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COMMENT

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Glomales rRNA gene diversity – all that glistens is not necessarily glomalean?

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The high levels of genetic diversity found in arbuscular mycorrhizal fungi (AMF) and the discovery of multiple ribosomal RNA gene sequences in single spores of these fungi show this to be a fundamentally different group of Eukaryota (Rodriguez et al. 2001; Rodriguez et al., unpublished). In addition, the obligate biotrophic nature of AMF means, in most cases, that the spores used for molecular analysis are obtained from "open" pot cultures or from field soils and not from axenic cultures. There is, therefore, a very high probability of amplifying contaminating fungal DNA during PCR amplifications. The first publicised examples of this were the papers of Hijri et al.(1999) and Hosny et al.(1999). In both these papers, a lack of out-groups and failure to compare sequences with existing sequence databases using BLAST caused non-AMF sequences to be incorrectly attributed to AMF (Glomales) and to be entered as such into the literature and databases until corrected recently (Redecker et al. 1999; Schüßler 1999). Despite the publication of numerous examples of the occurrence of multiple ribosomal sequences in single spores and isolates of AMF and acknowledgement of the dangers of contaminating DNA (Redecker et al. 1999; Schüßler 1999), many papers continue to be published without adequate critical examination of the data. Without doubt, many of these errors result from the "publish or else" attitude in short-term research projects and contracts. However, mislabelling of samples can also lead to the erroneous submission of data to international databases and consequent incorrect construction of phylogenies.

The first major problem is the lack of appropriate out-groups to secure the origin of the sequences. Recent publications by Pringle et al. (1999) and Millner et al. (2001) both contain phylogenetic trees of ribosomal sequences obtained from AMF spores without reference to out-groups from outside the Glomales. Pringle et al. (1999) acknowledged that BLAST analysis of some 5.8S genes indicated clustering with a wide range of fungi, including Basidio- and Ascomycetes. Nevertheless, these sequences were entered into the databases, with the implication that they are part of the genome of a named AM fungus, *Acaulospora colossica* (see figure in Electronic Supplementary Material for accession numbers). Out-groups must be included to show the true taxonomic affiliation of sequences as far as is possible (see figure in Electronic Supplementary Material for examples available in the databases) and sequences of uncertain taxonomic identity should not be attributed to the genome of their "host" but recorded as of indeterminate origin. A similar situation has arisen with the recent inclusion of *Acaulospora lacunosa* (AF006512) and *Entrophospora contigua* (AF005060, AF005061) sequences into the sequence databases. Analysis of the *A. lacunosa* and *E. contigua* sequence AF005060 shows them to be 99.3% identical along the full length of the ITS1, 5.8S gene and ITS2 and 95.8% identical to a basidiomycete *Stereum annosum*. By way of comparison, two closely related AMF, *Glomus coronatum* (X96844) and *Glomus mosseae* (X96828), are only 92.4% identical in this region. It is usually impossible to identify sufficient homologous base positions between taxonomically distant groups of fungi in the ITS1 and ITS2 regions to allow accurate alignment; however, the alignment of these two sequences with *S. annosum* is startlingly good. The second *E. contigua* sequence (AF005061) also appears to be a basidiomycete sequence quite unrelated to the AMF

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(see figure in Electronic Supplementary Material). There is, therefore, little doubt that these sequences represent contamination of the DNA extraction by non-AMF fungi, yet they are now erroneously entered into the databases as AMF sequences.

An example of the second problem category mentioned earlier is the sequence ''*G. clarum* BEG14 (accession number AJ276083)'', recently published and submitted to GenBank (Schüßler et al. 2001). When checked in the online BEG database, the isolate BEG14 is an isolate of *G. claroideum* not *G. clarum*, whilst the sequence reference AJ276083 associated with it in GenBank referred to an isolate of *G. lamellosum*. This was partially corrected on 31 May 2001. This also applies to accession number U36592 labelled in the same article as coming from *G. clarum* but in the database as *Glomus* sp. This can only lead to much confusion amongst scientists looking to compare sequences from these AMF in their work.

In concluding, we believe these issues are of sufficient importance to be drawn to the attention of AMF researchers and journal editors. We would like to make it clear that in using examples of work and errors here we are not criticising the authors and their entire research/study but only wish to draw attention to how these errors, if uncorrected, can lead to confusion in the research of other groups. A more thorough review of papers containing sequence data by qualified molecular biologists could help prevent the dissemination of such errors prior to publication. We would also like to encourage researchers in these fields to utilise the culture collections of The International Bank for the Glomales (BEG, http://www.ukc.ac.uk/biosciences/beg/) or INVAM (http://invam.caf.wvu.edu/) or similar like-minded repositories or collections. The BEG has 20 freely available and well-categorised AMF isolates and includes an up-to-date genetic archive, which is a primary source for checking the authenticity of newly acquired sequences, since all the sequence data originate from BEG cultures of known provenance identified as far as possible by experts.

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