D (2006) Is kinetoplastid pan-editing the result of an evolutionary balancing act? *IUBMB Life* 58, 91–96.

Speijer D, Breek CK, Muijsers AO, Hartog AF, Berden JA, Albracht SP, Samyn B, Van Beeumen J and Benne R (1997) Characterization of the respiratory chain from cultured *Crithidia fasciculata*. *Molecular and Biochemical Parasitology* 85, 171–186.

Speijer D, Lukeš J and Eliáš M (2015) Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. *Proceedings of the National Academy of Sciences of the United States of America* 112, 8827–8834.

Steinert M (1960) Mitochondria associated with the kinetonucleus of *Trypanosoma mega*. *The Journal of Biophysical and Biochemical Cytology* 8, 542–546.

Stoltzfus A (1999) On the possibility of constructive neutral evolution. *Journal of Molecular Evolution* 49, 169–181.

Stuart KD, Schnaufer A, Ernst NL and Panigrahi AK (2005) Complex management: rNA editing in trypanosomes. *Trends in Biochemical Sciences* 30, 97–105.

Sturm NR and Simpson L (1990a) Kinetoplast DNA minicircles encode guide RNAs for editing of cytochrome oxidase subunit III mRNA. *Cell* 61, 879–884.

Sturm NR and Simpson L (1990b) Partially edited mRNAs for cytochrome b and subunit III of cytochrome oxidase from *Leishmania tarentolae* mitochondria: rNA editing intermediates. *Cell* 61, 871–878.

Sturm NR, Vargas NS, Westenberger SJ, Zingales B and Campbell DA (2003) Evidence for multiple hybrid groups in *Trypanosoma cruzi*. *International Journal for Parasitology* 33, 269–279.

Sturm NR, Zamudio JR and Campbell DA (2012) SL RNA biogenesis in kinetoplastids: a long and winding road. In Bindereif A (ed.), *RNA Metabolism is Trypanosomes*. Berlin Heidelberg: Springer-Verlag, pp. 29–48.

Suematsu T, Zhang L, Aphasizheva I, Monti S, Huang L, Wang Q, Costello CE and Aphasizhev R (2016) Antisense transcripts delimit exonucleolytic activity of the mitochondrial 3' processome to generate guide RNAs. *Molecular Cell* 61, 364–378.

Sunter JD, Varga V, Dean S and Gull K (2015) A dynamic coordination of flagellum and cytoplasmic cytoskeleton assembly specifies cell morphogenesis in trypanosomes. *Journal of Cell Science* 128, 1580–1594.

Svobodová M, Zídková L, Čepička I, Oborník M, Lukeš J and Votýpka J (2007) *Sergeia podlipaevi* gen. nov., sp. nov. (Trypanosomatidae, Kinetoplastida), a parasite of biting midges (Ceratopogonidae, Diptera). *International Journal of Systematic and Evolutionary Microbiology* 57, 423–432.

Swart EC, Serra V, Petroni G and Nowacki M (2016) Genetic codes with no dedicated stop codon: context-dependent translation termination. *Cell* 166, 691–702.

Szempruch AJ, Sykes SE, Kieft R, Dennison L, Becker AC, Gartrell A, Martin WJ, Nakayasu ES, Almeida IC, Hajduk SL and Harrington JM (2016) Extracellular vesicles from *Trypanosoma brucei* mediate virulence factor transfer and cause host anemia. *Cell* 164, 246–257.

Szöör B, Haanstra JR, Gualdrón-López M and Michels PA (2014) Evolution, dynamics and specialized functions of glycosomes in metabolism and development of trypanosomatids. *Current Opinion in Microbiology* 22, 79–87.

Tait A (1980) Evidence for diploidy and mating in trypanosomes. *Nature* 287, 536–538.

Tait A, Buchanan N, Hide G and Turner CM (1996) Self-fertilisation in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* 76, 31–42.

Teixeira MM, Borghesan TC, Ferreira RC, Santos MA, Takata CS, Campaner M, Nunes VL, Milder RV, de Souza W and Camargo EP (2011) Phylogenetic validation of the genera *Angomonas* and *Strigomonas* of trypanosomatids harboring bacterial endosymbionts with the description of new species of trypanosomatids and of proteobacterial symbionts. *Protist* 162, 503–524.

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

Tibayrenc M (1998) Genetic epidemiology of parasitic protozoa and other infectious agents: the need for an integrated approach. *International Journal for Parasitology* 28, 85–104.

Tibayrenc M and Ayala FJ (1988) Isozyme variability in *Trypanosoma cruzi*, the agent of Chagas' disease: genetical, taxonomical, and epidemiological significance. *Evolution* 42, 277–292.

Tibayrenc M and Ayala FJ (1991) Towards a population genetics of microorganisms: the clonal theory of parasitic protozoa. *Parasitology Today* 7, 228–232.

Tibayrenc M and Ayala FJ (2012) Reproductive clonality of pathogens: a perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. *Proceedings of the National Academy of Sciences of the United States of America* 109, E3305–E3313.

Tibayrenc M and Ayala FJ (2013) How clonal are *Trypanosoma* and *Leishmania*? *Trends in Parasitology* 29, 264–269.

Tibayrenc M and Ayala FJ (2015) The population genetics of *Trypanosoma cruzi* revisited in the light of the predominant clonal evolution model. *Acta Tropica* 151, 156–165.

Tibayrenc M and Ayala FJ (2017) Is predominant clonal evolution a common evolutionary adaptation to parasitism in pathogenic parasitic protozoa, fungi, bacteria, and viruses? *Advances in Parasitology* 97, 243–325.

Tibayrenc M, Kjellberg F and Ayala FJ (1990) A clonal theory of parasitic protozoa: the population structures of *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas*, and *Trypanosoma* and their medical and taxonomical consequences. *Proceedings of the National Academy of Sciences of the United States of America* 87, 2414–2418.

Tibayrenc M, Neubauer K, Barnabe C, Guerrini F, Skarecky D and Ayala FJ (1993) Genetic characterization of six parasitic protozoa: parity between random-primer DNA typing and multilocus enzyme electrophoresis. *Proceedings of the National Academy of Sciences of the United States of America* 90, 1335–1339.

Turner CM, Sternberg J, Buchanan N, Smith E, Hide G and Tait A (1990) Evidence that the mechanism of gene exchange in *Trypanosoma brucei* involves meiosis and syngamy. *Parasitology* 101(Pt 3), 377–386.

Turner CM, Hide G, Buchanan N and Tait A (1995) *Trypanosoma brucei*: inheritance of kinetoplast DNA maxicircles in a genetic cross and their segregation during vegetative growth. *Experimental Parasitology* 80, 234–241.

Týč J, Votýpka J, Klepetková H, Šuláková H, Jirků M and Lukeš J (2013) Growing diversity of trypanosomatid parasites of flies (Diptera: Brachycera): frequent cosmopolitism and moderate host specificity. *Molecular Phylogenetics and Evolution* 69, 255–264.

Ullu E and Tschudi C (1993) 2'-O-methyl RNA oligonucleotides identify two functional elements in the trypanosome spliced leader ribonucleoprotein particle. *Journal of Biological Chemistry* 268, 13068–13073.

Urwyler S, Studer E, Renggli CK and Roditi I (2007) A family of stage-specific alanine-rich proteins on the surface of epimastigote forms of *Trypanosoma brucei*. *Molecular Microbiology* 63, 218–228.

Uttaro AD (2014) Acquisition and biosynthesis of saturated and unsaturated fatty acids by trypanosomatids. *Molecular and Biochemical Parasitology* 196, 61–70.

Uzureau P, Uzureau S, Lecordier L, Fontaine F, Tebabi P, Homble F, Grelard A, Zhendre V, Nolan DP, Lins L, Crowet JM, Pays A, Felu C, Poelvoorde P, Vanhollebeke B, Moestrup SK, Lyngso J, Pedersen JS, Mottram JC, Dufourc EJ, Perez-Morga D and Pays E (2013) Mechanism of *Trypanosoma brucei gambiense* resistance to human serum. *Nature* **501**, 430–434.

van der Spek H, van den Burg J, Croiset A, van den Broek M, Sloof P and Benne R (1988) Transcripts from the frameshifted MURF3 gene from *Crithidia fasciculata* are edited by U insertion at multiple sites. *EMBO Journal* **7**, 2509–2514.

van Hellemond JJ, Opperdoes FR and Tielens AG (1998) Trypanosomatidae produce acetate *via* a mitochondrial acetate:succinate CoA transferase. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 3036–3041.

van Luenen HG, Kieft R, Mussmann R, Engstler M, ter Riet B and Borst P (2005) Trypanosomes change their transferrin receptor expression to allow effective uptake of host transferrin. *Molecular Microbiology* **58**, 151–165.

van Schaftingen E, Opperdoes FR and Hers HG (1985) Stimulation of *Trypanosoma brucei* pyruvate kinase by fructose 2,6-bisphosphate. *European Journal of Biochemistry* **153**, 403–406.

Vanhamme L and Pays E (1995) Control of gene expression in trypanosomes. *Microbiological Reviews* **59**, 223–240.

Vanhollebeke B, De Muylder G, Nielsen MJ, Pays A, Tebabi P, Dieu M, Raes M, Moestrup SK and Pays E (2008) A haptoglobin-hemoglobin receptor conveys innate immunity to *Trypanosoma brucei* in humans. *Science* **320**, 677–681.

Vertommen D, Van Roy J, Szikora JP, Rider MH, Michels PA and Opperdoes FR (2008) Differential expression of glycosomal and mitochondrial proteins in the two major life-cycle stages of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **158**, 189–201.

Vickerman K (1976) Comparative cell biology of the kinetoplastid flagellates. In Vickerman K and Preston TM (eds), *Biology of Kinetoplastida*, vol. **1**. London: Academic Press, pp. 35–130.

Votýpka J, Maslov DA, Yurchenko V, Jirků M, Kment P, Lun ZR and Lukeš J (2010) Probing into the diversity of trypanosomatid flagellates parasitizing insect hosts in South-West China reveals both endemism and global dispersal. *Molecular Phylogenetics and Evolution* **54**, 243–253.

Votýpka J, Suková E, Kraeva N, Ishemgulova A, Duží I, Lukeš J and Yurchenko V (2013) Diversity of trypanosomatids (Kinetoplastea: Trypanosomatidae) parasitizing fleas (Insecta: Siphonaptera) and description of a new genus *Blechomonas* gen. n. *Protist* **164**, 763–781.

Votýpka J, Kostygov AY, Kraeva N, Grybchuk-Ieremenko A, Tesařová M, Grybchuk D, Lukeš J and Yurchenko V (2014) *Kentomonas* gen. n., a new genus of endosymbiont-containing trypanosomatids of Strigomonadinae subfam. n. *Protist* **165**, 825–838.

Votýpka J, d'Avila-Levy CM, Grellier P, Maslov DA, Lukeš J and Yurchenko V (2015) New approaches to systematics of Trypanosomatidae: criteria for taxonomic (re)description. *Trends in Parasitology* **31**, 460–469.

Weir W, Capewell P, Foth B, Clucas C, Pountain A, Steketee P, Veitch N, Koffi M, De Meeus T, Kabore J, Camara M, Cooper A, Tait A, Jamonneau V, Bucheton B, Berriman M and MacLeod A (2016) Population genomics reveals the origin and asexual evolution of human infective trypanosomes. *eLife* **5**, e11473.

Weng J, Aphasizheva I, Etheridge RD, Huang L, Wang X, Falick AM and Aphasizhev R (2008) Guide RNA-binding complex from mitochondria of trypanosomatids. *Molecular Cell* **32**, 198–209.

Westenberger SJ, Barnabe C, Campbell DA and Sturm NR (2005) Two hybridization events define the population structure of *Trypanosoma cruzi*. *Genetics* **171**, 527–543.

Wheeler RJ, Gluenz E and Gull K (2015) Basal body multipotency and axonemal remodelling are two pathways to a 9 + 0 flagellum. *Nature Communications* **6**, 8964.

Wickstead B, Ersfeld K and Gull K (2004) The small chromosomes of *Trypanosoma brucei* involved in antigenic variation are constructed around repetitive palindromes. *Genome Research* **14**, 1014–1024.

Xong HV, Vanhamme L, Chamekh M, Chimfwembe CE, Van Den Abbeele J, Pays A, Van Meirvenne N, Hamers R, De Baetselier P and Pays E (1998) A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell* **95**, 839–846.

Yang X, Figueiredo LM, Espinal A, Okubo E and Li B (2009) RAP1 is essential for silencing telomeric variant surface glycoprotein genes in *Trypanosoma brucei*. *Cell* **137**, 99–109.

Yoffe Y, Zuberek J, Lewdorowicz M, Zeira Z, Keasar C, Orr-Dahan I, Jankowska-Anyszka M, Stepinski J, Darzynkiewicz E and Shapira M (2004) Cap-binding activity of an eIF4E homolog from *Leishmania*. *RNA* **10**, 1764–1775.

Yoffe Y, Leger M, Zinoviev A, Zuberek J, Darzynkiewicz E, Wagner G and Shapira M (2009) Evolutionary changes in the *Leishmania* eIF4F complex involve variations in the eIF4E-eIF4 G interactions. *Nucleic Acids Research* **37**, 3243–3253.

Yurchenko V and Kolesnikov AA (2001) Minicircular kinetoplast DNA of Trypanosomatidae. *Molecular Biology (Mosk)* **35**, 3–13 (in Russian).

Yurchenko V, Hobza R, Benada O and Lukeš J (1999) *Trypanosoma avium*: large minicircles in the kinetoplast DNA. *Experimental Parasitology* **92**, 215–218.

Yurchenko V, Lukeš J, Jirků M, Zeledon R and Maslov DA (2006a) *Leptomonas costaricensis* sp. n. (Kinetoplastea: Trypanosomatidae), a member of the novel phylogenetic group of insect trypanosomatids closely related to the genus *Leishmania*. *Parasitology* **133**, 537–546.

Yurchenko V, Lukeš J, Xu X and Maslov DA (2006b) An integrated morphological and molecular approach to a new species description in the Trypanosomatidae: the case of *Leptomonas podlipaevi* n. sp., a parasite of *Boisea rubrolineata* (Hemiptera: Rhopalidae). *Journal of Eukaryotic Microbiology* **53**, 103–111.

Yurchenko V, Votýpka J, Tesařová M, Klepetková H, Kraeva N, Jirků M and Lukeš J (2014) Ultrastructure and molecular phylogeny of four new species of monoxenous trypanosomatids from flies (Diptera: Brachycera) with redefinition of the genus *Wallaceina*. *Folia Parasitologica* **61**, 97–112.

Yurchenko V, Kostygov A, Havlová J, Grybchuk-Ieremenko A, Ševčíková T, Lukeš J, Ševčík J and Votýpka J (2016) Diversity of trypanosomatids in cockroaches and the description of *Herpetomonas tarakana* sp. n. *Journal of Eukaryotic Microbiology* **63**, 198–209.

Záhonová K, Kostygov A, Ševčíková T, Yurchenko V and Eliáš M (2016) An unprecedented non-canonical nuclear genetic code with all three termination codons reassigned as sense codons. *Current Biology* **26**, 2364–2369.

Zaitseva GN and Salikhov TA (1972) Comparison of the ribosomes of cytoplasm and bipolar body (endosymbiont) in the cells of the zooflagellate *Strigomonas oncopelti*. *Doklady Akademii Nauk* **205**, 457–460 (in Russian).

Zaitseva GN and Salikhov TA (1973) Effect of chloramphenicol and cycloheximide on protein synthesis in the bipolar body (endosymbiont) of *Strigomonas oncopelti* cells. *Izvestiia Akademii Nauk Sssr. Seriia Biologicheskaia* **2**, 252–259 (in Russian).

Zíková A, Kopečná J, Schumacher MA, Stuart K, Trantírek L and Lukeš J (2008a) Structure and function of the native and recombinant mitochondrial MRP1/MRP2 complex from *Trypanosoma brucei*. *International Journal for Parasitology* **38**, 901–912.

Zíková A, Panigrahi AK, Dalley RA, Acestor N, Anupama A, Ogata Y, Myler PJ and Stuart K (2008b) *Trypanosoma brucei* mitochondrial ribosomes: affinity purification and component identification by mass spectrometry. *Molecular and Cellular Proteomics* **7**, 1286–1296.

Zíková A, Verner Z, Nenarokova A, Michels PAM and Lukeš J (2017) A paradigm shift: the mitoproteomes of procyclic and bloodstream *Trypanosoma brucei* are comparably complex. *PLoS Pathogens* **13**, e1006679.

Zinoviev A and Shapira M (2012) Evolutionary conservation and diversification of the translation initiation apparatus in trypanosomatids. *Comparative and Functional Genomics* **2012**, 813718.

Zinoviev A, Leger M, Wagner G and Shapira M (2011) A novel 4E-interacting protein in *Leishmania* is involved in stage-specific translation pathways. *Nucleic Acids Research* **39**, 8404–8415.