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Opinion

New Approaches to Systematics of Trypanosomatidae: Criteria for Taxonomic (Re)description

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While dixenous trypanosomatids represent one of the most dangerous pathogens for humans and domestic animals, their monoxenous relatives have frequently become model organisms for studies of diversity of parasitic protists and host–parasite associations. Yet, the classification of the family Trypanosomatidae is not finalized and often confusing. Here we attempt to make a blueprint for future studies in this field. We would like to elicit a discussion about an updated procedure, as traditional taxonomy was not primarily designed to be used for protists, nor can molecular phylogenetics solve all the problems alone. The current status, specific cases, and examples of generalized solutions are presented under conditions where practicality is openly favored over rigid taxonomic codes or blind phylogenetic approach.

Classification of Trypanosomatids

The protists classified into the family Trypanosomatidae (Euglenozoa: Kinetoplastea) represent a diverse and important group of organisms. These parasites utilize two general lifestyle strategies. **Dixenous** species (see Glossary) shuttle between invertebrates (mainly insects and leeches) and vertebrates (including humans) or plants, while their **monoxenous** relatives are restricted to invertebrates. There is substantial support for the hypothesis that the dixenous life cycle emerged from the monoxenous one independently for representatives of the genera *Trypanosoma*, *Leishmania*, and *Phytomonas* [1–3].

Despite recent advances, the taxonomy and systematics of Trypanosomatidae is far from being consistent with the known phylogenetic affinities within this group [4]. Early descriptions of these parasites were based on light microscopy, as the only method available prior to the advent of electron microscopy in the 1960s and 1970s. The traditional taxonomic system of trypanosomatids that dominated the field for decades was established at the twilight of this period. In essence, it used just two main traits: presence of particular morphotypes and properties of the life cycle [5,6]. At that time, the morphotypes were defined by the features observable under the light microscope: cell morphology, intracellular arrangement of the kinetoplast, nucleus and flagellar pocket, and the presence or absence of a single flagellum [7,8]. The advancements in electron microscopy in the 1970s to 1980s did not lead to a breakthrough in the field, nor did it help essentially with the classification, although several important discoveries were made. Besides description of cytoskeleton organization, flagellar pocket, and kinetoplastid structure,

Trends

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We are eliciting a discussion about an updated procedure in trypanosomatid systematics, as traditional taxonomy was not primarily designed to be used for protists.

We present the current status, specific cases, and examples of generalized solutions under conditions where practicality is openly favored over rigid taxonomic codes or blind phylogenetic approach.

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the main interest was focused on the β-proteobacterial endosymbiont that was found in the species currently classified as *Angomonas* and *Strigomonas* spp. [9]. Moreover, the apparently widespread viruses that can infect trypanosomatids were first identified using ultrastructural analysis [10]. In the meantime, biochemical and nutritional differences were also suggested as useful taxonomic markers [8]. Despite being considered not as precise as modern ones, these approaches (e.g., based on isoenzyme mobility: **MON** zymodeme number) are still widely used in the *Leishmania* community [11,12]. The electron microscopy era lasted for about 20 years until molecular methods entered the field in the 1980s. After a relatively short period of restriction fragment length polymorphism-based analyses and related methods [13–15], molecular sequences rapidly became indispensable [16–18]. Nucleotide sequence data, which contain hundreds or even thousands of informative characters, began to be used for building phylogenetic trees, allowing inferring of evolutionary relationships. Genetic loci routinely used in these analyses are 18S SSU (small subunit) rRNA and SL (**spliced leader**) RNA genes, along with gGAPDH (glycosomal glyceraldehyde 3-phosphate dehydrogenase) and internal transcribed spacers (ITSs) 1 and 2 [4,19,20]. Other markers, such as minicircle-derived or heat-shock protein gene sequences have been also proposed but their usage remains limited for a few particular cases only [21,22].

However, it is our opinion that this period is now reaching its conclusion. While single gene phylogenetic analyses continue appearing in the literature, the most competitive journals began requiring sequences of several genes for a given organism; preferably from both the mitochondrial (and/or plastid) and nuclear genomes. The main reasons for this requirement are that single-gene-based phylogenies are often misleading or poorly supported and single genes are prone to methodological sequencing errors. Trees built from the concatenated sequences of several genes are usually substantially more robust, since the larger datasets contain more phylogenetic information. Such multi-gene analyses are facilitated by the rapidly dropping cost of sequencing and the implementation of increasingly powerful computational methods.

In the foreseeable future, it can be anticipated that low cost and high speed of obtaining draft-quality sequences of whole genomes will enable next generation sequencing to become a routine method for generating concatenated datasets sufficient for production of more robust and accurate phylogenetic trees. Moreover, using KEGG (Kyoto Encyclopedia of Genes and Genomes at http://www.genome.jp/kegg/) and analogous databases, metabolic pathways can be reconstructed, providing a wealth of information about the lifestyle of the studied organism.

There is certainly no shortage of species descriptions in trypanosomatids, and the number of described taxa significantly grew after 1990, when a catalog of over 350 species was compiled [23]. Unfortunately, most previously described taxa were confounded by the then commonly accepted 'one host – one parasite' paradigm. However, a varying level of host–parasite specificity, from broad to strict one (as exemplified by *Crithidia brevicula* that can use a range of suitable hosts and *Blechomonas* spp. that is restricted to Siphonaptera insects, respectively [24,25]), is now an undeniable fact, indicating the host specificity alone cannot be sufficient for species recognition and formal description. In addition, the frequent occurrence of mixed infections is often underappreciated [26–29]. It is plausible to suggest that in many instances, obtained cultures do not represent the dominant components of natural infections. Thus, dependence of culture for genomic analysis is a real impediment. Unfortunately, single-cell genomics, the only solution available now to alleviate this problem, is currently applicable to larger protists only [30], but we can assume that the situation will change soon.

In the current taxonomic system based on a combination of traditional classification and phylogenetic reconstructions, the family Trypanosomatidae contains three formally described subfamilies corresponding to major phylogenetic **clades** within the Trypanosomatidae family [4]:

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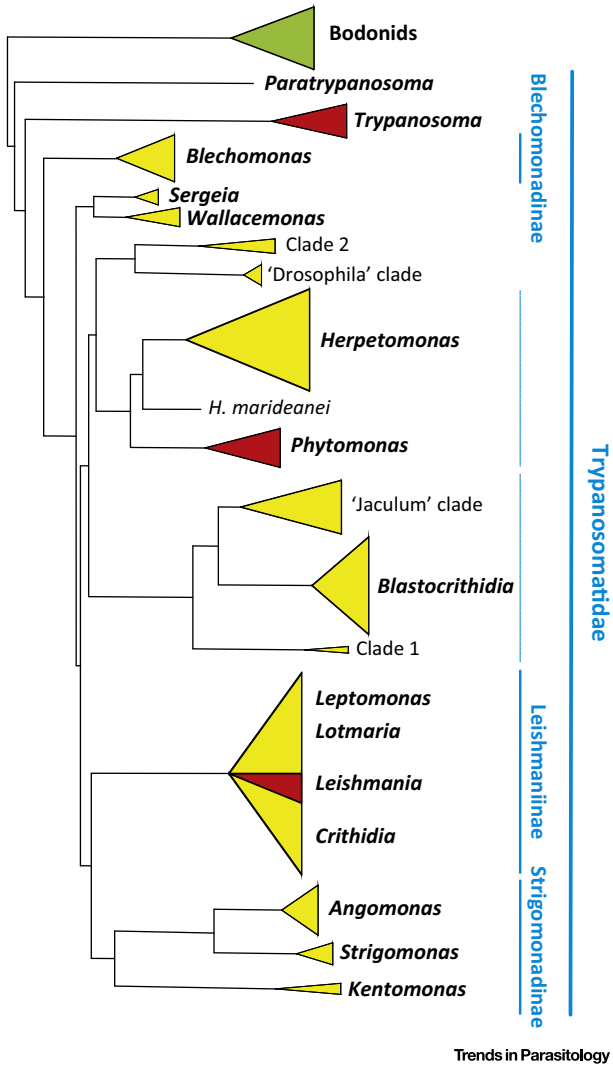

Figure 1. Bayesian Phylogenetic Tree Demonstrating Evolutionary Relationships among Trypanosomatids Based on Small Subunit rRNA Sequences. Green color depicts bodonids species used as an outgroup. Yellow and red colors represent monoxenous and dixenous parasites, respectively. All monoxenous and majority of dixenous species were included in the analysis. Three formally recognized subfamilies (Blechomonadinae, Leishmaniinae, and Strigomonadinae) are indicated. Potential groups to be united into new subfamilies are marked by broken lines. Clades 1, 2, *Drosophila* (parasites isolated from *Drosophila* spp.), and jaculum (trypanosomatids clustering together with *Leptomonas jaculum*) are recovered in all the recent phylogenetic reconstructions and consist mostly of the environmental samples.

Leishmaniinae uniting one dixenous genus *Leishmania* with three monoxenous genera *Crithidia*, *Leptomonas*, and *Lotmaria* [31,32]; Strigomonadinae containing three monoxenous genera *Angomonas*, *Kentomonas*, and *Strigomonas* [19]; and Blechomonadinae accommodating the monoxenous genus *Blechomonas* [25] (Figure 1). In addition, several other clades are associated with the genus level; yet, as these taxa also represent major clades, their taxonomical status should be elevated. These are dixenous genera *Trypanosoma* and *Phytomonas* [33,34], and monoxenous genera *Blastocrithidia*, *Herpetomonas*, *Sergeia, Paratrypanosoma*, and *Wallacemonas* [24,35,36]. The remaining **monophyletic** clades still await formal description since no cultivatable isolates are available thus far (Figure 1 and [4]).

Traditional versus Phylogenetic Classification System

A viable classification system should define taxa that are mutually exclusive and unambiguous, and at the same time should be simple and easy to use. In addition, a modern natural system combines molecular phylogenetic data with a mostly morphology-based traditional system, in which taxon names are still defined by a type, while it classifies organisms based on their inferred evolutionary relationships. The current classification of Trypanosomatidae is far from ideal and the following issues significantly complicate establishing of a consistent and practical set of rules:

Glossary

Choanomastigote: developmental stage or the morphological form in the lifecycle of the genus *Crithidia* characterized by barley-shape cells with a wide flagellar pocket and kinetoplast DNA prenuclear or adjacent to the nucleus.
Clade: a group consisting of an ancestor and all its descendants. A clade is monophyletic by definition.
Dixenous parasite: a parasite with a life cycle split between two host species; for example, as during insect-mediated transmission among vertebrates.
Endomastigote: developmental stage or the morphological form in the life cycle of the genus *Crithidia* characterized by round to oval cells with a short flagellum convoluted around the nucleus and not extending outside a flagellar pocket.
Hapantotype: when the type specimen of an extant protist consists of two or more individuals representing distinct stages of its life cycle and is collectively treated as a single entity.
Monophyletic: a monophyletic group includes an ancestor and all its descendants, or any two or more groups that share a common ancestor. Members of monophyletic groups are typically characterized by shared derived characteristics (synapomorphies).
Monoxenous parasite: a parasite that is restricted to a single host (invertebrate or vertebrate) during its life cycle.
Montpellier (MON) system: a standardized method for typing *Leishmania* using multilocus enzyme electrophoresis analysis of 15 different enzymes.
Paraphyletic: a paraphyletic group consists of an ancestor and the majority of its descendants. In other words, this is a monophyletic group from which one or more of its members are excluded to form separate groups (e.g., due to not sharing a particular derived trait that is present in the remaining members of the paraphyletic group).
Spliced leader (SL) RNA: a class of short transcripts (~100 nucleotides in length) which participate in mRNA maturation in kinetoplastids. An SL RNA molecule includes a conserved mini-exon of 39 nucleotides in length which is transferred onto the 5′ end of each mRNA by *trans*-splicing. SL

Box 1. The Curious Case of the Genus *Wallacemonas*

The illustrative example of how the proposed approach to taxonomy works concerns two taxonomical entities: *Crithidia brevicula* Frolov et Malysheva, 1989 [63] and the genus *Wallacemonas* Kostygov et Yurchenko, 2014. The taxonomical confusion started in the early 1990s, at the end of the period when classical systematics dominated the field. Initially described under the name *Proteomonas* (turned out to be preoccupied by a cryptophyte alga), this taxon was later renamed *Wallaceina* [64,65]. *Wallaceina* (formerly *Crithidia*) *brevicula* was designated as a type species and the genus comprised one more species, *Wallaceina inconstans*. Both species were morphologically distinct from typical crithidias **choanomastigotes**, with **endomastigotes** being the predominant morphotype, and in some cells, the flagellum looped around the nucleus. Over the years several additional isolates, related to *Wallaceina*, have been described. Importantly, all these cases were later confirmed as components of the mixed infections of wallaceinas with either *Blastocrithidia* or *Leptomonas* [66]. Not long ago, this polyphyletic genus consisted of two clades: a highly supported group of isolates within the subfamily Leishmaniinae along with three species (OTUs) of the collosoma clade considered as a true *Wallaceina* [19]. Our recent analysis proved that all Leishmaniinae-bound *Wallaceina* spp. are just different isolates of the same species renamed back to its original name *Crithidia brevicula* Frolov et Malysheva, 1989. Since the name *Wallaceina* was invalidated, species of the collosoma group had to be accommodated into the newly erected genus *Wallacemonas* [24].

RNA genes are arranged as clusters of multiple tandem repeats (0.2–1.0 kb in length). The intergenic regions of the repeats are nearly identical within the same species but are highly variable between species.

i. Absence of morphological differences for many phylogenetically distinct taxa [4,37–39]. This could lead to repetitive name changes if morphology is given the leading role in classification (Box 1).
ii. Multiple codes in use. Historically, classification of Trypanosomatidae was governed by The International Code of Zoological Nomenclature, ICZN. However, this Code is not applicable for all protists, as many of them fell under the jurisdiction of The International Code of Nomenclature for Algae, Fungi, and Plants, ICN (Box 2). To complicate matters even further, The International Code of Phylogenetic Nomenclature, known as the PhyloCode [40,41], is also used.
iii. Veterinary and medical systematics. For good reasons, medically and veterinary relevant trypanosomatid parasites receive most attention. The clinicians tend to use a simple system with emphasis on pathology-based features for species definitions (see below). In essence, this highly pragmatic approach exaggerates a set of certain phenotypic traits and (nearly) ignores the rest. To reconcile this with a broader approach, the traditional classification has to compromise.

Box 2. A Tale of Two Codes

Two codes governing the description of new organisms have been more or less independently established: The International Code of Nomenclature for Algae, Fungi, and Plants (ICN; formerly called The International Code for Botanical Nomenclature, ICBN) and The International Code of Zoological Nomenclature (ICZN) for animals. For historical reasons, protists traditionally fell either under the jurisdiction of the ICBN/ICN if they were algae or fungi, or under the jurisdiction of the ICZN if they were protozoa. However, the classification of protists could not accommodate both the ICN and the ICZN, as they are simultaneously incompatible. Two classification systems for protists also imply that, in some special cases (higher taxon), names should be changed according to the phylogenetic position (algae, fungi, or protozoa). These ambiguity issues have been discussed several times [67,68] and can be exemplified by unicellular organisms which have been recently recognized as members of Fungi or their close relatives, for example, the genus *Pneumocystis* and the particular species *Pneumocystis jirovecii* versus *Pneumocystis jiroveci*. Formerly a protozoan parasite, its name was regulated by the ICZN and spelled *P. jiroveci* (Jirovec; inflect *jiroveci*). Now as a member of Fungi, it fell under the jurisdiction of the ICN and spells *P. jirovecii* (the family name Jirovec was Latinized as Jirovecius; inflect *jirovecii*). According to the ICN article 60c.1(b), *P. jirovecii* is the correct spelling. However, confusion by both authors and editors is demonstrated by the fact that both names are almost equally used in the current scientific literature. Under these circumstances, taxonomists suggest that the final acceptance of a change must come from the scientific community and be based on usage.

Microsporidia represent another example and their relationship to Fungi created several taxonomical problems. For instance, the genus name *Perezia* Léger et Dubosq, 1909 (Fungi: Microsporidia) became a junior homonym of the plant *Perezia* La Gasca, 1811 (Viridiplantae: Asteraceae), and in such a case, the genus name of microsporidian would have to be replaced (once a valid name when considered a protozoan governed under the ICZN should be transferred to the authority of the ICN). However, this problem was finally solved by the special decree of ICN which excludes microsporidian from its jurisdiction and names of Microsporidia are constantly governed by the ICZN. Any organism once considered an animal continues to compete in homonymy with other ICZN-regulated taxa even if, as in this particular case, the organism is now considered to be related to Fungi. This explanation is not accepted by all scientists; however, there is broad consensus that it is unhelpful to create new homonyms, even if the homonyms would be separated by code boundaries.

iv. Mainly asexual propagation. One of the major hurdles for systematic nomenclature and taxonomy is the species concept and its definition for asexual organisms [42–44]. Molecular systematics attempts to solve this problem by introducing operational taxonomic units (OTUs), which are defined by DNA similarity levels, as species proxies. Without a clearly defined threshold, any given population of (asexual) organisms may be subdivided into smaller and smaller OTUs, up to individual isolates (asexual lines). Yet, several examples provided below illustrate that it is not possible to establish generally accepted boundaries.

v. Absence of the name-bearing types for many previously described trypanosomatid species. In the traditional approach, taxon names are defined by a type, which can be a specimen (or group of specimens) or a taxon of lower rank, and a diagnosis, which represents a statement intended to supply characters that differentiate the taxon from others with which it is likely to be confused. The fixation of the name-bearing type of a nominal taxon provides the objective standard of reference for the application of the name it bears. Without such a standard it is difficult to implement the comparison of the newly collected organisms to the 'old' named species (Box 3).

Box 3. The Collections

The name-bearing type is the keystone element for classification, systematics, nomenclature, or taxonomy since it is considered as the reference specimen defining a species. Unfortunately, for most trypanosomatid species the type material is missing and comparisons of the newly collected organisms with what has been described before is usually impossible. Whatever the nature of the type material (see the supplementary material online), its preservation to ensure its perenniality is crucial for next generations of scientists. Museums or similar governmental institutional collections that are opened to scientists are the ideal place to archive such type materials. Deposits in such reference collections must be a systematic demand expressed by the editors for publication of species description. Indeed, material entry in a collection implies attribution of an inventory number obliging the institution to ensure its preservation. One of the main problems encountered for many protist species described before 1950 was that type materials were usually kept by the authors in their personal collections. In the best cases, a donation of the collection to the museum or similar institution after their death avoided the type material becoming untraceable or lost. Unfortunately, examples of slide collections of renowned scientists thrown away into garbage cans for futile reasons are not rare. The nature of the preserved type materials was changing over time, however, for protists, it mainly constituted slides or smears as exemplified by the species of *Leptomonas drosophilae* described by E. Chatton and E. Alilaire in 1908 [69] (Figure I). **Hapantotypes** (smears, inventory number: mnhn-IR-1970-2) of this species were rediscovered in the personal collection of E. Chatton after donation to the National Museum Natural History of Paris, France, in the 1970s. Along with classical smears or slide specimens, the type material can be diversified with transmission or scanning electron micrographs, fixed (histology) blocks, alive or cryopreserved cultures, and DNA sequences and isolated DNA. The advantage is that under one inventory number scientists can have an access to a large panel of information or materials related to a species (Figure S1 in the supplementary material online). This does not imply that the ancient archived protist collections should be considered as obsolete or just of historical interest. The original type material is still useful for comparison of morphology and for nucleic acid isolation from stained, methanol- or formalin-fixed specimens [70,71].

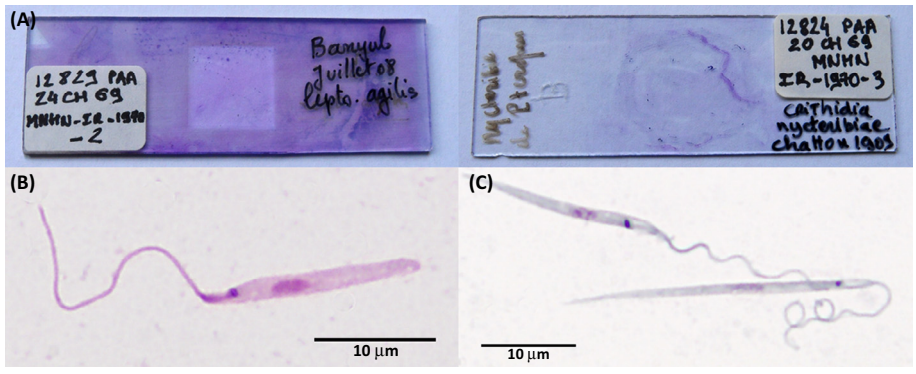

Figure I. Preserved Type Material in the Museum Collections. **(A)** Hapantotypes of monoxenous trypanosomatids *Leptomonas agilis* and *Crithidia nycteribiae* described by E. Chatton in 1909 from a reduviid heteropteran bug *Harpactor iracundus* Scop. and a Nycteribiidae bat fly *Cyclopodia sykesi* Westwood, respectively. **(B)** *Leptomonas agilis* Chatton 1909 and **(C)** *Leptomonas drosophilae* Chatton & Alilaire 1908 isolated from a fly *Drosophila confusa* Staeger. Microphotographs were taken from Giemsa-stained hapantotype slides. Scale bar is 10 µm.

Suggestions for an Easy-to-Use System

The issues mentioned above have prompted us to revise the traditional approach to classification of Trypanosomatidae. Governed by a set of rigid rules, it was originally established to provide a framework for description of individual OTUs as new species. Unfortunately, such rigid classification systems can only work if the rules are universally applied. As mentioned above and discussed below, this has not always been feasible or even possible. So, the scientific community remains suspended on this issue – on the one hand, the rules of the ICZN remain in effect, while on the other hand, they are ignored when perceived as nonapplicable. Information recommended and usually included into the current taxon (re)description is summarized in the supplementary material online.

The main motivation behind this article is to elicit discussion about an updated procedure for taxonomic (re)descriptions of Trypanosomatidae in a time when the traditional approach should converge with the one based on molecular phylogenetics. The traditional taxonomy has not been designed to be used for protists, but nor has been modern molecular phylogenetics. Below, we summarize the current status, describe specific situations and, when possible, present examples of general solutions. If the proposed system is accepted by the scientific community, such a dynamic and flexible approach would significantly simplify and streamline future (re)description of species and genera. In particular, we propose to:

(i) Keep the existing names, whenever possible. This is essential for medically or veterinary important species, such as *Trypanosoma* and *Leishmania*. From this point of view, merging several veterinary and medically distinct species of African trypanosomes into just one, *Trypanosoma brucei*, was probably not a well chosen solution [45], as those differ significantly in the disease manifestation, host, and vector specificity, geographical distribution, and principal molecular features [46–49]. In this particular case, to avoid further confusion, we suggest keeping these names separately as species and subspecies of the *T. brucei* complex: *T. brucei* with its three subspecies *T. b. brucei*, *T. b. rhodesiense*, *T. b. gambiense; Trypanosoma evansi*, and *Trypanosoma equiperdum*.

(ii) Maintain and extend the use of subgenera. Until now, those have been used only for two dixenous genera, *Trypanosoma* and *Leishmania*. Such an approach satisfies both taxonomists and practitioners and helps avoid unnecessary confusion of hitherto known names. A similar system for other medically relevant protists is used as well (for example, *Plasmodium*) [50]. Adoption of this scheme for monoxenous representatives of the subfamily Leishmaniinae [31] and designation of three newly suggested subgenera, *Leptomonas*, *Crithidia*, and *Lotmaria* of the genus *Leptomonas*, and probably several additional subgenera in near future would straighten their taxonomy and classification. Another good candidate is the dixenous genus *Phytomonas* accommodating a number of species differing by their host specificity and tissue location [34,51].

(iii) Affiliate new well-characterized isolates of trypanosomatids with old named species, whenever possible. In cases where the identity of a previously described species remains obscure (e.g., if the species was described by nonevident morphology and/or host specificity only, and the name-bearing types, such as slides suitable for DNA extraction or well associated culture are missing), the original name could be assigned to a carefully selected and well-characterized cultivable isolate that can be reasonably assumed to represent the original species. This was an approach used for re-description of the broadly distributed and well-known *Leptomonas pyrrhocoris* [52]. It allows keeping the existing historical names and, at the same time, provides a source for biological comparison with described species. These problems are general for all protists where there is no type material preserved. In addition to many cases of monoxenous trypanosomatids, this approach can be well illustrated by the taxonomic vagaries of avian trypanosomes. Firstly described from raptors, shrikes, and corvids in 1885, neither slides nor any other identifying material accompanied the original description of *Trypanosoma avium*. Later, trypanosomes of other bird orders were arbitrarily assigned to this species and deposited as such to American Type Culture Collection (ATCC). Recent analyses revealed that isolates designated as *T. avium* clearly belong to several species with different host specificity [53]. Assigning the name *T. avium* to one of the recently well-characterized avian trypanosomes kept in culture, accompanied by the re-description

of the species and establishing of the neotype blood smear, while describing the others as new species or leave them unnamed (see the next point), would be a reasonable solution.

Moreover, the same principal approach can be used at a generic level. If the identity of the type species of a genus remains vague or obscure, a well-characterized species shall replace it as the name-bearing type. For example, a speciose genus *Leptomonas* has a type species *Leptomonas bütschlii* Kent, 1880. However, this organism has never been re-encountered and its original description is vague to the point that it might even be a misnomer not related to trypanosomatids. We propose to appeal to the ICZN with an idea to establish a new type species for *Leptomonas*, preferably one already well defined by genomic data. One obvious candidate for such a role is *L. pyrrhocoris*.

(iv) Keep the parent (superordinate) name. For the lower taxonomic level (down to individual clonal lines) retention of the ID codes (for example, names of the environmental samples or clonal isolates) is sufficient. In the event that certain formally unnamed organism (e.g., genotype) subsequently becomes associated with distinct biological information (becomes known as pathogen, host or vector specificity is demonstrated, etc.), it may be assigned a proper formal name. This fact alone would not make it necessary to assign formal names to other groups at the same hierarchical (e.g., genetic) level. The problem can be exemplified by several monoxenous trypanosomatids [25,52], as well as by the cases of wild and domestic ungulate trypanosomes. Currently, these are lumped into a large species complex named *Trypanosoma theileri – Trypanosoma melophagium* [54]. Different isolates from two separate clades, *Tth*I and *Tth*II, clearly represent distinct OTUs [55]. However, it would be still premature to attach species names to these OTUs without additional information about host and vector specificity, etc. Indeed, within the clade *Tth*II, formal recognition of *T. melophagium* is fully warranted due to the knowledge of its specific host and vector. Although we are aware of the risk of creating a **paraphyletic** taxon, as an acceptable provisional solution, we propose that one or more internal clades within the *T. theileri – T. melophagium* group have a status of a separate species, while at this point, the same hierarchical level is not necessarily reciprocated in other clades (Figure 2).

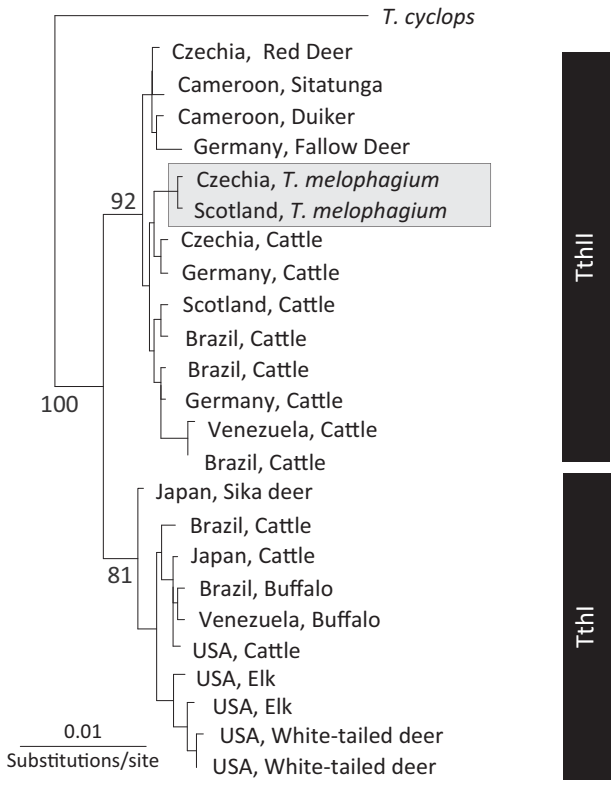

Figure 2. A Portion of the Small Subunit-Based Phylogenetic Tree Inferred by Maximum Likelihood Method Using V7–V8 18S rRNA Sequences, Focused on *Trypanosoma theileri – Trypanosoma melophagium* Species Complex (Subgenus *Megatrypanum*). Phylogenetic clades *Tth*I and *Tth*II (derived from *T. theileri*) are labeled. Three species names are in use in the literature: *T. theileri* (for parasites of cattle and buffalos in both lineages *Tth*I and *Tth*II), *T. melophagium* (for parasites of sheep transmitted by keds in *Tth*II), and *T.* cf. *cervi* (for parasites of wild ungulates in both lineages). Only the formal recognition of *T. melophagium* is fully justified (grey box) due to the knowledge of its specific host and vector. Other isolates on the tree are identified by their countries of isolation and specific hosts. Scale bar indicates number of substitutions per site.

In additional to the above-mentioned approach, we recommend using the abbreviation 'cf.' or 'type XY' for newly described OTUs that are phylogenetically related to the already described species. The abbreviation cf. is derived from the Latin word 'confer' and is usually used in systematics to define a taxon whose designation is uncertain because of some practical difficulties, such as poor preservation of the specimen. These labels can be removed when the distinct status of OTUs becomes evident due to additional life-cycle or molecular data.

(v) Establish molecular thresholds (a percentage of sequence identity for several well-characterized genetic loci) that should be used for new species delineation. Although threshold identity of some relevant molecular markers including barcode genes would be useful as a relevant guideline for species delimitation and description, we feel that such an established molecular taxonomy system, with almost no subjectivity and respect to biological traits, may fail in some ways, and thus it cannot be placed above the other approaches (see below). However, the usage of the phylogenetic position as a diagnosis is fully sufficient (see the supplementary material online). A 90% similarity threshold was established for the SL RNA gene [56]. Some other examples and markers are discussed elsewhere [4].

(vi) Accept a few well-established exceptions. *T. cruzi* includes six (or seven) discrete OTUs (*Tc*I to *Tc*VI), which show pronounced differences commensurable with the status of distinct species (if compared to the genus *Leishmania*, the phylogenetic distances of *T. cruzi* genotypes could even correspond to different subgenera), yet, their establishment would create a counterproductive confusion mainly for physicians [57,58]. Species and subspecies of the *T. brucei* complex, genetically almost identical, are still kept separately as they are host and vector specific, develop distinct molecular relationships with their hosts, reflected by different pathologies (see above) [45]. Members of several *Leishmania* species complexes, namely *Leishmania donovani*, *Leishmania hertigi*, and *Leishmania mexicana*, are for practical reasons, related to their life cycles and distinct pathologies they cause, often regarded as separate species. For example, *L. donovani* is split into *L. donovani* and *Leishmania infantum* (the latter named *Leishmania chagasi* in Latin America) [59–62].

In the cases of *T. brucei* and several *Leishmania* species complexes, differences in their transmission cycles and in clinical manifestations of the disease outweighed obvious genetic similarities, yet in the case of *T. cruzi*, the opposite took place.

Outstanding Questions

Can the flexible system proposed here unite the rigid taxonomy and modern phylogenetic approaches?

Will such a system be widely accepted by taxonomists, laboratory scientists, doctors, and veterinarians?

Will we see an overhaul of the current binominal systematics of trypanosomatids?

Concluding Remarks

Although the exact meaning of the species concept in relation to trypanosomatids remains elusive (see the supplementary material online), our approach to classification and taxonomy of this group allows for moving forward in spite of this and other hurdles. We openly favor practicality over srigid taxonomic codes (none of them was tailored for protists anyway) or a strictly phylogenetic approach, and embrace the compromise route taken by the current taxonomy of medically important trypanosomatids. By extension this flexible approach to medically and veterinary irrelevant but ecologically and phylogenetically important monoxenous trypanosomatids, distinct OTUs can be (provisionally) labeled and, subsequently, formally named as species. All sorts of biological information (host and/or vector specificity, morphological traits, phylogenetic position, and/or presence of unique genes) would lead to this destination (see Outstanding Questions).

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Supplemental Information

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