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Letters Response

Response to Li *et al.* and Shaw: Return of the ring – opportunities to challenge a hypothesis

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We appreciate the comments of Li *et al.* in this issue about our article in *Trends in Parasitology*. However, there are two reasons why we do not share their opinion that *Trypanosoma equiperdum* strains should not be considered to be *Trypanosoma evansi* without examining kinetoplast maxicircles.

First, the value of kinetoplast maxicircles for distinguishing both species is questionable because of the existence of dyskinetoplastic strains in the *Trypanozoon* subgenus, which was even stated in one of the previous articles by the same research group [1]. The use by Li *et al.* of PCR on NADH dehydrogenase to distinguish *T. evansi* from *T. equiperdum* might be invalid because, using this approach, Swiss Tropical Institute Basel (STIB) 818 is classified as a *T. equiperdum* strain, whereas our results and those of others cluster this strain into the *T. evansi* group [2–4].

Second, Li *et al.* support their approach with unpublished results, including reference to two strains (STIB 841 and STIB 842) for which no data are publicly available. These stabilates from the cryobank of the Swiss Tropical Institute Basel (http://www.sti.ch/) originate from the University of Bordeaux (http://www.u-bordeaux2.fr/), which had only Bordeaux *Trypanosoma* antigen type (BoTat) and Onderstepoort Veterinary Institute (OVI) in its collection by the 1980s. Thus, STIB 841 and STIB 842 are probably BoTat and OVI, or two variants of BoTat. Hence, the data from Li *et al.* confirm our classification, except (again) for STIB 818.

Furthermore, we do not fully agree with the statement by Li *et al.* about the *Trypanozoon* subgenus that 'several biochemical and molecular methods fail to distinguish among them'. This does not hold for *Trypanosoma brucei gambiense* type I, at least, which – as determined by all techniques used so far – is separate from the other *Trypanozoon* members [5,6]. The same could be true for *T. evansi* type B, which differs from classical *T. evansi* with regard to its minicircles only, and is not a *T. brucei* because it does not differentiate into procyclic forms [7,8]. Could *T. evansi* type B belong to a new *T. brucei* subgroup, as we suspect is the case for the *T. equiperdum* OVI and BoTat strains? Unfortunately, our repeated requests to obtain the four unique strains of *T. evansi* type B [held at the Kenya Agriculture Research Institute (http://www.kari.org), which was formerly known as the Kenya Trypanosomiasis Research Institute] for use in our investigations have been unsuccessful.

Jeffrey Shaw's comments in this issue about mislabeled strains are relevant to the preceding discussion. Similar problems and confusion have also arisen in leishmaniasis typing. In 1990, a World Health Organization [WHO (http://www.who.int/en/)] expert panel published a list of *Leishmania* reference strains, later called the WHO references [9]. These strains are available from reference cryobanks in which regular typing ensures their identity. However, the same strains are also being circulated worldwide, with the same label but without quality control. In the context of possible mistaken identities, it might be worthwhile implementing some controls: (i) for evaluating biological parameters, one should work only with strains that are provided directly by a reference bank and that have a sufficiently documented history and DNA print; and (ii) these 'reference' strains should be included in molecular characterization assays.

In conclusion, the introduction of molecular techniques has made the biological species concept problematic in some cases, especially in kinetoplastid parasites that undergo predominantly asexual reproduction. There is an urgent need for a consensus about how to merge the 'ancient' biological species parameters such as host range, vector and induced pathology (disease) with the 'new' molecular parameters. Therefore, we invite all

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researchers to join efforts by sharing all available *T. equiperdum*, *T. evansi* and *T. evansi* type B strains and by analyzing them using as many techniques as possible, including the detection of maxicircle DNA, to determine the taxonomic position of the strains.

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