

	Monday 6th
	Large Theatre
11:00-11:05	Welcome to 8 th biennial conference of the Systematics Association Juliet Brodie
	SYMPOSIUM: ALGAL SYSTEMATICS – WHERE NEXT? Chair: Juliet Brodie
11:05-12:00	KEYNOTE 1. Exploring the amazing world of algae, from cultures to single cells Debashish Bhattacharya
12:00-12:30	2. Horizontal gene transfer in algae John A. Raven
12:30-14:00	LUNCH
14:00-14:30	3. Genomic approaches to algal evolution: help or hindrance? John Bothwell
14:30-15:00	4. The rappemonads and their sisters: new and diverse additions to the algal tree of life Thomas A. Richards
15:00-15:30	5. Phylogeography of seaweeds: significance of cryptogenic species Frédéric Mineur
15:30-16:00	COFFEE
16:00-16:30	6. Glacial survival and unique genetic lineages under threat from current global climate change Jim Provan
	CONTRIBUTED TALKS Chair: Juliet Brodie
16:30-16:50	7. Towards an understanding of phylogenomic relationships in the Bangiales (Rhodophyta) Juliet Brodie
16:50-17:10	8. New roles for systematics in next generation biology David Patterson
17:30-19:00	WELCOME RECEPTION

	Tuesday 5th
	Large Theatre
	SYMPOSIUM:
	NEXT GENERATION SYSTEMATICS: STUDYING EVOLUTION AND DIVERSITY IN AN ERA OF UBIQUITOUS GENOMICS Chairs: James Cotton, Peter Olson, Joseph Hughes
9:00–9:30	9. A genome-sequence tree for Nematoda based on hundreds of genomes Mark Blaxter
09:30-10:00	10. Using phylogenomics to resolve the fungal tree of life David Fitzpatrick
10:00-10:30	11. Pathogenomics: investigating bacterial microevolution through second generation genome sequencing Simon Harris
10:30-11:00	COFFEE
11:00-11:30	12. Plant systematics in the age of genomics revisited Dennis Stevenson
11:30-12:00	13. Insights into flatworm evolution through comparative genomics Magdalena Zarowiecki
12:00-12:30	14. Ultimate barcoding - next generation sequencing meets high throughput mitogenomics Peter Foster
12:30-14:00	LUNCH
14:00-14:30	15. Next-generation biodiversity analysis Mehrdad Hajjibabei
14:30-15:00	16. Ecological differentiation of eukaryotic microbial lineages using 454 sequencing David Bass
15:00-15:30	17. Analysing metagenomic data to estimate diversity Chris Quince
15:30-16:00	COFFEE
16:00-16:30	18. Next generation sequencing in population genetics of gall wasps Graham Stone
16:30-17:00	19. Ongoing evolution in allopolyploid genomes examined

	<p>using next-generation sequencing</p> <p>Richard Buggs</p>
17:00-18:00	<p>KEYNOTE</p> <p>20. The role of NGS for integrative approaches of evo-devo and population genetics</p> <p>Ralf Sommer</p>
18:00-19:30	<p>POSTERS AND DRINKS RECEPTION (sponsored by Cambridge University Press)</p>

Wednesday 6 th		
	Large Theatre	Small Theatre
9:00-10:00	HOW-TO TALK 21. Clustal Omega and multiple sequence alignments Des Higgins Chair: James Cotton	
	CONTRIBUTED TALKS – THEORY AND METHODS FOR PALAEOONTOLOGICAL DATA Chair: Matthew Wills	CONTRIBUTED TALKS – PLANT SYSTEMATICS AND EVOLUTION Chair: Nina Rønsted
10:00-10:20	22. Stratigraphic congruence through geological time and across higher taxa: what factors explain the variation? Anne O'Connor	24. Snowdrops and snowflakes - falling slowly into place Nina Rønsted
10:20-10:40	23. Morphological disparity and clade shapes through time Martin Hughes	25. Phylogenetic studies of the aloes (<i>Aloe</i> L., Xanthorrhoeaceae) Olwen Grace
10:40-11:10	COFFEE	
	CONTRIBUTED TALKS – ANIMAL SYSTEMATICS AND EVOLUTION Chair: Julia Day	CONTRIBUTED TALKS – PLANT SYSTEMATICS AND EVOLUTION CONT. Chair: Julien Massoni
11:10-11:30	26. Biogeography and diversification in Africa: a case study from a species rich catfish clade Julia Day	30. Evolutionary history of a 10,000-species clade of angiosperms: reconstructing the phylogeny of magnoliids as a whole Julien Massoni
11:30-11:50	27. Evolutionary ecology of Lake Tanganyika's Mastacembelid eels Katherine Brown	31. Comparative transcriptomics and genomics: determining the regulators of petal spot development and the evolution of this specialised floral trait within the species complex <i>Gorteria diffusa</i> Rachel Walker
11:50-12:10	28. Distinguishing signal, noise and bias in early animal evolution: The difficult problem of the sponge phylogeny Roberto Fueda	32. Testing the impact of calibration on molecular divergence times using a fossil-rich group: The case of <i>Nothofagus</i> (Fagales) Hervé Sauquet
12:10-12:30	29. Reconciling morphological and molecular classifications: an	33. How to resolve conflict between taxonomy and phylogeny in basal living

	investigation of Hemiasterellidae (Demospongiae, Porifera) Christine Morrow	chitons (Mollusca: Ployplacophora) Julia Sigwart
12:30-	LUNCH EXCURSIONS	

	Thursday 7th
	Large Theatre
	SYMPOSIUM:
	ARTHROPOD SYSTEMATICS: ARE MORPHOLOGY, PALAEOLOGY AND MOLECULES COMING TOGETHER? Chairs: Davide Pisani, Omar Rota-Stabelli and Ronald Jenner
	SESSION 1: THE ARTHROPODS AND THEIR OUTGROUPS
9:00—9:45	KEYNOTE 34. What have we learned from 25 years of animal molecular systematics? Max Telford
9:45-10:15	35. Neural characters and arthropod systematics Angelika Stollewerk
10:15-10:30	36. MicroRNA and phylogenomics reveal velvet worms as the arthropod sister group Lahcen Campbell
10:30-11:00	COFFEE
	SESSION 2: THE ARTHROPODS AND THEIR OUTGROUPS CONTINUED
11:00-11:30	37. On the position of myriapods: insights from Onychophora (velvet worms) Georg Mayer
11:30-12:00	38. Molecular arthropod phylogeny: Early relationships and divergence times Thorsten Burmester
12:00-12:20	39. Ten years later: Phylogenetics utility and limitations of mitochondrial gene order Dennis Lavrov
12:20-13:00	DISCUSSION What is the outgroup to the arthropods? Is Mandibulata definitive?
13:00-14:00	LUNCH
	SESSION 3: THE PANCRUSTACEA PROBLEM(S)
14:00-14:30	40. Sources of deep-level phylogenetic signal in 62 protein-coding nuclear genes from arthropods Jerome Regier
14:30-15:00	41. Phylogenomic and evolutionary aspects of Pancrustacea and Remipedia –the hope and headache of “phylogenomics” Björn von Reumont

15:00-15:30	DISCUSSION What is the outgroup to the hexapods?
15:30-16:00	COFFEE
	SESSION 4: MOLECULES AND FOSSILS...
16:00-16:30	42. The Devil in the detail: modern morphological methods allow the confident placement of fossil arachnids as molecular calibration points Jason Dunlop
16:30-17:00	43. Morphological cladistics and arthropod phylogeny Greg Edgecombe
17:00-17:20	44. Are morphology-based cladograms of arthropods really less robust than those of vertebrates? Matt Wills
17:20-18:00	DISCUSSION Why is there a persistent gap in the arthropod, particularly myriapods, fossil record?
18:00-18:45	KEYNOTE 45. Vistas in arthropod evolution: throughout phylogeny, and beyond Alessandro Minelli
19.30 for 20.00	CONFERENCE BANQUET Belfast City Hall

Friday 8 th		
	Large Theatre	Small Theatre
	SYMPOSIUM: ADVANCES IN USING MUSEUM SPECIMENS AND ANCIENT DNA IN SYSTEMATICS RESEARCH Chairs: Patricia Lee and Samantha Mohun	
9:00-9:30	46. Extracting DNA from museum collections of dried eggshells Patricia Lee	
9:30-10:00	47. DNA damage repair and amplification from formalin fixed specimens Samantha Mohun	
10:00-10:30	48. Museum archaeobotanical collections as a source of biomolecular information for archaeogenetic analysis Oliver Smith	
10:30-11:00	COFFEE	
11:00-11:30	49. Postglacial recolonisation mode of North American <i>Amara alpina</i> (Carabidae: Coleoptera) using museum and ancient DNA Peter Heintzmann	
11:30-12:00	50. Developing museum molecular collections: "A global repository for schistosomiasis research" Fiona Allen	
12:00-12:30	51. Biobanking at the NHM London Jacqueline MacKenzie-Dodds	
12:30-14:00	LUNCH	
	CONTRIBUTED TALKS – ADVANCES IN USING MUSEUM SPECIMENS AND ANCIENT DNA IN SYSTEMATICS RESEARCH Chair: Christoph Hahn	CONTRIBUTED TALKS – THEORY AND METHODS OF PHYLOGENETICS Chair: Mark Wilkinson
14:00-14:20	52. Museomics for ectoparasites recovered from historical fish collections – lessons from <i>Gyrodactylus</i>	56. Fast Supertree Construction Mark Wilkinson

	Christoph Hahn	
14:20-14:40	<p>53. The DNA Bank Network – A platform for open access to voucher referenced DNA samples and data worldwide</p> <p>Gabriele Droege</p>	<p>57. Convergent evolution at the sequence level – batty and malarial examples</p> <p>James Cotton</p>
14:40-15:00	<p>54. Quantitative morphometric assessment of the disparity of Lake Tangayika's endemic gastropod fauna</p> <p>Olivia Cheronet</p>	<p>58. Rocks, Soils and Breaks: A new method for discovering and identifying biotic breaks</p> <p>Malte Ebach</p>
15:00-15:20	<p>55. Herbarium DNA: extraction optimization, damage assessment and use.</p> <p>Freek Bakker</p>	<p>59. <i>Nullius in Calculo</i>: On the Explicitness and Reproducibility of Cladistic Analyses</p> <p>Ross Mounce</p>
15:20-15:30	<p>CLOSING REMARKS AND PRIZE-GIVING</p> <p>Juliet Brodie</p>	

ORAL ABSTRACTS

1. Exploring the amazing world of algae, from cultures to single cells

Debashish Bhattacharya¹, Hwan Su Yoon^{2,3}, Dana C. Price¹, Eun Chan Yang², Cheong Xin Chan¹

1 Department of Ecology, Evolution and Natural Resources, Rutgers University, New Brunswick, NJ 08901, USA. 2 Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME 04575, USA. 3 Department of Biological Sciences, Sungkyunkwan University, Suwon 440-746, South Korea
E-mail: bhattacharya@aesop.rutgers.edu

Much of our understanding of the natural world has come from experiments done on model species manipulated under controlled laboratory conditions. The advent of modern high-throughput genomics and bioinformatics has allowed researchers to explore genetic diversity in natural systems, opening the way for exploring organism biology *in situ*. Here I will discuss new work from our lab on the genomes of key algal groups that help us understand plastid and eukaryote evolution and then I will introduce work that has focused on single cells to elucidate their biology. This latter approach, referred to as single cell genomics allows the study of prey and pathogens associated with protists in "real time" and may ultimately provide the opportunity to study endosymbiosis and horizontal gene transfer on a cell-by-cell basis.

2. Horizontal Gene Transfer in Algae

John A. Raven

University of Dundee at SCRI, Errol Road, Invergowrie, Dundee
E-mail: j.a.raven@dundee.ac.uk

Horizontal gene transfer (HGT) is now known to be widespread in algae. HGT is detected by marked discrepancies between phylogenies based on sequences of different genes. When other explanations have been eliminated, HGT is the most probable explanation. More cases will presumably come to light as more organisms are sequenced and tree generation becomes more robust. Only genes not subject to HGT reflect the phylogeny of the organism as well as the phylogeny of their genes. rRNA genes reflect organism phylogeny, while Rubisco genes have been subject to HGT and can only be used in constructing organismal phylogenies when HGT has been taken into account. The best-known mechanism of HGT in algae is that of the primary and subsequent endosymbioses, followed by gene loss and integration of some of the remaining endosymbiont genes into the exhabitant genome, distributing the capacity for oxygenic photosynthesis to a range of eukaryotes. Sequence analyses have revealed the genetic remains of plastids from a previous secondary endosymbiosis of a green alga in diatoms. The distribution of Rubisco can in part (Form ID in red algae and plastids from

secondary endosymbioses of a red alga) be explained by a secondary endosymbiosis, but perhaps not the initial occurrence of Form ID Rubisco in the red algae or of Form II Rubisco in the dinoflagellate-apicomplexan clade of alveolates. Alternatives to endosymbiosis include transfer by phagotrophy where the ingested organism never became a symbiont, or transfer by viruses such as is known to occur with cyanophages. Functional outcomes of HGT range from the obvious, i.e. the acquisition of oxygenic photosynthesis by eukaryotes, to the uncertain, e.g. the significance of replacing Form ID Rubisco by Form II Rubisco in the dinoflagellate-apicomplexan clade.

3. Genomic approaches to algal evolution: help or hindrance?

John Bothwell

Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast, BT9 7BL

E-mail: j.bothwell@qub.ac.uk

It is a commonplace that genomic technology can offer biologists new ways of looking at old problems (cf. our sister symposium: Next Generation Systematics - studying evolution and diversity in an era of ubiquitous genomics). Put simply, genomic approaches can offer a broader view of the genetic events which are associated with, and which may cause, life's diversity. In this talk, I will consider the extent to which algal systematics - specifically, questions of evolutionary history and adaptation - can benefit from genomic techniques developed in other model organisms. How cost-effective are these genomic approaches and do they favour hypothesis generation to the detriment of hypothesis testing? Using case studies drawn from the past decade of genomic work in the brown algae - particularly patterns of gene family loss and gain and the importance of sex chromosome evolution in brown algal speciation - we will discuss the evolutionary and ecological scales over which genomic perspectives should be considered in algae, and identify the barriers which still stand in the way of a fully developed genomic toolbox for the brown macroalgae.

4. The rappemonads and their sisters: new and diverse additions to the algal tree of life

Thomas A. Richards

Department of Zoology, Natural History Museum, London.

E-mail: t.a.richards@exeter.ac.uk

Sequencing of environmental DNA samples has radically expanded our understanding of the complexity of the tree of life. These methodologies have focused on sequencing small subunit (SSU) rRNA gene markers from environmental DNA samples. Eukaryotic algae provide two chances to investigate their evolutionary complexity using these methods because

researchers have successfully targeted both the nuclear and chloroplast SSU rRNA genes. In this presentation I briefly summarise progress in this field and then describe the discovery of a new algal form that we call the rappemonads. This group was identified using environmental DNA methods focusing on plastid SSU rDNA sequences. This diverse and uncultured eukaryotic algae forms a sister group to the haptophytes branching within the hacrobia 'supergroup'. Environmental sampling demonstrates that this algal group is present in multiple globally dispersed marine and freshwater environments. Using fluorescent *in situ* hybridisation (FISH) microscopy we demonstrate that these algae possess between 2-4 plastid organelles. Additional sequencing projects have now demonstrated that the rappemonads have previously unidentified relatives; further expanding our understanding of the diversity of plastid bearing microbes. Using these data I therefore pose the question, how many more major branches on the algal tree of life remain unsampled?

5. Phylogeography of seaweeds: significance of cryptogenic species

Frédéric Mineur and Christine A. Maggs

School of Biological Sciences, Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom
E-mail: f.mineur@qub.ac.uk

In the light of global change, biological invasions are considered as one of the most important threats to biodiversity. Over the 20th century, introduction events have increased exponentially. A higher frequency of vector movements and new transport pathways are clearly responsible for that trend. Introductions of exotic species of seaweeds have been recorded for two centuries. However, on a global scale, transport pathways have been in place at least since the Age of Discovery (15th century). Therefore, distribution of many organisms has probably been affected over a long period. Before the advent of molecular techniques, many species were believed to be cosmopolitan. During the last 10 years, advances in phylogeography have helped to discriminate sibling species and to distinguish natural distributions from human-mediated introductions. However, some taxa remain cryptogenic (i.e. neither native nor exotic). Using some macroalgal examples, we will discuss how these issues could be resolved, for instance by using old collection material and by developing molecular clocks.

6. Glacial survival and unique genetic lineages under threat from current global climate change

Jim Provan

School of Biological Sciences, Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom
E-mail: J.Provan@qub.ac.uk

The current-day distributions of many species have been shaped primarily by climatic oscillations during the Quaternary period. Phylogeographic studies have indicated that many species persisted through the ice ages in low latitude refugia, and recolonized formerly glaciated areas following the retreat of the ice. In genetic terms, this should be reflected in a decrease in levels of diversity from low to high latitudes, but this paradigm of “southern richness and northern purity” has recently been challenged by the discovery of “cryptic” northern refugia. Furthermore, in cases where species have persisted in multiple refugia which have not contributed equally to the recolonization process, these “isolated” refugia may harbour unique genetic variation found nowhere else across the species’ range. Consequently, no general consensus exists on the distribution of genetic diversity across species ranges. I will present evidence from a growing number of studies on intertidal algae that suggests that many rear-edge populations represent reservoirs of unique genetic variation. Given that current global warming is causing measurable shifts in the distributions of many of these species, characterised by the extinction of rear-edge populations, the loss of these populations is likely to have a disproportionate impact on the range-wide genetic diversity of many species.

7. Towards an understanding of phylogenomic relationships in the Bangiales (Rhodophyta)

Juliet Brodie¹ and Agnes Mols Mortensen²

¹Natural History Museum, Department of Botany, Cromwell Road, London, SW7 5BD, UK. ²Department of Biological Sciences, G32 Spaulding Life Science Center, University of New Hampshire (UNH), Durham, NH 03824, USA.

E-mai: j.brodie@nhm.ac.uk

The sequencing of whole genomes is heralding a new era of phylogenomics, with the possibility of using multi-gene analyses to test phylogenetic hypotheses that have been generated previously using one or a few gene analyses. This could be particularly valuable in the red algae where phylogenetic relationships have, until the application of molecular techniques, been based essentially on comparative morphology. Because of the morphological variation in the red algae, including cryptic species, convergence and in some cases heteromorphic life histories, molecular taxonomies have led to complete taxonomic revisions at the species and higher levels, including the creation of new orders. However, this has been limited by the number of molecular markers available. In 2008, a Joint Genome Institute project began to sequence the entire genome of the red alga *Porphyra umbilicalis*, the first for the Bangiales. In this talk, we will explore the taxonomic implications based on initial data from this project.

8. New roles for systematics in next generation biology

David J Patterson

MBL, Woods Hole, Massachusetts 02543, USA

E-mail: dpatterson@mbl.edu

In the next generation of biology, an increasing number of subdisciplines are expected to adopt the model of molecular biologists - that of sharing data. The change is motivated by the need for large amounts of data to answer large questions, such as those relating to changing abundances and distributions of species. Such questions require access to the data acquired over two and a half centuries, now held in a diversity of digital and non-digital forms, and rarely acquired with data-sharing in mind. The trend to digitize data, and make increasing amounts available for re-use is being made possible by advances in biodiversity informatics. A new community of data-centric biologists is beginning to assemble around these open (virtual) data pools. They need new ways to organize, analyze and visualize data if new insights and discoveries are to emerge. Data in the pool will need to be indexed and organized through widely agreed systems of metadata and ontologies. The system of Linnaean names is one of the most pervasive systems of metadata. Systematics offers ontological frameworks for such metadata. The use of taxonomy as an organizational framework for biodiversity data requires resolution of the 'many-names-for-one-taxon' and 'one-name-for-many-taxa' problems, managing different perspectives of 'what is a taxon' and representing taxa in competing classifications and phylogenies. Projects such as the Global Names Architecture are now putting such elements in place. GNA is an international effort to establish an open name-based cyberinfrastructure that can act as a virtual layer joining together numerous nomenclatural, taxonomic, phylogenetic, and biodiversity initiatives on the web, enriching them and providing new services that link together distributed data to make them available for those who collect or need information about taxa. As a result, systematics will find a new relevance in the field of biodiversity informatics.

9. A genome-sequence tree for Nematoda based on hundreds of genomes

Mark Blaxter and colleagues

Institute of Evolutionary Biology, School of Biological Sciences, The University of Edinburgh

E-mail: mark.blaxter@ed.ac.uk

The phylum Nematoda has only 24,000 described species, but meiofaunal surveys suggest that the true alpha-diversity may be in the range of 3-10 million taxa. In terms of individuals, it is likely that nematodes outnumber even arthropods. Nematodes occupy many niches, including those of aggressive animal and plant parasites that cause significant human health problems and agricultural losses. Understanding of the phylogeny of Nematoda thus ranks as an important issue, whether from a total biosphere or local interest viewpoint. Currently the major effort in nematode phylogenetics has been

focussed on the nuclear small subunit ribosomal RNA gene (nSSU rRNA). Over 4000 nSSU rRNA sequences for nematodes are available, and recent phylogenies have analysed up to 1200 different taxa. However the use of nSSU rRNA has perhaps reached its limit, with more taxa than characters (and many more taxa than informative characters), and the adverse effects of systematic bias (this is but one gene) compromising efforts to resolve the deeper branching patterns. Limited forays into multi-gene supermatrix analysis, particularly in nematodes related to *Caenorhabditis elegans*, have yielded better resolution. We are therefore leveraging the thousand nematode genomes initiative (<http://www.nematodegenomes.org/>) to build datasets of many genes covering many species. Using the phylogenomics workbench iPhy, our nematode transcriptome database NEMBASE and the genome sequences emerging from next generation sequencing we can address deep issues of nematode phylogeny.

10. Using phylogenomics to resolve the fungal tree of life

David Fitzpatrick

National University of Ireland, Maynooth
E-mail: david.fitzpatrick@nuim.ie

Traditional methods of systematics based on morphology of vegetative cells, sexual states, physiological responses to fermentation and growth tests have been used to assign fungal species to particular genera and families. Recent phylogenetic studies have shown that higher-level relationships amongst these groups are less certain and are best elucidated using molecular techniques. Today single-gene phylogenies have established many of the accepted relationships between fungal organisms. While there are undoubted benefits associated with using single genes, these analyses are dependent on the gene having an evolutionary history that reflects that of the entire organism, an assumption that is not always necessarily true. Currently there are over 100 fungal genomes currently sequenced. As a result of their relative small size and advances in sequencing techniques it is expected this number will increase dramatically in the coming years. Recently, novel phylogenomic methods that combine all available sequence data in a genome have been developed. This presentation will explore the wealth of fungal genomic data currently available and investigate the methods employed to perform genome scale fungal phylogenetic analyses.

11. Pathogenomics: investigating bacterial microevolution through second generation genome sequencing

Simon Harris

Wellcome Trust Sanger Institute
E-mail: sh16@sanger.ac.uk

The phenomenal output of second-generation sequencing machines allows whole-genome sequencing of multiple bacterial genomes in a single run. Using indexing protocols, deep coverage of 96 genomes per run is possible. This data can provide phylogenetic and epidemiological data at unprecedented levels of detail, allowing us to study bacterial populations at the genomic level. In this talk I will present recent data from collections of a number of bacterial pathogens at global and local levels, showing that the same data set has the potential to identify transmission events between countries and between patients, as well as giving information on evolutionary parameters and selection pressures acting on pathogen genomes

12. Plant systematics in the age of genomics revisited

Ernest K. Lee¹, Angélica Cibrián-Jaramillo^{1,2,3}, Sergios-Orestis Kolokotronis¹, Manpreet S. Katari³, Alexandros Stamatakis⁴, Michael Ott⁴, Joanna C. Chiu^{3,5}, Damon P. Little², W. Richard McCombie⁶, Robert A. Martienssen⁶, Gloria Coruzzi³, Rob DeSalle¹, and Dennis Wm. Stevenson².

¹Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, New York, USA. ²Cullman Program in Molecular Systematics, The New York Botanical Garden, Bronx, New York, USA.

³Center for Genomics and Systems Biology, Department of Biology, New York University, New York, New York, USA. ⁴Department of Computer Science, Technische Universität München, Munich, Germany. ⁵Department of Entomology, University of California, Davis, California, USA. ⁶Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.

Darwin famously described the sudden appearance and rapid diversification of flowering seed plants (angiosperms) from primitive gymnosperms as an “abominable mystery”. We are concerned with the processes that have lead to plant diversification, in particular with the genetic basis of plant diversity and adaptation. Here we explore the genomic origins of plant diversification using 22,833 sets of orthologs from the nuclear genomes of 150 plant species in 101 genera, analyzed in a combined analysis. We have found clear support for some of the nodes long problematic in seed plant phylogenetics such as the placement of Gnetales at the base of the gymnosperms. Furthermore, we used a novel phylogenomic approach to identify overrepresented functional gene categories at major nodes in our tree, revealing critical genes important to angiosperm diversification. Our analysis suggests that RNA interference (RNAi) played a significant role in the divergence of monocots from other angiosperms, a prediction that has experimental support in *Arabidopsis* and rice. In another example, the second largest subunit of RNA polymerase IV and V (NRPD2) played a prominent role in the divergence of gymnosperms, consistent with maternal control of small RNA in the seeds of flowering plants and the emergence of double fertilization in angiosperms.

13. Insights into flatworm evolution through comparative genomics

Magdalena Zarowiecki¹, James A. Cotton¹ and Peter D. Olson²

¹Wellcome Trust Sanger Institute. ²Department of Zoology, The Natural History Museum
E-mail: mz3@sanger.ac.uk

In many respects genomes represent the final frontier in organismal systematics and arguably the largest source of heritable characters for estimating evolutionary histories. Comparative genomics is still in its infancy, however, and thus the potential impact of whole genome data to the field of Systematics is hard to predict. Moreover, although next generation sequencing (NGS) will ameliorate current limitations in data availability, the utility of genomic data to comparative biology will remain strongly dependent on the degree to which such data have been well annotated. In flatworms (phylum Platyhelminthes), whole genome characterization was initiated long before the advent of NGS, driven by the importance of free-living forms as models of regeneration combined with the medical/veterinary importance of parasitic forms. Thus the genomes of a handful of free-living (i.e. *Schmidtea mediterranea* and *Macrostomum lignano*) and parasitic (*Schistosoma mansoni*, *Echinococcus multilocularis*, *E. granulosus* and *Hymenolepis microstoma*) species are currently available for analysis. We start by examining the phylogenetic utility of a highly conserved suite of core, single-copy eukaryotic genes (ie. CEGMA) used for training gene-finding models, using monophyly of the parasitic species, and of the tapeworms, as benchmarks. We then consider the evolution of gene families across the group, investigating to what extent the presence, absence and abundance of genes acts as a low-homoplasy marker for evolutionary relationships. Finally, we discuss how the availability of relatively complete, well-annotated genomes allows functional insights into non-model organisms, with some examples from our work on the development biology of tapeworms.

14. Ultimate barcoding - next generation sequencing meets high throughput mitogenomics

DTJ Littlewood, Andrea Waeschenbach and Peter G. Foster

Department of Zoology, The Natural History Museum
E-mail: p.foster2@gmail.com

The sequencing of mitochondrial (mt) genes as suitable markers for metazoan systematics and ecology, has a relatively long history in the relatively short time since molecular systematics came to the fore. A number of genes, such as *cox1*, *cytb*, *rrnL* and *rrnS*, have become very popular markers, since regions of high sequence conservation interspersed with regions of high variability have allowed for near 'universal' primers to be developed and applied to each and every animal phylum. Additionally, initiatives such as the Barcoding of Life, have added incentives to increase the sampling of partial *cox1* as an aid to species diagnostics. Meanwhile, researchers aiming

to increase the number of phylogenetically informative sites in their molecular data sets have pursued the characterisation of complete mitochondrial genomes (mtDNA), in addition to engaging in the pursuit of additional nuclear markers. The growing number of complete mtDNAs available for metazoans has had a striking effect on systematics: providing means to improve models of molecular evolution, the recognition of novel synapomorphies, the evaluation of within and between species variation across entire mt genomes, and perhaps most importantly resolving ambiguous phylogenetic nodes. Significantly, the growing database of mtDNAs allows for more tractable PCR-based methods of amplifying novel mtDNAs. Coupled with NGS technology the sequencing and characterisation of novel mtDNAs is now cheaper, but the challenge remains how to multiplex hundreds of taxa simultaneously without having to tag individual amplicons. We present a novel methodology for multiplexing and explore its prospects for ultimate barcoding, where entire mtDNAs are sequenced and characterized.

15. Next-generation biodiversity analysis

Mehrdad Hajjibabei

Biodiversity Institute of Ontario and Department of Integrative Biology,
University of Guelph
E-mail: mhajibab@uoguelph.ca

Biodiversity analysis is a key component of evolutionary investigations and ecological surveys. With the advancement of automated DNA sequencing devices, much work has been done in the past decade for understanding biodiversity by using sequence-based approaches such as in molecular phylogenetics and DNA barcoding. On-going assembly of DNA barcode reference libraries and a well-resolved Tree of Life provide the basis for a wide range of studies involving DNA-based molecular systematics. The use of recently introduced next-generation sequencing (NGS) approaches in biodiversity science has the potential to further extend the usability of DNA information in biodiversity science in an unprecedented scale. Our work has been focused on optimizing the use of NGS in biodiversity monitoring from bulk environmental samples in freshwater benthic macroinvertebrate communities--commonly used as bioindicator taxa--as well as in passively sampled terrestrial arthropods, soil, and water. Using experimental designs involving direct comparison of morphology-based, Sanger sequencing DNA barcoding, and NGS bulk sequencing of selected markers we investigated important issues such as PCR primer bias, identification accuracy versus sequence throughput and choice of marker. Although considerable effort will be required to further develop and optimize NGS molecular tools to robustly identify species, their abundance and phylogenetic diversity from bulk environmental samples, our work demonstrates the potential of NGS approaches in revolutionizing large-scale biodiversity studies.

16. Ecological differentiation of eukaryotic microbial lineages using 454 sequencing

David Bass

Department of Zoology, The Natural History Museum, London

E-mail: david.bass@zoo.ox.ac.uk

We used a range of more or less specific PCR primers to amplify microbial eukaryotic ribosomal DNA from large numbers of independent environmental samples from a diversity of habitats - including freshwater lakes and rivers, arable and set-aside soils, and estuarine gradients. We then used 454 sequencing to deeply sequence the amplicon set from each primer-sample combination. We will use these data to determine which abiotic and biotic factors are the strongest determinants of species turnover in the highly sampled freshwater lake systems, both across time and at different trophic levels. The results will reveal many other aspects of microbial eukaryotic biodiversity and ecology, for example genotype distribution along an estuarine gradient at high phylogenetic resolution, assessing differences between heavily cultivated and set-aside arable land, and the development of biofilms in polluted and non-polluted rivers.

17. Analysing metagenomic data to estimate diversity

Chris Quince

School of Engineering, Glasgow University, Glasgow, G128LT, UK

E-mail: quince@civil.gla.ac.uk

In many environmental genomics applications a homologous region of DNA from a diverse sample is first amplified by PCR and then sequenced. The next generation sequencing technology, 454 pyrosequencing, has allowed much larger read numbers from PCR amplicons than ever before. This has revolutionised the study of microbial diversity as it is now possible to sequence a substantial fraction of the 16S rRNA genes in a community. However, there is a growing realisation that because of the large read numbers and the lack of consensus sequences it is vital to distinguish noise from true sequence diversity in this data. Otherwise this leads to inflated estimates of the number of types or operational taxonomic units (OTUs) present. We have developed, AmpliconNoise, an algorithm that is capable of reducing pyrosequencing error rates, and Perseus a chimera identifier. Using artificial data sets of known diversity we demonstrate that together these algorithms can accurately determine OTU number. We then apply them to bacterial 16S rRNA pyrosequencing data sets from diverse environments: Arctic soils, deep-sea vents, oligotrophic lakes and sewage works. Presenting results on the proportion of the diversity attributable to noise in each case. We also calculate abundance distributions. Surprisingly although overall diversities are reduced the distributions still remained highly skewed to rare species at least in some of the environments. Finally we used Bayesian

statistics to extrapolate the abundance distribution of rare species to predict the true diversities which showed substantial variation across environments.

18. Next generation sequencing in population genetics of gall wasps

Konrad Lohse, Graham N Stone, Jack Hearn, Mark Blaxter, Nick Barton

Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh

E-mail: graham.stone@ed.ac.uk

Although evolutionary histories of genes and species have long been seen as branching trees, two recent developments have massively enhanced what branching genealogies can reveal. First, there has been a quantum leap in availability of sequence data through development of nextgen sequencing technologies. Second, advances in coalescent theory allow increasingly informative inference of population histories from a sample of genes. Here we discuss application of these advances to the rapidly growing field of phylogeography, which uses observed patterns of genetic diversity among populations to infer their history. In addition to questions of broader significance (such as human origins), phylogeography is central to biodiversity management (inferring origins and centres of genetic diversity, colonisation routes and rates of dispersal between regions and impacts of natural barriers). Accurate inference of population structure is of increasing importance as biological communities are disassembled or reconstructed through human activity. Furthermore, realistic null models that can account for population history are crucial when identifying loci under selection from genome wide scans. Most phylogeographic studies use data for small numbers of loci in a single species, an approach that is limited in two fundamental ways. First, because evolution generates a highly random distribution of genealogies, analyses of a few loci are inadequate to infer the history of the whole population. However, sampling many loci has so far been prohibitively difficult for non-model taxa. Second, focus on a single taxon, or on sets of co-occurring but ecologically unlinked taxa, has limited the use of phylogeography in addressing broader ecological questions. Nextgen sequencing removes both limitations, by allowing easy development of datasets of many thousands of loci for any species. The way is now open to generate highly-resolved population histories for ecologically-linked sets of species (e.g. predator/prey, plant/herbivore), and to use phylogeographic datasets to address one of ecology's greatest ongoing debates: how natural communities are assembled. By showing what can be achieved with small samples, we also highlight what can be done with existing archives of biological material, potentially adding significant value to past research.

19. Ongoing evolution in allopolyploid genomes examined using next-generation sequencing

Richard JA Buggs, S Renny-Byfield, IE Jordan-Thaden, L. Viccini, S.

Chamala, AR Leitch, PS Schnable, WB Barbazuk, PS Soltis, DS Soltis

School of Biological and Chemical Sciences, Queen Mary, University of London

E-mail: r.buggs@qmul.ac.uk

Hybridization and polyploidization are ubiquitous in the evolution of plants, but tracing the origins and subsequent evolution of the constituent genomes of allopolyploids has until now been challenging. Most allopolyploid species are 'non-model' organisms, and genome doubling greatly complicated genetic analyses. Recent and ongoing advances in DNA sequencing technology provide opportunity to analyze very many genetic markers in multiple individuals, and for the first time understand on a genomic scale the evolutionary processes that are ongoing in allopolyploid genomes. Here we review the application of these technologies to the study of duplicated gene loss and expression in *Tragopogon* allopolyploids, and transposon evolution in *Nicotiana* allopolyploids. These approaches can be easily and cheaply applied to many other plant species.

20. The role of NGS for integrative approaches of evo-devo and population genetics

Ralf Sommer

Max-Planck Institute for Developmental Biology, Tübingen

E-mail: ralf.sommer@tuebingen.mpg.de

During the last two decades, evolutionary developmental biology (evo-devo) has established itself as an important new branch for the study of evolutionary patterns and processes. Evo-devo can provide insight into the mechanistic changes that result in evolutionary novelty. Beside the many successful applications of evo-devo in animals and plants, there is a need for a better integration of evo-devo with population genetics and evolutionary ecology. Work at the interface between these disciplines can bring different research traditions closer together and requires an interdisciplinary toolkit. I argue that next generation sequencing will play a pivotal role in first, moving evolutionary biology into the next decades and second, in bridging the different research traditions in contemporary evolutionary biology.

21. Clustal Omega and multiple sequence alignments

Des Higgins

Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.

E-mail: des.higgins@ucd.ie

Multiple Sequence Alignments (MSA) are used to take a set of related DNA or protein sequences and line them up so as to make them easy to compare to

each other. Most MSAs are made using a range of related heuristics that involve clustering the sequences and building an alignment that follows the clusters. These methods have served us well for the past 20 years but are now starting to creak. I will describe a new program called Clustal Omega which can make alignments of any number of sequences. It gives good quality alignments in reasonable times and has extensive features for adding new sequences to or for exploiting information in existing alignments. It is available for download from www.clustal.org in a command-line driven format (Linux style) for proteins only. It is also available for on-line use from the EBI and from Galaxy.

22. Stratigraphic congruence through geological time and across higher taxa: what factors explain the variation?

Anne O'Connor

Department of Biology & Biochemistry, 4 South, The University of Bath,
Claverton Down, Bath BA2 7AY
E-mail: aoc23@bath.ac.uk

Many studies report congruence between the order in which taxa branch within cladograms and their stratigraphic sequence in the fossil record. While strong congruence is taken to support both the accuracy of cladograms and the fossil record, poor congruence many reflect inaccurate trees, a misleading fossil record, or both. It has been demonstrated that published measures of stratigraphic congruence are logically and empirically biased by parameters such as tree size and topology, the temporal extent of clades and the mean size of ghost ranges. Statistical modeling methods were used to assess the influence of these parameters on a commonly-cited measure of stratigraphic congruence, the Gap Excess Ratio (GER), for a dataset of 650 published cladograms. Approximately 73% of the observed variance in the GER was explained for by the chosen parameters, with the mean duration of ghost ranges consistently found to be the most influential. In agreement with previous studies, arthropods have poorer congruence than other groups, likely a product of their preservation potential. Furthermore, congruence varies through time, with lowest values in the Early Palaeozoic and Cenozoic and highest values in the Mesozoic, mirroring the temporal distribution of ghost ranges, a trend also reported in large-scale empirical studies. However, once sources of bias are factored out there are no clear residual trends either through geological time or across higher taxa.

23. Morphological disparity and clade shapes through time

Martin Hughes, Sylvain Gerber, Matthew Wills

Biology and Biochemistry, University of Bath, Claverton, Bath, BA2 7AY
E-mail: m.hughes@bath.ac.uk

Morphological diversity (or disparity) is conceptually and empirically distinct from taxonomic diversity. 'Diversity' provides an index of the number (and possibly relative abundance) of species or higher taxa in a sample. 'Disparity', by contrast, attempts to quantify the relative magnitude of differences in morphology between taxa. Parallel plots of diversity and disparity through time for major clades can reveal striking incongruities. Most of the case studies published in the last twenty years concern invertebrate groups radiating in the Palaeozoic (e.g., Crinoids, Blastozoans, Gastropods). These clades often have low diversity but high or maximal disparity early in their evolution. This implies that a small number of taxa rapidly colonises a large fraction of the 'morphospace' that will subsequently be exploited by the clade throughout its history.

It is not clear, however, whether this apparent tendency towards early high/maximal disparity pertains across all higher taxa and at all times through the Phanerozoic. In order to address this, we have calculated disparity curves for 90 vertebrate and invertebrate groups based upon published, discrete character matrices. We attempt to control for inconsistencies of taxonomic sampling inherent in such data, as well as variations in temporal resolution. Disparity profiles for individual clades can be summarised in terms of their centre of gravity (relative top or bottom heaviness). Preliminary results suggest that there are no significant trends in these statistics through the Phanerozoic. This finding also holds when Palaeozoic and post-Palaeozoic time bins are treated separately. At a finer temporal and taxonomic scale, however, the data suggest the influence of mass extinctions upon some disparity profiles. For example many of the dinosaur clades examined are *relatively* top heavy, coinciding with the end Cretaceous event.

24. Snowdrops and snowflakes - falling slowly into place

Nina Rønsted¹ & Aaron P. Davis²

¹Natural History Museum of Denmark, Sølvgade 83, DK-1307 Copenhagen, Denmark²Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK.
E-mail: nronsted@snm.ku.dk

Snowdrops (*Galanthus*, 19 spp.) and snowflakes (*Leucojum*, 2 spp. and *Acis*, 10 spp.) are cherished garden plants and welcome harbingers of spring. *Galanthus* is the most traded wild bulb species in the world. Despite their popularity and economic importance many species in these genera are not at all easy to tell apart, and both the tribal and infrageneric classifications are uncertain.

We present a molecular phylogenetic study of *Galanthus* and related genera (Eurasian tribes of Amaryllidaceae subfamily Amaryllidoideae) to clarify circumscription of tribe Galantheae, and as a basis for a new infrageneric classification of *Galanthus*. Sequences of nuclear encoded nrITS, and plastid encoded *matK*, *trnLF* and *ndhF* were analyzed using bayesian inference and maximum parsimony. Phylogenetic analyses of all sequenced DNA regions support circumscription of tribe Galantheae s.s. including only the genera *Galanthus*, *Leucojum* and *Acis*. Phylogenetic analyses of

Galanthus, based on nuclear ITS sequences, provide a well resolved topology allowing the identification of seven major clades, some of which do not correspond to recent classifications based on morphology. The plastid regions provide far less resolution, and show significant incongruence with the nuclear tree topology.

Both new and ancient hybridization events may be inferred and several *Galanthus* species are in need of recircumscription. New morphological and genomic synapomorphies are identified and biogeographical patterns are highlighted. On the basis of phylogenetic relationships a new classification for *Galanthus* is emerging: the snowdrops are falling slowly into place.

25. Phylogenetic studies of the aloes (*Aloe* L., Xanthorrhoeaceae)

Grace, O. M.^{1, 2*}, Dzajic, A.³. & Rønsted, N.¹

¹Natural History Museum, University of Copenhagen, Sølvgade 83, Copenhagen 1307, Denmark. ²Jodrell Laboratory, Royal Botanic Gardens, Kew, TW9 3AB, Surrey, United Kingdom. ³Faculty of Pharmaceutical Sciences, Universitetsparken 2, Copenhagen 2100, Denmark
E-mail: o.grace@kew.org

Aloes are an iconic feature of the landscape throughout their range across Africa, the Arabian Peninsula, Madagascar and eastern Indian Ocean islands, and are known the world over as a source of natural products and a feature of succulent plant collections. *Aloe* (Xanthorrhoeaceae, subfamily Asphodeloideae) comprises over 620 leaf-succulent species and is steadily expanding as new taxa continue to be described, although many of these remain unplaced in the current infrageneric classification. Many species of *Aloe* are threatened, but taxonomic confusion adds to the complexities of effective conservation and sustainable use. The molecular systematics of the genus is an emergent area of study in this otherwise well-described group. *Aloe* has been consistently recovered as a paraphyletic group with neighbouring genera *Gasteria* and *Haworthia* in previous studies using DNA sequence data. Within the genus, relationships defined by molecular characters have conflicted with existing classifications, and branch tips have been poorly resolved. In the current study, we have considerably expanded the sampling to approximately a third of *Aloe* spp., representing the full morphological and geographical diversity of the genus, and both the nuclear and plastid genomes. Plastid (matK) and nuclear (ITS) sequence data accumulated to date have yielded phylogenetic trees of comparable topology but limited bootstrap support. Bayesian posterior probabilities for branches in trees of the combined data partitions, however, identify several well-supported clades within a monophyletic alooid clade. These preliminary analyses suggest that geographical structure and overlap with at least some existing morphogroups may be expected as our phylogenetic study of *Aloe* expands. New insights into the molecular systematics of aloes will have important practical implications in their conservation, beyond informing the alpha taxonomy of the genus.

26. Biogeography and diversification in Africa: a case study from a species rich catfish clade

Julia J. Day

University College London, Department of Genetics, Evolution & Environment,
Gower Street
E-mail: j.day@ucl.ac.uk

Complete species-level phylogenies are rare due to the difficulty of acquiring all members of a group. However, such studies based on molecular data are highly fruitful since they allow investigation into the general causes of diversification and how diversification rates vary within a clade. Aside from cichlid fishes, densely sampled phylogenies for African fish groups are lacking. As such, I have selected the considerably species rich African catfish genus *Syndontis* (Siluriforms: Mochokidae), since the group contains over 120 species and has a Sub-Saharan freshwater distribution, including the Nile. Their broad distribution enables examination of biogeographic scenarios, and whether diversification rates vary between regions. A multi gene species level phylogeny, based on mitochondrial and nuclear genes (3584 bp) and including ~80% of overall diversity across all river basins is presented. Multiple methods of phylogenetic inference, including Bayesian molecular dating are applied to these data. The resulting concatenated phylogeny recovers two main lineages, the ancestors of which are reconstructed as West and Central African respectively, having diverged ~17 Mya. These lineages subsequently diverged soon afterwards, with the former lineage revealing a disjunct East/West distribution. Conversely, Nilotic and Southern African taxa are polyphyletic, however, molecular dating reveals that these taxa are recent colonisers to these areas, having diverged ~2.5 Mya. The biogeographic history and diversification rates of *Syndontis* will be compared to other aquatic and terrestrial African groups.

27. Evolutionary ecology of Lake Tanganyika's Mastacembelid eels

Katherine J. Brown, Lukas Rüber and Julia J. Day

Dept of Genetics, Evolution and Environment, University College London,
Gower Street, London, WC1E 6BT
E-mail: katherine.brown@ucl.ac.uk

Lake Tanganyika (LT) is the oldest of the African Rift Lakes and is one of the most diverse freshwater ecosystems on Earth. The endemic species flocks that occur in LT, such as cichlids, crabs, catfish and spiny eels, provide unique comparative systems for the study of patterns and processes of speciation. Spiny eels (Teleostei: Mastacembelidae) form a species flock in LT of 14 currently described endemic species. A dated molecular phylogeny has demonstrated that the LT spiny eels are the result of a single colonisation event ~7-8 Ma, indicating within lake diversification, and subsequent rapid

radiation in to five main lineages. This seemingly rapid diversification is one of the features indicative of an adaptive radiation (*sensu* Schluter). To test adaptive radiation hypotheses, rapidly diversifying species flocks should also demonstrate phenotypic, physiological and/or ecological segregation, which is of particular significance when species occur in sympatry. In order to investigate ecological factors of this purported adaptive radiation, we have analysed dietary niche partitioning of up to 11 sympatric species, using stable isotope signatures of carbon ($\delta^{13}\text{C}/\delta^{12}\text{C}$) and nitrogen ($\delta^{15}\text{N}/\delta^{14}\text{N}$). We have found evidence for dietary niche partitioning, and therefore an ecological role in the diversification of this species flock.

28. Distinguishing signal, noise and bias in early animal evolution: The difficult problem of the sponge phylogeny

Roberto Feuda, Erik A. Sperling, Kevin J. Peterson and Davide Pisani

Biology Department National University of Ireland Maynooth
E-mail: robertofeuda@gmail.com

Early animal relationships are still hotly debated, and three main hypotheses have been proposed in the last few years. The first suggests that the sponges represent the monophyletic sister group of all the other Metazoa. The second suggests that sponges plus the Coelenterata and perhaps the Placozoa represent the sister group of all the other Metazoa (an hypothesis named Diploblastica), and the third suggests that sponges are paraphyletic, with the Demospongiae representing the sister group of all the other Metazoa, and a Homoscleromorpha representing the sister group of the Eumetazoa. Recently evidence was provided suggesting that Diploblastica could be dismissed as the result of paralogy, alignment errors and tree reconstruction artifacts. However, it is still unclear whether sponges represent the monophyletic or the paraphyletic sister group of Eumetazoa. Here, we shall show results of reanalyses of two recently published data sets (Pick et al. 2010 and Philippe et al. 2009) and illustrate how the support for alternative sponge phylogeny changes when compositional heterogeneity, and other potential sources of phylogenetic bias are taken into consideration. Our results confirm that Diploblastica is a phylogenetic artifact. In addition to that they also provide evidence suggesting that sponge monophyly might also represent a tree reconstruction artifact as previously postulated by Sperling et al. (2009). In any case, our results indicate that current evidence to resolve the phylogeny of the sponges is scant and that the problem of posed by the sponge phylogeny cannot be considered resolved yet.

29. Reconciling morphological and molecular classifications: an investigation of Hemiasterellidae (Demospongiae, Porifera)

Christine C. Morrow^{a*}, Bernard E. Picton^b, Frederic Mineur^a, Christine A. Maggs^a and A. Louise Allcock^c.

^a School of Biological Sciences, MBC, 97 Lisburn Road, Queen's University, Belfast, BT9 7BL. ^b National Museums Northern Ireland, 153 Bangor Road, Holywood, BT18 0EU, Northern Ireland, UK. ^c Dept. of Zoology, Martin Ryan Marine Science Institute, Galway, University Road, Galway, Ireland.
E-mail: christinemorrow@gmail.com

An analysis of the phylogenetic relationships of the demosponge family Hemiasterellidae based on partial 28S rRNA and mitochondrial CO1 sequences was carried out. Monophyly of Hemiasterellidae as currently defined was not supported. Rather than finding a single clade comprising all hemiasterellids, we obtained a well-supported clade including members of some hemiasterellid genera (*Paratimea* spp. and *Stelligera* spp.) and *Halicnemis* spp. (Heteroxyidae). This clade was distant from *Adreus fascicularis* and *Axos cliftoni* (Hemiasterellidae). Based on morphology it is likely that *Adreus* and *Axos* will group with *Hemiasterella*, however the type species *H. typus* was not available for DNA extraction and therefore we cannot identify the 'true' hemiasterellid clade.

An undescribed species of *Paratimea* was discovered with an unusual type of spicule that has also been found in *Halicnemis verticillata*. This close relationship between *Paratimea* spp. and *Halicnemis* spp. was anticipated by Topsent in 1893 when he classified *Paratimea constellata* as a species of *Halicnemis*. It is suggested that the asters in *Paratimea* and *Stelligera* may be homologous with the acanthoxea in *Halicnemis*.

The approach of using molecular derived phylogenies as a basis for re-interpreting morphology is shown to be useful in groups such as sponges where there is a paucity of morphological characters and the origin and direction of their evolution is uncertain.

30. Evolutionary history of a 10,000-species clade of angiosperms: reconstructing the phylogeny of magnoliids as a whole

Julien Massoni, Hervé Sauquet

Université Paris-Sud 11, Lab. Écologie, Systématique, Évolution (ESE),
CNRS UMR 8079, bât. 360, 91405 Orsay, France
E-mail: julien.massoni@u-psud.fr

Magnoliids are an early-diverging clade of angiosperms consisting of four orders (Piperales, Magnoliales, Laurales, and Canellales) and about 10,000 species. Current understanding of deep relationships within magnoliids relies mostly on higher-level analyses of angiosperms with limited sampling of magnoliids. In an effort to address evolutionary questions in this clade, we assembled a large molecular data set of three plastid markers (*rbcL*, *matK*, and *ndhF*) and 205 species representing >75% of magnoliid genera. Phylogenetic analyses of this combined data set yielded well resolved trees consistent with previous higher-level and family-level studies. In particular, magnoliids appear to consist of two large clades: [Piperales+Canellales] and [Laurales+Magnoliales]. On the other hand, our analyses resolved a number of long-standing polytomies (e.g., the position of Magnoliaceae within

Magnoliales). These results highlight the importance of a large taxonomic and molecular sample to resolve both deep and shallow phylogenetic relationships within magnoliids. This study is the first step toward a robust and accurate phylogenetic framework needed to reconstruct patterns of floral evolution and diversification in this clade.

31. Comparative transcriptomics and genomics: determining the regulators of petal spot development and the evolution of this specialised floral trait within the species complex *Gorteria diffusa*

Walker, Rachel H.¹, Brockington, S. F.¹, Rudall, P. J.² and Glover, B. J.¹

¹Department of Plant Sciences, University of Cambridge, Downing Street, CB2 3EA. ²Jodrell Laboratory, Royal Botanic Gardens, Kew, TW9 3AB
E-mail: rhw37@cam.ac.uk

Petal spots have evolved across a phylogenetically broad range of angiosperms and – in at least some cases – function in the attraction of pollinators. Spots develop through the accumulation of a contrasting pigment in a group of cells of the petal epidermis; in a few species the spots also exhibit elaborated epidermal cell morphologies. To study the evolution and development of petal spots, we have chosen a species that displays both elaborated epidermal cells and variation in overall petal spot morphology – the South African daisy *Gorteria diffusa* Thunb. (Asteraceae). The morphology of the *Gorteria* petal spot is complex, being composed of three distinct cell types and a concentrated deposition of anthocyanin arranged across a structure (ray floret) that consists of several congenitally fused petals. *Gorteria diffusa* exists as several sympatric but geographically identifiable groups of populations that occur within a narrow range in the Northern Cape of South Africa. We term these populations “morphotypes”. The morphotypes have distinct phenotypes in terms of spot morphology, including pigment content, cell shape and number and position of spots on the capitulum. These morphotypes therefore provide natural variation with which to compare the expression patterns and function of key regulators of petal spot morphology. Our approach utilises next-generation sequencing technologies in two ways: (1) We have used 454-sequencing to generate comparative transcriptomic datasets to identify putative regulators of petal spot development. This has identified several differentially expressed candidate genes, including MYB and bHLH transcription factors that are known to regulate epidermal cellular processes. Further characterisation of these regulators will allow us to formulate a model for the pathway of petal-spot development, ultimately defining the molecular events that have led to the evolution of this floral feature. (2) We are using RAD-sequencing to generate population-level markers to fully understand the complex population structure of these morphotypes. This will inform our understanding of genetic differentiation within morphotypes and allow us to explore floral and petal spot variation in association with gene flow between morphotypes.

Keywords: *Gorteria diffusa*, petal-spot development, comparative transcriptomics, 454 sequencing, RAD-sequencing.

32. Testing the impact of calibration on molecular divergence times using a fossil-rich group: The case of *Nothofagus* (Fagales)

Hervé Sauquet, Simon Y. W. Ho, Maria A. Gandolfo, Gregory J. Jordan, Peter Wilf, David J. Cantrill, Michael J. Bayly, Lindell Bromham, Gillian K. Brown, Raymond J. Carpenter, Daphne M. Lee, Daniel J. Murphy, J. M. Kale Sniderman and Frank Udovicic

Université Paris-Sud 11, Lab. Écologie, Systématique, Évolution (ESE),
CNRS UMR 8079, bât. 360, 91405 Orsay, France
E-mail: herve.sauquet@u-psud.fr

Temporal calibration is widely recognized as critical for obtaining accurate divergence-time estimates using molecular dating methods, but few studies have evaluated the variation resulting from different calibration strategies. Depending on the information available, researchers have used primary calibrations from the fossil and geological records or secondary calibrations from previous molecular dating studies. Fossil data can vary substantially in accuracy and precision, presenting a difficult choice when selecting appropriate calibrations. Here, we test the impact of eight plausible calibration scenarios for *Nothofagus* (Nothofagaceae, Fagales), a plant genus with a particularly rich and well-studied fossil record. To do so, we reviewed the phylogenetic placement and geochronology of 38 fossil taxa of *Nothofagus* and other Fagales, and identified minimum age constraints for up to 18 nodes of the phylogeny of Fagales. Molecular dating analyses were conducted for each scenario using maximum likelihood (RAxML + r8s) and Bayesian (BEAST) approaches on sequence data from six regions of the chloroplast and nuclear genomes. Using either ingroup or outgroup constraints, or both, led to similar age estimates, except near strongly influential calibration nodes. Using 'early but risky' fossil constraints in addition to 'safe but late' constraints led to older age estimates. In contrast, using secondary calibration points yielded drastically younger age estimates. This empirical study highlights the critical influence of calibration on molecular dating analyses. Even in a best-case situation, with many thoroughly vetted fossils available, uncertainties remain and appear larger than have previously been acknowledged. For example, our estimates for the crown-group age of *Nothofagus* varied from 13 to 113 Ma across our full range of calibration scenarios. We suggest that increased background research should be made at all stages of the calibration process to reduce errors wherever possible, from obtaining the latest geochronological data on the fossils to critical re-assessment of their phylogenetic position.

33. How to resolve conflict between taxonomy and phylogeny in basal living chitons (Mollusca: Polyplacophora)

Julia Sigwart

Queen's University Belfast, School of Biological Sciences, Marine Laboratory
E-mail: j.sigwart@qub.ac.uk

Modern polyplacophoran molluscs (chitons) are unambiguously divided into two clades. The earliest-derived living chitons form the order Lepidopleurida, which contains ten nominal genera. However, the vast majority of described species (approximately 120 of 150 valid taxa) are in the genus *Leptochiton*. These species are predominantly found in deep sea habitats but also include shallow-water members, and are globally distributed with species known in all oceans. Recent work to assess the phylogeny of Lepidopleurida with combined molecular and morphological analyses shows that *Leptochiton sensu stricto* is restricted to the North Atlantic and Mediterranean. The rest of the genus is divided into three clades. New analyses aim to define morphological descriptors to support the subdivisions of *Leptochiton* either *a posteriori*, and/or via additional morphocladistic analyses. A new expanded dataset of 91 discrete, 24 continuous, and 4 independent sets of landmark morphometric data were analysed for 44 ingroup taxa using TNT. Morphological synapomorphies for the clades of interest remain elusive. These analyses revealed interesting information about character evolution in the group and are also an interesting test case for the utility of new methods to incorporate landmark morphometric data in cladistic studies.

34. What have we learned from 25 years of animal molecular systematics?

Max Telford

Department of Genetics, Evolution and Environment, Darwin Building, Gower St, London, WC1E 6BT
E-mail: m.telford@ucl.ac.uk

It is close to 25 years since a paper by Field et al. appeared in Science describing the use of small subunit ribosomal RNA sequences to address the phylogenetic relationships of the animals. I will discuss the progress in reconstructing animal phylogenies since this founding study, the sources of error in phylogenetic trees and the consequences of the new molecule-based view of animal relationships. I will focus in particular on the evolutionary importance of instances of secondary absence of characters - both molecular and morphological.

35. Neural Characters and Arthropod Phylogeny

Angelika Stollewerk

School of Biological and Chemical Sciences, Queen Mary, University of London

E-mail: a.stollewerk@qmul.ac.uk

One of the controversial debates on euarthropod relationships centres on the question if insects, crustaceans and myriapods (Mandibulata) share a common ancestor or if myriapods group with the chelicerates (Myriochelata). The debate was stimulated recently by studies in chelicerates and myriapods which show that groups of neural precursors segregate from the neuroectoderm generating the nervous system, while in insects and crustaceans the nervous tissue is produced by stem cells. Do the shared neural characters of myriapods and chelicerates represent derived characters that support the Myriochelata grouping? Or do they rather reflect the ancestral pattern? Analysis of neurogenesis in a group closely related to euarthropods, the onychophorans, shows that similar to insects and crustaceans, single neural precursors are formed in the neuroectoderm, potentially supporting the Myriochelata hypothesis. However, we have shown recently that the nature and the selection of onychophoran neural precursors are distinct from euarthropods. In my talk I will dissect the morphological and molecular processes of neurogenesis in all arthropod groups. I will critically evaluate the neural characters that have been used to support the various theories on arthropod relationships and suggest an evolutionary sequence of arthropod neurogenesis which is in line with the Mandibulata hypothesis.

36. Minuscule tardigrades, microRNAs and phylogenomics reveal velvet worms as the Arthropod sister group

Lahcen Campbell, Omar Rota-Stabelli ,Trevor Marchioro, Stuart Longhorn, Gregory Edgecombe, Max Telford, Herve Philippe, Lorena Rebecchi, Kevin Peterson, Davide Pisani.

Department of Biology, National University of Ireland Maynooth
E-mail: lahcencampbell@gmail.com

Morphological data traditionally recovers Onychophora (Velvet worms), Tardigrada (Water bears) and Arthropoda (e.g. crabs, wasps, centipedes) within the monophyletic group Panarthropoda on the basis of morphology (i.e. presence of walking appendages). However, studies of molecular sequence data provide support for alternative placements of Tardigrada within the Ecdysozoa; most often grouping tardigrades closer to the cycloneurialian clade Nematoda (round worms). This result is suspicious however, as the branches associated with both the Tardigrades and the Nematoda are very long, suggesting the nematode-tardigrade grouping might represent a long-branch attraction (LBA) artifact. In order to test the hypotheses of tardigrades relationships, we have analysed two independent genomic data sets: (1) a large EST data set and, (2) microRNAs for all relevant taxa, including newly sequenced microRNAs for Tardigrada and Onychophora. Using careful experimental manipulations – such as: comparisons of model fit, signal dissection, and taxonomic sampling – we were able to show that support for a Nematoda plus Tardigrada group derives from the phylogenetic artifact of long

branch attraction (LBA). Our small RNA libraries fully support our EST analysis, as no microRNAs were found to link Tardigrada with Nematoda, while a single microRNA (miR-276) was found to be shared by all panarthropod groups. Each data set yielded congruent results, thus providing compelling evidence in favor of monophyletic Panarthropoda. To corroborate our findings, similar methodologies applied to our EST's were applied to a ribosomal RNA (rRNA) data set, which also produced congruent results. Thus, our findings suggest that Onychophora is indeed the sister group to Arthropoda, with Tardigrada being the sister group of Arthropoda and Onychophora. Our findings should therefore provide a solid foundation to fully understand the process of "arthropodization".

37. On the phylogenetic position of myriapods: insights from Onychophora (velvet worms)

Georg Mayer

Animal Evolution & Development, Institute of Biology, University of Leipzig
E-mail: gmayer@onychophora.com

The phylogeny of the four major arthropod groups (Chelicerata, Myriapoda, Crustacea and Hexapoda) is currently under debate. In particular, the position of myriapods is contradictory as they are regarded as either the closest relatives of crustaceans and hexapods (Mandibulata hypothesis) or of chelicerates (Paradoxopoda/Myriochelata hypothesis). Both alternatives have received support from recent analyses of morphological and molecular data sets; hence, an unambiguous decision between them is difficult. In this talk, I discuss pros and cons of the two competing hypotheses and provide an embryologist's point of view on the position of the Myriapoda within the Arthropoda.

38. Molecular arthropod phylogeny: Early relationships and divergence times

Thorsten Burmester

University of Hamburg, Institute of Zoology and Zoological Museum
E-mail: thorsten.burmester@uni-hamburg.de

Arthropods are the most diverse animal phylum, but still uncertainties exist on both their exact position within the Protostomia and the relationships among arthropod taxa. We employed next generation pyrosequencing to obtain large numbers of expressed sequence tags (ESTs) from the priapulid *Halicryptus spinulosus*, the scorpion *Pandiunus imperator*, the harvestman *Phalangium opilio*, the sunspider *Gluvia dorsalis*, the pseudoscorpion *Chelifer cancroides*, the whip scorpion *Mastigoproctus giganteus*, the whip spider *Euphrynichus bacillifer*, the centipede *Scolopendra subspinipes* and the diplopod *Polyxenus lagus*. Phylogenetic analyses employed >100 selected orthologous genes and

included more than 100 arthropod species. First results show that Tardigrades are not closely related to the arthropods, but form a common clade with the Nematoda. Arachnids were found paraphyletic because the Acari (tick and mites) occupy a basal position within the Euchelicerates. The position of the Myriapoda still remains unresolved. A molecular clock approach suggests that arthropods emerged ~620 million years ago (MYA). Onychophorans and euarthropods split ~600 MYA, Pancrustacea and Myriochelata ~570 MYA, Myriapoda and Chelicerata ~560 MYA, and 'Crustacea' and Hexapoda ~515 MYA. Endopterygote insects appeared ~390 MYA. In addition, we specifically applied hemocyanin sequences to solve the relationships among arthropods. Hemocyanins are respiratory proteins that assemble into oligomers with clear orthology. We show that sea spiders (pycnogonids) are sister group to euchelicerates, a sister group position of whip spiders and whip scorpions (confirming the taxon Pedipalpi), as well as a sister group relationship of Symphyla and Diplopoda. Most importantly, hemocyanins suggest that the hexapods, the most diverse and successful animal taxon, originated from remipede-like crustaceans.

39. Ten Years Later: Phylogenetic utility and limitations of mitochondrial gene order

Dennis Lavrov

EEOB, Iowa State University
E-mail: dlavrov@iastate.edu

Mitochondria – the energy producing organelles present in most eukaryotic cells – contain their own genome (mt-genome or mtDNA), separate from that of the nucleus. Approximately 10 years ago there were about 100 completely sequenced animal mt-genomes and high expectations for the phylogenetic utility of mitochondrial gene orders. At present (May 2011) there are 2315 complete animal mt-genomes in the NCBI Genomes database but relatively few recent studies utilize mitochondrial gene order as a phylogenetic character. Here we explore the reasons for this apparent paradox and discuss three possible factors that can hamper the use of mitochondrial gene order data for phylogenetic inference: 1) the lack of bioinformatics support; 2) the inadequacy of models for gene order evolution; 3) the limited amount of phylogenetic signal contained in the gene order data. We developed a suite of computer programs to handle and analyze the gene order data. We use these tools to analyze the gene orders in the 377 genomes currently available in the Panarthropoda group and explore the information present in the gene order data for the panarthropod phylogeny.

40. On the position of myriapods: insights from Onychophora/velvet worms

Jerome C. Regier, Andreas Zwick, Derrick Zwickl

Institute for Bioscience and Biotechnology Research, University of Maryland,
College Park, MD 20742, USA
E-mail: regier@umd.edu

A higher-level phylogeny of arthropods based on analysis of a data set consisting of sequences from 62 distinct protein-coding nuclear genes from 75 diverse arthropods and 5 (and separately, 10) outgroup species has recently been published (doi:10.1038/nature08742). Using several phylogenetic approaches, most of the relationships within Mandibulata, but not Chelicerata, were strongly supported, including six newly named groups within Pancrustacea, and Symphyla + Pauropoda (both Myriapoda). The sister-group of Hexapoda was identified as Remipedia + Cephalocarida, or "Xenocarida". Using that same data set, we have addressed several questions about the basis of the conclusions. 1) Do single genes support the higher-level groupings? 2) How many data overall are required to achieve strong support? 3) How does the rate of gene evolution affect node support? 4) What are the relative contributions of synonymous and nonsynonymous change? 5) Does nucleotide compositional heterogeneity affect the outcome? 6) Does data partitioning and separate modeling improve the result? 7) Why do a few high-interest nodes have substantially higher bootstrap values with nucleotides than amino acids, even though both sorts of characters yield very similar ML topologies? Addressing these questions will form the basis of our oral presentation.

41. Phylogenomic and evolutionary aspects of Pancrustacea and Remipedia –the hope and headache of “phylogenomics”

Björn M von Reumont

Adenauerallee
E-mail: bmvr@arcor.de

The reconstruction of the evolutionary history of crustaceans is still difficult addressing internal relationships, but also revealing the likely crustacean sistergroup to Hexapoda. Most molecular studies corroborate Pancrustacea with paraphyletic crustaceans supporting Copepoda, Branchiopoda or recently Remipedia + Cephalocarida as sistergroup to Hexapoda. Neuroanatomical and hemocyanin data support a clade Remipedia + Hexapoda. A major challenge is the conflict between morphological and molecular data, which is not trivial to resolve. A likely scenario of euarthropod evolution persists a dispute, in which the Remipedes form a key taxon as the possible link to Hexapoda conquering land habitats. In this phylogenomic approach first 454 data of Remipedia were included. Genes were selected from the unreduced dataset applying the matrix reduction software MARE. Additionally two different ortholog gene sets based on the HaMStR approach were compared. The largest datamatrix comprises 866,417 aa positions and 1,866 genes. All reconstructed topologies highly support Remipedia as sistergroup to Hexapoda. The results further support recent studies concluding that critical data evaluation is crucial and new

methods are essential for prospective phylogenetic analyses. Until now, the handling of long branch taxa is still problematic. This and further issues, e.g. the gene selection and the identification of informative genes are essential to improve our understanding of molecular crustacean evolution and phylogenomic data. Anyhow, to enlighten pancrustacean evolution, it is still crucial to collect more crustacean key taxa (e.g. Cephalocarida). The presented analysis includes first 454 data of leptostracan species, until now, an overhead of decapod EST sequences is published, while data for non-decapods are very sparse.

42. The Devil in the detail: modern morphological methods allow the confident placement of fossil arachnids as molecular calibration point

Jason Dunlop, Russell Garwood and Dave Penney

Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity at the Humboldt University Berlin, 10115 Berlin, Germany.
E-mail: jason.dunlop@mfn-berlin.de

The paleontological record offers robust evidence for minimum ages of cladogenesis by documenting the oldest example(s) of any given group. Yet a problem has always been the reliability of these fossil identifications; as well as a philosophical approach about whether extinct animals should invariably be assigned to new higher taxa, or simply lumped together with their closest-looking living relatives. To increase the accuracy with which fossil data can be used to calibrate divergence ages in a given (molecular) phylogeny, we need (a) comprehensive catalogues of what fossil taxa are actually out there, and (b) an objective assessment about the reliability of published referrals – particularly where modern families or genera are involved. Under ideal circumstances we can achieve a robust degree of ‘taxonomic equivalence’, in which fossils are placed using many of the apomorphies used in the taxonomy of living species. Drawing primarily on recently published data relating to arachnids, examples are shown of how computed tomography in particular can, and will, be invaluable for dating the origins of major arthropod taxa. Tomographic reconstructions of three-dimensional fossils (particularly specimens in nodules or inclusions in amber) can and do yield character sets which would be almost impossible to recover using traditional (optical) methods of study. It is argued here that these images and movies are not merely aesthetic, but represent a key means of accurately dating lineages, and of calibrating evolutionary trees.

43. Morphological cladistics and arthropod phylogeny

Gregory D. Edgecombe

Department of Palaeontology, The Natural History Museum, Cromwell Road,
London SW7 5BD, UK
E-mail: g.edgecombe@nhm.ac.uk

The growth of molecular datasets via denser taxon sampling and broader gene sampling has not obviated the need for accurate morphological character formulation. Morphology remains the basis for including fossils in arthropod phylogeny, offers character data of unrivalled complexity, and comprises the set of observations that most investigators are attempting to explain when undertaking a phylogenetic analysis. Most morphological contributions to arthropod phylogeny are comparative studies of single character systems, now often informed by new imaging technology and gene expression, or compilations of characters for select clades; few attempts are made to generate a morphology-based cladogram for Arthropoda as a whole. An analysis that samples terminals used in multi-locus sequence analyses for their morphological features illustrates the challenges of arthropod phylogeny based on morphology, including the choice between coding groundpatterns versus exemplars, the usage of supraspecific terminals versus single species, and the historical legacy of coding characters only in select (pre-conceived) clades, e.g., coding “hexapod characters” as unknown or inapplicable in crustaceans. The notion that morphology and molecules are fundamentally in conflict, or that arthropod phylogeny is “anything goes” chaos, is a holdover from the 1990s. Parsimony analysis of a 395-character dataset for extant taxa retrieves major clades that are congruent with current molecular topologies, including Tetraconata rather than Articulata, and Mandibulata rather than Paradoxopoda. Fossils do not substantially alter the interrelationships of extant arthropod groups, but they inform on the sequence of character acquisition (notably between long branches such as Onychophora versus Euarthropoda), and no compelling theoretical or empirical reasons justify their exclusion from phylogenetic analyses.

44. Are morphology-based cladograms of arthropods really less robust than those of vertebrates?

Matthew A Wills, Andrea Cobbett, Clive Moncrieff, Anne O’Connor, Ross Mounce, Sylvain Gerber

Department of Biology & Biochemistry, University of Bath, BA27AY
E-mail: m.a.wills@bath.ac.uk

Received wisdom holds that many groups of arthropods are particularly morphologically homoplastic and difficult to resolve phylogenetically. Here we test this assertion by comparing 90 arthropod cladograms with 300 cladograms of vertebrate taxa. Conventional indices of homoplasy, including the homoplasy index ($HI = 1 - CI$), are influenced by dataset parameters (i.e., the number of taxa and possibly the number of characters). The Homoplasy Excess Ratio is much more suitable for comparing levels of homoplasy between datasets, but requires an intensive permutation procedure and is therefore very rarely reported. Regression models including dataset

dimensions, taxonomic level and taxonomic group as predictors reveal that homoplasy in arthropod cladograms is significantly greater than in vertebrate trees. Moreover, measures of tree support including the total support index (ti) are significantly lower for arthropod than vertebrate cladograms, reflecting inferior Bremer support at internal nodes. Initial results suggest that small perturbations in the taxon sample have a greater impact upon the relationships in arthropod trees than those of their vertebrate counterparts. This may partially reflect differences in the adequacy of the available (living and fossil) taxon samples in the two groups. Indeed, when arthropod cladograms are plotted onto stratigraphic range charts of first fossil occurrences, the ghost ranges inferred are often extensive, and stratigraphic congruence (GER*, MSM etc.) is frequently poor. This contrasts with the excellent congruence in many vertebrate groups. The difference may partially reflect the particular concentration of important arthropod fossils in a limited number of exceptional sites. Finally, we present preliminary results comparing arthropod trees inferred variously from more and less fossilisable characters. Reassuringly, these data partitions often do not yield significantly different trees, which speaks for the utility of even incomplete fossils for increasing the taxon sample.

45. Vistas in arthropod evolution: throughout phylogeny, and beyond

Alessandro Minelli

Department of Biology, University of Padova, via Ugo Bassi 58 B, I 35131
Padova, Italy
E-mail: alessandro.minelli@unipd.it

A predictable consequence of recent and sometimes revolutionary advances in arthropod phylogeny is an in-depth but still very incomplete revisitation of many important aspects of comparative morphology and developmental biology of these metazoans. In turn, a better appreciation of trait evolvability, along with revised definitions of key concepts (e.g., segment), can contribute to improve compilation and analysis of data matrices. Examples of critically important phylogenetic hypotheses (and of the evolutionary problems for which these are relevant) include the ecdysozoan affinities of the Arthropoda (the nature of the segment and the existence of the acron), membership of stem group Arthropoda (head evolution), the plausibility of the Mandibulata vs. Myriochelata hypothesis (internalization of appendages), the monophyly of Myriapoda, Hexapoda, and Palaeoptera (water to land transitions), the internal interrelationships among the major clades of Diplopoda and Chilopoda (plausibility of Williston's law), the internal phylogeny of the Scolopendromorpha (eye evolution), the phylogenetic position of the Strepsiptera (morphological vs. developmental complexity), and the internal phylogeny of the Epimorpha (segment number evolution).

46. Extracting DNA from museum collections of dried eggshells

Patricia L.M. Lee & Robert R. Prys-Jones

Department of Biosciences, College of Science, Swansea University,
Singleton Park, Swansea SA2 8PP, UK
E-mail: p.l.m.lee@swansea.ac.uk

We present a comprehensive protocol for extracting DNA from egg membranes and other internal debris recovered from the interior of blown museum bird eggs. A variety of commercially available DNA extraction methods were found to be applicable. DNA sequencing of PCR products for a 176 bp fragment of mitochondrial DNA was successful for most egg samples (> 78%) even though the amount of DNA extracted was significantly less than that obtained for bird skin samples. For PCR and sequencing of snipe (*Gallinago*) DNA, we provide eight new primers for the 'DNA barcode' region of COI mtDNA. In various combinations, the primers target a range of PCR products sized from 72 bp to the full 'barcode' of 751 bp. Not all possible combinations were tested with archive snipe DNA, but we found a significantly better success rate of PCR amplification for a shorter 176 bp target compared with a larger 288 bp fragment (67% versus 39%). Finally, we explored the feasibility of whole genome amplification (WGA) for extending the use of archive DNA in PCR and sequencing applications. Of two WGA approaches, a PCR-based method was found to be able to amplify whole genomic DNA from archive skins and eggs from museum bird collections. After WGA, significantly more archive egg samples produced visible PCR products on agarose. However, overall sequencing success did not improve significantly.

47. DNA damage repair and amplification from formalin fixed specimens

Samantha Mohun

Department of Zoology, Natural History Museum, Cromwell Road, London,
SW7 5BD
E-mail: samm@nhm.ac.uk

During the past two decades, the demand for the use of archival collections for DNA-related applications has increased. Specimens from Natural History Collections (NHC) are a readily available source of material from organisms in remote habitats. Further pressure is due to the advances in Next Generation Sequencing techniques which require good quality and a large quantity of DNA. However, much of this archived material has been either fixed in different chemicals (primarily formaldehyde) or been stored for a time under suboptimal conditions limiting its utility for DNA studies i.e. ancient DNA. Thus the need to access samples stored in museums is critical, and making the use of archival collections is increasingly important. Unfortunately, much of this material has been either fixed in DNA-damaging chemicals (primarily formaldehyde or its derivatives) or stored for a time under suboptimal conditions limiting its utility for DNA studies. Formalin-preservation of tissue has numerous direct and indirect impacts on the structure of DNA:

covalent cross-linking, irreversible denaturation, modification, and fragmentation.

There is a great need therefore to optimise and validate procedures for the recovery and analysis of DNA from aged (formalin preserved) zoological tissue specimens using convenient technology.

A museum-industry partnership to develop, optimise and validate procedures for the recovery and analysis of DNA from museum specimens using Whatman FTA® technology will be described as well as a review of current molecular and storage methods that optimise extraction and amplification yields, reverse DNA damage, and obtain sequence data from small amounts of DNA.

48. Museum archaeobotanical collections as a source of biomolecular information for archaeogenetic analysis

Oliver Smith, Rafal Gutaker, Sarah Palmer, Robin Allaby

University of Warwick School of Life Sciences, Wellesbourne, Warks
E-mail: o.smith.1@warwick.ac.uk

Many ancient samples used for research into genetic archaeobotany are sourced from museum or herbarium collections, and with good reason. As with any and all archaeological specimens, the reliability of contextual information is key to optimizing technical aspects of scientific analysis and ensuring accurate conclusions to research. Museum collections include material from which age, provenance and depositional environment (i.e. original burial conditions) can be verified and logged. Aside from being useful information regarding the individual sample, consistency of information across multiple specimens appropriates sample selection for detailed comparative analysis.

Precautions taken in museums and herbariums to preserve biological material (such as environmental controls, appropriate (i.e. tissue-specific) storage materials or minimally-destructive conversion of depositional environment) ultimately allow detailed archeogenetic analysis to take place. To that end, museum collections can be seen as 'banks' of biomolecular information, representing a wide temporal and geographical range and providing the means to answer specific questions. Archaeobotanically important areas of study such as plant population diversity, movements, domestication and adaptation can be addressed due to this combination of availability and information accuracy.

For example, our current project on flax population diversity leading to domestication in Europe utilizes pre and post 'green revolution' material sourced from multiple museum and herbarium collections across Europe. This material regularly yields sufficient DNA to amplify and sequence key regions associated with evolution and domestication traits.

Further questions pertain to current topical issues such as food security. Samples from other collections suggest that the extreme aridity of southern Egypt's paleoclimate may induce locally-grown key domesticates to exhibit stress-responses. DNA from cotton is of high enough quality to allow

high-throughput metagenomic sequencing, while RNA from ancient barley grain is of sufficient quantity and quality to allow similar sequencing of short regulatory RNAs to investigate localized adaptation to drought.

49. Postglacial recolonisation mode of North American *Amara alpina* (Carabidae: Coleoptera) using museum and ancient DNA

Peter D. Heintzman¹, Ian Barnes¹ and Scott A. Elias²

¹School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, TW20 0EX, UK. ²Department of Geography, Royal Holloway University of London, Egham, Surrey, TW20 0EX, UK
E-mail: Peter.Heintzman.2009@rhul.ac.uk

Predicted future climatic change is likely to result in the colonisation of new regions by species. Historical colonisation events can be used to predict the mode and tempo of these future events. This study investigated the mechanism by which the arctic beetle *Amara alpina* recolonised Canada at the end of the last ice age, utilising information from both modern and ancient representatives. Previous work had suggested that recolonising individuals originated from the western (Beringian) and southern (northern lower 48 US states) refugia. The results described here show a more complex pattern of recolonisation than originally thought. A distinctive haplogroup has been identified in Canada, which did not originate from either of the two aforementioned refugia. This raises the possibilities of a refugium in northeast North America or a postglacial immigration event to North America by individuals from Europe. Additionally, the genetic composition of the Beringian population shows surprising constancy through time, a result in contrast to the major turnover events seen in the mammalian megafauna over this period.

50. Developing museum molecular collections: A global repository for schistosomiasis research

Fiona Allan, David Rollinson & Aidan Emery

Department of Zoology, Natural History Museum, Cromwell Road, London, SW7 5BD
E-mail: fiona@nhm.ac.uk

The Natural History Museum, London (NHM) is undertaking a new initiative to develop the collections facilities of its Wolfson Wellcome Biomedical Laboratories (WWBL) as a global repository for schistosomiasis-related material.

The WWBL has a long history of field-based research into schistosomiasis, and is a World Health Organization Collaborating Centre for the identification of schistosomes and their intermediate snail hosts. Over the years, WWBL has built up substantial research collections of schistosomes, mostly through laboratory passage and storage in liquid nitrogen. Twelve species of

schistosomes are represented in this material, originally collected from 28 countries, with specimens held in liquid nitrogen dating back to 1983. Additionally, material is held in ethanol, both purposely for molecular biology, and also historically as in the case of an important and extensive collection of African freshwater snails compiled from 1947, which was the working collection used by the late Chris Wright and David Brown, authorities on African freshwater snails. Specimens in the collections have been used extensively for many retrospective studies, including genetic marker development, population genetic analysis and taxonomic research.

51. Biobanking at the NHM London

Jacqueline MacKenzie-Dodds

Department of Zoology, Natural History Museum, Cromwell Road, London, SW7 5BD

E-mail: jm@nhm.ac.uk

Molecular research at the NHM plays a major role in resolving questions on the diversity of life. Plants and animals are collected for traditional dry or alcohol preservation, and as a long term resource for molecular analysis. Molecular products are also extracted from 'traditional' collections. The NHM is developing new 'molecular collections' (in parallel to the 'traditional' collections) for future use beyond its own research programmes, making them accessible to the wider community and ensuring their long term value to science. Developments in the techniques of molecular biology, including next generation DNA sequencing, evaluation of DNA damage and repair, and epigenetics, create exciting research opportunities and drive forward the need to preserve biological samples for study by future generations.

Until now all NHM molecular collections have been managed by individual scientists and their research teams. New infrastructure, management and curation will consolidate and provide centralised accommodation for all existing and future archival molecular collections and integrate their development and management with the traditional collections. To ensure this, the NHM has built a central specialised storage facility within easy access of the NHM's research teams and the traditional collections.

Future-proofing the facility requires international legal compliance, identification of optimal storage methods, global standards, best practice and incorporation of new science and IT technologies. Exchange of knowledge in all these areas among partner institutions is vital to promote global sharing of genetic data.

52. Museomics for ectoparasites recovered from historical fish collections – lessons from *Gyrodactylus*

Hahn, C., Zeyl, E., Bakke, T.A., Harris, P.D. & Bachmann, L.

National Center for Biosystematics, Natural History Museum, University of Oslo, Norway
E-mail: christoph.hahn@nhm.uio.no

Gyrodactylus v. Nordmann, 1832 (Platyhelminthes; Monogenea) is a genus of viviparous ectoparasites infecting teleost fish species throughout the world. The ~450 described species are expected to only represent about 2% of the worldwide diversity. *Gyrodactylus salaris* is known as a major pathogen of Atlantic salmon in Norway which, apart from its economic importance, has imposed significant ecological burdens upon freshwater ecosystems. *G. salaris* may have evolved by a host shift from the related *G. thymalli* on grayling. Despite extensive research neither morphological nor molecular analyses have yet identified the origin of the host shift. To reconcile the host patterns with the complex *Gyrodactylus* phylogenies and to disentangle host-switches from co-evolutionary events is an ambitious task, particularly as human impacts caused regional extinction of former abundant freshwater fish species. The impact of stocking on autochthonous fish populations is also an important issue in conservation biology.

Historical changes in fish diversity are well documented in ichthyological collections, but so far very few attempts have been made to explore the ectoparasites unwittingly collected together with their hosts. We apply museomics approaches to fish ectoparasite diversity and we intend to add a temporal dimension to the understanding of the current distribution of *Gyrodactylus* in European watercourses. Until now gyrodactylids could be identified from fish material from the Natural History Museums Vienna (Austria), Paris (France) and Oslo (Norway) including parasites from historical fish collected in the late 19th century. Most of the recovered parasites still allow for morphological species identification, e.g., *Gyrodactylus* specimens from salmon collected in 1876 and grayling in 1880 were identified as *G. derjavinoidea* and *G. thymalli*, respectively. Furthermore, we have successfully amplified and sequenced the intergenic spacers of the nuclear ribosomal gene cluster and the mitochondrial cytochrome oxidase I gene from this material.

53. The DNA Bank Network – A platform for open access to voucher referenced DNA samples and data worldwide

Droege, G., Zetzsche, H. & Gemeinholzer, B.

DNA Bank Network, Botanic Garden and Botanical Museum, Freie Universität Berlin, Koenigin-Luise-Str. 6-8, 14195 Berlin, Germany
E-mail: g.droege@bgbm.org

Accessible voucher specimens are the only reliable basis to verify the species identity of molecular sequences published in databases such as GenBank, EMBL, DDBJ, or BOLD. Although the deposition of voucher specimens in public research collections for taxonomic descriptions is mostly general routine an equal diligence in molecular analysis concerning the deposition of DNA voucher samples is the exception rather than the rule.

The scope of the DNA Bank Network (www.dnabank-network.org) is to facilitate access to genetic resources as well as to related data including digital voucher images. Furthermore we encourage scientists to deposit their DNA samples and voucher specimens in DNA banks and natural history museums after publication. Having such material available is an asset for users in all areas of biological research who are pinched for time or have a lack of expertise to make such an investment. While DNA banks focusing on DNA from organismic samples can make their accessions available via a shared portal scientists can search for and order voucher referenced DNA and tissues.

The Network also provides a unique opportunity to document biospecimens as well as derived DNA samples in online accessible databases. The data architecture of the DNA Bank Network is based on the GBIF (Global Biodiversity Information Facility, www.gbif.org) concept and makes use of the same specimen databases. Furthermore the Network has developed the open source software DNA Module to manage DNA data and has proposed a standard for transfer of DNA specific data.

Here we present a) the IT tools of the DNA Bank Network for data management, b) the best practice to deposit samples and data, c) the Network's data model to enable access to DNA collections, specimen and sequence data networks, and d) the requirements for institutions to become a partner of the DNA Bank Network.

54. Quantitative morphometric assessment of the disparity of Lake Tangayika's endemic gastropod fauna

Olivia Cheronet¹, Jonathan Todd², Ellinor Michel³ and Jonathan Krieger⁴

¹ The Natural History Museum, London SW7 5BD, and Imperial College London, London SW7 2AZ. ² The Natural History Museum, Department of Palaeontology, London SW7 5BD. ³ The Natural History Museum, Department of Zoology, London SW7 5BD. ⁴ Royal Botanic Gardens, Kew, Richmond, TW9 3AB
E-mail: o.cheronet@nhm.ac.uk

Lake Tanganyika's cerithioid gastropod radiation is often claimed to be the most diverse monophyletic freshwater gastropod radiation. We tested this assertion with a geometric morphometric assessment of morphospace occupation, quantifying gross shell shape in the context of a current phylogeny, and contextualising the results in a broader gastropod morphospace.

We used coordinate-point extended-eigenshape (CP-EES) analyses (a new method described in Figueirido et al, 2011) to characterise morphology, and determined within-group variation, among-group variation and morphospace occupation density using disparity metrics. In addition, we contrasted results from the Tanganyikan radiation with morphospace occupation of two other diverse fossil radiations: cerithioids from the East

African Albertine Rift Valley and gastropods from the South American Pebas Formation.

Morphospace occupied by the Tanganyikan fauna represents a region of relatively uniform growth with few adult modifications (notable shifts in geometry associated with sexual maturity). Much of the unoccupied space appears biologically less favourable, involving extreme adult modifications. Although correlation with phylogeny is generally poor, a few groupings are nevertheless evident. A negative relationship between within-taxon morphological disparity and taxonomic diversity suggests higher levels of experimentation within species-poor clades.

Morphospace occupation was largely similar among comparative groups. Pebas Formation cochliopids occupy a quasi-identical space to the Tanganyikan radiation. No radiation of similar magnitude was observed in the Albertine Rift Valley cerithioids. Thus, one could hypothesise a common mechanism for filling morphospace by the Tanganyikan and Pebas radiations, with incumbent taxa and species-poor taxa accommodating themselves in morphospace through greater shell morphological experimentation. It nevertheless remains evident that the Tanganyikan radiation is morphologically extremely variable.

55. Herbarium DNA: extraction optimization, damage assessment and use.

Martijn Staats, Robyn Cowan, Tiina Sarkinen, James Richardson, Argelia Cuenca Navarro, Gitte Petersen, Ole Seberg, Ursula Eberhardt & Freek T. Bakker

Biosystematics Group, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
E-mail: freek.bakker@wur.nl

We present the results of a Joint Research Activity within the SYNTHESYS2 programme that focuses on optimization extraction and preservation of 'Museum DNA'. As JRA4, we assessed the relative performance of commonly-used extraction methods and characterised DNA post-mortem damage in herbarium tissue. Success rates for DNA extraction, including anion exchange, CTAB/chloroform, silica, and magnetic charge, was measured in terms of yield, purity and PCR amplification success. Tests were performed on a panel of 48 herbarium vouchers, representing both phylogenetic and phytochemical Angiosperm diversity. Silica-based extractions and standard *Taq* DNA polymerase enzymes were found to perform best. Results for a fungal herbarium panel are underway.

We are compiling PCR success from herbarium specimens from most Angiosperm families. The end product will be a searchable and updatable data base containing all available published and unpublished (negative) results. This data base will serve as a valuable tool to the herbarium DNA community, both as a trouble-shooting archive and as a basis for further optimising the use of herbarium DNA.

Results are presented on an assessment of DNA damage in the form of miscoding lesions in old herbarium specimens of *Lonicera*, *Ginkgo*, *Liriodendron*, and *Laburnum*, using 454-sequencing. We assessed DNA degradation as a result of strand breaks and apuric sites by qPCR for multiple regions in cpDNA, mtDNA and nuclear DNA. Using pairs of fresh and (up to 114 year) old herbarium specimens of the same individual we quantitatively assess post-mortem DNA damage, directly after herbarium fixation, as well as after long-term storage. Results indicate that most DNA damage occurs directly after fixation, and that there is no evidence for preferential degradation of organelle versus nuclear genomes. Increased levels of C→T/G→A transitions were observed in old herbarium DNA, representing 21.8% of observed miscoding lesion. We interpret this type of post-mortem DNA damage-derived modification to have arisen from the hydrolytic deamination of cytosine during long-term storage.

56. Fast Supertree Construction

Mark Wilkinson & Peter G. Foster

Department of Zoology, The Natural History Museum, London
E-mail: m.wilkinson@nhm.ac.uk

One of the purported advantages of supertree methods is their speed. Fast methods could be very important for constructing large scale phylogenies in reasonable time. Whereas supermatrix approaches involve time consuming searches of tree space, supertree methods that can avoid this heuristic could, in principle, be much faster. However, most described supertree methods also depend upon searches of tree space and thus offer no real advantage over simultaneous analysis of supermatrices. In contrast, quartet joining is a recently described heuristic supertree method that uses logic rather than an optimality criterion to place leaves on a growing tree and thus avoids searches of tree space. We describe the method and its current implementation, and present some results of comparisons with the matrix representation with parsimony (MRP) method which is currently the most widely used supertree method. Funded by BBRSC grant BBG 0247071.

57. Convergent evolution at the sequence level – batty and malarial examples

Liu Yang, Claire Chewapreecha, Kalina Davis, Emma Teeling, Stephen Rossiter, James A. Cotton

Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
E-mail: james.cotton@sanger.ac.uk

Cases of convergent evolution — where different lineages have evolved similar traits independently — are common and have proven central to our

understanding of evolution by natural selection. Until recently, however, convincing examples of adaptive convergence at the sequence level have been exceptionally rare, leading to the idea that the enormous 'sequence space' available to proteins is so large that proteins can evolve closely similar function and structure without evolving similar sequence. Recently, new examples of sequence-level convergence have been published, partly due to new approaches for detecting unusual patterns of sequence evolution. Prestin is a motor protein involved in high-frequency hearing in the mammalian auditory system. We have found evidence of convergent evolution in Prestin between different groups of echolocating bats, and between bats and cetaceans, and show that these convergent substitutions are likely to have been driven by positive natural selection. Intriguingly, we also find evidence for convergent substitutions in echolocating bats in some other genes relating to the auditory system. In a separate study, we have used similar approaches to look genome-wide for sequence convergence between the two lineages of primate-infective malarial parasites, in an attempt to identify loci involved in host specificity. We find at least one strong candidate, but there are important limitations to the study so far that limit the power of this analysis.

58. Rocks, Soils and Breaks: A new method for discovering and identifying biotic breaks

Malte C. Ebach, Giovanni di Virgilio & Shawn Laffan

School of Biological, Earth & Environmental Sciences, University of New South Wales, Australia.

E-mail: mcebach@unsw.edu.au

It is currently difficult for biogeographers, macro-ecologists and geographers to visualise and assess actual biotic breaks. The lack of detailed maps, diagnostic descriptions and methods to quantify these breaks means that most researchers are left with ad hoc areas.

Geobiotic Polyphasic Consilience (GPC) combines taxic distributions and abiotic structures in order to discover the processes that constrain biotic distributions. What makes GPC unique is its premise of life and Earth evolving together and that abiotic factors such as geography, soil chemistry, surface geology and hydrology all influence biotic distributions.

The aim of GPC is to find and map breaks within biotic distributions and identify their structures and geographical processes. This is done using geospatial analysis software (ArcGIS and Biodiverse) to spatially analyse the biotic distributions and identify areas of high spatial turnover of biota. The results can then be overlaid with other spatial data such as from topographical, geological or geographical maps to identify putative causes and controls. In this way, actual biotic breaks can be visualised without the need for ad hoc area definitions, instead letting the data define the break.

59. Nullius in Calculo: On the Explicitness and Reproducibility of Cladistic Analyses

Ross CP Mounce

Biology & Biochemistry Dept., University of Bath, BA2 7AY
E-mail: Ross.Mounce@gmail.com

The result of a Maximum Parsimony cladistic analysis *should* be repeatable. Yet I present here numerous examples of recent papers in which the results contained therein cannot be replicated, given only the content of the paper, supplementary materials and links. Barriers to study replication include (1) absence of requisite information, (2) typesetting errors, and even (3) author error. I argue that these problems, many of which are easily-spotted, should not be appearing in peer-review published papers with such regularity.

Inspired by the Royal Society's motto, I humbly suggest that reviewers and editors not only examine the words of papers, but also the underlying data and calculations: *Nullius in Verba, Nullius in Calculo*. Furthermore, I believe the reporting of phylogenetic analyses would greatly benefit from increased Standardization following community-agreed criteria c.f. MIAPA (Leebens-Mack et al, 2006), and data deposition in appropriate data archives specifically designed to accommodate phylogenetic data e.g. TreeBASE or MorphoBank. In addition to problems with reproducibility, I also detail problems with explicitness of method reporting. A manual examination of over 300 recently published incongruence length difference (ILD) tests provides evidence to suggest that method sections are rarely sufficiently explicit in their detail to exactly replicate the methods used to generate reported results. In particular I suggest that authors should be encouraged to explicitly state how 'gaps' are coded and treated, and which branch collapsing rules are followed in analyses. Different settings can and do generate different results, therefore all such important settings should be explicitly stated.

Improvements in computing and the Internet offer us an excellent opportunity to digitally enrich the quality and legacy of the science we publish – we just need to embrace some small beneficial changes to the way we publish.

POSTER ABSTRACTS

P1. DNA barcoding reveals cryptic species and helps to resolve taxonomy in character poor bryophyte groups

David Bell, David G. Long, Alan D. Forrest, Michelle L. Hollingsworth, Hans H. Blom and Peter M. Hollingsworth

Royal Botanic Garden, 20A Inverleith Row, Edinburgh, EH3 5LR
E-mail: dbell@rbge.ac.uk

We present a study of DNA barcoding in a group of European liverwort species as an example of the utility of DNA barcoding for revealing cryptic species and resolving taxonomy in difficult bryophyte groups. DNA barcoding of a group of four European liverwort species from the genus *Herbertus* was undertaken using three plastid (*matK*, *rbcL* and *trnH-psbA*) and one nuclear (ITS) marker. The DNA barcode data were effective in discriminating among the sampled species of *Herbertus*, and contributed towards the detection of a previously overlooked European *Herbertus* species, *H. norenius* sp. nov. This species shows clear cut differences in DNA sequence for multiple proposed barcode regions and is also morphologically distinct. The DNA barcode data were also useful in clarifying taxonomic relationships of the European species with some species from Asia and North America. In terms of the discriminatory power of the different barcode markers, ITS was the most informative region, followed closely by *matK*. All species were distinguishable by ITS alone, *rbcL*+*matK*, and various other multi-marker combinations.

P2. Ichthyophiid caecilian amphibians – a non-adaptive radiation out of India

David J. Gower, Anna Papadopolou

Department of Zoology, The Natural History Museum, London, SW7 5BD, UK
E-mail: d.gower@nhm.ac.uk

With approximately 50 species, the South and Southeast Asian Ichthyophiidae Taylor is the largest of the nine currently recognised families of caecilian amphibians (Gymnophiona). Previous molecular phylogenetic work concluded that ichthyophiids' distribution far beyond former Gondwanan areas could best be explained by an Out of South Asia dispersal following accretion of the Indian subcontinent. However, geographic and taxonomic sampling was very limited, leaving open the possibility that ichthyophiids reached Southeast Asia by an alternative route or that the previously detected signal was a back-dispersal rather than the primary event.

We present results from new analyses that sampled some 160 ichthyophiids from across their entire range, including multiple populations of

all three genera. Phylogenies and timetrees were inferred from mitochondrial and nuclear DNA sequence data. Results confirm support for the Out of South Asia hypothesis. Neither *Ichthyophis* or *Caudacaecilia*, nor unstriped or striped members of these genera are monophyletic, and *Ichthyophis* is paraphyletic with respect to *Uraeotyphlus* as well as *Caudacaecilia*. Species-level diversity (a point of previous debate) is high, with several new taxa likely in need of description.

In Southeast Asia ichthyophiid genetic diversity is largely geographically structured, with some regions having multiple, relatively distantly related lineages. Morphologically, and probably ecologically, *Ichthyophis* and *Caudacaecilia* are not particularly diverse, and there is no evidence for a greatly episodic diversification of these genera. Ichthyophiidae seems to represent a non-adaptive radiation. Implications for Southeast Asian diversification and biogeography are briefly discussed.

P3. A Next-Generation Approach for Environmental Biomonitoring

Jennifer Spall, Shadi Shokralla, Mehrdad Hajibabaei

Department of Integrative Biology/Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada
E-mail: jspall@uoguelph.ca

Environmental biomonitoring programs have traditionally relied on the use of bioindicator taxa to infer the health of ecosystems. In freshwater river systems, these taxa frequently include aquatic macroinvertebrate larva from benthic samples (Bonada et al., 2006). Morphological identification of aquatic larva is tedious, time-consuming and often inaccurate. Identification beyond the taxonomic level of family requires an entomology expert and species-level classification is often impossible (Bonada et al., 2006). Moreover, research has shown that the variation in species richness of one group can only predict a mere 10-11% of the species richness of another group (Lawton et al., 1998). Traditional DNA barcoding based on Sanger sequencing technology can help identification of species, but this approach is not adequate for sequencing bulk benthic samples. These samples regularly contain hundreds, if not thousands of individuals. Sequencing each of these individuals in parallel is simply beyond the scope of traditional sequencing technologies (Hajibabaei et al., 2011). Next-generation platforms, however, have the ability to discern each individual and determine its genomic sequence independently from the sequences of others. We have seen that 454-Roche can accurately identify all species present in a bulk environmental sample with an abundance of at least 1% (Hajibabaei et al., 2011). Using multiple locations that have been used for traditional environmental assessment along the Humber River, just East of Toronto, Canada as our sampling sites, we have collected several samples of benthic macroinvertebrates for analysis with 454 sequencer. The results obtained from this analysis will be compared with available biomonitoring data from these sites and will allow us to develop a framework by which consistent

and accurate biomonitoring programs can be carried out to their full potential using next generation sequencing technology.

References

- Bonada, N., Prat, N., Resh, V. H., & Statzner, B. (2006). Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. *Annual review of entomology*, 51, 495-523.
- Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. a C., & Baird, D. J. (2011). Environmental Barcoding: A Next-Generation Sequencing Approach for Biomonitoring Applications Using River Benthos. (C. R. Voolstra, Ed.) *PLoS ONE*, 6(4), e17497.
- Lawton, J. H., Bignell, D. E., Bolton, B., Bloemers, G. F., Eggleton, P., Hammond, P. M., et al. (1998). Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. *Nature*, 391(6662), 72-76.
-

P4. Surveys on biodiversity of *Ulva* (Chlorophyta, Ulvales) in the North Adriatic sea (Mediterranean, Italy)

K. Sciuto^{*}, M.A. Wolf^{*}, C. Andreoli, I. Moro

^{*} These authors contributed equally to this work

via U. Bassi 58/B, 35131 Padova, Italy

E-mail: katia.sciuto@unipd.it

Taxonomy has originally been based on morphological features. In the last two decades the use of DNA barcoding has enabled the identification of new species and the systematics of many groups. This has been extended also to photosynthetic organisms, such as cyanobacteria, microalgae, seaweeds, and plants. In fact, for the high degree of morphological plasticity exhibited by many members of these taxa, several characters traditionally considered diagnostic have revealed ambiguous and so useless.

The genus *Ulva* Linnaeus (Ulvophyceae, Ulvales) is cosmopolitan in its distribution, with species occurring in all aquatic habitats from freshwater through brackish to fully saline environments. Members of this genus show a very simple morphology and a certain degree of phenotypic plasticity, heavily influenced by environmental conditions, making difficult the delineation of species by morphological features alone (Loughnane et al., 2008).

In spite of this, most of the studies dealing with *Ulva* biodiversity in Italian waters have been based only on morphological characters. For this reason we have recently started surveys on *Ulva* biodiversity in the North Adriatic Sea (Mediterranean, Italy).

As maritime traffic is considered one of the major causes of species introduction, for example through ballast waters, hull fouling, and ship sea chests, we have focused on three places for sampling: Venice Lagoon, Chioggia inlet, and Lido of Venice. The first two are greatly affected by naval traffics, while the last one is much less influenced by this phenomenon. The molecular analyses, carried out using the *rbcL* and *tufA* genes as molecular markers, revealed the presence of six different species with

overlapping morphologies: *U. compressa*, *U. californica*, *U. pertusa*, *U. rigida*, and two probable new taxa. Some of the recognized species have not been reported up to now in the investigated areas.

P5. DNA barcoding on Mediterranean Leaf Beetles (Coleoptera, Chrysomelidae), preliminary results

M. Montagna, D. Sassi, G.C. Lozzia

DIPAV/DIPSA, University of Milan, Via Celoria 10 – I 20133 Milan
E-mail: matteo.montagna@unimi.it

International Barcode of Life project aims to obtain a complete molecular "catalogue of life" in order to simplify identification of samples and to create a world DNA-sequences databank. Nowadays, this purpose is becoming even more important every day considering the rising rates in species extinction. DNA barcoding is based on identification of individuals of different taxa using a molecular approach. The main problem of DNA barcoding is being able to correctly evaluate the capability of molecular markers to discriminate between different species. For barcode purpose, an ideal molecular marker must present high interspecific variability and high intraspecific identity.

The Mediterranean Region is considered a hot spots for species biodiversity. So far, a species list of the mediterranean Leaf Beetles (Coleoptera, Chrysomelidae) has not been generated, but it is estimated in 1300 species. The purpose of the project is to test the DNA barcoding approach and create a DNA-sequence databank of mediterranean Chrysomelidae. One hundred ninety specimens belonging to 132 species were collected from different populations; DNA was extracted following standard Barcoding protocols. A fragment of 658 bp of cytochrome oxidase subunits I (COI) gene (primers LepF1/LepR1) was amplified by PCR. The obtained sequences database was tested, estimating pairwise genetic distance, in order to assess the presence of the "identity percentage gap" between intra- and inter- specific variation. The mean value of genetic distance within species is 2.04% (SE 0.41%), within genus is 20.31% (SE 0.079). Maximum Likelihood and Bayesian inference methods have confirmed the absence of introgression.

Our preliminary results show that DNA barcoding approach for mediterranean Chrysomelide is coherent with the morphological identification and it can be considered a valuable and complementary tool in species identification.

P6. Towards large-scale second-generation sequencing of full mitochondrial genomes in the Araneae

Briscoe, A.G.¹, Goodacre, S.², Masta, S.³, Taylor, M.I.¹, Hurst, G.⁴, Arnedo, M.⁵, Creer, S.¹

¹Molecular Ecology and Fisheries Genetics Laboratory, Bangor University, Bangor LL57 2UW, ²Institute of Genetics, Queens Medical Centre, University of Nottingham NG7 2UH, ³Department of Biology, Portland State University, Portland, Oregon, USA, ⁴Biological Sciences, University of Liverpool, Liverpool L69 3BX, ⁵Departament Biologia Animal, Universitat de Barcelona, Barcelona, Spain
E-mail: bsp808@bangor.ac.uk

Mitochondrial DNA is firmly embedded in contemporary molecular systematics, but more accurate phylogenetic reconstruction is often impeded by low sequence volume and number of loci. Second generation sequencing may resolve this issue, offering the potential of analysing multiple, whole mitochondrial genomes to resolve complex phylogenetic relationships. However, while the potential of second-generation mitogenomics is evident it has yet to be realised on a large scale, since most 454 Roche mitogenomic studies present either incomplete genomes or have focused on large, nominally intraspecific datasets.

My research is focused on the continued development of 'universal' protocols facilitating the rapid creation of mitochondrial whole-genome interspecific datasets in the Araneae (True spiders). The Araneae are a diverse order containing over 40,000 described species and have been the focus of relatively few molecular based studies, especially from a higher-level molecular systematic perspective, and very little is known about inter-family relationships. In order for the approaches to be truly interspecific multiple methods including single fragment long-range PCR and rolling circle amplification (RCA) have been optimised. To date this has resulted in the amplification of mitochondrial genomes from taxa spanning over 30 of the 109 currently recognised families that comprise the Araneae.

P7. Developing maximum likelihood and Bayesian supertrees

Wasiu Akanni, Davide Pisani and Peter Foster

NUI Maynooth, Co. Kildare, Ireland
E-mail: WAAKANNI13@GMAIL.COM

Very little work has been done on the development of a supertree method in the Likelihood and Bayesian frameworks. Recently, it has been proposed that Maximum Likelihood (ML) supertrees could be developed by using an exponential probability distribution to model the probability that the input trees could be erroneous. When the tree-to-tree distances used in the ML computation are calculated using the Symmetric Difference, the ML supertree has been shown to be equivalent to a Majority Rule Consensus Supertree, and hence, exactly as the latter, the ML supertree must have the desirable property of being a median tree – with reference to the input set. In addition, the ability to estimate the likelihood of supertrees, will allow the implementation of Bayesian MCMC approaches, which have the advantage of

allowing for a natural estimation of the support for the nodes on the recovered supertree. We are developing the first software for the estimation of Maximum Likelihood and Bayesian supertrees. The program is being written in Python and will also exploit the capabilities of already available software, i.e. P4.

Here, we shall reanalyze the dataset of Holton and Pisani (2010) and present the first Bayesian Genomic Supertrees generated using our new software. In addition, we shall compare these supertrees with those derived using other common supertree methods (e.g. Matrix Representation with Parsimony and Average Consensus). Our software is still lacking many important functions. However, when completed, we expect it not only to be able to recover supertrees using MCMC but also to implement a variety of tests that makes use of the recovered supertree (e.g. diversification rate analyses), or those that evaluate if the likelihood of the recovered supertree is better than that of other possible alternative (supertree equivalent of the common test of two trees – e.g. Kishino-Hasegawa test).

P8. Advances in using wet museum collections of molluscs for molecular phylogenetics

Renita Danabalan, Björn Stelbrink & Thomas von Rintelen

Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der Humboldt-Universität zu Berlin, Invalidenstr. 43, 10115 Berlin, Germany
E-mail: bjoern.stelbrink@mfn-berlin.de

Museum collections constitute a DNA archive for research allowing the monitoring of genetic diversity for, at least, the last 250 years. While the majority of taxa present no big challenge for DNA extraction, molluscs contain a significant amount of mucopolysaccharides (mucus). This compound inhibits the subsequent amplification DNA, when standard methods of DNA extraction are applied. Thus, the cost effective Cetyl trimethylammonium bromide (CTAB) extraction method is commonly used on these organisms. However, this protocol requires the use of β -mercaptoethanol (a noxious chemical component); it is time consuming and is not suitable in a high-throughput laboratory. Additionally, it is found to be ineffective for museum specimens. Therefore systematic tests were conducted on a range of available kits and methods for DNA extraction from molluscs both fresh and preserved. The effect of adding β -mercaptoethanol on DNA yield and PCR success was also assessed.

It was found that DNA purity and PCR success using **Solid Phase Reverse Immobilization** technology (magnetic beads) matches that of the CTAB protocol. In addition, magnetic bead based protocols can easily be run on semi(robots) allowing for the full automatization of DNA extraction. The results also show that the addition of β -mercaptoethanol does not have any effect on extraction success and can thus be omitted allowing the flexibility of working on a smaller scale and promotes a safer work environment.

These initial results show that it is possible to extract DNA effectively and efficiently without compromising the quality of the output; thus enabling laboratories to assess the value of existing museum collections of molluscs, a group comprising at least 200,000 species and millions of specimens in European institutions.

Further analysis will include the setup of a database for monitoring success rates and problem identification in a large-scale test in several European collections with different storage conditions and duration.

P9. Emonocot: biodiversity informatics for monocot plants

Wilkin, P., Barker, A., Baker, E., Baker, W.J., Bone, R., Clark, B., Godfray, H.C.J., Kelly, J., M., Kitching, I.J., Mayo, S.J., Phillips, S., Scoble, M.J., Simpson, D.A., Smith, V.S., Trias-Blasi, A., Villalba, S. & Weber, O.

Herbarium, Library, Art & Archives, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK

E-mail: a.triasblasi@kew.org

The eMonocot project which started in late 2010, aims to change the methods by which biodiversity information is delivered to its users as taxonomy develops into an eScience. The project is creating an authoritative web-based treatment of monocot plants by generating ~70,000 outline species pages based on the World Checklist of Monocotyledons (www.kew.org/wcsp/monocots). These will be supported by keys and taxon pages to all the families of monocots and ~2000 genera in 8 major families. Comprehensive species pages will include European monocots (~2000 species), "Sampled Red List Index" monocots (1500 species) and Cyripedioideae (slipper orchids) (~130 species) with over 20,000 species having enhanced content. Further taxa will be delivered via "Scratchpads" (<http://scratchpads.eu>), community websites for taxonomists geared to uploading and presenting a wide variety of taxonomic data. Existing monocot web resources such as CATE-Araceae (www.cate-araceae.org), Palmweb (www.palmweb.org) and Grass Base (www.kew.org/data/grasses-db.html) represent important building blocks around which the eMonocot system will be built. The project will be linked to other resources maintained by key international stakeholders in biodiversity informatics. eMonocot will generate software tools to permit future taxonomic research to be web-based, enabling monocot scientists anywhere in the world to participate, creating social structures and working practices to facilitate and manage these global interactions. This global participation will be critical in developing and sustaining both the data of eMonocot and the communities that will generate and enhance it.

P10. Variation of meristic characteristics of the wild Population of the Brown trout in Lar National Park, Iran.

Sara Ghorbani¹, Asghar Abdoli², Kiavash Golzarian¹, Amir Abbas Mashhadi Ahmai³, Bahram H Kiabi¹

1: Faculty of Biological Science, Shahid Beheshti University, Evin, Tehran.

2: Environmental Sciences Research Institute, Shahid Beheshti University, Evin, Tehran. 3: Department of Environment, Tehran, Iran

E-mail: sara_borzi@yahoo.com

A study was conducted to determine meristic characteristics of the wild population of brown trout (*Salmo trutta*) in Lar National Park from 2009 to 2011. Fish specimens were captured (obtained) by Electro fishing in 10 stations during summer and autumn 2009. Meristic characteristics are among taxonomic features used to identify fish species, or to make intra or inter species comparisons. Though those characteristics are considered fixed through the fish life history, they may be affected by some physical and chemical factors during embryonic period. In previous studies of meristic characteristics in Brown trout (*Salmo trutta*) even in different parts of the world or in different fresh water basins in Iran, there were not any significant differences in majority of the characteristics between various stations. But in the present study in Lar National Park, Iran, significant differences have been detected in nine characteristics including branched dorsal rays, un branched dorsal rays, branched pectoral rays, un branched pectoral rays, branched pelvic rays, un branched pelvic rays, Branched and un branched anal rays and gill rakers between specimens collected from ten stations ($P < 0.5$). The observed differences between specimens can be attributed to different environmental features of stations, e.g., the selected stations differed in water temperature, salinity and PH. Besides, *Salmo trutta* is considered a polygenetic species. And the fact that in this project we detected variations which are not reported from populations existing in other parts of the world might be due to having wild population in the study area.

P11. Morphometric study of the European cherry fruit fly (*Rhagoletis cerasi*) populations of Tehran and Alborz province in Iran

Farjamfar, M, J. Nozari.

Department of plant protection, college of Agriculture, University of Tehran, Iran-Karaj

E-mail: mahsa.farjamfar@yahoo.com

The European cherry fruit fly, *Rhagoletis cerasi* is one of the most important insect pests of sweet and sour cherries in Iran such that chemical control has become an essential tool in cherry production. The mated female flies lay their eggs inside pericarp and under fruit skin when different ripening cultivars starts to become red. The aim of this study was to explore morphological variation among *R. cerasi* populations in different geographical parts of Tehran and Alborz province in Iran using multivariate analysis of distance

measurements among morphological landmarks. The cherry fruit fly populations were collected from two different localities in Alborz and Tehran province. Then twelve morphological characters were measured on twenty individuals of larva. Some of the most important characters as well as body length, spiracles distance in caudal region to each other, mandible's distance, mandible's palps distance, etc. Finally the result showed that there are not significant differences between two populations of *R. cerasi* and the overall conclusion is that morphological divergence cannot separate *R. cerasi* populations from each other and for population separation, other study methods such as molecular markers and DNA sequencing must be used.

P12. Biobanking at the NHM London

Jacqueline Mackenzie-Dodds

Natural History Museum, London
E-mail: jm@nhm.ac.uk

Molecular research at the NHM plays a major role in resolving questions on the diversity of life. Plants and animals are collected for traditional dry or alcohol preservation, and as a long term resource for molecular analysis. Molecular products are also extracted from 'traditional' collections. The NHM is developing new 'molecular collections' (in parallel to the 'traditional' collections) for future use beyond its own research programmes, making them accessible to the wider community and ensuring their long term value to science. Developments in the techniques of molecular biology, including next generation DNA sequencing, evaluation of DNA damage and repair, and epigenetics, create exciting research opportunities and drive forward the need to preserve biological samples for study by future generations.

Until now all NHM molecular collections have been managed by individual scientists and their research teams. New infrastructure, management and curation will consolidate and provide centralised accommodation for all existing and future archival molecular collections and integrate their development and management with the traditional collections. To ensure this, the NHM has built a central specialised storage facility within easy access of the NHM's research teams and the traditional collections.

Future-proofing the facility requires international legal compliance, identification of optimal storage methods, global standards, best practice and incorporation of new science and IT technologies. Exchange of knowledge in all these areas among partner institutions is vital to promote global sharing of genetic data.