The adaptive significance of leaf size and shape variation in *Jamesbrittenia* (Scrophulariaceae: Manuleae)

T.E. Moore, G.A. Verboom, M.D. Cramer

Department of Botany, University of Cape Town, Private Bag X1, Rondebosch 7701, South Africa

The genus *Jamesbrittenia* (Scrophulariaceae: Manuleae) is widespread in South Africa, where it occupies a diversity of environments and shows striking variation in leaf size and shape. This leaf form variation may reflect adaptation for leaf thermoregulation or water conservation in contrasting habitats. To evaluate these competing hypotheses, we correlated leaf form variation in *Jamesbrittenia* against a series of environmental variables (soil nutrients, rainfall and temperature). We also compared rates of water and heat loss in potted plants representing a sample of *Jamesbrittenia* species of variable leaf size and shape. Although species showed significant variation in leaf temperature under experimental conditions, this variation was not correlated with leaf size. Whilst water loss per leaf area was greater in smaller-leaved species, the shoots of larger-leaved species showed higher rates of water loss overall due to their higher leaf area per shoot length. Consistent with these results, interspecific leaf size variation in a clad of summer-rainfall perennial species was strongly correlated with rainfall received during the driest time of the year, even when comparisons were phylogenetically controlled. In contrast, leaf size was poorly correlated with environmental variables in the predominantly annual, winter-rainfall clad. Since reduced leaf size leads to reduced leaf area per shoot length in *Jamesbrittenia*, the evolution of smaller leaves represents an adaptation for reduced water loss during the dry season.

doi:10.1016/j.sajb.2008.01.082

Molecular phylogenetics of the genera *Pimelea* and *Thecanthes* (Thymelaeaceae)

M.C. Motsi^a^, B. Rye^b^, M. Van der Bank^c^, T.E. Moore, G.A. Verboom, M.D. Cramer

^a^Molecular Systematic Laboratory, Department of Botany and Plant Biotechnology, University of Johannesburg, South Africa

^b^Department of Conservation and Land Management, Western Australian Herbarium, Australia

*Pimelea* and *Thecanthes* are two closely related genera belonging to the family Thymelaeaceae. *Pimelea* has 108 species endemic to Australia and New Zealand, while *Thecanthes* is a small genus of five species occurring in Australia and extending into the Philippines. The monophyly of *Pimelea* and reconstruction of high-level and species-level relationships using a combination of plastid and nuclear sequences will be presented. The molecular results so far indicate that the Australian genus *Pimelea* is nested within the South African *Guadua* and the small tropical genus *Thecanthes* is nested within *Pimelea*. The validity of the seven sections by Rye (1988) in *Pimelea* will also be presented.

doi:10.1016/j.sajb.2008.01.083

Isolation of high molecular weight genomic DNA from *Sclerocarya birrea* subsp. *caffra*, a high-polyphenolic content plant

M. Moyo, J.F. Finnie, J. Van Staden

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

The isolation of intact, high molecular weight and PCR amplifiable genomic DNA from plants with high polyphenols and polysaccharides is often a challenge in molecular biology applications. A modified cetyltrimethylammonium bromide (CTAB) protocol has been optimized for the extraction of genomic DNA from leaves of *Sclerocarya birrea* subsp. *caffra* seedlings. Previous work on the plant does not make reference to the quantity and quality of DNA. The quantity of genomic DNA isolated using the modified CTAB protocol ranged from 179.16 to 408.72 µg/g of fresh weight. This DNA quantity was extracted from 400 mg and 100 mg of initial sample weight per 500 µL of extraction buffer, respectively. The quality of DNA as measured by the A260/A280 ratio was 1.76 to 2.00. The DNA purity was further confirmed by gel electrophoresis of the PCR amplification products obtained using OPB05 and OPB06 arbitrary primers. In conclusion, the isolated DNA has good spectral qualities, is suitable for PCR amplification and can be used for molecular biology applications such as amplified fragment length polymorphism and DNA cloning.

doi:10.1016/j.sajb.2008.01.084

Seed germination studies of medicinal plant *Alepidea natalensis*

R.B. Mulaudzi, M.G. Kulkarni, R.A. Street, J.F. Finnie, J. Van Staden

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

*Alepidea natalensis* is a highly threatened medicinal plant species. The rhizome of this species is traditionally used as a popular remedy for colds, coughs, chest complaints, asthma, influenza, diarrhoea and abdominal cramp. The plant is extensively used, necessitating the improvement of cultural practices. Seed germination of *A. natalensis* is not well understood, therefore the effect of different temperatures, light conditions, cold stratification, smoke-water and plant growth regulators were investigated. At 20 and 25 °C, there was 78% germination with a mean germination time (MGT) of 12 days. None of the seeds germinated at 35 °C. Potassium nitrate improved the germination of *A. natalensis* seeds in comparison to GA3 and Kinetin at 10^-4 M. Smoke–water and cold stratification enhanced the germination of *A. natalensis* seeds.

doi:10.1016/j.sajb.2008.01.085

Tutti Frutti in *Lepidium* — Comparative analysis of fruit dehiscence/indehiscence in Brassicaceae

K. Mummenhoff^a^, G. Theissen^b^, A. Mühlhausen^a^, M. Smoke^b^

^a^Universität Osnabrück, FB Biologie/Botanik, Barbarastrasse 11, 49076 Osnabrück, Germany

^b^Friedrich-Schiller-Universität Jena, Genetik, Philosophenweg 12, 07743 Jena, Germany

Recent developmental genetic studies on fruit dehiscence in *Arabidopsis thaliana* provided detailed information about the anatomy and the regulating network underlying the dehiscence (opening) of fruits. To evaluate a general pattern of fruit opening and the impact of lignification on the dehiscence process we examined the anatomy and lignification patterns of fruit valve margin cells in dehiscent and indehiscent fruits of wild Brassicaceae species. We have identified a convenient study system consisting of *Lepidium campestre* (dehiscent fruits) and *L. appelianum* (indehiscent fruits). Both species are closely related and they are diploid (2n = 2x = 16). Fruit dehiscence in *Arabidopsis* is initiated by differentiation of three specialized cell types in the fruit valves, i.e., the lignified endocarp layer b, the lignified valve margin cells, and the dehiscence zones. As in *Arabidopsis* wild type fruits, well-developed dehiscence zones are apparent on both sides of the replar vascular bundles in *Lepidium campestre* fruits. Furthermore, lignified valve margin cells and endocarp layer b cells are dissimilar. As with *Arabidopsis* mutant (indehiscent) fruits, no valve margin cells and no dehiscence zones are formed in *L. appelianum* fruits. Instead, a continuous strip of lignified cells stretches from one replar vascular bundle to the other on the opposite side of the fruit. Thus, the anatomy of dehiscent and indehiscent *Lepidium* fruits corresponds to the *Arabidopsis* wild type (dehiscent) and mutant (indehiscent) fruit types, suggesting that orthologous genes are involved in both
Pollen diversity in Cyperaceae

A. Nagels¹, A.M. Muasya², S. Huysmans³, A. Vrijdaghs³, E. Smets³, S. Vinckiers³
¹Laboratory of Plant Systematics, Institute of Botany and Microbiology, Kasteelpark 31, BE-3001 Leuven, Belgium
²Botany Department, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa
³National Herbarium of the Netherlands, Leiden University Branch, PO Box 9514, 2300 RA Leiden, The Netherlands

Pollen grains vary in shape and morphology and distribution of orbicules in 51 genera representing all tribes and subfamilies. The fragment pollen collapse when acetylated and we explore alternative methods of preparing samples. Cyperaceae pollen grains vary in shape from spheroidal to periporate. Orbicules are always present, varying in shape and ornamentation. The palynological data are interpreted in a phylogenetic context.

doi:10.1016/j.sajb.2008.01.087

Antibacterial, antifungal and anti-inflammatory effects of medicinal concoctions used in South Africa

A.R. Ndhlala, J.F. Finnie, J. Van Staden
Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

Nine traditional South African herbal concoctions used to treat a range of diseases were tested for their claimed antibacterial, antifungal and anti-inflammatory (cyclooxygenase-1 and -2) activities. Antibacterial activity was evaluated using two Gram-positive bacteria, Bacillus subtilis (ATCC number 6051), Staphylococcus aureus (ATCC number 12600) and two Gram-negative bacteria Escherichia coli (ATCC number 11775), Klebsiella pneumoniae (ATCC number 13883) using the micro-dilution assay. Four out of the nine concoctions showed no activity against the tested bacteria while five showed activity. The ability of the concoctions to inhibit fungal growth was tested using the micro-dilution assay. Four of the concoctions showed no activity against the tested fungi while five showed activity. The ability of the concoctions to inhibit fungal growth was tested using the micro-dilution assay. Four of the concoctions showed no activity against the tested fungi while five showed activity.

doi:10.1016/j.sajb.2008.01.088

Bulb evolution in Oxalis revisited: the link between anatomy and phylogeny

K.C. Oberlander⁴, L.L. Dreyer⁵, D.U. Bellstedt⁶
⁴Department of Botany and Zoology, Stellenbosch University, Private Bag XI, Matieland 7602, South Africa
⁵Department of Biochemistry, Stellenbosch University, Private Bag XI, Matieland 7602, South Africa

Southern Africa Oxalis is understudied due to a combination of extreme morphological variation, a large number of species, poorly resolved species boundaries and often incomplete herbarium specimens. Previous studies have indicated the presence of a large polytomy at the base of the southern African clade, which could have been caused by a rapid radiation in the genus, as has been documented for several other CFR taxa. Here we present a phylogenetic reconstruction of southern African Oxalis, representing three quarters of currently recognised species, using DNA sequence data from both plastid and nuclear partitions. As currently recognized taxonomic sections are retrieved as being para- or polyphyletic, we propose a new phylogenetic classification for the genus. We argue for an informal temporary classification due to 1) the completely artificial nature of the current classification, which makes the attempt to rescue sectional names meaningless; 2) the poor resolution and support values for several large clades; 3) the presence of many well-supported clades without clear morphological synapomorphies. In order to resolve the age of the southern African clade, and to determine the rapidity of the radiation, divergence–time analyses, using both semi-parametric and Bayesian approaches were conducted. When the age of the family Oxalidaceae is inferred from a larger-scale Oxalidales data set with an enforced maximum age equal to the age of the eudicots, the age of crown group southern African Oxalis is dated at late Oligocene/early Miocene (±23 mya). This represents the oldest absolute age for the crown-group southern African lineage. The age of the southern African clade will probably be found to be younger when more realistic age constraints are used.

doi:10.1016/j.sajb.2008.01.089

Effects of recovery environment modifications on Eucalyptus grandis in vitro axillary buds injured during cryopreservation

K. Padayachee⁷, M.P. Watt⁸, N. Edwards⁹, D.J. Mycock⁸
⁷School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg 2050, South Africa
⁸School of Biological and Conservation Sciences, University of KwaZulu Natal, Durban, South Africa
⁹Trahar Biotechnology Centre, Mondi Business Paper, Hilton, South Africa

Recovery of plant cells and tissues injured during cryopreservation may be influenced by the post-thaw environment to which the material is exposed. For example, free radical production and enzyme injury may be worsened by exposure of thawed tissues to high light intensities. The components of the recovery medium on which the buds are plated post-thawing may also affect the extent of injury, since ion homeostasis mechanisms are likely to be affected in frozen and thawed buds. In addition, plating thawed buds on a recovery medium containing high concentrations of inorganic nutrients could also result in the accumulation of ions to toxic levels due to impaired regulatory mechanisms. Similarly, free radical injury may be minimized by maintaining thawed material in darkness for one or a few days, as this would inhibit photosynthesis and reduce free radical generation, while ion accumulation and subsequent toxicity may be reduced by reducing the concentration of some or all of the inorganic nutrients in the recovery medium. Therefore, during attempts to cryopreserve Eucalyptus grandis in vitro axillary buds, post-thaw conditions were regarded as an important criterion for recovery. In this regard, various parameters, including modifications to the recovery medium and the influence of light were investigated. The trends observed suggested that dark incubation and a reduction in NH4NO3 and KNO3 in the recovery medium prolonged survival.

doi:10.1016/j.sajb.2008.01.090