

Report To ASBS re: Hansjörg Eichler Research Grant Funding

Candidate: Ryonen Butcher
PhD student- Department of Botany,
University of Western Australia, Nedlands, WA 6907

I was awarded a 1998 Eichler Research Grant of \$1000 to assist my PhD research into the taxonomy and phylogeny of the South-Western Australian endemic *Synaphea* (Proteaceae: Conospermeae). The grant was used to facilitate an examination of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) and the molecular characters were intended to supplement a cladistic data set in which there is a paucity of morphological and anatomical characters.

Additionally, ITS 1 and ITS 2 characters were envisaged as being a contrasting source of phylogenetic information for this study, which will concentrate on the resolution of the infrageneric classification of *Synaphea*, especially within section *Synaphea* which contains the majority of species (44 of the 50 currently described) loosely united by their possession of a shortly emarginate stigma.

The funding was used to purchase chemicals, equipment and services required for the extraction, purification, amplification and sequencing of DNA. This included the construction and trial of region specific primers, the assessment and determination of suitable polymerase chain reaction (PCR) and sequencing protocols of the ITS 1 and ITS 2 regions for 14 taxa. The small selection of taxa represented a range of morphologically diverse as well as similar forms within *Synaphea*, and the sequence information derived from these was aligned and compared to resolve taxonomic and phylogenetic queries.

Sequencing problems were encountered in the ITS 1 region in all but one of the taxa sampled, with secondary structure of DNA causing the sequence trace to collapse to nothing after a run of repeated Gs approximately one-third of the way through the read. Consequently I was unable to obtain complete sequence data for this region. Potential solutions to this problem include the addition of chemicals to the sequencing reaction mix which will denature secondary structure and allow the read to continue uninterrupted, or sequencing up to this collapsed area from the opposite direction and piecing the two separate reads together as best as possible. Comparatively, sequence data from the ITS 2 region has been relatively unproblematic to obtain but has yielded a very small number of phylogenetically informative characters, with some morphologically distinct taxa possessing no base pair polymorphism in this entire region! Failure to obtain good ITS 1 characters so far is an unfortunate development, as this region has been found to possess both more base pair INDEL (insertion-deletion) and substitution characters than ITS 2. These results indicate that the ITS region may not be the most suitable for phylogenetic studies in *Synaphea* and it may be necessary to sequence a different locus of the genome which has been found to be more variable than ITS in other taxa (e.g. 5S intergenic spacer or the ETS region of the 45s rDNA repeat unit) in order to elucidate relationships within this group.