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# Species concepts for trypanosomes: from morphological to molecular definitions?

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## Abstract

The way species and subspecies names are applied in African trypanosomes of subgenera *Trypanozoon* and *Nannomonas* is reviewed in the light of data from molecular taxonomy. In subgenus *Trypanozoon* the taxonomic importance of pathogenicity, host range and distribution appear to have been inflated relative to actual levels of genetic divergence. The opposite is true for subgenus *Nannomonas*, where current taxonomic usage badly underrepresents genetic diversity. Data from molecular characterisation studies are revealing a growing number of genotypes, which may represent distinct taxa. Unfortunately few of these genotypes are yet supported by sufficient biological data to be recognized taxonomically. But we may be missing fundamental epidemiological information, because of our inability to distinguish these trypanosomes in host blood morphologically or in tsetse by their developmental cycle. Molecular taxonomy has led the way in identifying these new genotypes and now offers the key to elucidating the biology of these organisms.

## The concept of a species

In our daily lives we are surrounded by animals and plants that belong to distinct and quite clearly demarcated species. Even little children know that dogs and cats, or apples and pears, are different, and so we come to expect that different species should look different. Yet there is no biological imperative dictating this. To paraphrase an eminent entomologist, the stripes on the legs of mosquitoes are not there for the taxonomist's benefit to facilitate the identification of different species. On the other hand, there may be striking phenotypic variations between individuals of the same species, as exemplified by dogs and other domestic species, that are considered to have no taxonomic relevance.

Taxonomy is traditionally based on morphological differences, but identification of species by morphology is not without pitfalls. How do taxonomists decide what level of morphological difference defines a species? Biologists believe in the concept that (eukaryote) species are defined by the ability of individuals to mate and produce viable and fertile offspring. In practice, this is never actually put to the test in the majority of cases. Instead, a taxonomist with expert knowledge of the group of organisms, extrapolates from detailed information on a few species to make judgements on the group as a whole. The key taxonomic characters defining species will vary from group to group. Unfortunately, the biological species concept offers no guidance on the taxonomy of asexually reproducing organisms.

How does taxonomy based on molecular characters fit into this conceptual framework? Underlying molecular taxonomy is the idea that non-interbreeding populations

**Table 1: Characteristics of species within subgenus *Trypanozoon* See text for explanation.**

| Species | Distribution | Host range | Transmission | Pleomorphic | Differentiation to procyclics | Kinetoplast DNA | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Maxicircles | Heterogeneous minicircles | Homogeneous minicircles |
| *Trypanosoma brucei* | Tropical Africa | Wild & domestic mammals | Tsetse – cyclical transmission | + | + | + | + | - |
| *Trypanosoma evansi* | North Africa, Asia, S. America | Camels, horses, dogs, bovids | Bloodsucking flies – mechanical transmission | - | - | - | - | + |
| *Trypanosoma equiperdum* | Cosmopolitan | Horses | Transmitted via copulation | - | - | +/- | - | + |

will diverge genetically. Therefore genetically similar individuals belong to the same species. The difficulty arises in determining what level of similarity defines a species, and just how long ago the event took place that separated 2 lineages. Just as with morphological characters, it takes expert knowledge of the extent of variation within and between known species in the group of organisms to make a judgement on what level of difference constitutes a new species. Again the key taxonomic characters (genes) used for each group of organisms may differ, and even when the same gene is used, for example the 18S ribosomal RNA gene, different levels of variation may prove significant in defining species.

So much for theory – how does this work in practice? It is illuminating to compare taxonomic ideas for two subgenera of African tsetse-transmitted trypanosomes. In subgenus *Trypanozoon*, taxonomy has been based largely on pathogenicity, distribution and host range. How do these criteria compare to observed levels of genetic divergence? For subgenus *Nannomonas*, data from molecular characterisation studies are revealing a growing number of distinct genotypes. Does each of these genotypes represent a distinct biological entity and does it matter?

## Species in subgenus *Trypanozoon*

It is generally accepted that subgenus *Trypanozoon* is divided into 3 species: *Trypanosoma brucei*, *T. evansi* and *T. equiperdum*, with *T. brucei* further subdivided into 3 subspecies defined by pathogenicity, distribution and host range [1]. Bloodstream form trypanosomes of the 3 species are morphologically indistinguishable, save for the occurrence of short-stumpy forms in *T. brucei*. Confusingly, the trait of pleomorphism can be lost in laboratory isolates of *T. brucei*, and they then become indistinguishable from the monomorphic species, *T. evansi* and *T. equiperdum*.

At the functional level pleomorphism reflects the ability of *T. brucei* to develop in its vector, the tsetse fly, and this is in turn dependent on possession of a complete and functional set of genes for mitochondrial operation. The mitochondrial genome is contained in the maxicircle DNA of the kinetoplast of *T. brucei*, together with the set of minicircle-encoded genes necessary for editing the maxicircle transcripts so they can be correctly translated. These features define *T. brucei*, and their absence defines *T. evansi* and *T. equiperdum* (Table 1). Neither *T. evansi* or *T. equiperdum* is cyclically transmitted by tsetse (although tsetse potentially could transmit *T. evansi* mechanically), and indeed, neither species is capable of cyclical development. *T. evansi* lacks a mitochondrial genome and its kinetoplast contains only a homogeneous set of minicircles. The few isolates of *T. equiperdum* examined also have missing kinetoplast DNA. One Chinese strain of *T. equiperdum* had maxicircles just over half the size of those of *T. brucei* and homogeneous minicircles like *T. evansi* [2]. Two other laboratory strains of *T. equiperdum* also had homogeneous minicircles; one had full-size and one reduced size maxicircles [3,4]. Examination of nuclear DNA polymorphisms by isoenzymes, RFLP, karyotype, minisatellite or phylogenetic analysis has shown no obvious differences between *T. evansi*, *T. equiperdum* and *T. brucei* [2,5–8]

In a sense then, *T. evansi* and *T. equiperdum* can both be regarded as natural mutants of *T. brucei*. Do they deserve separate species status? Arguably yes, because both satisfy the biological species definition above of non-interbreeding populations. Since genetic exchange in *T. brucei* takes place during cyclical development in the tsetse fly [9], this excludes participation of either *T. evansi* or *T. equiperdum*.

## Subspecies of *Trypanosoma brucei*

*Trypanosoma brucei* consists of 3 morphologically indistinguishable subspecies, all of which started as full species [1]. The demotion to subspecies came about after recogni-

**Table 2: Characteristics of subspecies and subgroups within *Trypanosoma brucei* See text for explanation**

| Subspecies | Distribution | Host range | Transmission | Growth in rodents | Genetic variability | Presence of SRA gene |
|---|---|---|---|---|---|---|
| *Trypanosoma brucei brucei* | Tropical Africa | Wild & domestic mammals, not humans | Tsetse | Fast | +++ | - |
| *Trypanosoma brucei rhodesiense* | East Africa | Humans, wild & domestic mammals | *Morsitans* group tsetse | Fast | ++ | + |
| *Trypanosoma brucei gambiense* Group 1 | West & Central Africa | Humans, wild & domestic mammals | *Palpalis* group tsetse | Slow | + | - |
| *Trypanosoma brucei gambiense* Group 2 | Ivory Coast | Humans, wild & domestic mammals | *Palpalis* or *morsitans* group tsetse? | Fast | ++ | - |

tion that the biological differences between the 3 species were not that significant and could mostly be explained by host range variation and geographical distribution (Table 2). Molecular evidence has not changed that view for *T. b. brucei* and *T. b. rhodesiense*. These 2 subspecies share the same range of genetic polymorphisms [8,10–13] and have been demonstrated to interbreed in the laboratory [14]. Most compellingly, it has now been demonstrated that *T. b. brucei* and *T. b. rhodesiense* can differ by as little as the expression of a single gene [15–17]. In fact there is a greater level of genetic variation between different *T. b. brucei* isolates than between *T. b. brucei* and *T. b. rhodesiense* [13,18,19].

The majority of *T. b. gambiense* isolates form a homogeneous group (group 1) that stands apart from the rest of the *T. brucei* group, because of its restricted range of genetic polymorphisms and limited antigenic repertoire [20–24]. It is clear that there is a much greater genetic distance between *T. b. gambiense* group 1 and other *T. brucei* subspecies than between *T. b. brucei* and *T. b. rhodesiense*. *T. b. gambiense* group 1 conforms to the classical concept of *T. b. gambiense* as a slow growing parasite in experimental rodents in contrast to the typically fast growing *T. b. brucei*/*T. b. rhodesiense* phenotype. If *T. b. gambiense* group 1 is genetically isolated, there may be a case for reinstating it as the species *T. gambiense*. But several questions need to be answered first.

Importantly, can *T. b. gambiense* group 1 undergo genetic exchange with *T. b. brucei*? These experiments are not easy, because *T. b. gambiense* group 1 is not readily transmitted through the *morsitans* group flies, which are commonly kept as laboratory colonies. It is unlikely that *T. b. gambiense* group 2 isolates represent genetic hybrids of *T. b. gambiense* group 1 and *T. b. brucei*, although they have the human infectivity of the former and the virulence and fly transmissibility of the latter. *T. b. gambiense* group 2 isolates shared only a single microsatellite marker with sympatric group 1 isolates [8]. *T. b. gambiense* groups 1 and 2 are also unlikely to have the same mechanism of human serum resistance. We know that neither group possesses the *SRA* gene, which confers human infectivity on *T. b. rhodesiense* [15,17,25,26], but whereas *T. b. gambiense* group 1 shows solid resistance to human serum, human serum resistance in *T. b. gambiense* group 2 varies with parasite passage as in *T. b. rhodesiense* [27].

We already know that *T. b. gambiense* group 1 isolates have a restricted range of genetic polymorphisms and the smallest genomes within the *T. brucei* species complex [20,28]. But what level of divergence does this represent? Perhaps a genome-wide comparison of *T. b. gambiense* group 1 and *T. b. brucei* will provide an answer.

## Species in subgenus *Nannomonas*

Trypanosomes of subgenus *Nannomonas* are defined by their developmental cycle in the tsetse fly, which involves the midgut and proboscis. As bloodstream forms these trypanosomes are the smallest of the Salivaria, but there is considerable morphological variation, both in dimensions (length and maximum width), and in features such as body shape, prominence of the undulating membrane and presence of a free flagellum [1]. Coupled with variation in host range and pathogenicity, this morphological variation led to the description of many species and variants in the past. However, these fine distinctions were later disregarded and traditionally subgenus *Nannomonas* is split into 2 species: *Trypanosoma congolense*, which has a wide range of ungulate hosts, and *T. simiae*, for which pigs are regarded as the most important host [1]. This simplifies clinical diagnosis: if you find a small trypanosome in the blood of a sick ox, goat or sheep, it will be *T. congolense*, while in pigs with acute trypanosomiasis it will be *T. simiae*.

This simple picture may be set to change. A number of hitherto cryptic subgroups have been discovered by molecular characterisation during the past 20 years or so. Only one of these has been described in sufficient detail to warrant acceptance as a new species, *T. godfreyi* [29]. Molecular characterisation reveals that *T. congolense* is divided into 3 subgroups, savannah, forest and kilifi (or Kenya coast), while the *T. simiae* group comprises *T. simiae*

**Table 3: Characteristics of species and genotypes within subgenus *Nannomonas*. See text for explanation**

| Species | Genotype | Distribution | Host range | Transmission – recorded high prevalence | Growth in rodents | Satellite DNA repeat size | KDNA minicircle size | Miniexon repeat size |
|---|---|---|---|---|---|---|---|---|
| *Trypanosoma congolense* | savannah | Tropical Africa | Wide range of ungulates & other mammals | *morsitans*, *palpalis* and *fusca* groups | + | 369 bp | 850 bp | 760 – 1500 bp |
| | forest | West & Central Africa | Pigs, goats. cattle, dogs, Other? | *palpalis* group | + | ~350 bp | 850 bp | ~800 bp |
| | kilifi or Kenya coast | East Africa | Cattle, sheep, goats. Not pigs Other? | *morsitans* group | + | 368 bp | 700 bp | ~600 bp |
| *Trypanosoma simiae* | | Tropical Africa | Suids | *Morsitans*, *palpalis* and *fusca* groups | - | 521 bp | 900 bp | 630 bp |
| | tsavo | East Africa | Suids | *morsitans* and *fusca* groups | - | ~540 bp | - | 508 bp |
| *Trypanosoma godfreyi* | | Tropical Africa | Suids | *morsitans* and *fusca* groups | - | 373 bp | 800, 750 bp | ~500 bp |

and *T. simiae* tsavo (Table 3) [29–35]. Each subgroup possesses a unique satellite DNA sequence, and these sequences have been exploited for the development of specific DNA probes and PCR tests [36–39]. The ability to identify these subgroups accurately opens the way for systematic studies of their host range, distribution, pathogenicity, etc. The satellite DNA repeats are sensitive tools for identification, because they are highly reiterated in the genome, forming the bulk of the minichromosomes. The equivalent satellite DNA repeat of *T. brucei* is conserved throughout subgenus *Trypanozoon* [40,41]. This suggests either that the satellite repeats evolve very rapidly in subgenus *Nannomonas* compared to subgenus *Trypanozoon*, or that divergence between subgroups in subgenus *Nannomonas* is greater than that between subdivisions in subgenus *Trypanozoon*.

Few attempts have been made to assess the level of genetic divergence within subgenus *Nannomonas*. Total DNA hybridisation showed that *T. congolense* savannah and kilifi subgroups were only distantly related compared to species within subgenus *Trypanozoon* [42]. A survey of nuclear and kinetoplast DNA polymorphisms in 5 species/subgroups (*T. congolense* savannah, forest and kilifi, *T. simiae*, *T. godfreyi*) revealed differences in the size of miniexon repeats and kDNA minicircles and maxicircles (Table 3) [43]. In this study, the most closely related trypanosomes were *T. congolense* savannah and forest [43], which share 71% similarity in satellite DNA sequence compared to an average 40–45% similiarity in the rest of the subgenus [44]. The *T. congolense* kilifi subgroup was as divergent from other *T. congolense* subgroups as from *T. simiae* or *T. godfreyi* [43]. The gene for the major surface glycoprotein, glutamate and alanine rich protein or GARP, is well conserved among *T. congolense* subgroups; the amino acid sequences of *T. congolense* savannah and forest strains differed by 4–5%, compared to about 16% from kilifi subgroup [45]. More divergent *GARP* genes have also been identified in *T. simiae* and *T. godfreyi* [46]. In agreement with the variation seen in *GARP* genes, phylogenetic analysis based on the 18S ribosomal RNA gene divides subgenus *Nannomonas* into 2 major clades: (1) *T. congolense* savannah, forest and kilifi subgroups, (2) *T. simiae*, *T. godfreyi* and *T. simiae* tsavo (previously designated *T. congolense* tsavo [31,34]. The absolute nucleotide differences between 18S ribosomal RNA genes in subgenus *Nannomonas* are larger than those in subgenus *Trypanozoon* [34].

In summary, there is compelling molecular evidence of far greater levels of genetic divergence within subgenus *Nannomonas* compared to subgenus *Trypanozoon*. However, biological criteria to support these "molecular taxa" are scarce. The inability of several of these trypanosomes to grow in experimental rodents has precluded the isolation of bloodstream forms from mammalian hosts or tsetse mouthparts in the field, so host range and distribution data are incomplete (Table 3). The use of PCR identification of tsetse infections has led to recognition that some of these new genotypes are extremely widespread and prevalent in the field, e.g. [47–51]. What contribution do these trypanosomes make to livestock disease? Are these various genotypes responsible for assumed "strain" differences in drug response, virulence or fly transmission dynamics? Can these new genotypes be correlated with the old morphological criteria and species designations? We really need the biology to catch up with the molecular taxonomy to answer these questions.

## Conclusions

There is no consistency in the way species and subspecies names are applied in subgenera *Trypanozoon* and *Nannomonas*. In subgenus *Trypanozoon* the taxonomic importance of pathogenicity, host range and distribution appear to have been inflated relative to actual levels of genetic divergence. Taking all evidence into account, it is arguable that *T. b. gambiense* group 1 should be reinstated as the

species *T. gambiense,* leaving *T. b. rhodesiense* and *T. b. gambiense* group 2 as host range variants of *T. b. brucei*.

In comparison, current taxonomic usage badly underrepresents diversity in subgenus *Nannomonas*. Data from molecular characterisation are revealing a growing number of genotypes, which may represent distinct taxa. Unfortunately few of these genotypes are yet supported by sufficient biological data to be recognized taxonomically. But we may be missing fundamental epidemiological information, because of our inability to distinguish these trypanosomes in host blood morphologically or in tsetse by their developmental cycle. Molecular taxonomy has led the way in identifying these new genotypes and now offers the key to elucidating the biology of these organisms.

## Competing interests

None declared.

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