

# Young Systematics

Home
Awards
Meetings
Newsletters
Publications
Membership
About The
Association
Biennial 2003

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Royal Botanic Gardens
Kew Richmond
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The **Young Systematist's Forum** represents an exciting, annual setting for postgraduate students and young postdoctoral researchers to present their data, often for the first time, to a scientific audience interested in taxonomy, systematics and phylogenetic inference. The meeting provides an important opportunity for budding systematists to discuss their research in font of their peers, and hopefully supervisors too, within a supportive environment.

#### The Abstracts for 2003 appear below

#### **Oral Presentations**

#### The evolution of pollen structures in caesalpinioid legumes

Hannah Banks Micromorphology Group, RBG Kew, Richmond, Surrey.

Pollen has enduring value in providing diagnostic features towards assessments of relationships between plants. The pollen of over 900 samples representing all 161 genera of legume subfamily Caesalpinioideae has been examined using LM, SEM, and (selectively) TEM. Many factors affect pollen structure: phylogenetic inheritance, random mutations, pollination syndromes, functional efficiency (during dehydration, rehydration, and germination), recognition system requirements, and protection from environmental conditions (e.g. abrasion, crushing, desiccation). The impact of some of these factors on caesalpinioid pollen structures is discussed. Assessing homologies of structures, and determining those of parallel or convergent evolution, is necessary to assist the understanding of: 1) relationships between taxa and 2) the evolution of pollen

morphology in the group. A synopsis of the current understanding of legume pollen structures is given in the context of providing phylogenetically informative characters.

### Phylogenetic relationships in Nicotiana (Solanaceae) based on glutamine synthetase sequences

James J. Clarkson<sup>1</sup>, Sandra Knapp<sup>2</sup> and Mark W. Chase<sup>1</sup>

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Nicotiana is a complex genus, composed of both diploid and allopolyploid species. We present a phylogenetic tree with almost complete species sampling, based on nuclear glutamine synthetase sequences, a single copy gene which is not subject to gene conversion in Nicotiana. Allotetraploids, therefore have two loci, which correspond to the two parental species. Sequences from the allotetraploids can be extremely similar to extant diploids, indicating that the hybrids are recent, (eg. N. tabacum) or highly divergent from all extant diploids, representing ancestral forms (eg. the progenitors of N. section Suaveolentes).

Results of analysis of this dataset are compared to two previous molecular matrices (plastid & nuclear ribosomal) gathered for the same set of species. All show exactly the same relationships for the diploid species. The patterns of maternal inheritance in allopolyploids are shown by a combined plastid dataset (consisting of the regions *trnL* intron, *trnL-F* spacer, *trnS-G* spacer, *matK* and *ndhF*). However, trees based on the internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA) show gene conversion to one parental type (which can be converted in both directions).

## Winner of Best Presentation Does a tree-like phylogeny only exist at the tips in the prokaryotes?

Christopher J. Creevey Bioinformatics and Pharmacogenomics Laboratory, National University of Ireland, Maynooth, Co. Kildare, Ireland.

The extent to which prokaryotic evolution has been influenced by horizontal gene transfer (HGT) is unclear. Here we use supertree methods to ask whether a definitive prokaryotic

phylogenetic tree exists and whether it can be confidently inferred using orthologous genes. We analysed two datasets, one spanning the deepest divisions of prokaryotic relationships and one spanning the relatively recent gamma-proteobacteria using species for which complete genomes are available. Compatibility between gene-trees spanning deep relationships is only slightly better than random. Contrastingly, a strong, almost perfect, phylogenetic signal exists in gamma-proteobacterial genes. We conclude that deep-level prokaryotic relationships are either difficult to infer due to systematic biases or are not tree-like because of extensive HGT, hidden paralogy or both. Although we used two small datasets, this approach will help decide the extent to which we can say that there is a prokaryotic phylogeny and where in the phylogeny a cohesive signal exists.

#### Phylogeny and evolution of the Juncaceae

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Relationships within *Juncus* and *Luzula* and among the rest of Juncaceae are still unclear. In addition to creating hypotheses of relationships within Juncaceae and testing the classification of the sections, the goals of this study were to (1) assess relationships within *Juncus* and *Luzula*; (2) confirm monophyletic groups; (3) determine the position of small Andean genera within the *Juncus* clade, and (4) clarify relationships between the Juncaceae and the Cyperaceae. I conducted separate analyses of the *rbcL* data set and the *trnL* intron plus *trnL-trnF* intergenic spacer along with a combined data analysis.

Parsimony analyses revealed several well-supported clades. The traditionally distinguished genus *Luzula* is monophyletic while *Juncus* is non-monophyletic. *Juncus trifidus* appears in separate position on the tree, as a sister group to *Luzula* or as a basal taxon to the Juncaceae according to *trnL-F* data. A southern hemisphere clade contains not only the five South American genera, but also the South African *J. lomatophylus* and *J. capensis*. Within *Juncus* and *Luzula* the monophyly of a number of groups was demonstrated while it was questioned for others. There is no support for the exclusion of *Oxychloë* from the vicinity of *Patosia* or even from the Juncaceae based on either plastome data or morphology. The data demonstrate the Juncaceae to be a monophyletic group.

Genetic variation and ancient divergence between

#### freshwater and marine Protozoa

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The theory of microbial ubiquity states that 'all small things are everywhere, the environment selects', because of their astronomical numbers in the environment and because there are no barriers to free dispersal. Many free-living protist morphospecies are found in marine and freshwater environments, as well as soils and recently, screening of environmental samples has detected protist sequences from extreme environments. We have isolated and identifed morphospecies of bodonid flagellates and *Goniomonas* from several global sites and have sequenced their 18S rDNA genes using PCR primers specific for those groups and are analysing their genetic diversity and global distribution. For bodonids and goniomonads, marine and freshwater sequences cluster according to 'ecotype', which suggest that there are physiological barriers to free dispersal, and that multiple divergences between marine and freshwater morphospecies occurred several million years ago. Further analysis of bodonids using heat shock protein 90, supports this divergence between marine and freshwater 'species'.

### How to swim sideways: a systematics perspective on flatfish evolution

Jennifer Jackson

Bayesian techniques were used to resolve questions about the monophyly and interfamilial relationships of flatfishes. Sequence data from three nuclear genes (Rag-1, Rhodopsin, TMO4C4) and two mitochondrial genes (12S rRNA and 16S rRNA) was analysed. The efficacies of different types of data partitioning and ways of best discriminating alternative Bayesian hypotheses are discussed, with respect to both treeness and the Deviance Information Criterion.

### The phylogeny of liverworts - with special attention to the traditional "basal" leafy liverwort lineages

Xiaolan He-Nygrén, Inkeri Ahonen, Aino Juslén, David Glenny, Sinikka Piippo Division of Systematic Biology, University of Helsinki. Present address; Nationaal herbarium Nederland, Utrecht university branch, W.C. van Unnikgebouw, Heidelberglaan

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In order to reconstruct a first comprehensive molecular phylogeny of leafy liverworts, *rbc*L, *rps4*, and *trnL-F* regions of chloroplast genome were sequenced. Altogether 123 plant species, including 99 liverworts (46 families and 76 genera), two hornworts, 13 mosses, six vascular plants, and three green algae were investigated. The data was analysed with direct optimization and parsimony analysis based on static alignment with different combination of parameter values.

Our results show that liverworts are a monophyletic group, which diverged first from the rest of the land plant lineages. Blasia pusilla alone is resolved as a sister to the rest of the liverworts. Complex thalloid and simple thalloid liverworts are monophyletic as well as leafy liverworts. Within leafy liverworts Pleurozia-Metzgeria-Noteroclada clade is sister to rest of the species, which are further divided into two major groups. Different apical cell modifications reflect major evolutionary transformations in our phylogeny. The bush-like phylogeny of leafy liverworts based on previous morphological studies is denied. Erect and radial growth forms are derived rather than primitive opposed to earlier hypotheses.

### Phylogenomic Analysis Of The Translational Machinery: The Cytoplasmic Ribosomal Proteins In Arthropods.

Stuart J. Longhorn<sup>1,2,3</sup>, Kosmas Theodorides<sup>1,3</sup>, Peter G. Foster<sup>2</sup>, and Alfried P. Vogler<sup>1,3</sup>
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Ribosomal proteins (RPs) are vital components in the translational machinery of the cell. In eukaryotes, the utility of these genes for estimating taxonomic relationships is largely unknown. Using a transcriptomic (EST) approach to generate sequences, we investigated the ability of RP genes to reconstruct the placement and relationships of the mega-diverse Holometabolan insects. We sequenced 20 cDNA libraries of Coleoptera (beetles) plus single libraries from 5 other insect orders. RP data from these plus from Arthropods with public EST data were assembled at both protein and nucleotide level. The behaviour of a 40 RP gene dataset will be shown with regards to the inter-relationships of the Holometabolan insects, and will introduce results from a 10 RP gene analysis including the order Strepsiptera. Results are suggested to reflect how the complete set of approximately 80 genes may behave. A 25 RP gene set demonstrates the utility of these genes in the

Metazoan phylogeny, suggesting against support for the nematode-arthropod clade (ecdysozoa).

### Biogeography, diversity and systematics of the Jurassic ammonite *Badouxia*

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Ammonites are the classic index fossil, widely distributed and rapidly evolving. Most species of *Badouxia* on the other hand, are endemic to the eastern Pacific. Badouxia is present in many areas of North and South America. In addition, there is an account of a species of Badouxia from the Austrian Alps and another questionable report from northeast Asia. This study examines the Badouxia fauna from the Taseko Lakes map area, British Columbia. The type species of *Badouxia* was originally defined in this area. New results from this study suggest the existing paratypes of the type species are actually a new species. In this paper, I use measurements of shell geometry and ornamentation to delineate eight new species of Badouxia. I recognize sexual dimorphism in several species based on bimodal size distribution and differences in ornamentation. These results will form part of a future stratocladistic analysis of Hettangian ammonites.

#### Runner up, best presentation Evolution and systematics of *Aglaia* (Meliaceae): inferences from DNA sequence data and secondary metabolites

A. N. Muellner, R. Samuel, M. W. Chase, C. M. Pannell and H. Greger Institute of Botany and Botanical Garden, Dept. of Higher Plant Systematics and Evolution, University of Vienna, Rennweg 14, A-1030 Vienna, Austria.

Aglaia Lour. (angiosperm family Meliaceae, order Sapindales) is an arborescent genus occurring in the tropics of S.E. Asia, the Pacific islands and N. Australia. It comprises more than 100 species and presents more taxonomic problems in species delimitation than any other genus of the family. This resulted in the adoption of a wide species concept, by which taxa contain considerable morphological variation. We performed maximum parsimony and Bayesian analyses of sequence data from two regions (nuclear ITS and plastid *rps16* intron) to estimate phylogenetic relationships within genus *Aglaia* and its relations to the other genera of tribe *Aglaieae*. Based on 67 newly

sequenced accessions of *Aglaieae*, three taxa of Guareae and two taxa of Melieae (outgroup), this study provides the first reassessment of the current circumscription of *Aglaieae* and *Aglaia*, and of sections and species concepts. DNA data are compared to recently collected data on chemical profiles of the respective taxa.

#### Numerical taxonomy study of the genus *Alcea*

Maneezhe Pakravan Alzahra University, Iran.

Alcea is a mainly South West Asian genus with about 70 species in the world. It contains 34 species in Iran. The classification of the genus in the world is critical. In this research, a numerical taxonomic study of 33 quantitative and qualitative characters was conducted using different clustering and ordination methods. The results of clustering methods such as UPGMA, single linkage and Ward show two main clusters. The first cluster comprises species of the section apterocarpa and the second cluster species of pterocarpa. Also closed species occurred in closed branches of phenogram. Ordination of studied taxa on the first three principal component axes completely confirmed the clustering results.

Finally based on the obtained phenogram the relationships between species of the genus Alcea are discussed.

### The Colonization of Land by Animals: A Molecular Perspective

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A better understanding of how organisms colonized land in Earth history will provide insights into the evolution of terrestrial life in general. The geologic record provides evidence of terrestrial prokaryotes in sedimentary rocks as old as the Archean. However, fossil evidence for terrestriality among complex multicellular organisms is not seen until much later in the Paleozoic. This study represent an attempt to better define the relationships and times of origin of arthropods to better understand the colonization of land by animals. The results obtained seem to suggest that although Deuterostomia diverged from Arthropoda in deep Precambrian times (~1 Ga), and the radiation of arthropods occurred in the Precambrian, arthropods are most likely to have colonized land in the lower Palaeozoic (possibly during the Ordovician).

#### Floral mimicry in oncidioid orchids

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Oncidioid orchids (subtribe Oncidiinae) demonstrate an extraordinary radiation, comprising over 1700 species in tropical America. We evaluate the role floral mimicry has played in this radiation in the Neotropical forests of Costa Rica.

To study visual mimicry, we analyzed color distributions and floral reflectance of sympatric plants from 32 sites where an oncidioid orchid was central to the site. As bees are the most common pollinators in these communities and almost certainly the pollinators of oncidiod orchids, we measured floral color reflectance from 300-700 nm. Preliminary analyses of the data indicate that two basic types of orchid mimicry are in operation: (i) the genera *Oncidium* and *Erycina* strongly reflect both yellow and ultra-violet light, a characteristic shared only with *Byrsonima crassifolium* (Malpighiaceae); and (ii) other oncidioid orchids tend to converge on the rest of the community without displaying any unique differences.

### Seed protein analysis in relation to taxonomic of Persian *Linum* species

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Seed storage protein analysis was carried out on twelve species of *Linum* in Iran with the aim to illustrate species inter-relationships and to evaluate the taxonomic treatment proposed for genus. Placement of species in different sections according to cluster analysis of protein data was highly in agreement with previous phenetic morphological based studies. Grouping of species supported the proposed memberships of Linum species in Iran, and the SDS-PAGE profile of seed

protein proved to be useful to be included together with other molecular markers at sun-generic level in biosystematics Of the genus *Linum*.

### Phylogeny, rates of evolution and estimating divergence time events in cephalopod molluscs

Jan Strugnell

The Class Cephalopoda (phylum Mollusca) contains octopuses, squids, cuttlefish and Nautilus. Although most families of living cephalopods are well-defined, phylogenetic relationships among them are controversial. To resolve these relationships three mitochondrial (16S rRNA, 12S rRNA, COI) and three nuclear (pax-6, octopine dehydrogenase and rhodopsin) genes were sequenced from 40 taxa. partitioned Bayesian analysis techniques were used to generate the first ever robust cephalopod phylogeny.

#### Runner up, best presentation Concepts, morphology and molecules: delimiting species in the New Caledonian kauris (*Agathis*, Araucariaceae)

**Timothy Waters** 

Over the past twenty years, a growing amount of attention has been focused on the conceptual and theoretical aspects of species delimitation and species concepts, even as the main focus of systematic endeavour has moved away from species-level revisionary taxonomy and towards higher-level phylogenetics.

I will explore the uses of population aggregation analysis as an implementation of the phylogenetic species concept *sensu* Cracraft, for both morphological and molecular data in the tropical conifer genus *Agathis* in New Caledonia. The group is currently accepted as containing five species, but geographically focused collecting and large samples of serially homologous structures suggest a reduction in the number of species recognized to four. Preliminary molecular data are more difficult to analyse in the same framework, and the possibility of a realistic species concept, applicable both in theory and practice to all forms of data, is questioned.

Flatworm taxonomy revisited? Molecular phylogeny of the 'Typhloplanoida' and its implications for the phylogeny of the Rhabdocoela (Platyhelminthes).

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'Typhloplanoida' and 'Dalyellioida' are free-living Platyhelminthes from marine and fresh water habitats. These taxa have never been proved to be monophyletic, nor have the relationships within both groups ever been considered. The primary aim of our study is to elucidate the relationships of and within the 'Typhloplanoida'. The cladistic analysis, based on complete 18S rDNA sequences of 41 species of 'Typhloplanoida', and 49 species from other major groups of Platyhelminthes, revealed some surprising results: (1) nor 'Typhloplanoida', nor 'Dalyellioida' are monophyletic but form a strongly supported clade together, the sisterclade of the Kalyptorhynchia (formerly considered 'Typhloplanoida'); (2) limnic 'Dalyellioida' + limnic 'Typhloplanoida' + marine 'Typhloplanoida' form a strongly supported clade, the sisterclade of the marine 'Dalyellioida'; (3) 'Dalyellioida' is not the sister taxon of the Neodermata (as thought before). Further implications for the platyhelminth taxonomy will be discussed.

#### **Poster Presentations:**

**Colombian EBA Project: Research in Biodiversity, Systematics and Conservation in the Colombian Andes.** 

Blanca C. Huertas H.<sup>1</sup>, &Thomas M. Donegan<sup>2</sup>

<sup>1</sup>MSc student, Imperial College &Natural History Museum. London. <sup>2</sup> ProAves Foundation, Medellín, Colombia.

Evolutionary relationships of palaeognathous birds, combining morphological characters and information from fossils.

Leona Leonard
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Palaeognathous birds and their inconclusive phylogenetic history have been a source of speculation among systematists

for over a century. The interrelationships of all the birds (extant and extinct forms) must be determined in a definitive manner to allow us to further explore the underlying questions of the geographical point of diversification and the loss of flight for these large, primarily terrestrial birds. It is by examining morphological features of the skeletons of ratites (ostrich and living relatives and their extinct allies- moa, elephant bird and lithornithids), as well as the flighted tinamou, that we may ascertain the primitive and derived characters of this group. Traditionally, palaeognaths are defined by their very unique palate and general skull morphology, but this study also addresses characters from the postcranial aspect of the skeleton. The resulting cladistic analysis will allow a comprehensive view of characters from complete skeletons and, for the first time, fossil material. It will refine the current hypothesis on the relationships of the entire palaeognathous group.

### Hybridization and introgression in *Carduus crispus* and *C. personata*

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Carduus crispus L. and C. personata (L.) Jacq. are two of the thistle species growing in the Czech Republic. Carduus crispus is common in the whole area of the CR, mainly along river streams. It is absent in mountain areas, although there exist a few localities with secondary occurrence. C. personata is spread mainly in mountain and subalpine grade in Bohemian and Moravian mountains. In lowlands it can grow along rivers. They can occur in the same localities (not often) and then their hybridization is possible. The hybridization was studied using: karyology, flow-cytometry, artificial hybridization, pollen analysis and morphometric analysis.

The chromosomal number of *Carduus crispus* is 2n=16, of *C. personata* 2n=22. Hybrids of F1 generation have 2n=19 chromosomes. This count corresponds with parental combination n=8 and n=11. Hybrids have lower pollen viability but they are not sterile. Backcross experiments and interbreeding obtained seeds of F2 and B1 generation with different chromosome counts (2n=18, 2n=19 and 2n=20). Both species, *C. crispus* and *C. personata*, have huge morphological variability although some discriminating features were found. The hybrids are intermediate between parental species. However, it exists a few overlapping plants with both of parent species. This overlap is possible to explain as introgressive hybridization.

#### Winner of Best Poster Cladistic analysis of the tribe Muscini (Diptera, Muscidae)

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The tribe Muscini exhibits a wide variety of morphologically and ecologically diverse forms, including their reproductive strategy (female oviparous or larviparous), larval feeding habits (saprophagous, coprophagous, carnivorous) and adult colouration (metallic, pale, yellowish). This group, with 22 genera and about 350 species around the world, is regarded among the most basal clades within the Muscidae and is characterised by a number of diagnostic features.

The aim of the present study is to reconstruct the phylogenetic relationships among the genera of Muscini using mainly adult morphological characters, and larval characters wherever available.

The preliminary results indicate that: (1) the monophyly of Muscini is supported; (2) the monophyly of some genera (e.g. *Polietes, Morellia, Eudasyphora*) is doubtful; (3) the New World and Old World species of *Morellia* do not form a unique clade; and (4) several clades are congruent with regard to the basal position of the genera endemic to Neotropical, Afrotropical and Australasian regions, which suggest the origin of the Muscini could be related to Gondwana.

> Abstracts from previous years 2004 | 2003 | 2002 | 2001 | 2000 |

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12 of 12