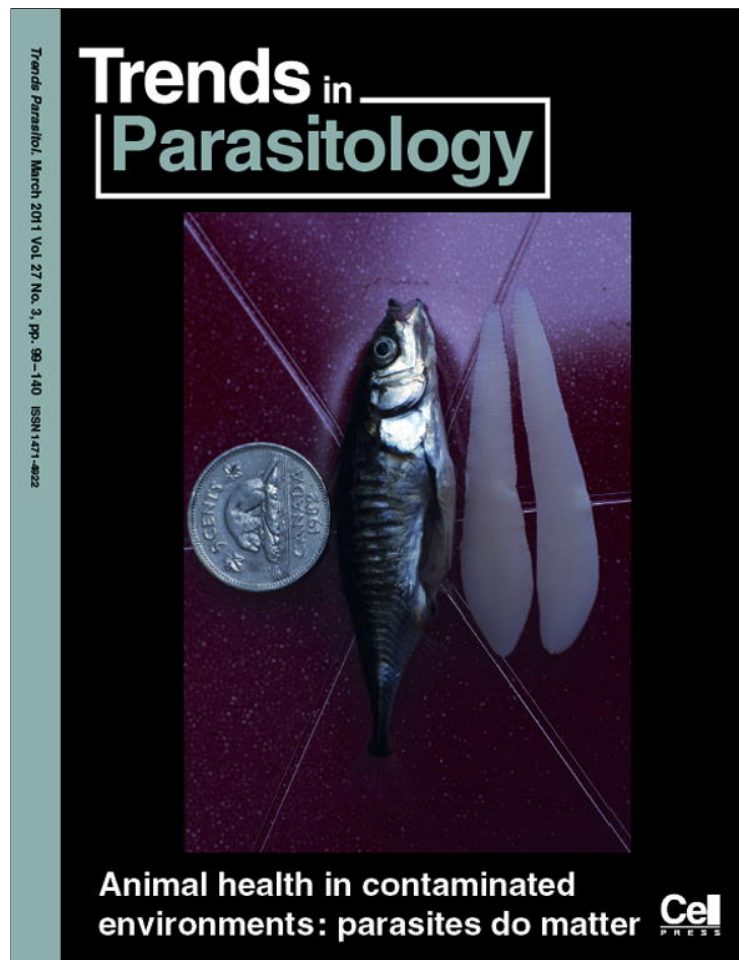


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Resolving relationships between Australian trypanosomes using DNA barcoding data

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The review of Thompson *et al.* [1] is a timely assessment of the threats to Australian wildlife posed by parasites, either by moving hosts within Australia or by introducing novel organisms. However, the presented phylogenetic tree (modified from [2]), showing the relationships between trypanosomes of Australian mammals, is misleading, and raises wider questions about how to best analyse such sequence data for identification of unknown organisms (DNA barcoding) and for species discovery. Many of the sequences used to construct the tree originate from recent PCR-based surveys, which have greatly increased our knowledge of trypanosome diversity in both native and introduced Australian mammals [2–6]. Indeed, at least four unrelated groups are now known from Australian marsupials, with new hosts including woylies [2], koalas [4], wallabies [3], potoroos, and quokkas [6]. Furthermore, three exotic species are now known, *Trypanosoma theileri* from cattle [7]; *Trypanosoma lewisi* from rats [2]; and *Trypanosoma nabiasi* from rabbits [8], which are likely to have been introduced to Australia by humans with their host species.

The presented phylogenetic trees [1,2] give the impression that *T. lewisi* (a cosmopolitan rat trypanosome) is closely related to a trypanosome of a kangaroo first characterised by Noyes *et al.* [9]. However, there is strong evidence from trees based on 18S rRNA and glyceraldehyde phosphate dehydrogenase (*gGAPDH*) genes that the kangaroo trypanosome is more closely related to *Trypanosoma cruzi* (the causative agent of Chagas disease) than to *T. lewisi* [10,11]. The tree presented in Averis *et al.* [2] is constructed using the small (approx. 500 bp) V7–V8 region of 18S rDNA, the region of choice for most sequence-based surveys of trypanosome diversity. However, this region is unsuitable for resolving relationships between distantly related trypanosomes, as it evolves quickly, and sequences from distantly related taxa cannot be aligned with confidence. As such, the most variable regions are often excluded from analyses; indeed, only 77 characters (of the approx. 2 kb gene) were included in the analyses of Averis *et al.* [2]. Furthermore, these analyses [2] excluded the majority of trypanosome diversity and key outgroup taxa, which would be required for a robust analysis. With such fast evolving sequences, elimination of more distant taxa can make it easier to identify sites of positional homology in an alignment, a useful practice for resolving relationships between closely related taxa. However, as shown by a number of 18S rDNA-based studies, resolution of relationships between distantly related trypanosomes (such as those from diverse Australian marsupials) requires broad taxon coverage to ensure correct inference of evolutionary relationships [12].

This raises the question of how to best analyse such short sequences. Averis *et al.* [2] also used BLAST for analysis of their short sequences. This approach is arguably more appropriate to an initial analysis of such sequence data, as it uses the full length of the sequence generated and enables comparison to all available sequences in a database (rather than a restricted subset). If sequences are found to be similar to those from reference taxa, phylogenetic analysis can then be restricted to a subset of closely related taxa. However, if sequences differ considerably from those of reference taxa (indicating the presence of novel species), accurate phylogenetic placement requires longer 18S rDNA sequences and/or sequences from other genes such as *gGAPDH*, together with careful taxon selection and inclusion of suitable outgroups.

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