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Letters

Heligmosomoides polygyrus: one species still

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We are responding to a recent article in which Behnke and Harris apply a different species name to the laboratory nematode commonly termed *H. polygyrus*, to distinguish it from the 'wild' parasite found in European *Apodemus* mice [1]. We believe this renaming to be certainly premature and quite probably incorrect. At this stage, we consider there is a convincing case for retaining the subspecies taxonomy that classifies the laboratory parasite *H. polygyrus bakeri*.

The argument for renaming cites differing morphological, ecological, and molecular traits between isoforms of *H. polygyrus*, most of which are reflected in the existing subspecies nomenclature. The available evidence is weak and there is considerable subjectivity in the discussion. The morphological features described by Behnke and Harris reflect biological divergence, but show considerable quantitative variation between individuals. These and other measures do not provide a clear-cut distinction and are not diagnostic of either population. Moreover, quantitative morphology is confounded as parasite age and nutritional environment are not necessarily comparable. Other characteristics (such as 'greater protein content') are completely nonquantitative and based upon a superficial analysis.

An interesting issue raised is the host-specificity of the laboratory and 'wild' isolates. Quinnell *et al*. [2] reported that wild isolates of *H. polygyrus* are far less infective to inbred *Mus musculus* than the laboratory strain, perhaps because the latter has been selected for infectivity to laboratory mice over many decades. Quite possibly the laboratory strain is poorly infective to *Apodemus* for the same reason. These authors also show that wild isolates will establish at a low level in laboratory mice, albeit at $<10\%$ of the inoculum. Hence, it would be possible to 're-adapt' this isolate into laboratory mice, to establish if the characteristics of *H. polygyrus bakeri* can be reproduced within a substrain of *H. polygyrus polygyrus.* Plus, hybridization experiments can be undertaken, once isolate-specific molecular markers are identified.

A further plank of the authors' case is that ribosomal ITS and mitochondrial CO1 sequences are too diverse for a single species [3]. We do not believe this conclusion to be justified. In our current transcriptomic and genomic sequencing of laboratory-maintained *H. polygyrus bakeri,* we find sequence variation of the same order (3–10% of

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nucleotide positions) as reported in the ITS and CO1 loci, and substantial sequence divergence between different *H. polygyrus bakeri* specimens is apparent (J. Urban and E. Hoberg, personal communication). Because unexpectedly high levels of variation have been found within single isolates of the related strongylid nematode species *Nippostrongylus brasiliensis* and *Haemonchus contortus*, there is no clear quantitative threshold for defining taxonomic levels within this nematode family.

Moreover, when we searched our draft *H. polygyrus bakeri* genomic dataset with the database entries for *H. polygyrus polygyrus* ITS-1, one (AY332649) showed 99% identity, slightly higher in fact than the entry for *H. polygyrus bakeri* itself (AY332648). For ITS-2, Cable *et al*. [3] did not define the *H. polygyrus polygyrus* 'consensus sequence' upon which their analysis is based, but with AM409084 we recorded 88–97% identity scores to different genomic contigs, similar to the 88–96% identity obtained when searching with *H. polygyrus bakeri* ITS-2 (AY333382). Thus, the high level of *H. polygyrus polygyrus* ITS sequence variation is equally apparent in *H. polygyrus bakeri* and hence differences at this locus are not informative.

At the CO1 locus, several different *H. polygyrus polygyrus* sequences were reported [3]. The Nottingham laboratory strain of *H. polygyrus bakeri* (DQ408627) matches our *H. polygyrus bakeri* dataset, and differs by ~8% from most *H polygyrus polygyrus* sequences. On this basis, Behnke and Harris conclude that *H. polygyrus bakeri* diverged from its conspecific relative ~3 million years ago. However, the Guernsey isolate of *H. polygyrus polygyrus* (DQ408633) differs from various British isolates to an even greater exent (~9%), seriously calling into question the use of the CO1 locus in this context, especially because Guernsey became an island relatively recently.

The introduction of high-throughput sequencing will greatly expand the sequence dataset in the next few years. This should include various laboratory populations thought to have all originated, via London, from a Californian source (J. Urban and E. Hoberg, personal communication) as well as putative ancestral populations circulating in the wild. Without such data, it is not possible to adjudicate on the taxonomic divisions of *Heligmosomoides*. It is our firm view that, taking into account the questionable nature of the evidence, the community should continue to regard the different isolates as subspecies, in a manner which will avoid confusion, retain continuity, and allow for future refinements through sequencing and biological testing.

What would be the best practice now, and for the future? All will agree, clarity and precision are paramount. Fortunately, past work with the laboratory strain is easily identifiable, but we propose (as suggested previously, when these authors last renamed the parasite [4]) that future publications on this isolate should use *H. polygyrus bakeri*. Equally, work with the wild isolates should use *H. polygyrus polygyrus*. We would not favour using '*H. polygyrus*' alone for the wild isolate, to avoid confusion with past publications on the laboratory strain.

At this time, we would also discourage authors from using the '*H. bakeri*' name for *H. polygyrus bakeri* because: (i) it prejudges the outcome of future molecular and biological investigations, and (ii) if it is decided to retain the existing subspecies structure, '*H. bakeri*' will be discarded, and its literature is in jeopardy of being ignored. The future use of subspecies names will also make it clear that older papers that used only *H. polygyrus* without an added subspecies epithet could be describing either laboratory or wild isolates, details of which will be found in the methods sections of each article.

Acknowledgements

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References

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