

PHYLOGENETIC ANALYSES

Over the last few years we have made much progress in understanding the phylogenetic relationships between the major liverwort lineages (Crandall-Stotler et al., in press; Forrest & Crandall-Stotler 2004, 2005). We have created an eight-locus (nuclear 18S and partial 26S, mitochondrial partial *nad5* [primers K2-L] and chloroplast *rbcL*, *rps4*, *psbA*, *atpB* and *trnL-F* regions) dataset for ca. 100 liverwort species, focusing on the simple thalloids (Metzgeriidae) (Forrest & Crandall-Stotler, 2004, 2005, unpublished data); in collaboration with researchers at Duke University (NC, U.S.A.), the Royal Botanic Gardens Edinburgh (Scotland) and the University of Göttingen (Germany), we are developing a further five locus dataset for a subset of these regions (*rbcL*, *rps4*, *psbA*, 26S and *nad5*) for ca. 200 species across all liverwort lineages, which we will present at the XVII International Botanical Congress in Vienna 2005.

Our results suggest that the liverworts are split into five well-supported lineages: 1. *Haplomitrium*/Treubiaceae; 2. Blasiaceae/complex thalloids, 3. Aneuraceae/Metzgeriaceae/*Pleurozia*; 4. all other leafy liverworts, and 5. Pallaviciniales/Fossombroniales (Fig. 1 and unpublished data). These results are broadly congruent with those from studies being conducted in other laboratories.

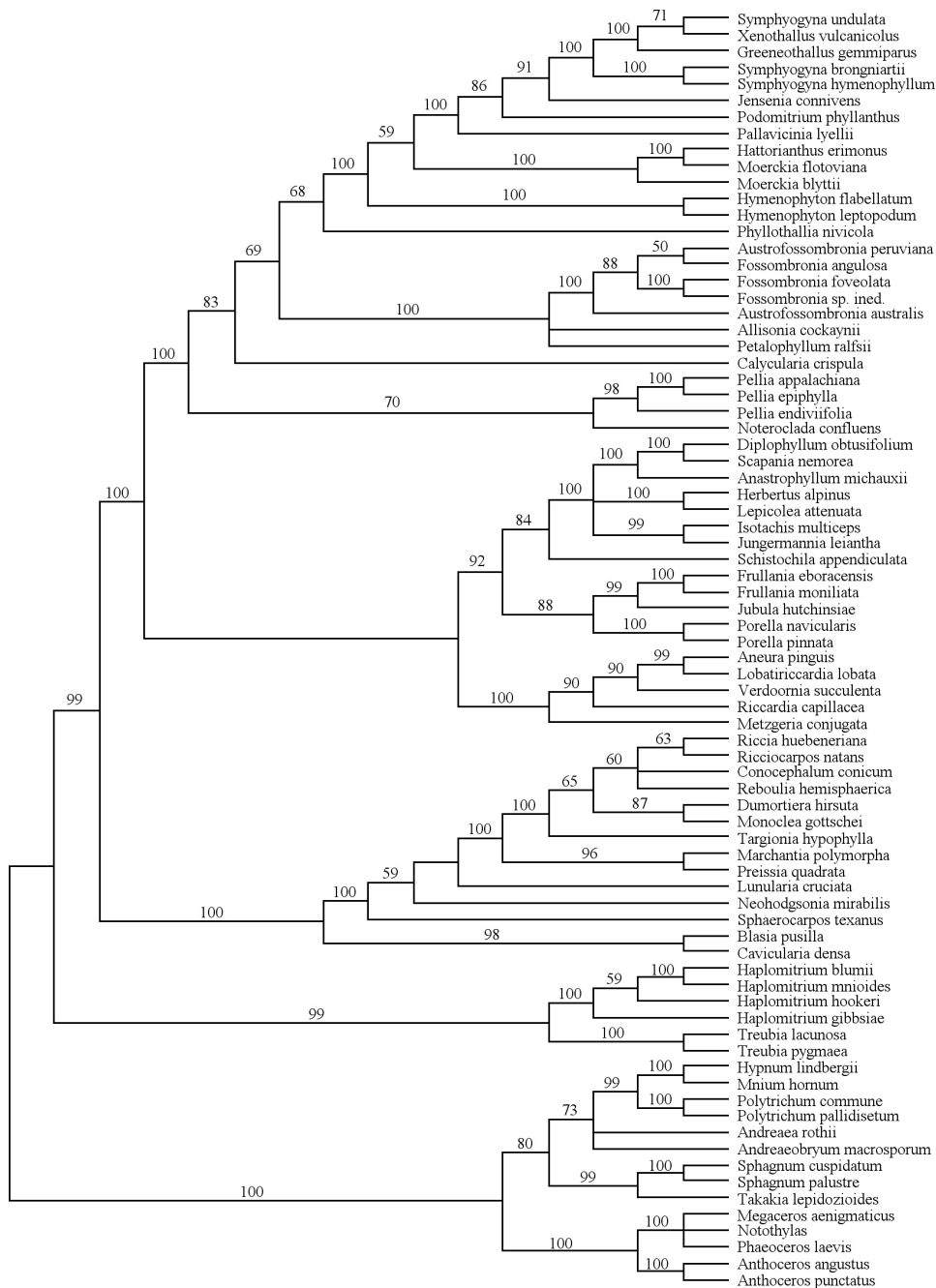


Fig. 1. Strict consensus of 24 minimum length trees generated in a maximum parsimony analysis of eight loci. Numbers above the branches are bootstrap support values above 50%.

A study to investigate the applicability of a further region, chloroplast ITS, to reconstructing these deep level liverwort relationships is currently underway in conjunction with a Masters student at SIUC, Dylan Kosma.

Because we are constructing data matrices of morphological characters to be analysed in conjunction with the molecular sequence data, we are able to make inferences about the direction of morphological evolution within the liverworts (Crandall-Stotler et al., in press and unpublished data). With a sound phylogenetic framework in place, a robust new classification of the liverworts can be produced.

Our research at SIUC focuses on the simple thalloid liverworts, and we are currently involved in producing family- or order-level phylogenies for several of the major clades in this group. In collaboration with Dr Virginia Freire (University of Wisconsin, Steven's Point) and Dr Chris Cargill (Australian National Botanic Gardens, Canberra) we have developed a sub-generic molecular dataset for the Fossombroniales (chloroplast *trnL-F*, *rbcL* and *rps4*, and nuclear ITS) (e.g. Forrest et al. 2003); we are also developing matrices for the Metzgeriales (chloroplast *rbcL* and *rps4*) (Forrest & Crandall-Stotler unpublished data), the Pelliaceae (chloroplast *rbcL* and *rps4*) (Forrest & Crandall-Stotler unpublished data) and the Pallaviciniales (chloroplast *rbcL*, *rps4*, *psbA* and *trnL-F*) (Forrest et al., 2004).

There is a lot of potential for phylogeographic studies in this group, with many extremely widespread species, and several interesting transoceanic disjunctions. However, before such questions can be addressed below the species level, species level phylogenies are required, to be able to assess whether the disjuncts truly represent monophyletic units. At the genus level, we have undertaken studies of the monophyletic but widely disjunct simple thalloid *Jensenia* (Pallaviciniaceae), using sequence data from the nuclear 26S region, mitochondrial *nad5* gene and intron regions, and chloroplast *rbcL*, *rps4*, *psbA*, *trnT-F* and *atpB-rbcL* regions (Forrest et al. 2004; in press); the extremely low levels of sequence divergence within this genus suggest that species are recent (Fig. 2), and that their distribution does not, therefore, represent Gondwanan relictualism, but rather long distance dispersal. This runs contrary to traditional views of the group's history.

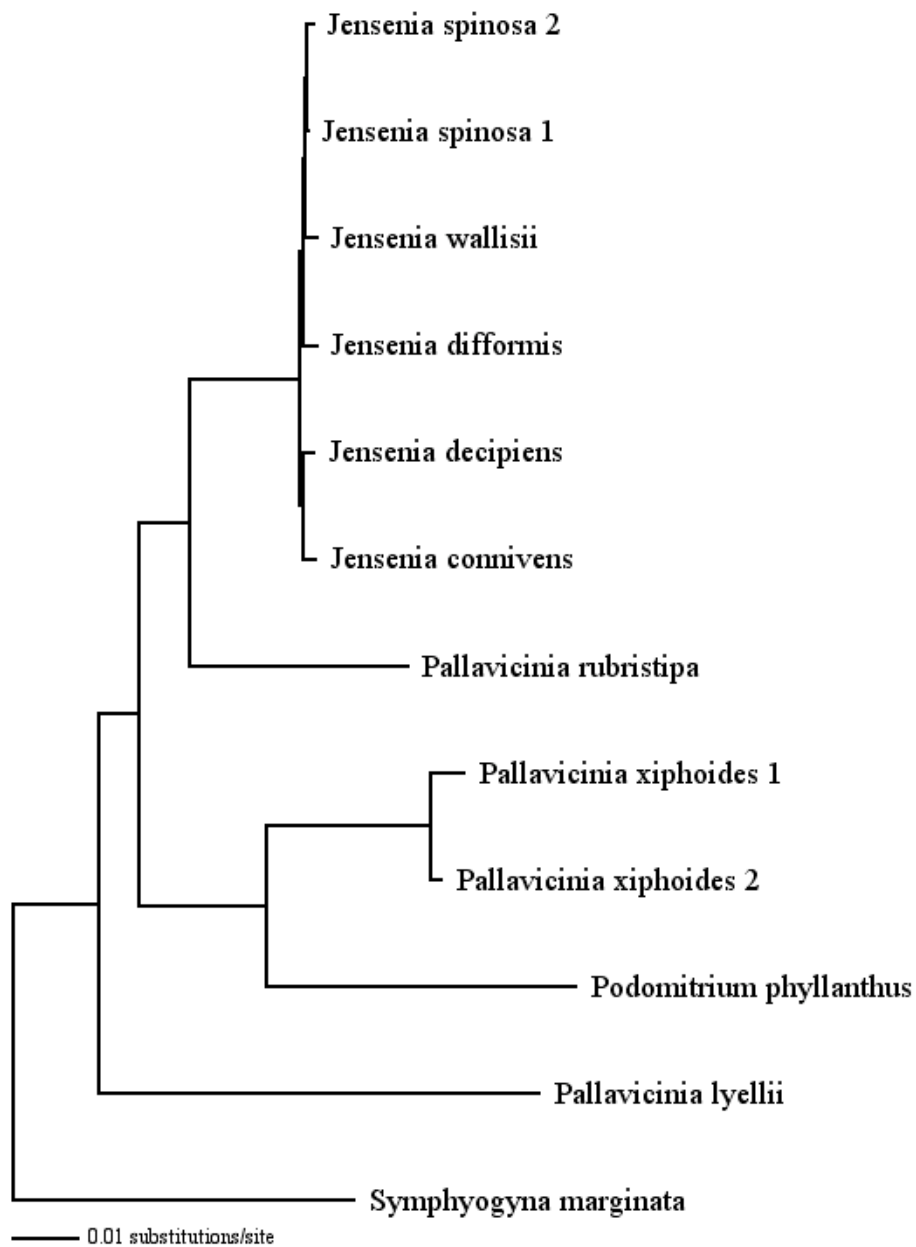


Fig. 2. Maximum likelihood phylogram of relationships within the simple thalloid liverwort genus *Jensenia*, constructed using DNA sequence data from eight loci. Note the extremely short branch lengths within the genus. The *Jensenia* collections used in this study are from New Zealand, Venezuela, Chile and New Guinea, while the *Pallavicinia xiphoides* collections are from New Zealand's North and South Islands.

How important the role of long distance dispersal is in the distribution of other liverwort lineages, however, remains to be tested. An eight locus phylogeny of *Haplomitrium*, a genus that we have recently shown to belong to the earliest-diverging liverwort lineage (Crandall-Stotler et al., in press; Forrest & Crandall-Stotler 2004), is in preparation in collaboration with colleagues at DUKE and RBGE; preliminary data (Forrest et al., unpublished data) suggest that this genus contains several long-separated lineages that may indeed reflect ancient geological history, and imply limited dispersal.

Molecular sequence data is therefore providing us with new and robust information about liverwort relationships at many levels of the phylogeny, from the species up to the phylum. Only with this sort of data can we finally begin to unravel such questions as what characters can be construed as 'primitive' within the lineage, or how efficient liverwort dispersal might be.