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Review

Evolution of parasitism in kinetoplastid flagellates

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ABSTRACT

Kinetoplastid protists offer a unique opportunity for studying the evolution of parasitism. While all their close relatives are either photo- or phagotrophic, a number of kinetoplastid species are facultative or obligatory parasites, supporting a hypothesis that parasitism has emerged within this group of flagellates. In this review we discuss origin and evolution of parasitism in bodonids and trypanosomatids and specific adaptations allowing these protozoa to co-exist with their hosts. We also explore the limits of biodiversity of monoxenous (one host) trypanosomatids and some features distinguishing them from their dixenous (two hosts) relatives.

Contents

1. Emergence of parasitism: setting (up) the stage

With a certain degree of simplification, when the frequency of eukaryotic parasites encountered in vertebrate and invertebrate hosts is considered, probably only apicomplexans surpass kinetoplastid protists in abundance and diversity, and only parasitic nematodes seem to have a broader host range [1,2]. Kinetoplastids are evolutionarily more ancestral compared to the majority of other groups of parasitic protists, widespread and adaptable, which is an apparent reflection of their extremely successful life style. A recent taxonomy places Kinetoplastea along with its three sister groups (Euglenida, Symbiontida and Diplonemea) into Euglenozoa that belongs to the Discicristata, a group of protists unified by a striking feature–discoidal mitochondrial cristae [3] (Fig. 1). Euglenida are phototrophic or less frequently phagotrophic, the latter life strategy being characteristic for all known symbiontids and diplonemids [3]. Accordingly, parasitism must have emerged uniquely in the kinetoplastid lineage. It is an exciting challenge to identify genetic changes and/or inventions underlying this dramatic switch to a parasitic life style; however, it has to be postponed until the whole genomes for these sister clades of kinetoplastids are available.

Phylogenetic evidence strongly supports the early-branching of Prokinetoplastina within Kinetoplastea. This tiny group harbors only two known representatives – *Ichthyobodo* and *Perkinsela* (Fig. 2) [4,5]. While *Ichthyobodo* (also called *Costia*) is a bi-flagellar ectoparasite of fish, *Perkinsela* (also known as PLO, parasome and *Perkinsiella*) resides directly in the cytoplasm of certain amoebae parasitizing the gills of fish. This aflagellar kinetoplastid seems

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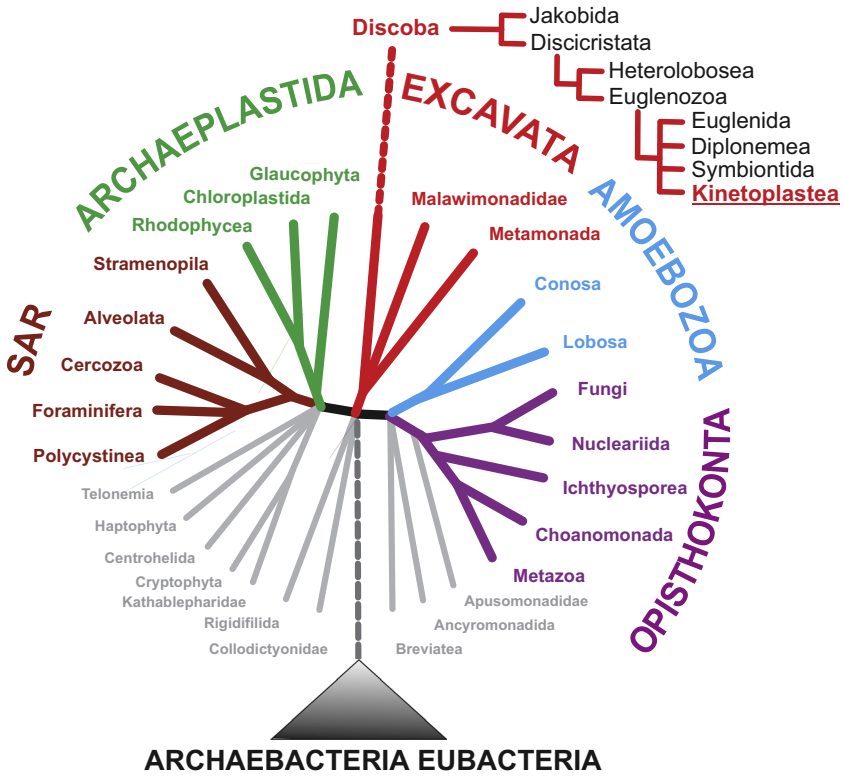

Fig. 1. A view of general eukaryote phylogeny reflecting the recent classification (based on [3]) and highlighting the taxonomic position of Kinetoplastea.

to behave like an organelle, invariably located close to the host nucleus and dividing synchronously with the host cell [6]. Based on DAPI staining, mitochondrial (=kinetoplast [k]) DNA of *Perkinsela* seems to be much more abundant than its nuclear DNA [7]. It will be exciting to investigate whether the extremely tight relationship with the amoeba host is reflected in the kDNA and nuclear genome of *Perkinsela*. Due to its robust branching at the basis of the Kinetoplastea clade, it is tempting to interpret the endosymbiont-like intracellularity of *Perkinsela* as some ancestral form of parasitism *via* which the kinetoplastid invaded first hosts. However, the absence of flagella, which are otherwise present in all sister clades (euglenids, symbiontids and diplonemids) as well as in all derived lineages, qualifies *Perkinsela* as a unique case of parasitic reductionism.

All the remaining bodonids fall into Metakinetoplastina, a group further subdivided into four clades (Neobodonida, Parabodonida, Eubodonida and Trypanosomatida) (Fig. 2), of which only the latter is obligatory parasitic [3,4]. Mutual relationships within the bodonids are far from being firmly established, yet it is obvious that they acquired parasitic life style independently more than once. Still, only a handful of parasitic bodonids is known, whereas some free-living species are virtually omnipresent and ecologically highly significant [5,8]. Members of the genera *Trypanoplasma* and *Cryptobia* parasitize fish and snails [9,10], respectively. *Azumiobodo hoyamushi* causes economically important damage to cultured ascidians [11], while *Jarrellia attramenti* found in the blowhole of whales and dolphins [12] may rather be a commensal than a parasite (Fig. 2). For the purpose of this review, we will focus on flagellates belonging to Trypanosomatida as they embrace an absolute majority of parasitic species (see below).

2. Diversity *versus* taxonomy: closing the gap

The taxonomy of Trypanosomatida was originally defined by a set of morphotypes, which differ in respect to the mutual positions of the kDNA, nucleus and flagellar pocket, and the presence or derived loss of a single flagellum [13–16]. Extensive application of electron microscopy in studies of trypanosomatids did not add any important distinguishing features [17,18]. Since the advent of molecular methods it became obvious that neither the individual morphotypes nor their combination within a given life cycle hold any taxonomic value, as they are randomly distributed in the sequence-based phylogenetic trees [19]. Moreover, it seems plausible that there is a continuum of cell forms rather than eight distinct morphotypes.

Due to this dearth of morphological features, one has to resort to DNA sequencing in order to establish taxonomic position of a given trypanosomatid flagellate. There are two categories of genes of choice suitable for this purpose: the small subunit (SSU) rRNA and the glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) genes are informative for higher level taxonomy, and are usually sufficient for the genus-level ranking [20–22], while the sequences of the spliced leader (SL) RNA gene and the respective intergenic region allow distinguishing among individual species or even populations [23–27]. The growing number of species and strains, for which sequence data are available, revealed the artificial character of all previously described monoxenous (=one invertebrate host) genera, however, all three dixenous (=vertebrate or plant host and invertebrate vector) genera *Trypanosoma*, *Leishmania* and *Phytomonas* remain monophyletic and well supported (Fig. 3) [19,28].

One approach to close the gap between the outdated morphology-based taxonomy and the molecular-based cladistics that better reflects the relationships among trypanosomatids is to attach taxonomic units to the latter clades. Using this approach, some decades-old taxa rendered paraphyletic by molecular studies and hence invalidated, can be "recycled", *i.e.* used just for a single clade containing the type species of a given genus. This solution is taxonomically acceptable, and was successfully used in several instances so far [29–31]. In an alternative approach, novel clades

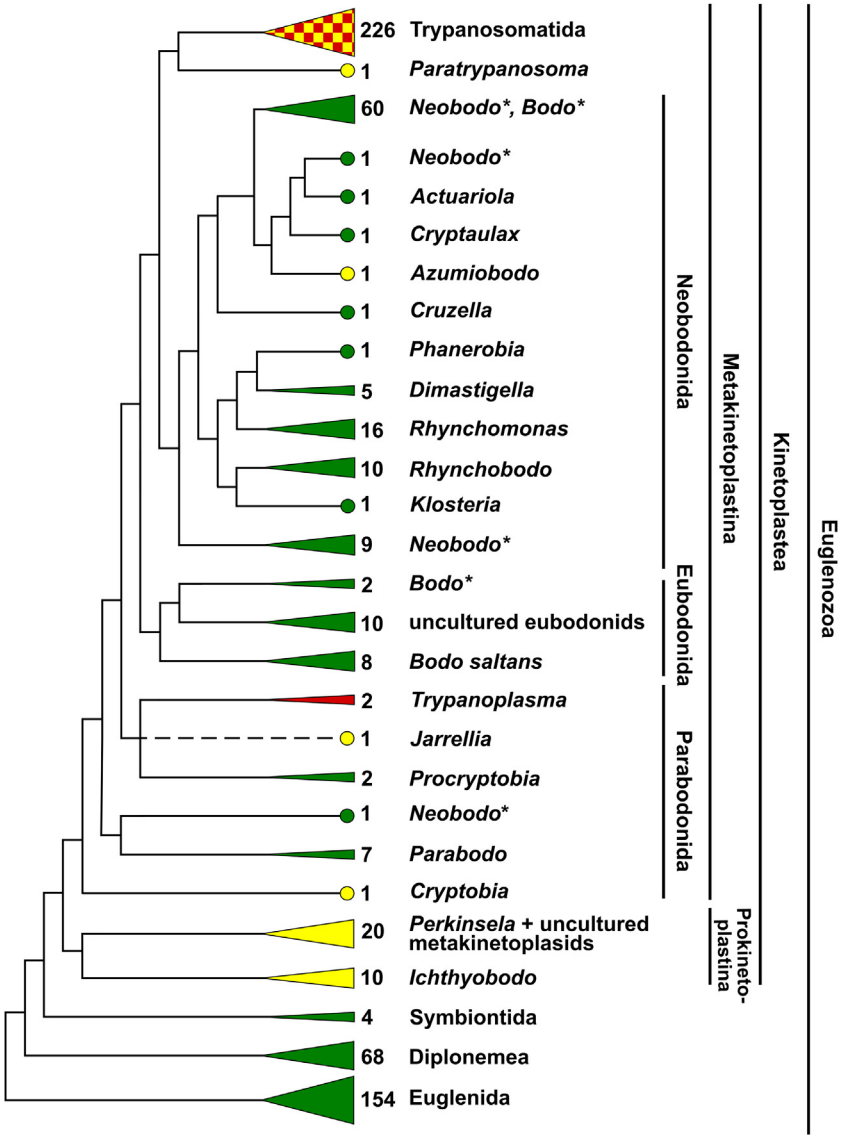

Fig. 2. Evolutionary relationships among bodonids based on SSU rRNA sequences. Numbers of available sequences representing individual species are shown as well as the new high level classification. Circles and triangles denote single and multiple known representatives of a particular clade, respectively. Dotted line indicates morphology-based position in the absence of sequence data. Green color depicts free-living species; yellow and red colors represent monoxenous and dixenous parasites, respectively. The intentionally collapsed clade of trypanosomatid flagellates (Trypanosomatida) is highlighted by a yellow-red checkerboard. The paraphyletic genera *Neobodo* and *Bodo* are labeled with asterisks.

that are consistently and highly supported in the phylogenetic reconstructions and contain at least several isolates are labeled with new generic names [32]. Based on the available sequence data, one can predict the existence of no more than two dozens of clades deserving the level of a genus, making a combination of these approaches not only feasible but also practical. The presently recognized taxonomic units are summarized in Fig. 3.

3. Diversity is not limitless: defining its extent

Defining the higher level taxonomy of Kinetoplastea is important for matching the new molecular data with a taxonomic framework constructed for over 100 years, but unfortunately it tells very little about the true diversity of these widespread protists. Bodonid SSU rRNA sequences obtained mostly in frame of environmental studies are summarized in Fig. 2. These bi-flagellar kinetoplastids are present in all aquatic ecosystems, yet they rarely abound and hence tend to be ignored. In the absence of extensive morphological analysis of bodonids it remains to be established whether the present taxonomy is robust or artificial. Some bodonid species are globally distributed [33], which supports the "everything is everywhere" paradigm formulated for the free-living protists by Fenchel and Finlay [34]. However, while morphological analyses seemingly support this hypothesis, we do not have enough genetic data yet to confirm it properly because many various isolates are indistinguishable from each other by morphology but significantly different in genetic analyses [8,33].

Much more data is available for the obligatory parasitic trypanosomatids, especially members of the dixenous genera. Due to the medical relevance of trypanosomes and leishmanias as causative agents of sleeping sickness, Chagas disease and leishmaniases, Trypanosomatida has been attracting most of the researchers' attention. Hence, at least some sequence information is available for over 100 *Trypanosoma* and 35 *Leishmania* species in the NCBI database. This allows their straightforward detection and determination, which is needed given the pathogenicity of many of them for humans and economically important vertebrates. Whole genomes have been sequenced for several strains/subspecies of

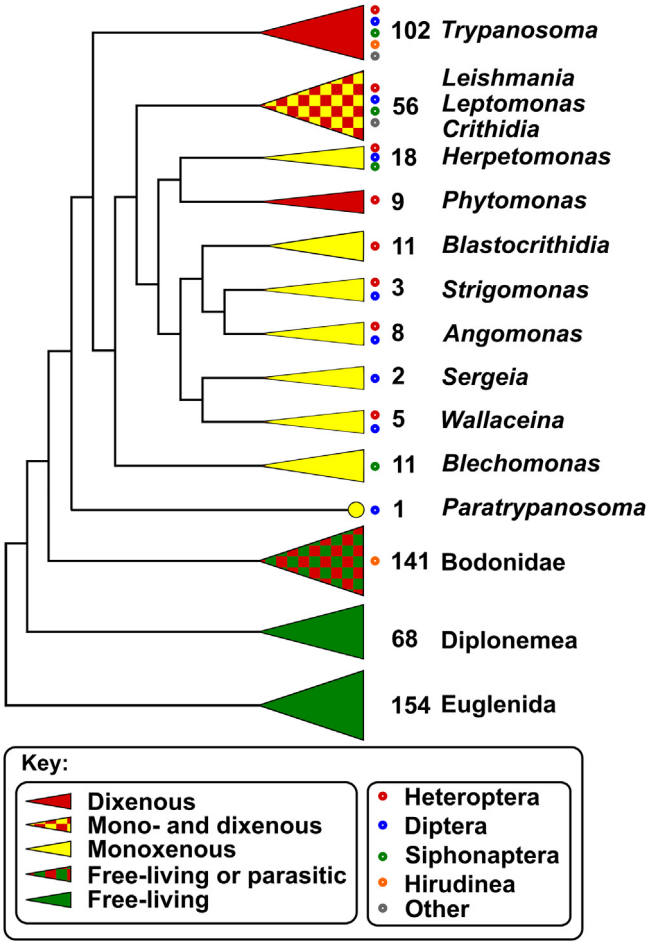

Fig. 3. Evolutionary relationships among trypanosomatids based on SSU rRNA sequences. Numbers of available sequences representing individual species are shown. Green color depicts free-living outgroups, the intentionally collapsed clade of free-living and parasitic bodonid flagellates (Bodonidae) is highlighted by a red-green checkerboard. Yellow and red colors represent monoxenous and dixenous parasites, respectively. The yellow-red checkerboard depicts a clade with mixed life cycles. Small colored circles depict major groups of invertebrate hosts.

T. brucei, *T. cruzi*, *T. congolense*, *T. vivax*, *L. major*, *L. donovani*, *L. infantum*, *L. braziliensis* and *L. mexicana* (www. http://tritrypdb.org/), and most recently also for two species of *Phytomonas* [35], with many more being in the pipeline. Due to the lack of economic significance, the monoxenous trypanosomatids of insects have been largely overlooked, with most species being described solely on the basis of their morphology and host specificity [36,37]. This has changed recently, and the SL and SSU rRNA sequences have been deposited for almost 150 species. Moreover, a draft-quality assembly of the whole genome of at least one monoxenous species, *Angomonas deanei* [38], and the unassembled reads of *Leptomonas seymouri* and the early model trypanosomatid *Crithidia fasciculata* are now available (http://www.sanger.ac.uk/resources/downloads/protozoa/).

The total number of extant monoxenous trypanosomatids might be staggeringly high given the extreme species richness of their insect hosts [39]. In order to tackle this potentially enormous landscape, we and our collaborators have recently established a system based on the so-called typing units (TUs), defined on the basis of >10% sequence divergence of the SL RNA gene [19,23,24,26,27,31]. A publicly available database which would contain the information of the host, location of infection, geographical origin, date of isolation, availability in culture *etc.* for each TU, is under preparation (J.V., J.L., V.Y. and D. A. Maslov, unpubl. data). We encourage the community to deposit information on all trypanosomatids isolated from insect and other invertebrate hosts into this shared database. Although much additional data is needed for more definitive conclusions, it is already obvious that several TUs (and likely species) are globally distributed [26], and some TUs are confined to a (very) narrow host range, while others are opportunists [24,25,29,32,40,41]. Now we know several heteropterid, siphonapterid and/or dipterid species that host more than a single TU, but it also appears that many insect orders are never or only rarely infected with trypanosomatids [19].

4. Acquisition of parasitic life style: the "big" transition

Species-rich and morphologically diverse euglenids and diplonemids are almost exclusively free-living, so this life style was likely the ancestral state of early kinetoplastids. The tree in Fig. 2 shows the generally accepted paraphyly of bodonids, and independent establishment of parasitic or commensalic life styles in five to six lineages (*Azumiobodo*, *Cryptobia*, *Ichthyobodo*, *Jarrellia*, *Perkinsela* and *Trypanoplasma*). Interestingly, with the sole exception of *Cryptobia helicis* [42], all parasitic bodonids remain confined to aquatic hosts.

Morphological and molecular evidence strongly support the origin of obligatory parasitic trypanosomatids from the clade of free-living *Bodo saltans* [5,43–45]. Until recently, the most basal branch of this clade was the genus *Trypanosoma*, supporting the scenario in which ancestral trypanosomatids established themselves in vertebrates already at an early stage, and only subsequently invaded insects *via* their blood-sucking fellows [46]. Such chain of events was challenged by fossil evidence in the form of a flagellate morphologically indistinguishable from the extant *Leishmania*, discovered in an amber-trapped blood-engorged mosquito (Fig. 4) [47,48]. This so-called *Paleoleishmania* dated to approximately 150 MYA was found among nucleated erythrocytes, prompting speculations that these originated from a dinosaur [47]. If correct, this finding would place the invasion of vertebrates by trypanosomatids to the early Cretaceous period at the latest. Phylogeographic data must be taken with caution as has been recently exemplified for trypanosomes of South American alligators and African crocodiles. While Crocodylidae and Alligatoridae split in mid Cretaceous (~90 MYA), their parasites are still closely related testifying to a much later separation due to the marine circumtropical dispersal at the Miocene/Pliocene boundary (~4–5 MYA) [49]. An ancestor of *Leishmania* probably developed dixenous life cycle in the warm-blooded vertebrates in late Cretaceous about 85 million years ago [50], around the time of divergence of mammalian orders and the first fossil records of its vector (Phebotominae) (Fig. 4). This event likely took place in Neotropics (present South America) in sloths, which have lower body temperature [51–53]. However, an alternative hypothesis places the marsupials in the spotlight: dixenous (*e.g. Trypanosoma cruzi* or *Leishmania* spp.) and monoxenous trypanosomatids of the genera *Crithidia*, *Leptomonas* and *Herpetomonas* can survive and multiply in their anal scent glands. Monoxenous species could pre-adapt there to the dixenous life cycle because within the scent glands parasites are protected from the host immune system and the body temperature is lower [54].

Recent description of the likely monoxenous *Paratrypanosoma confusum*, which constitutes a well-supported branch between the free-living *B. saltans* on one side and *Trypanosoma* plus all other trypanosomatids on the other side [28,45] supports a scenario, in which the ancestral flagellate first invaded insects or other invertebrate hosts and only subsequently, probably by blood feeding, entered vertebrates – a theory proposed by Léger in 1904 [55]. Comparative analysis of the genomes of *B. saltans* [43] and *P. confusum* (T.S. et al., unpubl. data) may shed key light on this dramatic change in life strategy.

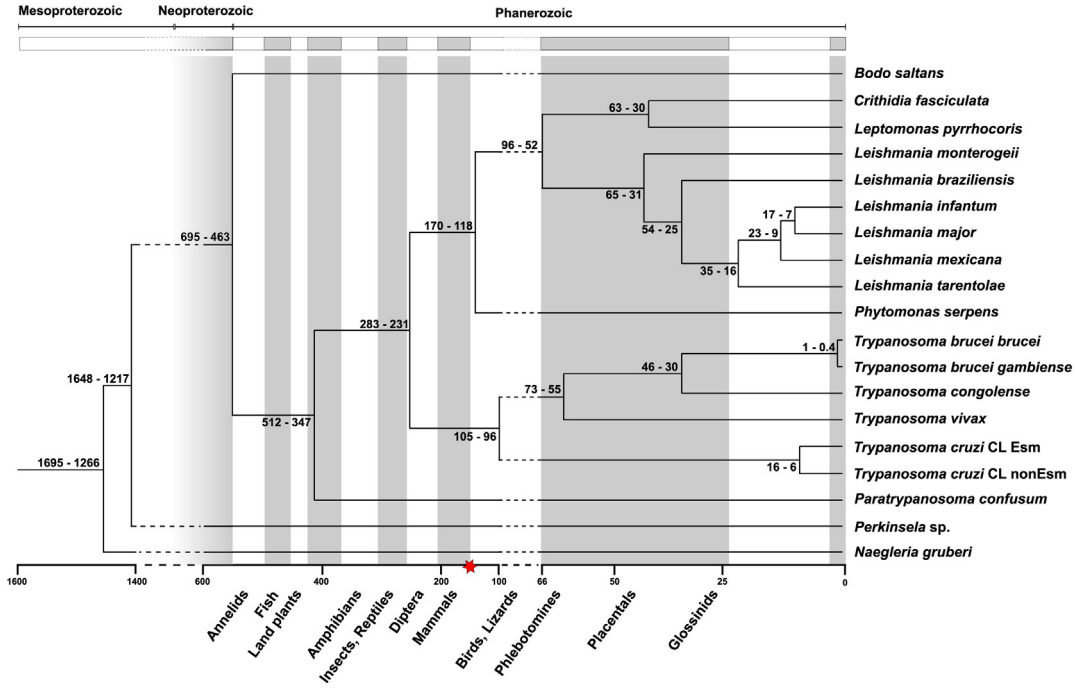

Fig. 4. Phylogenetic tree of kinetoplastid flagellates constructed under the clock model. Concatenated dataset of 42 proteins from 18 kinetoplastid species and *Naegleria* serving as an outgroup described previously, was used for molecular dating [28,98]. Divergence time estimates were inferred by the PhyloBayes 3.3f using CIR process [99] and birth-death prior with implemented soft bounds [100]. A star denotes single known fossil evidence of a kinetoplastid [47]. On the *x* axis absolute time scale in millions of years is shown along with the estimated emergence of host groups parasitized by kinetoplastids [101]. Nodes are at mean divergence and the numbers represent 95% confidence intervals.

Monoxenous trypanosomatids circulate among their insect hosts *via* contamination, coprophagy, necrophagy or predation, with the fecal transmission being probably the predominant way. Transmission among dipteran and hemipteran insects, which host over three-fourths of all described monoxenous species, is favored by their habit of feeding on rich organic sources that are often contaminated by excretions from infected specimens [16,19].

5. Acquisition of dixenous life style: the "small" transition

The origin of the dixenous life style has been discussed several times and the insect-early scenario is now generally favored, since the two-hosts genera *Leishmania* and *Phytomonas* are phylogenetically nested within clades of the single-host trypanosomatids (Fig. 3) [30]. Current phylogenies also almost invariably support a view, in which the derived dixenous life style evolved from the monoxenous one independently for each of the genera *Trypanosoma*, *Leishmania* and *Phytomonas* [19,28,45]. Moreover, it seems that some monoxenous trypanosomatids occasionally attempt a switch to dixeny, or what we call here a "small" transition, as there are several documented cases of mammals and birds being infected with *Herpetomonas megaseliae* and *Crithidia* sp., respectively [16].

More recently, a hypothesis claiming that the monoxenous trypanosomatids may explore new niches particularly in immunocompromised hosts has been put forward [56]. It stemmed from the observation that immunosuppressed HIV-positive patients were often found to host flagellates such as *T. cruzi* or *Leishmania* spp. [57,58], as well as presumably monoxenous species [59]. One such example is a close relative of *Blechomonas* (formerly *Leptomonas*) *pulexsimulantis* isolated from an HIV-positive individual in Brazil [60], which usually parasitizes fleas [32]. An independent line of evidence originated from studies of visceral leishmaniases or kala-azar, caused by the *Leishmania donovani* complex [61]. *Phlebotomus argentipes*, a sand fly vector implicated in the transmission of leishmaniases in Nepal, was estimated to be infected almost as often with the non-*Leishmania* species as with genuine *Leishmania* [62]. The only species recovered so far from co-infections with *Leishmania donovani* is *Leptomonas seymouri* originally isolated from a hemipterid bug [63–65]. The exact ratio between these two parasites is hard to estimate *in situ* because they are morphologically very similar [19,30]. Importantly, *Leptomonas seymouri* outgrows *Leishmania donovani in vitro* resulting in primary cultures enriched with this species [64]. Consequently, when the whole genomes of three strains isolated from kala-azar patients were analyzed, over 95% of sequencing reads belonged to *Leptomonas seymouri* [63]. This also explains another important mystery noted by us (V.Y. and J.V., unpubl. data), namely that several entries reported in GenBank as *Leishmania donovani* are, in fact, sequences of *Leptomonas seymouri*.

We posit that there are several species of monoxenous trypanosomatids capable of surviving within the warm-blooded vertebrate hosts. The evolutionary advantages of such exploration are obvious – potential new ecological niches may suit parasite's needs better and thus facilitate its prevalence and distribution. Nevertheless, everything comes with a price tag. In this particular case it means that parasites must adapt to new environmental conditions within vertebrates which are dramatically different from what they have experienced in insects. Molecular mechanisms of such adaptations are presently unknown. One obvious and critical factor is temperature, which in contrast to invertebrates is constantly high in the warm-blooded vertebrates. Observations discussed above imply that at least some monoxenous trypanosomatids may withstand a thermal shock. This has been proven experimentally for a hemipteran-derived *Crithidia* sp., which was capable of infecting chicken embryos [66]. Two other monoxenous species available in culture (*C. hutneri* and *C. luciliae thermophila*) are known to survive at elevated temperatures [67,68]. Moreover, *Leptomonas seymouri* can also tolerate temperatures of the range observed in

warm-blooded vertebrates (V.Y., J.V., and J.L., in prep.). The molecular nature of adaptations needed for a successful transition from the monoxenous to dixenous life cycle remains to be investigated.

The above-mentioned tendency of monoxenous flagellates to switch for the dixenous life style can be further exemplified by *Blastocrithidia culicis*, which colonizes not only mosquito's digestive tract and haemocoel, but also its salivary glands, raising the possibility of transmission to vertebrates by a mosquito bite [69]. However, there are only very few records of monoxenous species from the blood-sucking vectors of *Trypanosoma* (*e.g.* tsetse flies) and *Leishmania* (sand flies) [5]. The criteria generally used to incriminate vectors are: (i) presence in the same environment as the host; (ii) feeding on the host; (iii) development of the parasite to a transmissible infective stage; (iv) presence of the same parasite in wild-caught vectors and hosts, and finally, (v) transmission *via* a bite, ingestion, or feces. Trypanosomes can be transmitted by saliva (Salivaria), feces (Stercoraria), mechanically, and by ingestion of the vector. Mechanical transmission of some dixenous flagellates by a wide range of vectors has been demonstrated in the laboratory, yet this route does not seem to play a significant role under natural conditions [70]. Leishmanias use several ways of transmission to the vertebrate host: (i) members of the subgenus *Leishmania* are transmitted by inoculation through parasite regurgitation (back flow), although their occurrence in the sand fly salivary glands was also repeatedly reported; (ii) subgenus *Viannia* is transferred similarly, however, prediuresis (excreting urine to concentrate proteins of the bloodmeal while feeding on the host) is also considered as a possible route; (iii) subgenus *Sauroleishmania* is transmitted by defecation, prediuresis or by ingestion of the vector, and finally; (iv) some *Leishmania* (*Endotrypanum*) changes its hosts by contamination [71,72].

Most vectors of the dixenous genera *Trypanosoma*, *Leishmania* and *Phytomonas* are haematophagous or phytophagous members of the orders Hemiptera and Diptera, however other insects or invertebrates such as mites and leeches can also serve as vectors [73–75]. The list of new invertebrate vectors of dixenous species was extended by blood-sucking terrestrial leeches (Haemadipsidae) transmitting a new trypanosome lineage [76], while ticks (*Ixodes* spp.) and midges (Ceratopogonidae) have a potential to transmit *T. copemani* [77] and *Leishmania* spp., respectively [78]. Moreover, fleas (Siphonaptera) were recently shown to be surprisingly frequent hosts of a new clade of monoxenous trypanosomatids named *Blechomonas*. These holometabolous insects undergo a radical metamorphosis and since the only meal of adult fleas is blood, the infections must be established already at the larval stage, with the parasites accompanying the hosts throughout their metamorphosis [32]. In addition, free-living ciliates were occasionally shown to harbor trypanosomatids, potentially serving as their reservoirs [7]. Probably because of the long evolutionary association, the majority of trypanosomes have developed well-balanced relationships with their invertebrate hosts that allow mutual survival, although in some cases virulence was retained (*e.g. T. rangeli* and subspecies of *T. brucei*). Similar logic applies to the relationship between trypanosomes and their vertebrate hosts. Most species cause mild infections with no obvious symptoms, yet for very good reasons, most attention is given primarily to those highly pathogenic for humans and economically important mammals [79].

Dixenous hemoflagellates adapted their transmission to ecological conditions and environment of their hosts. Although *Trypanoplasma* and *Trypanosoma* are unrelated, the former being a bodonid and the latter a trypanosomatid, they both circulate in the blood of marine and freshwater fishes and both are transmitted exclusively by leeches [10,73]. Same principle applies to different avian trypanosomes, which use for their transmission a wide range of dipteran insects commonly attacking birds, such as black flies (Simuliidae), flat flies (Hippoboscidae), mosquitoes (Culicidae) and biting midges (Ceratopogonidae) [80,81]. Similarly, trypanosomes parasitizing bats are transmitted by blood-sucking bugs (Cimicidae and Triatominae) and/or sand flies (Phlebotominae) living within or close to their host's colonies. Infections usually occur when a grooming bat ingests an infected insect or its feces [82]. Other mammalian trypanosomes are transmitted by blood-sucking horse flies (Tabanidae) and sheep keds (Hippoboscidae) and bugs (Triatominae), as well as by fleas (Siphonaptera), which repeatedly defecate on the skin and fur while taking a blood meal [83].

6. Monoxenous life style: back again

The capacity for cyclic development of the Salivarian trypanosomes (species that terminate development in the salivary glands) depends on the suitability of available vectors. *Trypanosoma congolense*, *T. brucei gambiense*, *T. b. rhodesiense* and *T. b. brucei* are (fortunately) confined to the tsetse belt in sub-Saharan Africa, since no other vector than tsetse flies (Glossinidae) is capable of their transmission [84,85]. Two other members of the *T. brucei* species complex escaped the tsetse belt: *T.* (*b.*) *evansi* has adopted horse flies (Tabanidae), other insects (Stomoxyinae and Hippoboscidae) and even vampire bat (*Desmodus* spp.) as its apparently surrogate vectors, while *T.* (*b.*) *equiperdum* has avoided using vectors completely and switched to direct sexual transmission [86,87].

7. Adaptations to parasitism: are there any?

When applied to trypanosomatid parasites, the conventional thinking tries to explain all their unusual and complex features by positive selection during adaptations to parasitism. Unfortunately, this straightforward approach does not withstand rigid scrutiny, which became possible only lately when molecular data from the sister non-parasitic lineages appeared. Indeed, even distantly related lineages of Alveolata and Kinetoplastea share several otherwise (very) rare molecular traits such as *trans*-splicing, polycistronic transcription and mitochondrial gene fragmentation and RNA editing [88,89]. The situation is even more complicated because many of these oddities apparently arose by convergent evolution through the accumulation of neutral mutations rather than by pure selection [90]. As exemplified by RNA editing, the molecular mechanisms underlying it are fundamentally different in Alveolata and Kinetoplastea, leading to the only plausible conclusion that these pathways have evolved in convergence [88]. In summary, the molecular data accumulated so far testify that many if not all of the peculiar features attributed to kinetoplastids were independently "tried on" at least several times during protistan evolution. Indeed, none of the traits appears to have evolved as a specific adaptation to the parasitic life style, as all of the typical trypanosomatid features are in some form already present in the basal kinetoplastid group of free-living bodonids.

However, considering the impressive success of trypanosomatid parasites, there might have been important pre-adaptations. One such example is the kDNA that is present in multiple fundamentally different forms in the bodonids [91], while a single kDNA network highly conserved in terms of organization, gene content and order, is a unifying feature of all trypanosomatids [92,93]. The transition from the non-catenated pro-kDNA of free-living *B. saltans* to the catenated kDNA disk of parasitic *P. confusum* might represent an important transition that contributed to the success of trypanosomatids, in which no further diversification of the kDNA seems to have occurred. In the absence of more information on the diverse bodonid kDNAs, we cannot pinpoint the critical differences, other than the potential importance of catenation of circular DNA molecules at the expense of their supercoiling [91]. A consequence of this invention might be a highly organized and efficient

replication and maintenance of a single kDNA catenane, allowing major contraction of the invariably huge kDNA of bodonids, which likely outweighed the inefficiency of replication by redundancy.

Trypanosomatids might serve as a good model group for tracing the evolution of parasitism. Several whole genomes of the dixenous trypanosomatids are already available for analysis [94–97]. By analyzing the genomic information from their free-living bodonid relatives and early-branching trypanosomatid *P. confusum* [28], we shall be able to identify features shared between these organisms as well as those representing key differences, especially in terms of gene loss and/or gain. From the currently available data, most differences are associated with genes encoding metabolic and cell surface proteins [43].

Modern methods of genome analysis allow direct comparison of the gene content between different groups of Trypanosomatida. The underlining assumption of this approach is that there must be some genes responsible for adaptation to the dixenous life style. Those genes should exclusively be either present or absent in *Trypanosoma*, *Leishmania*, and *Phytomonas* as compared to their monoxenous kins. Such an analysis promises to bring novel insight into what drives flagellates to the more complex dixenous life cycle and is indeed underway (Pavel Flegontov, J.L., V.Y., and J.V., in prep.).

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